Arch. Environ. Contam. Toxicol. 37, 227–235 (1999)

ARCHIVES OF
Environmental
Contamination
and Toxicology
© 1999 Springer-Verlag New York Inc.

Developmental Effects of Urban Storm Water in Medaka (*Oryzias latipes*) and Inland Silverside (*Menidia beryllina*)

L. Skinner,[1] A. de Peyster,[2] K. Schiff[3]

[1]San Diego County Department of Environmental Health, P.O. Box 129261, San Diego, California 92112-9261, USA
[2]Graduate School of Public Health, San Diego State University, San Diego, California 92182, USA
[3]Southern California Coastal Water Research Project, 7171 Fenwick Lane, Westminster, California 92683, USA

Received: 20 August 1998/Accepted: 14 March 1999

Abstract. Stormwater runoff in a coastal urban area (San Diego County, CA) produced significant toxicity to early life stages of medaka (*Oryzias latipes*) and *Menidia* (*M. beryllina*). Exposure of embryos to lower concentrations (5 to 25%) increased the incidence of abnormal swim bladder inflation and other teratogenic responses, whereas higher concentrations resulted in mortality or failure to hatch. Comparisons of EC50s for mortality and failure to hatch with concentrations of individual chemical pollutants (including Cd, Cr, Cu, Pb, Ni, and Zn) revealed low correlations; however, the correlation with total metals was high (−0.84) and corresponded with sample exceedences of Water Quality Criteria (WQC) for Cd, Cu, Pb, and Zn. This strong association between developmental toxicity and toxic metal content of storm water compared favorably with developmental anomalies reported in other studies. Analytical chemistry data for pesticides that may have been in these samples were limited to selected pesticides found usually below detection limits. Greater toxicity of the watershed effluents sampled was generally associated with more developed land surface and less open space. Both medaka and *Menidia* were found to be useful for studying effects of stormwater on embryonic and early larval development.

A major impetus for this study of coastal San Diego County, CA, storm water was that San Diego Bay provides a habitat for many important fish species and also continues to have water quality problems (McCain *et al.* 1992) despite increasing regulation of known point sources of pollution. Some of the pollution observed undoubtedly comes from stormwater runoff, which may be responsible for up to 30% of surface water quality problems nationwide (US EPA 1992). Stormwater pollutant loads in southern California may rival those contributed by traditional point sources (Cross *et al.* 1992). Impaired reproductive success and embryo viability had been observed in indigenous fish species collected from other coastal areas of California (Cross and Hose 1988; Spies and Rice 1988), and specific contaminants in storm water in the San Diego Bay region had been identified in several previous studies (Conway and Gilb 1990; Gadbois 1992; Schiff and Stevenson 1996).

Fish embryo and larval development are especially sensitive to the adverse effects of pollution (McKim 1977). Teratogenic effects are highly correlated with pollutant loads (Hose *et al.* 1981). Common abnormalities include central nervous system defects, spinal deformities, circulatory system abnormalities (*e.g.,* tube heart, pericardial edema, and hemostasis), and developmental delay (Weis and Weis 1987). Medaka (*Orizias latipes*) were selected as the primary test species in this study because of the abundant information about their biology and sensitivity to environmental contaminants. Their eggs are large (1–1.5 mm) and have a transparent chorion, allowing for ease in following developmental changes. Medaka are freshwater Japanese ricefish of the family *Oryziidae* (order *Cyprinodontiformes*) and are sensitive to a variety of chemicals causing developmental toxicity, including methyl mercury (Dial 1978), pesticides (Solomon and Weis 1979; Marty *et al.* 1991), metals (Hiraoka and Okuda 1983), dioxin (Wisk and Cooper 1990), and n-nitroso compounds (Marty *et al.* 1990b). Contaminants such as these have all been found frequently in storm water. Some samples were tested using both medaka and inland silverside (*Menidia beryllina*). *Menidia* are used more commonly in water monitoring programs, including stormwater effluent impacts in receiving waters of other California bays (Katznelson *et al.* 1993) and have demonstrated sensitivity to developmental toxicants (Borthwick *et al.* 1985; Middaugh *et al.* 1986, 1987, 1988, 1993, Middaugh and Whiting, 1995; US EPA 1988).

