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Toxicity of Agricultural Subsurface Drainwater from the San Joaquin Valley, California, to Juvenile Chinook Salmon and Striped Bass

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Abstract.—Juvenile chinook salmon *Oncorhynchus tshawytscha* (40–50 mm total length, TL) and striped bass *Morone saxatilis* (30–40 mm TL) were exposed to serial dilutions (100, 50, 25, and 12.5%) of agricultural subsurface drainwater (WWD), reconstituted drainwater (RWWD), and reconstituted seawater (IO). Agricultural subsurface drainwater contained naturally elevated concentrations of major ions (such as sodium and sulfate) and trace elements (especially boron and selenium), RWWD contained concentrations of major ions that mimicked those in WWD but trace elements were not elevated, and IO contained concentrations of total dissolved salt that were similar to those in WWD and RWWD but chloride replaced sulfate as the dominant anion. After 28 d of static exposure, over 75% of the chinook salmon in 100% WWD had died, whereas none had died in other dilutions and water types. Growth of chinook salmon in WWD and RWWD, but not in IO, exhibited dilution responses. All striped bass died in 100% WWD within 23 d, whereas 19 of 20 striped bass had died in 100% RWWD after 28 d. In contrast, none died in 100% IO. Growth of striped bass was impaired only in WWD. Fish in WWD accumulated as much as 200 μg/g (dry-weight basis) of boron, whereas fish in control water accumulated less than 3.1 μg/g. Although potentially toxic concentrations of selenium occurred in WWD (geometric means, 158–218 μg/L), chinook salmon and striped bass exposed to this water type accumulated 5.7 μg Se/g or less. These findings indicate that WWD was toxic to chinook salmon and striped bass. Judging from available data, the toxicity of WWD was due primarily to high concentrations of major ions present in atypical ratios, to high concentrations of sulfate, or to both. High concentrations of boron and selenium also may have contributed to the toxicity of WWD, but their effects were not clearly delineated.

Agricultural subsurface (tile) drainwater from irrigated lands on the west side of the San Joaquin Valley, California, is routinely discharged into the lower San Joaquin River. Tile drainage systems have been installed on the valley floor to alleviate waterlogging of soils and allow flushing of salts from the crop root zone (California State Water Resources Control Board 1987; Gaines 1988). Currently, over 31,161 hectares of tile-drained lands contribute about 78,944,000 m^3/year of saline drainwater to the river (Gaines 1988). Dissolved salts in tile drainwater are dominated by major ions such as sodium and sulfate (California State Water Resources Control Board 1987). In addition to high concentrations of major ions, tile drainwater may contain chromium, mercury, selenium, and other trace elements at concentrations approaching or exceeding the maximum limits recommended by the U.S. Environmental Protection Agency (USEPA 1986, 1987) for protecting freshwater life.

Fish kills have been reported in the San Luis Drain (Saiki 1986; Saiki and Lowe 1987) and in other waters that receive tile drainage in the San Joaquin Valley (USEPA 1972–1975). These kills were usually attributed to anoxic conditions, excessive buildup of ammonia and hydrogen sulfide, misuse of agricultural chemicals, or a combination of these factors (USEPA 1972–1975; Brown 1985) rather than to toxic effects from dissolved salts and trace elements.

Fish from several locations in the San Joaquin Valley contain elevated concentrations of arsenic, boron, cadmium, copper, mercury, lead, and selenium (Saiki 1986; Rasmussen et al. 1987; Saiki and May 1988; White et al. 1988; Saiki and Palawski 1990). However, only selenium concentra-

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tions in fish appear to be influenced by inflows of tile drainwater into the San Joaquin River system; the highest concentrations are in fish from reaches receiving drainwater. Saiki (1986) and Saiki and Lowe (1987) reported that mosquitofish *Gambusia affinis* from the San Luis Drain and Kesterson Reservoir accumulated as much as 370 μg Se/g whole-body dry weight (hereinafter, all elemental concentrations in biota refer to whole-body dry weights); they also reported selenium concentrations up to 23 μg/g in green sunfish *Lepomis cyanellus* from Mud Slough, a west-side tributary of the San Joaquin River that flows adjacent to Kesterson Reservoir. These concentrations exceeded 12 μg/g, the estimated threshold concentration for selenium toxicity that can elicit reproductive failure in sensitive species such as centrarchids (Lemly and Smith 1987).

The California State Water Resources Control Board (1987) recently proposed several water quality objectives for the San Joaquin River basin to protect fishing and other beneficial uses of the river. However, none of the data reviewed by the board were from long-term toxicological studies of the effects of tile drainwater on chinook salmon *Oncorhynchus tshawytscha* or striped bass *Morone saxatilis*, two of the most important anadromous species in the San Joaquin River.

The primary objective of this study was to determine if disposal of tile drainwater into the San Joaquin River threatens juvenile life stages of anadromous fish. To address this objective, we hypothesized that survival, growth, and body condition of juvenile chinook salmon and striped bass are adversely affected during long-term exposure (up to 28 d) to the drainwater. A secondary objective was to identify the toxic chemical constituents in tile drainwater. We hypothesized that elevated concentrations of total dissolved salt in tile drainwater (concentrations can approach 25,000–30,000 mg/L) are toxic to juvenile fish. We also hypothesized that atypical ratios of major cations and anions in the drainwater are toxic to juvenile fish. Finally, we compared the concentrations of trace elements (boron, molybdenum, and selenium) in tile drainwater and fish with the toxic threshold concentrations reported by other investigators to determine if elemental concentrations were sufficiently elevated to be toxic to juvenile fish.

Methods

Chinook salmon fingerlings (40–50 mm total length, TL) were obtained from the Merced River Fish Facility (about 6 km south of Snelling in Merced County) on March 23, 1988; striped bass fingerlings (30–40 mm TL) were obtained from the Central Valleys Fish Hatchery (1.2 km northwest of Elk Grove in Sacramento County) on June 6, 1988. Both hatcheries are operated by the California Department of Fish and Game.

Fish were transported from the hatcheries to a laboratory in Los Banos, Merced County, in polyethylene bags filled with oxygenated hatchery water. To avoid unnecessary stress on fish, each bag was placed inside a covered ice chest that, in the case of chinook salmon, also contained crushed ice. Prior to the experiments, fish were acclimated in holding tanks for either 6 d (chinook salmon) or 10 d (striped bass). The holding tanks contained clean water from the hatcheries at either 12°C (chinook salmon) or 18°C (striped bass). During the acclimation period, fish were fed daily with a maintenance ration of BioDiet®[2] number 4 crumbles (BioProducts, Inc., Warrenton, Oregon).

Experimental design.—Tile drainwater (WWD) was collected from Sump 20 on the Westlands Water District (about 10 km south of Mendota in Fresno County). As judged by eight separate ranked measurements of boron, molybdenum, selenium, and conductivity made in 1986–1987 (T. M. Garvey, Westlands Water District, unpublished data), we identified this sump as the worst of 63 active sumps in the Westlands. Reconstituted drainwater (RWWD) and Instant Ocean® (IO) were prepared according to procedures described by Hamilton et al. (1989). Our goal was to formulate RWWD to the concentrations of major ions occurring in WWD and to formulate IO to the concentration of total dissolved salt occurring in WWD (Table 1).

Chinook salmon and striped bass were exposed to serial dilutions (100, 50, 25, and 12.5%) of WWD, RWWD, and IO. Dilution waters were obtained from critical habitats occupied by juvenile life stages of these two species. For tests with chinook salmon, dilution water was collected from the San Joaquin River at Crows Landing Road (SJR; about 23 km south of Mendota in Stanislaus County). For tests with striped bass, dilution water was collected from the Sacramento–San Joaquin Delta at the Tracy Fish Collection Facility

[2] Reference to trademarks and trade names of manufacturers does not imply government endorsement of commercial products.