The main objective of this study was to characterize more fully the toxicity of urban stormwater runoff to aquatic life using fish developmental toxicity as an endpoint. Previous studies have shown that urban runoff can have appreciable toxicity to aquatic organisms (Marsh 1993; KLI 1994; Schiff and Stevenson 1996) but few provide toxicity information on effects of runoff in early life stages of fish. The present study evaluated a broad spectrum of developmental events in two species of fish, ranging from egg lethality and failure to hatch to changes in larval morphology.

Portions of this work were presented at the 15th annual meeting of the Society of Environmental Toxicology and Chemistry, Denver, Colorado, 1994

Correspondence to: L. Skinner

Materials and Methods

Sampling

Sampling sites (Figure 1) included smaller drainages with a single predominant type of land use and mass loading sites, characterized by multiple uses of the land in these larger watershed areas (Table 1). Paradise Creek and Fish Market are small drainages classified as urban; Yarrow is an industrial site; Park is residential and Top Gun was a construction site. All other sites shown in Figure 1 (except Chula Vista Marina) are classified as mass loading sites. Levels of contaminants in sediment near the Chula Vista Marina pipe outflow were known to be low, so runoff samples collected at this outflow were expected to cause little toxicity. Selection of other sites to be sampled was based on previous surveys of contaminant accumulation at storm drainages (Conway and Gilb 1990). For example, samples from Fish Market pipe in San Diego and Paradise Creek in National City were expected to be high in heavy metals, polychlorinated biphenyls, and total polyaromatic hydrocarbons. Grab samples were collected from the smaller drainages at mid- to low tide during the middle of storms occurring from December 1993 through March 1994.

Sampling was also performed at mass loading sites draining the most populated areas into bays and coastal waters. These samples were collected using automatic flow-weighted compositing samples between January and May 1994 and were required by a National Pollutant Discharge Elimination System (NPDES) permit (40 CFR 122, 123). Table 1 details the types of land use and percentage of total watershed for each of the mass loading sites sampled.

Samples collected from mass loading sites were also analyzed for 129 priority pollutants required by this NPDES permit, including metals, volatile and semivolatile compounds, certain pesticides (aldrin, dieldrin, DDT and its metabolites, endosulfan, endrin, heptachlor and toxaphene, and their methylated or sulfated forms), and 16 routine water quality parameters including pH, ammonia, alkalinity, hardness, and dissolved oxygen. Un-ionized ammonia ranging from 0.001–0.1 mg/L was calculated from total ammonia measurements using standard conversions taking into account the pH of the sample (Hampson 1977). Other details of effluent sampling and analysis have been reported elsewhere (Schiff and Stevenson 1996). Two samples, one each from a residential and a mass loading site, were split and tested side by side using medaka to gather preliminary information about whether toxicity was likely to be caused by particulate or dissolved pollutants. These samples were divided before filtering one portion through a 1.6-µm Whatman glass-fiber filter.

Fish Development Bioassays

Procedures used in this study were generally similar to those used by others performing bioassays of environmental samples using medaka or *Menidia* embryos and larvae (Marty *et al.* 1990b, 1991; USEPA 1988, 1989; Benoit *et al.* 1991). Embryos were obtained from Aquatic Research Organisms, Inc. (Hampton, NH). Eggs were examined under a dissecting microscope to verify that they had been fertilized and were viable and at the appropriate stage for beginning exposures (clear chorion, round shape, progression to at least blastula stage). Test exposures were begun at the late gastrula to late neurula stage. Eggs were not used if they were either obviously dead, at a later developmental stage than the rest of the embryos, or completely coated with debris from the egg cluster or spawning substrate. Staging of medaka was based primarily on Kirchen and West (1976), although other sources were consulted. *Menidia* were staged based on Lagler *et al.* (1977). After cleaning, sorting, and staging, eggs were rinsed in a 5% sodium chloride solution, then rinsed again with embryo rearing medium (Kirchen and West 1976).