TABLE 1.—Concentrations (mg/L) of salts used to formulate reconstituted test waters. Actual concentrations were adjusted to the conductivities of waters they attempted to mimic.

Salts and conductivity	Reconstituted water[a,b]			
	RWWD	RSJR	RDELTA	IO
$CaCl_2$			53	
$CaSO_4 \cdot 2H_2O$	1,680	395		
KCl	42	13	4	
$MgSO_4 \cdot 7H_2O$	1,328	416	111	
NaCl	1,520	381	26	
$NaHCO_3$	196	235	91	
Na_2SO_4	11,115	59		
Instant Ocean				15,138
Conductivity (μS/cm at 25°C)	28,000	2,000	395	28,000

[a] RWWD, reconstituted drainwater; RSJR, reconstituted river water, RDELTA, reconstituted delta water; and IO, reconstituted seawater.

[b] For RWWD, RSJR, and RDELTA, salts were added to deionized water; for IO, salts were added to charcoal-filtered tap water.

(DELTA; in Contra Costa County about 13 km northwest of Tracy, San Joaquin County).

Supplemental controls for the chinook salmon tests included reconstituted river water (RSJR) and water from the Merced River Fish Facility (MR). Supplemental controls for the striped bass tests included reconstituted delta water (RDELTA) and water from the Central Valleys Fish Hatchery (EGH). Reconstituted river water was formulated to contain about the same concentrations of major ions as SJR, and RDELTA was formulated to contain about the same ionic concentrations as DELTA (Table 1).

Procedures.—Tests were conducted as 28-d static renewal exposures. Test chambers were placed in baths of continuously circulating refrigerated water, which maintained temperatures at 12°C for chinook salmon and 18°C for striped bass. Test waters were either collected or reconstituted every 4 d and aged for at least 24 h in refrigerated water baths before use. Photoperiods of both tests were standardized at 16 h light : 8 h dark.

Test chambers consisted of 38-L glass aquaria with clear Plexiglas® covers. The placement of aquaria in water baths was determined beforehand by a double-blind draw. All treatments and water types were duplicated.

Ten fish of similar length were placed into each test chamber. Fish were fed about 2% of their body weight twice daily with BioDiet number 4 crumbles, once in the morning and again in the afternoon. Analysis of six samples of BioDiet for trace element concentrations (μg/g) yielded the following (element, geometric mean, range): boron, 2.2, <2.5–3.5; molybdenum, 0.30, <0.34–0.49; and selenium, 1.6, 1.5–1.7. These values were similar to background concentrations in aquatic forage organisms from uncontaminated waters (Ohlendorf et al. 1986; Saiki and Lowe 1987). Each test chamber was cleaned daily, and about 80% of the test water was replaced.

Temperature, dissolved oxygen, pH, and conductivity were measured daily in each test chamber just before each water change. Whenever new batches of water were collected or reconstituted, we took water samples for measurement of bicarbonate, carbonate, and total alkalinity; calcium, magnesium, and total hardness; total dissolved salt; turbidity; ammonia; chloride; sulfate; sodium; and trace elements.

Survival of fish was monitored daily and dead fish were removed. On day 0, all fish were measured for TL and weight (W); on days 14 and 28, surviving fish were remeasured to estimate growth rates and relative condition factors ($K_n = W/a\text{TL}^n$; LeCren 1951). On day 28, all remaining live fish were killed by asphyxiation and saved for analysis of trace elements. Bioconcentration factors for trace elements were estimated by dividing the concentrations in fish by the mean concentrations in water to which the fish had been exposed.

Routine analyses of water quality.—Water temperature and dissolved oxygen concentrations were measured with a YSI® model 57 oxygen meter; pH, with either a VWR® model 55 Digital Mini-pH-Meter or a Cole–Parmer® model 5994 Digisense® pH meter; conductivity, with a LaMotte® Multirange model DA conductivity meter; and turbidity, with a LaMotte model BH-2 turbidity meter. Total alkalinity was determined by potentiometric titration to pH 4.5 (APHA et al. 1980). Total dissolved salt was measured by drying filtered water samples in a convection oven at 103–105°C (APHA et al. 1980).

Calcium, magnesium, and total hardness were determined from titration with a HACH® model HAC-DT Total-and-Calcium Hardness kit. Calcium and magnesium concentrations were estimated from standard conversion factors (HACH Co., Loveland, Colorado). Ammonia (as ammonia-nitrogen) was determined colorimetrically by the Nessler method (APHA et al. 1980); chloride, by mercuric nitrate buret titration (HACH); sulfate, by the SulfaVer® 4 method (HACH); and sodium, with an Orion® model 84-11 ROSS® sodium electrode.

TABLE 2.—Concentrations of selected major ions and related physicochemical variables in various water types for the chinook salmon tests. Statistics: M, geometric mean; R, range. Sample size for all water types: $N = 7$.

Variable	Statistic	Water type[a] WWD	RWWD	IO	SJR	RSJR	MR
Ammonia (mg/L)	M	1.6	1.4	3.8	0.49	0.28	0.036
	R	1.3–2.2	1.1–2.3	3.4–4.8	0.44–0.74	0.12–0.88	0.010–0.077
Calcium (mg/L)	M	456	288	241	88	86	2.5
	R	440–480	240–370	210–260	72–100	68–96	2.4–2.6
Chloride (mg/L)	M	1,240	978	7,330	253	239	2.3
	R	1,100–1,400	870–1,100	7,200–7,600	190–310	230–250	2.0–2.7
Magnesium (mg/L)	M	182	121	532	40	36	0.60
	R	160–200	98–160	490–560	27–49	32–42	0.51–0.68
Sodium (mg/L)	M	5,780	4,140	4,290	236	230	1.6
	R	4,700–7,300	3,100–5,900	3,800–5,200	170–280	210–270	1.3–1.8
Sulfate (mg/L)	M	13,300	9,170	1,120	316	391	0.00
	R	11,000–17,000	7,000–14,000	900–1,300	230–370	350–490	0.00–0.00
Total alkalinity (mg/L as $CaCO_3$)	M	187	131	276	176	137	14.9
	R	180–190	120–160	230–310	150–220	130–150	5.7–18
Total dissolved salt (mg/L)	M	20,500	14,300	16,000	1,160	1,280	28
	R	18,000–23,000	12,000–18,000	16,000–16,000	900–1,400	1,000–2,500	10–50
Total hardness (mg/L as $CaCO_3$)	M	1,890	1,220	2,780	381	364	8.8
	R	1,800–2,000	1,000–1,600	2,500–2,900	300–450	300–410	8.6–9.0
Turbidity (NTU)[b]	M	0.41	0.192	0.35	14.5	0.180	0.36
	R	0.21–0.74	0.045–0.30	0.25–0.51	4.5–52	0.020–0.30	0.22–0.46

[a] Water types are WWD, tile drainwater from Sump 20 in the Westlands Water District; RWWD, reconstituted drainwater; IO, reconstituted seawater; SJR, water from the San Joaquin River at Crows Landing Road; RSJR, reconstituted river water; and MR, water from the Merced River Fish Facility.
[b] Nephelometric turbidity units.

Trace element analyses and quality control.—Water samples collected for analysis of boron, chromium, copper, molybdenum, and selenium were filtered through 0.4-μm polycarbonate membranes, acidified to pH less than 2 with nitric acid (Ultrex® II Ultrapure Reagent, J. T. Baker Inc., Phillipsburg, New Jersey), then stored frozen (−10°C) in 500-mL acid-washed polyethylene bottles (I-Chem Research, Hayward, California). Fish samples collected for analysis of boron, molybdenum, and selenium were wrapped in polyethylene, double-bagged, then stored frozen (−10°C).

All water and fish samples were analyzed for trace elements by the National Fisheries Contaminant Research Center in Columbia, Missouri. Except for selenium, the concentrations of trace elements in water samples were analyzed without prior digestion; samples for analysis of selenium were digested with a persulfate oxidation–hydrochloric acid procedure. For determinations of chromium and copper, subsamples were diluted with equal volumes of 20% nitric acid and then analyzed with a Perkin–Elmer® model 5000 atomic absorption spectrophotometer equipped with a graphite furnace. Zeeman background correction was needed in the analysis of chromium because of the high salt content of some samples; conventional deuterium-arc background correction was used for the analysis of copper. Water samples were analyzed for boron and molybdenum with a Perkin-Elmer Plasma 40 inductively coupled argon plasma emission spectrometer; selenium was analyzed with a Varian SpectraAA-20 atomic absorption spectrophotometer equipped with a Varian VGA-76 hydride generator and a Varian PSC-56 programmable autosampler.

Fish samples were lyophilized to determine moisture content. Before analysis of boron, aliquots of the samples were digested with a nitric acid–hydrogen peroxide microwave procedure. For analysis of molybdenum, aliquots were digested with a nitric acid–hydrogen peroxide microwave procedure, followed by treatment with indium (a precipitating agent) to concentrate molybdenum from solution; the precipitate was then dissolved in concentrated nitric acid. Aliquots for selenium determination were digested with a nitric acid–magnesium nitrate dry-ash procedure. Boron and magnesium were determined by inductively cou-

TABLE 3.—Concentrations of selected major ions and related physicochemical variables in various water types for the striped bass tests. Statistics: M, geometric mean; R, range. Sample size for all water types: $N = 7$.

Variable	Statistic	Water type[a] WWD	RWWD	IO	DELTA	RDELTA	EGH
Ammonia (mg/L)	M	0.581	0.051	1.060	0.1080	0.066	0.093
	R	0.000–4.8	0.000–0.34	0.050–5.1	0.0025–0.38	0.000–0.22	0.000–0.43
Calcium (mg/L)	M	436	372	217	25	25	25
	R	440–440	350–400	190–240	18–34	16–38	22–27
Chloride (mg/L)	M	1,040	947	5,540	89	79	11.1
	R	970–1,100	930–970	4,800–6,200	67–120	52–120	9.5–12.0
Magnesium (mg/L)	M	131	126	432	14	14.4	16
	R	120–140	110–140	390–460	11–20	8.5–20	14–17
Sodium (mg/L)	M	4,400	4,380	3,210	56	50	18
	R	3,600–4,900	3,900–4,800	2,900–3,500	39–84	27–74	17–19
Sulfate (mg/L)	M	9,200	8,860	846	41	43	0.00
	R	7,400–10,000	8,300–9,600	750–900	28–72	20–64	0.00–0.00
Total alkalinity (mg/L as $CaCO_3$)	M	166	118	289	81	73	146
	R	150–170	120–120	230–370	73–94	43–110	150–150
Total dissolved salt (mg/L)	M	15,600	14,700	12,100	315	280	221
	R	14,000–17,000	15,000–15,000	12,000–13,000	250–450	140–390	180–260
Total hardness (mg/L as $CaCO_3$)	M	1,630	1,450	2,320	119	121	126
	R	1,600–1,600	1,400–1,500	2,200–2,400	96–160	74–180	120–130
Turbidity (NTU)[b]	M	0.24	0.37	0.41	47	0.082	0.171
	R	0.12–0.33	0.11–0.65	0.15–0.62	32–62	0.010–0.16	0.010–0.38

[a] Water types are WWD, tile drainwater from Sump 20 in the Westlands Water District; RWWD, reconstituted drainwater; IO, reconstituted seawater; DELTA, water from the Sacramento–San Joaquin Delta at the Tracy Fish Screen; RDELTA, reconstituted delta water; and EGH, water from the Central Valleys Fish Hatchery.
[b] Nephelometric turbidity units.

pled argon plasma emission spectrometry; selenium was determined by hydride generation atomic absorption spectrophotometry.

Spectrometers were calibrated with the following certified reference solutions: National Bureau of Standards (NBS) 3100 series; NBS water number 1643b; NBS Se 3149; NBS standard reference materials (SRM) 3134; NBS SRM 3107; International Atomic Energy Agency (IAEA) fresh water number W4; and USEPA waters number 1 and number 2. Recoveries of elements from these reference solutions were either within or very close to their certified ranges. One analytical problem during analysis of water samples was the considerable dilution required to reduce salt concentrations, which resulted in higher detection limits.

Statistical analyses.—Toxic effects on percent survival, growth, and body condition of fish were evaluated by analysis of variance (ANOVA). All data were either arcsine-transformed (if percentages) or logarithmically transformed. Depending on the hypothesis, treatment means were compared against the control mean with Dunnett's t-test, or means were simultaneously compared with the Tukey–Kramer honestly significant difference (hsd) test. Spearman rank correlation (r_s) analyses were used to evaluate relations among (1) exposure dilutions, (2) the survival, growth, and body condition of fish, (3) concentrations of trace elements and BCFs, and (4) water quality variables.

Results

General Water Quality

In contrast to seawater, which is dominated by sodium chloride, the ionic composition of WWD was dominated by sodium sulfate (Tables 2, 3). Sodium sulfate dominated in SJR and sodium chloride in DELTA.

Judging by measurements of various physicochemical variables (Tables 2, 3), we achieved appropriate ionic ratios for RWWD, RSJR, and RDELTA, and total dissolved salt concentrations for RSJR and RDELTA. However, the concentrations of total dissolved salt in both RWWD and IO were as much as 30% less than anticipated, probably because of incomplete dissolution or precipitation in the mixing tanks.

Test chambers were maintained at ± 1°C of the target values by refrigerated water baths. The concentrations of dissolved oxygen generally aver-

TABLE 4.—Dissolved concentrations (μg/L) of trace elements in various water types used in tests with chinook salmon and striped bass. Statistics: M, geometric mean; R, range. $N = 3$ for boron and molybdenum; $N = 21$ for chromium, copper, and selenium. Water types are identified in Tables 2 and 3.

Water type	Statistic	Trace element B	Cr	Cu	Mo	Se
		Chinook salmon				
WWD	M	48,800	25	4.0	766	218
	R	44,000–53,000	15–36	2.5–7.5	650–910	54–290
RWWD	M	<200	0.97	0.6	<50	0.35
	R	<200–<200	<0.80–2.4	<1.2–1.3	<50–<50	<0.40–0.90
IO	M	2,650	19.5	13	<50	1.1
	R	2,400–2,800	9.7–26	11–18	<50–<50	<0.40–2.2
SJR	M	1,170	0.69	2.0	<50	6.5
	R	900–1,600	<0.90–6.8	<2.5–4.0	<50–<50	4.8–8.1
RSJR	M	<200	0.61	0.86	<50	<1.29
	R	<200–<200	<0.80–2.8	<1.2–2.1	<50–<50	<0.60–<1.4
MR	M	<200	0.50	1.5	<50	0.24
	R	<200–<200	<0.90–1.4	<2.5–3.8	<50–<50	<0.40–0.40
		Striped bass				
WWD	M	49,300	24	4.3	663	158
	R	45,000–52,000	17–34	3.3–6.5	590–760	130–270
RWWD	M	<200	<1.5	<2.3	<50	0.26
	R	<200–<200	<1.5–<1.5	<2.3–<2.3	<50–<50	<0.50–0.50
IO	M	2,340	17.8	8.8	32	1.06
	R	2,100–2,600	5.3–32	7.5–12	<50–50	<0.50–2.6
DELTA	M	139	0.61	1.7	32	0.53
	R	<200–270	<0.60–2.5	<2.8–4.1	<50–50	<0.40–1.3
RDELTA	M	192	0.41	<2.3	46	<0.37
	R	<200–710	<0.60–1.4	<2.3–<2.3	<50–80	<0.30–<0.50
EGH	M	<200	5.1	<2.8	<50	<0.42
	R	<200–<200	3.4–6.9	<2.8–<2.8	<50–<50	<0.30–<0.50

aged 5–6 mg/L; pH ranged from 7.3 to 8.2. Conductivities varied according to water type and treatment.