Embryo-rearing medium (ERM) was also used as dilution and baseline control water. ERM contained NaCl, KCl, $CaCl_2$, and $MgSO_4$ (Kirchen and West 1976) and had a total salinity of 1.2 parts per thousand (ppt). *Menidia* have been shown to tolerate low salinities in the absence of other stressors with no complications (Middaugh *et al.* 1986). An initial test using water of varying salinities confirmed that *Menidia* could easily tolerate 1.3 ppt salinity. ERM was autoclaved, sealed, and kept for use as stock water. In order to provide adequate dissolved oxygen concentrations, ERM was aerated for 12–24 h prior to starting test exposures. The pH of ERM was also adjusted to 7.0–7.5 using sodium bicarbonate at a concentration of 4 g/L. Diluent water exposures were also performed to confirm that both medaka and *Menidia* controls had acceptable levels of successful hatch (at least 93%) without significant incidences of abnormalities.

Stormwater effluent samples were stored at 4°C and tested within 10 days after collection. Embryos were exposed in borosilicate glass scintillation vials, with 2 ml of test solution and 18 ml of headspace to maximize oxygen available for exchange (Marty *et al.* 1990a). Tests were static, nonrenewal exposures. Concentrations of stormwater used in this study were 5, 10, 25, 50, and 100% concentrations of effluent diluted with ERM. Eight embryos were placed in each exposure vial with four replicates for each concentration of effluent. Controls were exposed to ERM dilution water. Exposure vials were capped, sealed with Teflon tape, and opened only as necessary to remove dead embryos.

Exposure vials were maintained at 25–27°C and a 16:8 h light/dark cycle. The vials were inverted manually four times daily to simulate the movement of eggs in the natural environment. This movement aids in minimizing fungal growth and helps distribute chorionase, a hatching enzyme secreted by the embryo, over the interior surface of the chorion (Yamagami 1981). The estimated day prior to hatching or on hatching, usually between day 9 and 12 for medaka and day 7 and 9 for *Menidia*, the test solutions were replaced with fresh, aerated ERM in order to provide optimal conditions for successful swim bladder inflation. Several preliminary positive control experiments were conducted with both species of fish using a known teratogen, N-methyl-N-nitrosourea (MNU) to establish the sensitivity of these organisms to developmental toxicants and refine scoring methods. The relative sensitivity of the two fish species to stormwater effluents was also compared by exposure to split samples from three sites.

Scoring

Vials were coded before scoring so that the scorer did not know the effluent concentration or type of exposure (*e.g.*, stormwater effluent or diluent control). The scoring system used was adapted from one specifically designed for medaka (Shi and Faustman 1989) and was similar to those discussed by others (Solomon and Weis 1979; Marty *et al.* 1990b). The occurrence of an abnormality was noted for each individual with no attempt to grade the severity of the defect. Development was observed and recorded for egg, embryo, and larval stages by observing individuals in exposure vials placed under a dissecting scope at 40–80×. Embryos that died early in the exposure were identified by eggs with an opaque chorion (dead eggs). Later-stage embryo lethality was identified by lack of heartbeat. Failure to hatch was counted as embryos clearly remaining alive but failing to hatch by the end of the test. Occurrences of nine other adverse developmental endpoints were recorded in all embryos that hatched. The abnormalities scored were abnormal swim bladder inflation (included either normal hatching with partial swim bladder inflation or normal hatching without swim bladder inflation), pericardial edema, hemostasis, tube heart, hypopigmentation of the eyes, eyes of abnormal or unequal size, microcephaly, spinal abnormalities in the tail, and reduction in overall body size. Microcephaly was observed in only one larva exposed to one concentration of one stormwater sample. Eyes an unequal distance from the midline was also scored, but was not observed with any of the exposures. Most of these endpoints could actually be observed in stationary embryos before hatching and were