Dissolved concentrations (μg/L) of trace elements in the various water types ranged as follows: boron, <200–53,000; chromium, <0.60–36; copper, <1.2–18; molybdenum, <50–910; and selenium, <0.30–290 (Table 4). In general, concentrations of boron, chromium, molybdenum, and selenium were highest in WWD; the concentration of copper was highest in IO.

Chinook Salmon Tests

Survival of chinook salmon was significantly reduced (Dunnett's one-tailed t-test, $P \leq 0.05$) by exposure to 100% WWD but not to diluted WWD or to other water types. After 28 d of exposure, survival of chinook salmon averaged 23% in 100% WWD, compared with 100% in all other dilutions and water types. Deaths first occurred in 100% WWD on day 19, then progressed at a rate of about 1.6 fish/d through the end of the 28-d test. On day 28, survival of chinook salmon was significantly lower (Tukey–Kramer hsd test, $P \leq 0.05$) in 100% WWD than in 100% RWWD and 100% IO.

Lengths and weights of chinook salmon were significantly lower (Dunnett's one-tailed t-test, $P \leq 0.05$) after 14 d and 28 d of exposure to WWD and after 28 d of exposure to RWWD, but not after exposure to IO (Figures 1, 2). In WWD and RWWD, growth generally increased with dilution by SJR. On day 28 the lengths and weights were significantly less (Tukey–Kramer hsd tests, $P \leq 0.05$) for chinook salmon held in lower dilutions (i.e., 50% and 100%) of WWD and RWWD than in similar dilutions of IO; the lowest measurements were from fish in WWD. Chinook salmon held in most water types and treatments grew appreciably during the tests; however, on day 28, surviving fish held in 100% WWD averaged about 5% shorter and weighed 28% less than the averages for fish measured on day 0.

Relative condition factor of chinook salmon exhibited a dilution response on day 14 but not on day 28 in WWD and no response in RWWD or

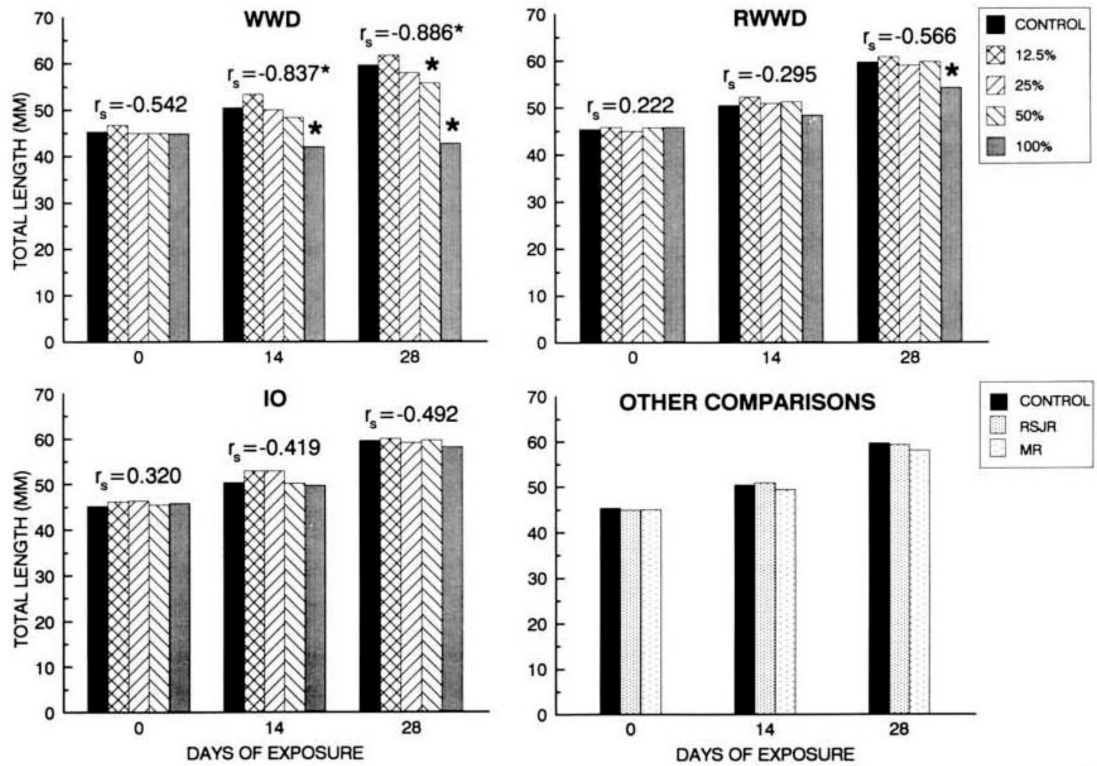

FIGURE 1.—Total lengths of chinook salmon fingerlings exposed to serial dilutions of three water types and controls. Spearman rank correlation coefficients (r_s) are given for relations of fish length to the strength of WWD, RWWD, and IO. Legend: WWD, tile drainwater; RWWD, reconstituted drainwater; IO, reconstituted seawater; CONTROL, dilution water from the San Joaquin River; RSJR, supplemental control composed of reconstituted river water; and MR, supplemental control from the Merced River Fish Facility (hatchery). Asterisk following an r_s value indicates a significant correlation (Student's two-tailed t-test; $P \leq 0.05$); asterisk above a bar indicates a significant difference from the CONTROL (Dunnett's one-tailed t-test, $P \leq 0.05$).

IO (Figure 3). However, on day 28, the K_n of chinook salmon held in 100% WWD was significantly lower (Tukey–Kramer hsd test, $P \leq 0.05$) than the K_n of chinook salmon held in either 100% RWWD or 100% IO. Fish held in 100% WWD readily ate food only during the first 8 d of the tests; thereafter, these fish mouthed the food and spit it out. During the final week, many fish appeared emaciated, and few showed any interest in feeding. In addition, all fish held in 100% WWD developed a blackish tinge, and many still exhibited well-defined parr markings. In contrast, chinook salmon held at higher dilutions of WWD and at all dilutions of other water types actively fed throughout the tests and appeared bright silver after 28 d.

Trace elements in chinook salmon that survived the 28-d exposure to WWD increased as dilution with SJR decreased (Table 5). In fish exposed to 100% WWD, boron averaged 192 μg/g, molybdenum averaged 0.31 μg/g, and selenium averaged 2.0 μg/g. By comparison, fish exposed to waters from control locations (e.g., SJR, MR) and from 100% RWWD and 100% IO all contained concentrations no greater than 5.8 μg B/g, 0.33 μg Mo/g, and 1.7 μg Se/g. Bioconcentration factors (BCFs) for the three trace elements were as follows (element, N, geometric mean, range): boron, 9, 2.9, 0.6–20.2; molybdenum, 9, 4.9, 0.5–8.3; and selenium, 10, 531.2, 8.8–5,338.8. The BCFs for boron and selenium concentrations in chinook salmon were inversely correlated ($P \leq 0.05$) with dissolved concentrations of these trace elements; the BCFs for molybdenum were not correlated ($P > 0.05$) with dissolved concentrations.