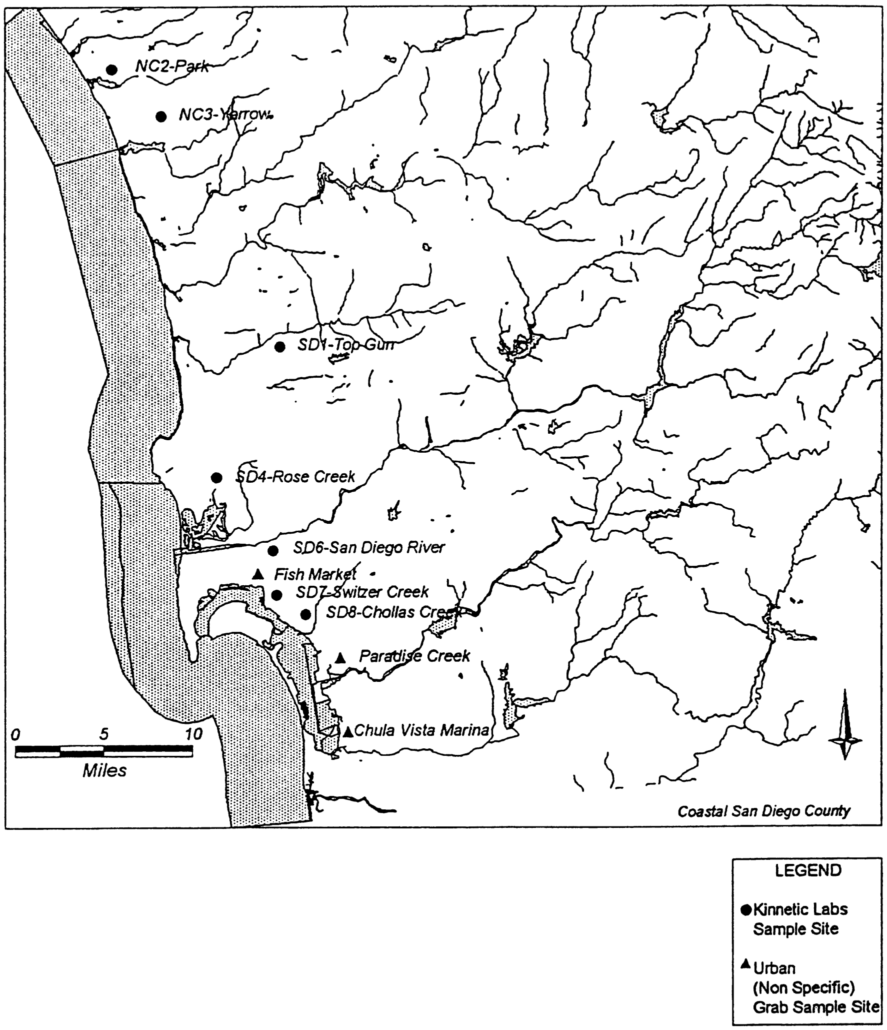

Fig. 1. Sample site locations

Table 1. Land use/land cover of watersheds for mass loading sample sites

Watershed	% Sampled*	Percent Land Use/Land Cover			
		Residential	Commercial	Industrial	Open
Rose Creek	100	16	15	8	60
San Diego River	99	30	8	4	57
Switzer Creek	100	45	22	2	31
Chollas Creek	55	63	15	2	20

* Percentage sampled out of the total acreage drained by the catchment
Adapted from Kinnetic Laboratories, Inc., 1994

counted as such if the embryo hatched. Scoring of hatched larvae that are constantly in motion was diminished by cooling exposure vials by placing them in a −10°C freezer for approximately 5 min, which was just enough to make it possible to note abnormalities like hatching with abnormal swim bladder inflation. Morphological abnormalities were ultimately counted only in larvae that hatched successfully; that is, even if a physical defect was observed in an embryo that died later before hatching the effect recorded for that individual would be embryo lethality. Frequency of response was always compared to that seen in diluent water controls.

Statistical Analysis

Responses in each of the four replicate test vials for a given concentration were averaged for a given concentration and sample to give a mean percent response for each endpoint. Results of blind and known control exposures were combined to give a baseline percent response for each endpoint. Data were normalized using the arcsine square-root transformation (US EPA 1989). Transformed data were analyzed by a one-way analysis of variance, followed by Tukey's honest significance difference analysis for pair-wise comparisons and a significance level of $p \leq 0.05$ using SPSS/PC+, 1994 version 4.1 (SPSS 1994).

The lowest observed adverse effect concentration (LOAEC) was identified as the lowest effluent dilution producing statistically elevated developmental toxicity over baseline controls. EC50s were estimated using simple linear regression. EC50s could not be calculated in all cases because either the variability was too high or the concentration response curve was biphasic. Responses for the two most frequently observed adverse effects, egg lethality and abnormal hatch, were compared with levels of 13 of the chemical constituents in composite mass loading site samples. Chemical concentrations were log transformed prior to analysis, as chemical data for effluent were log-normally distributed.

Correlations between toxicity (EC50s) and contaminant levels were calculated. Toxicity was also compared with exceedences of water quality criteria (WQC) (SWRCB 1991). WQC for five trace metal constituents (Cd, Cu, Ni, Pb, and Zn), based on hardness, were applied to chemical measurements of stormwater samples that were split for toxicity exposures. Samples were noted as significantly toxic, exceeding WQC, both, or neither. A sample was considered toxic if it produced either a statistically significant effect in any acute endpoint (egg lethality or failure of live eggs to hatch within the period of the test) or an elevated incidence of any teratogenic effect in larvae that hatched. A sample was classified as exceeding WQC if at least one trace metal concentration exceeded the accepted value.

Results

Figure 2 shows EC50s for the 10 stormwater samples that significantly elevated either medaka egg lethality or failure to hatch by at least one concentration of effluent. EC50s for egg lethality ranged from as little as 26% effluent at the Chollas Creek site, to no statistically increased egg lethality after exposure to even 100% effluent from the Park sampling site. Egg hatching within the expected time period was only scored in eggs that were still alive when the test was terminated. Thus, there were enough surviving eggs to determine an EC50 for failure to hatch only in samples shown toward the top of Figure 2, where only the high effluent concentrations were lethal (that is, roughly 80% effluent or greater in the Top Gun, Rose storm 2, and Park samples). EC50s calculated for failure to hatch when exposed to these three samples ranged from 27%, 41%, to 49% effluent, respectively. Very few surviving eggs were observed to hatch in samples with lower EC50s for egg lethality of 40% effluent or less. High egg lethality prevented rigorous calculation of EC50s for failure to hatch when scored only in surviving eggs. The most toxic effluent came from the Chollas Creek watershed sampling site which also had the highest percentage of developed land cover (80%) of the four mass loading sites examined (Table 1). High lethality was also noted in eggs exposed to samples from an industrial site (Yarrow) and the two urban sites (Paradise Creek and Fish Market). Samples from the Rose Creek mass loading site were highly variable, producing significantly elevated egg lethality at LC50s ranging from a low of 32% effluent to a high of 87% depending on the storm and the sampling method. Approximately 60% of the land cover draining to the Rose Creek watershed site is designated open space. Very little toxicity was associated with runoff from the much larger San Diego River watershed area, which has nearly the same percentage of open space as the Rose Creek watershed and roughly half the industrial and commercial area, but twice the residential area.

Table 2 shows results of scoring all samples for more subtle developmental abnormalities in medaka and *Menidia* that hatched, expressed as LOAECs. The types of morphological abnormalities seen in the embryos and larvae exposed to stormwater runoff in this region were similar to those reported in previous studies of fish exposed to individual pollutants. The most commonly observed statistically significant findings were larvae with incomplete or no swim bladder inflation (*i.e.*, abnormal hatch, 13/19 tests); spinal curvature (6/19); reduced overall size overall (5/19); larvae with eyes of abnormal or unequal size (5/19); and larvae with tube heart (1/19). Many other individual organisms appeared to have these and other developmental problems; however, if the incidence of a specific endpoint in a sample was not statistically elevated over controls at any effluent concentration tested then these findings were not considered significant here.