Striped Bass Tests

Survival of striped bass was affected by exposure to WWD and RWWD but not to IO (Figure 4). Moreover, dilution responses were evident in

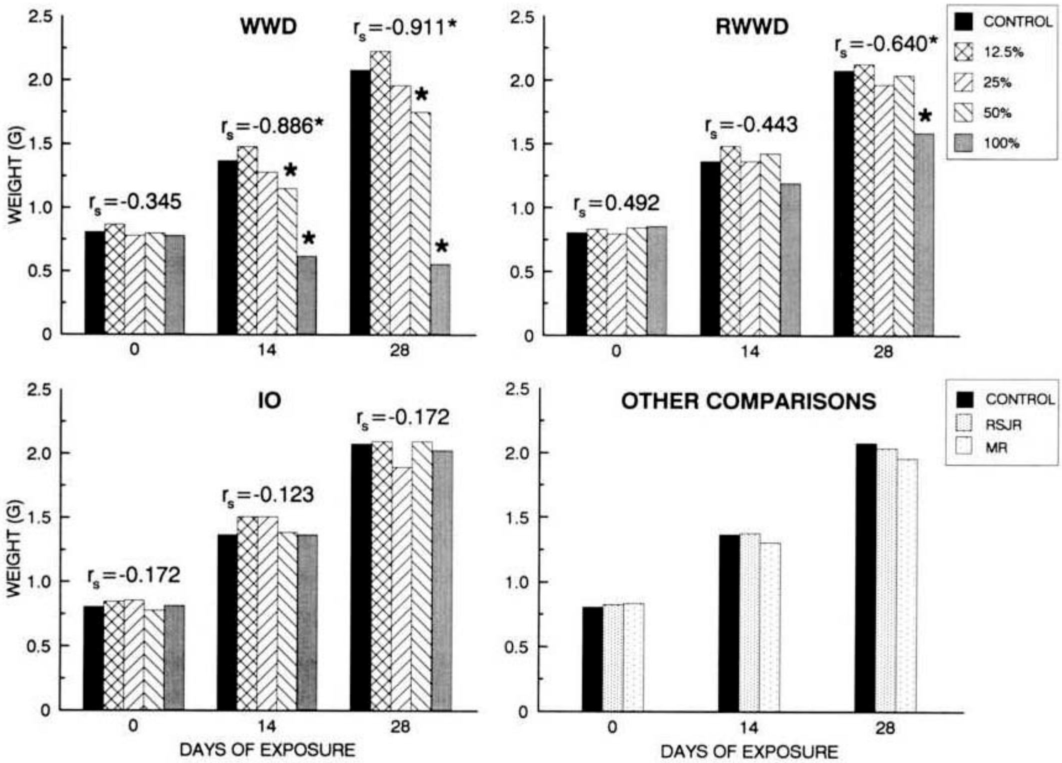

FIGURE 2.—Weights of chinook salmon fingerlings exposed to serial dilutions of three water types and controls. Legend is as in Figure 1, except r_s refers to fish weight versus water strength.

WWD and RWWD. After 28 d, the survival of fish in 100% WWD and 100% RWWD did not differ (Tukey–Kramer hsd test, $P > 0.05$); however, survival in these two water types was significantly lower ($P \leq 0.05$) than in 100% IO.

Growth and K_n of striped bass varied considerably (Figures 5–7) making detection of dilution–response patterns difficult. Because all fish exposed to 100% WWD died by day 23, no individuals from this treatment were measured for growth and K_n on day 28. Nevertheless, as indicated by data on day 14, striped bass in WWD exhibited a dilution response for weight (Figure 6) and K_n (Figure 7). In addition, whereas fish in diluted WWD and in all dilutions of RWWD and IO exhibited increases of 5% or more in length and 20% or more in weight between days 0 and 14, fish in 100% WWD grew only 3% in length and 4% in weight during the same time interval. On day 28, the K_n of fish in 100% RWWD was significantly (Tukey–Kramer hsd test, $P < 0.05$) lower than the K_n of fish in 100% IO.

Mean concentrations (μg/g) of trace elements in striped bass that survived 28 d in various dilutions of WWD ranged as follows: boron, 2.0–6.6; molybdenum, 0.23 to less than 0.33; and selenium, 1.4–1.4 (Table 5). Although striped bass in 100% WWD did not survive the 28-d exposure, fish that died after 15–23 d contained mean concentrations (μg/g) of elements as follows (N and range in parentheses): boron, 136 (6, 90–190); molybdenum, 0.22 (6, <0.31–0.48); and selenium, 1.3 (6, 1.1–1.4). Striped bass that survived 28 d of exposure to other water types contained the following concentrations (μg/g): boron, 2.2 to less than 3.1; molybdenum, 0.19 to less than 0.31; and selenium, 1.06–1.5. Bioconcentration factors for the three elements in fish that survived the 28-d-long tests were as follows (element, N, geometric mean, range): boron, 6, 5.2, 0.8–15.3; molybdenum, 6, 6.4, 4.6–9.9; and selenium, 7, 2,436.3, 749.5–6,045.1. The BCFs of all three elements in striped bass were inversely correlated ($P \leq 0.05$) with dissolved concentrations in water.

Discussion

Our study demonstrated that survival and growth were reduced among juvenile chinook

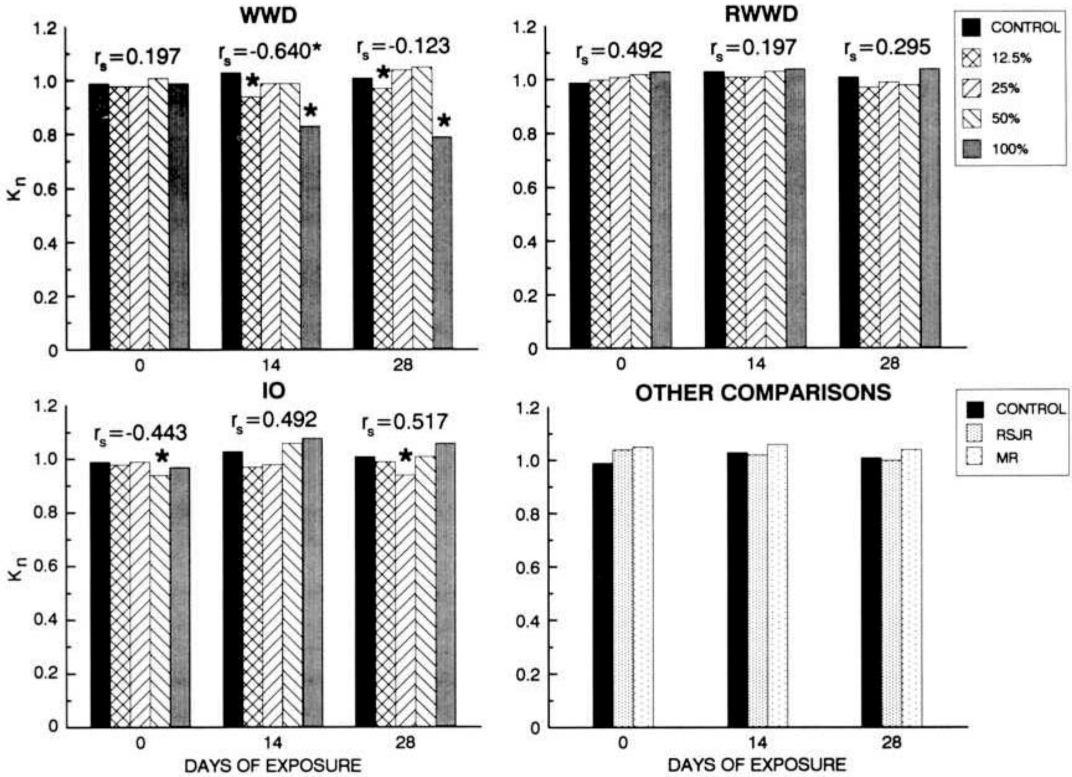

FIGURE 3.—Relative condition factors (K_n) of chinook salmon fingerlings exposed to serial dilutions of three water types and controls. Legend is as in Figure 1, except r_s refers to fish condition versus water strength.

salmon and striped bass exposed for 28 d to agricultural subsurface (tile) drainwater (WWD), which contained naturally elevated concentrations of major ions and trace elements. The toxicity of WWD was not directly related to high concentrations of total dissolved salt. Fish in reconstituted seawater (IO), whose concentrations of total dissolved salt were similar to those in WWD but where chloride replaced sulfate as the dominant anion, survived and grew well, whereas fish in WWD at lower salt concentrations (e.g., 50% WWD) experienced either some mortality (striped bass) or poorer growth (chinook salmon). Other researchers reported that juvenile life stages of chinook salmon (Wagner et al. 1969; Woo et al. 1978) and striped bass (Lal et al. 1977) can tolerate dilute seawater with dissolved salt concentrations as high as those in 100% WWD.