Table 2 highlights some differences in toxicity among the sites, storms sampled, and sampling methods. Fourteen of the 19 tests (74%) revealed significant increases in one or more adverse developmental effects in medaka or *Menidia* eggs or larvae. The variability in potency of these stormwater samples is readily apparent. The sample that was not expected to be toxic (Chula Vista) and samples from two other sites (Park composite and San Diego River) produced no significant developmental toxicity in medaka or *Menidia*. At the majority of sampling locations, any significant developmental defects observed generally occurred at concentrations ranging from 5% to 25%, as compared with the effluent concentrations of nearly 30% or more that were generally needed to cause significantly increased egg lethality or failure to hatch, shown in Figure 2. The most sensitive endpoint scored, swim bladder inflation, was recorded as abnormal in most of the tests. With the exception of the second storm sampled at the San Diego River site and tested in a medaka bioassay, the LOAECs producing abnormal swim bladder inflation generally ranged from 5–25%, while intermediate stormwater concentrations of 25 to 50% or greater tended to produce cardiovascular, musculoskeletal, and other morphological defects, as well as reduced overall size of surviving hatchlings. The unfiltered Park composite sample from this residential site produced no significant developmental toxicity in medaka, but the grab sample from the same storm caused abnormal swim bladder inflation at 25% effluent. The site with the greatest variety of adverse developmental endpoints was Rose Creek. As noted earlier, the acute toxicity of different samples from this site was highly variable (Figure 2). All developmental effects scored (except hypopigmentation) were seen in at least one of the three samples tested from Rose Creek. It seems reasonable to assume that multiple defects produced by this sample indicate the presence of multiple pollutants in the effluent from this watershed. Low toxicity to early life stages of fish was also seen in the San Diego River site samples, where the first storm sample produced no adverse effects, and the undiluted (100%) sample from this watershed composited during the second storm caused only a decrease in full swim bladder inflation and hemostasis in medaka and no observable effects in *Menidia*.

Table 2 suggests that medaka, a freshwater fish, and *Menidia*, an estuarine species, responded more or less similarly to these stormwater samples in that both showed effects in two of the three samples tested concurrently in splits from the same storms. EC50s for egg lethality and hatching success were essentially the same, but *Menidia* appeared to develop more subtle adverse effects at slightly lower effluent concentrations in the Chollas and Switzer Creek samples.

Samples from five storms collected at the mass loading sites were analyzed for up to 129 priority pollutants. The method of analysis was capable of detecting low ppb (0.02–1 μg/L) concentrations of pesticides and volatile and semivolatile organics, which were almost all nondetectable in these samples (not shown). Selected routine water quality parameters and concentrations of six metallic pollutants that were often found above detection limits are shown in Table 3. Total ammonia levels measured at the time the samples were collected from mass loading site effluents ranged from 0.1 to 1.0 mg/L. The majority of samples were at least moderately hard with $CaCO_3$ concentrations greater than 100 mg/L. This is important because hardness decreases bioavailability of metals, thereby