Although supporting data were not presented, Roberts et al. (1979) mentioned that both the concentration of dissolved salts (osmotic effects) and the composition (ratio) of its ions can influence the survival and reproduction of fish (and presumably other aquatic organisms). After exposing adult *Neomysis mercedis*, an opossum shrimp, to tile drainwater from the San Joaquin Valley and a reconstituted drainwater that contained only the major ionic components (calcium, magnesium, sodium, chloride, and sulfate), Bailey (1985) concluded that concentrations and ratios of major ions were probably responsible for short-term (<14-d) acute deaths in this species.

Galat et al. (1985) reported that Lahontan cutthroat trout *Oncorhynchus clarki henshawi* from brackish lakes in the western USA exhibited slight to moderate hyalin deposition in kidney tubules when concentrations of sulfate exceeded 2,000 mg/L and dissolved salts approached 5,000 mg/L. However, because anadromous salmonids are exposed to slightly higher concentrations of sulfate (2,700 mg/L) and much higher concentrations of total dissolved salt (nearly 35,000 mg/L) in typical seawater (Hem 1970) without suffering from severe hyalin degeneration, Galat et al. (1985) suggested that atypical ion ratios were probably responsible for the histopathological changes seen in trout from the brackish lakes. Burnham and Peterka (1975) reported that embryos and sac fry of fathead minnows *Pimephales promelas* experienced poor survival in brackish (conductivity, >12,000 μS/cm), sodium sulfate-type lake water, whereas those in brackish, sodium chloride-type lake water experienced much better survival. According to toxicity data tabulated by Hughes (1969, 1973), 1-month-old (30–52 mm TL) striped bass are also more sensitive to sodium sulfate (96-h LC50, 3,500 mg/L as sulfate) than to sodium chloride (96-h LC50, 5,000 mg/L as chloride). During our study, either the unusual ratios of major ions or the high concentrations of sulfate in WWD contributed to poor survival and growth of chinook salmon and striped bass, because fish in reconstituted drainwater (RWWD), which mimicked the major ion concentrations of WWD but lacked elevated trace elements, also exhibited poor survival and growth, whereas fish in IO showed no evidence of adverse effects.

Although mean values were not always significantly different, fish (especially chinook salmon) in 50% and 100% WWD experienced slightly poorer survival or growth than fish in similar dilutions of RWWD. These differences might have been due to one or more of the following conditions: higher concentrations of major ions in WWD than in RWWD (Tables 2, 3); toxic concentrations of trace elements in WWD that were absent from RWWD (Table 4); and other toxic but unmeasured variables in WWD that were absent from RWWD. We were unable to further evaluate the influence of high concentrations of major ions in WWD on the survival and growth of chinook salmon and striped bass because salts used to formulate RWWD either failed to dissolve completely or precipitated during storage in the mixing tanks. Also, we did not assess the effects of unmeasured variables in WWD.

The concentrations of several dissolved trace elements measured in 100% WWD (Table 4) indicate that only chromium (15–36 μg/L) and selenium (54–290 μg/L) exceeded ambient water quality criteria for the protection of aquatic organisms and their uses (USEPA 1986, 1987). However, as noted by the USEPA (1986), the criterion of 11 μg/L for chromium (4-d average concentration as hexavalent chromium that should not be exceeded more than once every 3 years) was established from chronic tests conducted mostly in soft water (total hardness, <50 mg/L as $CaCO_3$). Because the toxicity of hexavalent chromium decreases considerably as hardness increases (USEPA 1986), concentrations of chromium measured in 100% WWD, which is extremely hard (total hardness, 1,600–2,000 mg/L as $CaCO_3$; Tables 2, 3), were probably not acutely toxic.

The USEPA (1987) recommends a criterion of 5 μg/L (4-d average concentration that should not be exceeded more than once every 3 years) for acid-soluble selenium. However, this criterion rests on an assumption that fish and other predators in natural environments are exposed to selenium by

TABLE 5.—Whole-body concentrations (μg/g dry weight) of boron, molybdenum, and selenium in chinook salmon and striped bass surviving 28 d in serial dilutions of tile drainwater (WWD) and other undiluted (100%) water types (identified in Tables 2 and 3). Statistics: M, geometric mean; R, range. N = 6 except as follows (species, water type, element): chinook salmon, 100% WWD, B and Mo, N = 2; chinook salmon, 100% WWD, Se, N = 3; striped bass, 100% WWD and RWWD, B and Mo, N = 0; striped bass, 100% WWD, Se, N = 0; striped bass, RWWD, Se, N = 1.

Water type	Statistic	B	Mo	Se
		Chinook salmon		
12.5% WWD	M	10.3	<0.30	1.4
	R	6.4–16	<0.30–<0.30	1.3–1.4
25% WWD	M	12.4	0.21	1.5
	R	9.6–19	<0.30–0.59	1.4–1.7
50% WWD	M	19	0.17	1.9
	R	14–27	<0.30–0.36	1.5–5.7
100% WWD	M	192	0.31	2.0
	R	190–200	<0.30–0.67	2.0–2.1
RWWD	M	<1.6	0.17	1.4
	R	<1.6–<1.6	<0.30–0.31	1.3–1.5
IO	M	2.1	<0.32	1.4
	R	<1.6–5.8	<0.30–<0.33	1.3–1.5
SJR	M	<1.6	0.17	1.5
	R	<1.6–<1.6	<0.30–0.33	1.4–1.7
MR	M	1.2	<0.30	1.3
	R	<1.6–4.9	<0.30–<0.30	1.2–1.4
		Striped bass		
12.5% WWD	M	2.3	<0.31	1.4
	R	<3.8–5.3	<0.31–<0.31	1.2–1.5
25% WWD	M	2.0	<0.33	1.4
	R	<3.1–7.8	<0.31–<0.33	1.3–1.6
50% WWD	M	6.6	0.23	1.4
	R	4.3–9.4	<0.31–0.72	1.3–1.4
RWWD	M			1.5
IO	M	2.2	<0.31	1.06
	R	<3.8–4.0	<0.31–<0.31	0.43–1.4
DELTA	M	<3.1	0.19	1.3
	R	<3.1–<3.1	<0.33–0.40	1.1–1.4
EGH	M	<3.1	0.22	1.2
	R	<3.1–<3.1	<0.33–0.43	1.1–1.4

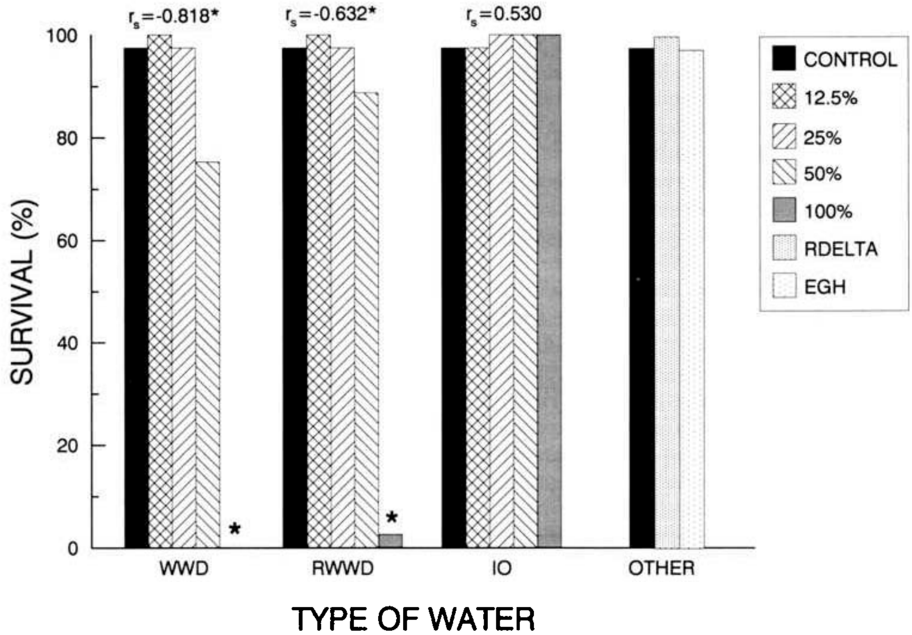

FIGURE 4.—Survival of striped bass fingerlings exposed to serial dilutions of three water types and controls. Spearman rank correlation coefficients (r_s) are given for relations of survival to the strength of WWD, RWWD, and IO. Legend: WWD, tile drainwater; RWWD, reconstituted drainwater; IO, reconstituted seawater; CONTROL, dilution water from the Sacramento–San Joaquin Delta; RDELTA, supplemental control composed of reconstituted delta water; and EGH, supplemental control from the Central Valleys Fish Hatchery. Asterisk following an r_s value indicates a significant correlation (Student's two-tailed t-test, $P \leq 0.05$); asterisk above a bar indicates a significant difference from the CONTROL (Dunnett's one-tailed t-test, $P \leq 0.05$).

ingesting forage organisms that have concentrated this element from water. Hamilton and Wiedmeyer (1990) reported that growth and survival of juvenile (2–3-g) chinook salmon were adversely affected by 90-d waterborne exposures to a 6:1 mixture of selenate and selenite (simulating the proportions found in tile drainwater) that collectively exceeded 35–70 μg/L. Palawski et al. (1985) reported that the 96-h LC50 of selenite for juvenile (63-d-old) striped bass exceeded 1,300 μg/L, whereas Klauda (1986) indicated that the 96-h LC50 of selenate for slightly older (72–76-d-old) striped bass was about 85,800 μg/L. In the studies of Palawski et al. (1985) and Klauda (1986), much lower concentrations of selenium might have been toxic if striped bass had been exposed longer to this element. Selenium concentrations measured in 100% WWD (Table 4) exceeded the toxic threshold reported by Hamilton and Wiedmeyer (1990), suggesting that this element was elevated and a possible source of chronic toxicity to fish (especially chinook salmon).

Although boron (44,000–53,000 μg/L) and molybdenum (590–910 μg/L) were measured in 100% WWD, ambient water quality criteria for these two elements are not available. However, both boron and molybdenum are relatively nontoxic (96-h LC50s exceeded 100,000 μg/L) to swim-up and advanced fry of chinook salmon (Hamilton and Buhl 1990). The USEPA (1986) reported that the minimum lethal dose of boron (administered as boric acid) to minnows (presumably cyprinids) was over 300 times higher than the concentrations measured in our samples of WWD. Eisler (1989) stated that aquatic organisms are comparatively resistant to molybdenum salts; adverse effects on growth and survival usually occurred at dissolved concentrations over 55 times higher than the maximum concentration measured in WWD. Collectively, these reports suggest that boron and molybdenum did not contribute greatly toward the overall toxicity of tile drainwater.

Chinook salmon and striped bass exposed to WWD accumulated high concentrations of boron but relatively little molybdenum or selenium (Table 5). Saiki and May (1988) suggested that freshwater fish generally contain less than 4 μg B/g, whereas fish exposed to WWD during the present

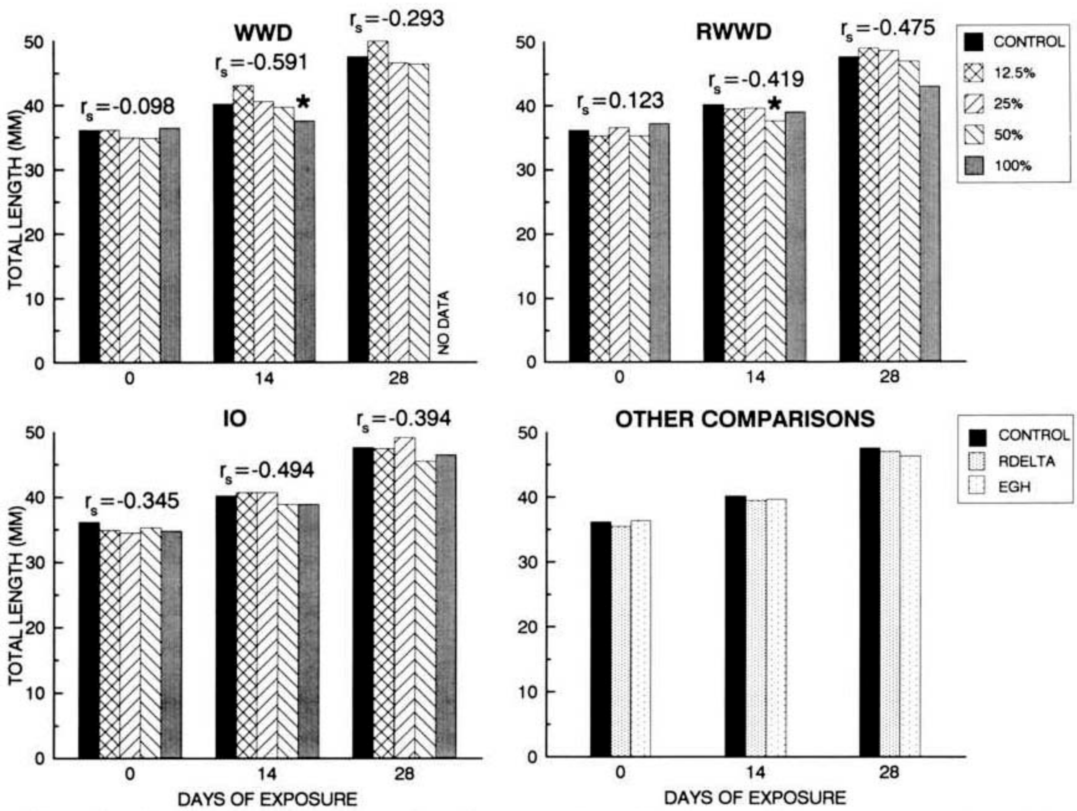

FIGURE 5.—Total lengths of striped bass fingerlings exposed to serial dilutions of three water types and controls. Legend is as in Figure 4, except r_s refers to fish length versus water strength.

study accumulated as much as 200 μg/g. Thompson et al. (1976) reported that alevins of coho salmon *Oncorhynchus kisutch* that succumbed during a 283-h exposure to boron (from sodium metaborate) measuring 328,000–656,000 μg/L accumulated about 900–1,560 μg/g (assumes 75% moisture) of the element. Thompson et al. (1976) reported BCFs ranging from 2.4 to 2.7, which are similar to the geometric means we estimated for chinook salmon (2.9) and striped bass (5.2). Thompson et al. (1976) speculated that high concentrations of boron in their coho salmon were caused by toxicity from this element. If they are correct, the high concentrations of boron in fish exposed to 100% WWD during our study indicate this element might have contributed to the overall toxicity of tile drainwater.

Fish are able to accumulate molybdenum and selenium in direct proportion to dissolved concentrations in the aqueous environment (Short et al. 1971; Ward 1973; Hodson et al. 1980; Bertram and Brooks 1986). However, Phillips and Russo (1978) reported that BCFs for many elements decrease as waterborne concentrations increase, a pattern we observed for both molybdenum and selenium during our study. Salmonids (and perhaps other euryhaline fish) have well-developed osmoregulatory systems that may allow them to excrete excess molybdenum efficiently, thereby maintaining relatively constant concentrations in their tissues (Ward 1973). According to Hodson and Hilton (1983), uptake of selenium from water by rainbow trout *Oncorhynchus mykiss* is very efficient at low waterborne concentrations, but uptake at high waterborne concentrations is limited, perhaps by saturation of gill absorption processes. Thus, the relatively low body burdens of molybdenum and selenium in chinook salmon and striped bass exposed to WWD were not unusual, because the kinetics of waterborne accumulation seemingly prevented fish from acquiring the high concentrations reported in some natural fish populations (which can accumulate these elements by foraging on contaminated foods; Eisler 1985,

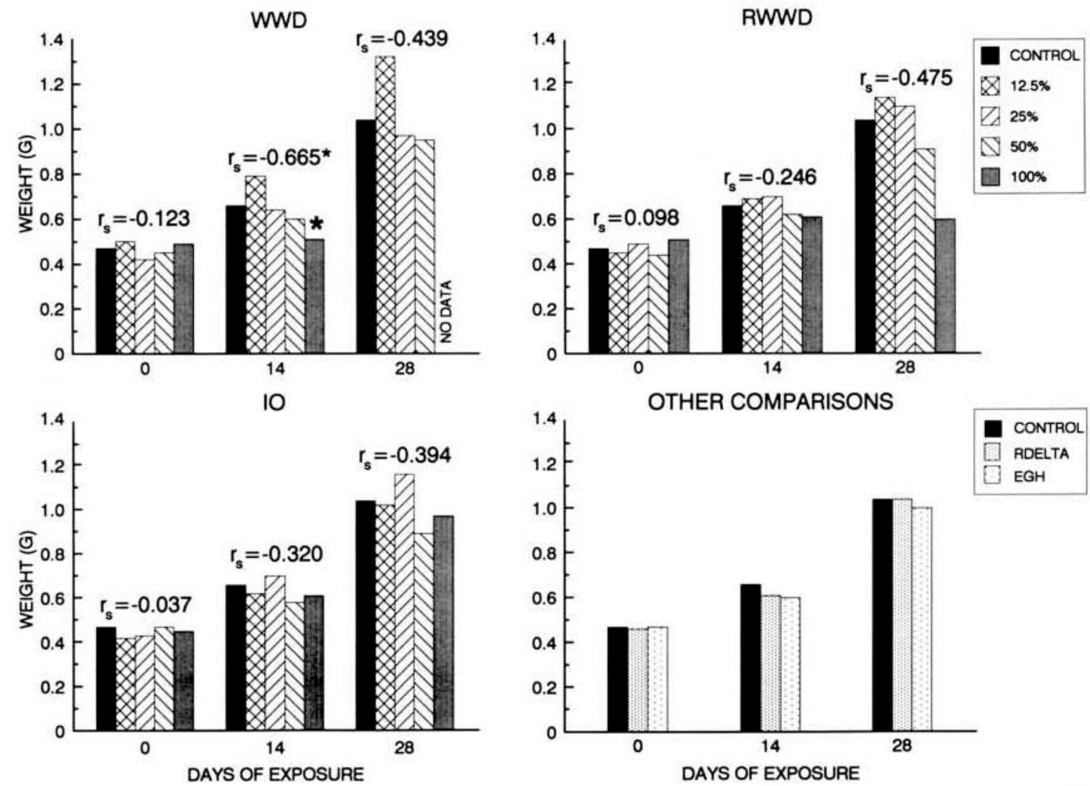

FIGURE 6.—Weights of striped bass fingerlings exposed to serial dilutions of three water types and controls. Legend is as in Figure 4, except r_s refers to fish weight versus water strength.

1989). In addition, the relatively short duration of our study (28 d) may have been insufficient for elemental concentrations in fish to reach steady state. Juvenile chinook salmon require at least 60 d to reach steady state with prevailing waterborne concentrations of selenium (Hamilton and Wiedmeyer 1990). Time periods for reaching steady state with dissolved concentrations of selenite and selenate generally have exceeded 28 d for other freshwater fish (e.g., Adams 1976; Gissel Nielsen and Gissel-Nielsen 1978; Sato et al. 1980; Lemly 1982; Bertram and Brooks 1986).

In conclusion, we demonstrated that tile drainwater from the San Joaquin Valley can be toxic to juvenile chinook salmon and striped bass. Moreover, the toxicity of tile drainwater is not a simple consequence of excessive concentrations of total dissolved salt. Tile drainwater in this study was toxic because fish were unable to tolerate the atypical ratios of major cations and anions constituting the dissolved salts, the high concentrations of sulfate, or both. Elevated concentrations of trace elements (especially boron and selenium) also may have contributed to the toxicity of tile drainwater, but their effects were not clearly delineated. To better document and understand the toxicity of tile drainwater to anadromous fish in the San Joaquin River system, we recommend implementation of a monitoring program that includes on-site or in situ toxicity tests for reaches that receive concentrated drainwater. We also recommend that regulatory agencies consider the potential toxicity from unusual ratios of major ions and high concentrations of sulfate in tile drainwater when formulating water quality objectives or standards.

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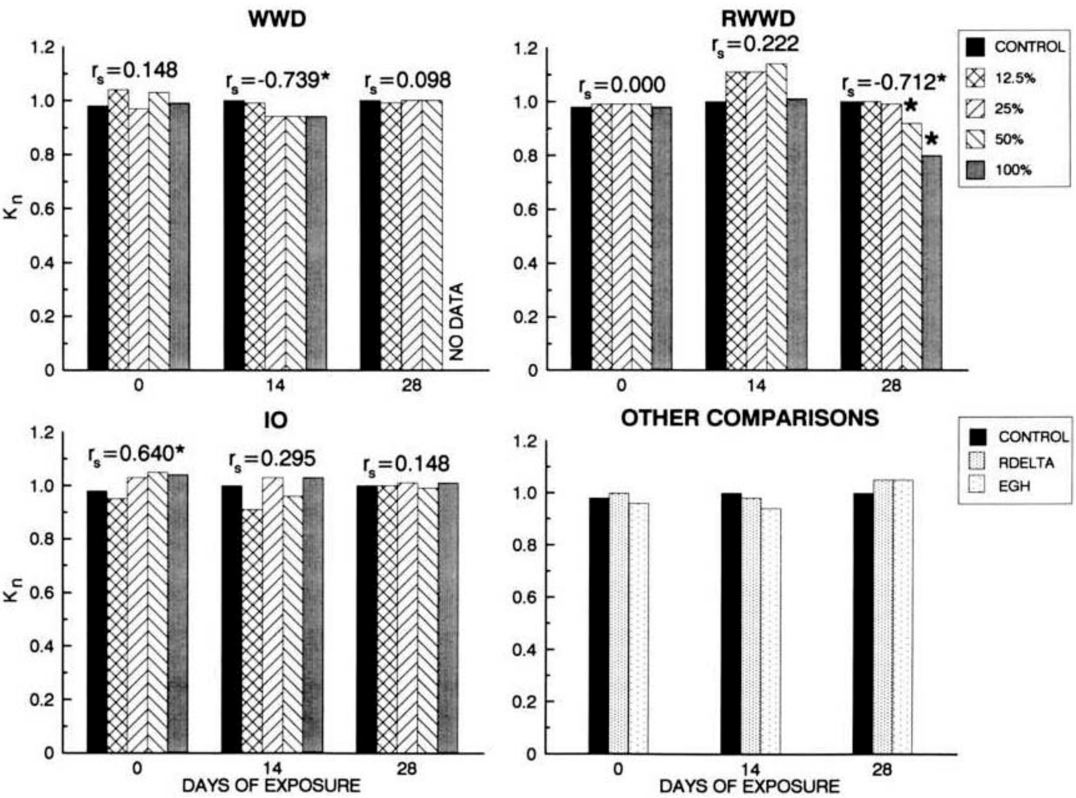

FIGURE 7.—Relative condition factors (K_n) of striped bass fingerlings exposed to serial dilutions of three water types and controls. Legend is as in Figure 4, except r_s refers to fish condition versus water strength.

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