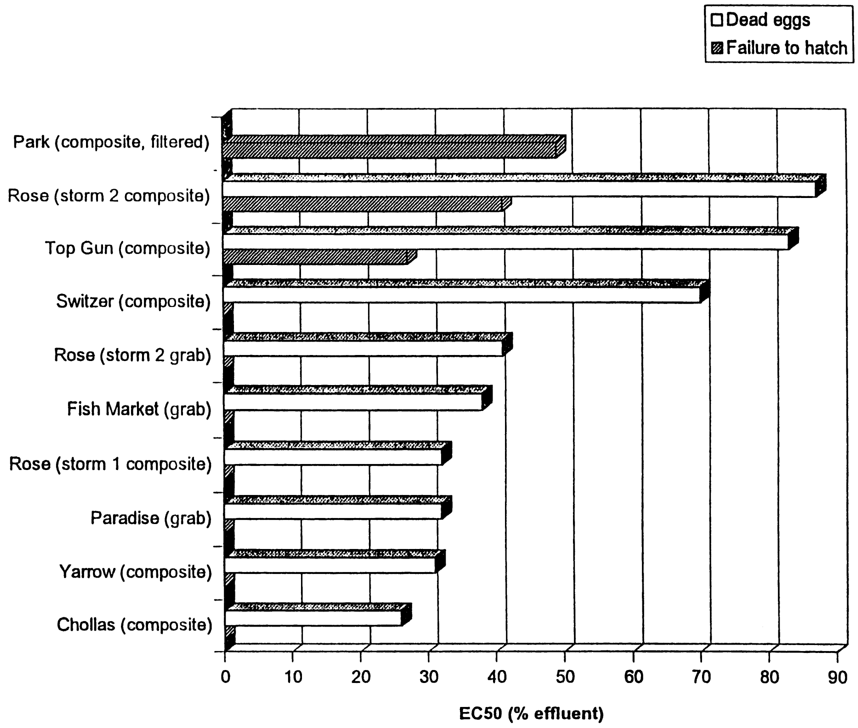

Fig. 2. Acute toxicity of stormwater samples to medaka eggs

Table 2. LOAECs (% storm water) causing developmental abnormalities in medaka and *Menidia* exposed to San Diego stormwater effluents

Sample Location and Type[a]	Abnormal Swim Bladder Inflation	Pericardial Edema	Hemostasis	Tube Heart	Hypopigmentation	Abnormal Eye Size	Spinal Curvature	Reduced Body Size
Medaka tests								
Chula Vista (g)	—[b]	—	—	—	—	—	—	—
Paradise (g)	5	—	—	—	—	—	—	—
Fish Market (g)	50	—	—	—	—	—	—	—
Yarrow (c)	5	—	—	—	—	—	—	—
Park (c)	—	—	—	—	—	—	—	—
Park (c, filtered)	—	—	—	—	—	—	—	—
Park (g)	25	—	—	—	100	—	25	50
Top Gun (g)	25	—	—	—	—	—	—	—
Rose (storm 1, c)	10	—	—	—	—	—	—	—
Rose (storm 2, c)	5	—	50	—	—	50	10	50
Rose (storm 2, g)	10	25	50	25	—	10	—	10
San Diego (storm 2, c)	100	—	100	—	—	—	—	—
Chollas (c, filtered)	25	—	—	—	50	—	25	—
Medaka and *Menidia* Comparison Tests								
San Diego River (storm 1 composite)								
Medaka	—	—	—	—	—	—	—	—
Menidia	—	—	—	—	—	—	—	—
Chollas Creek (composite)								
Medaka	—	—	—	—	—	10	10	
Menidia	5	—	—	—	25	10	10	25
Switzer Creek (composite)								
Medaka	25	—	—	—	—	—	—	—
Menidia	10	—	—	—	25	10	25	25

[a] (g)rab; (c)omposite

[b] Frequency observed not statistically different from control at any concentration tested

reducing their toxicity. Detection limits for metals shown in Table 3 were 0.2 µg/L for Cd; 1.0 µg/L for Cr and Pb; and 5.0 µg/L for Cu, Ni, and Zn. Chromium, lead, and zinc were above detection limits in all samples from mass loading sites; cadmium, copper, and nickel were also commonly found. Sum totals of metal pollutant levels ranged from 35 µg/L to 421 µg/L.

None of the correlations (r^2) between individual pollutant concentrations and the two developmental toxicity endpoints

Table 3. Analysis of stormwater from mass loading sites

Sample Location and Date	Un-ionized Ammonia (mg/L)	Hardness (mg/L)	Metals (µg/L) Cd	Cr	Cu	Pb	Ni	Zn	Total	Medaka Toxicity as Egg LD50 (% Effluent)
San Diego River										
2/17/94	0.005	240	0.3	2.1	8.8	24	<5.0	61	96	>100
3/24/94	0.006	200	<0.2	4.2	<5.0	7.4	<5.0	23	35	>100
Park										
2/4/94	0.003	325	0.6	2.0	15	16	5.0	180	219	>100
Rose Creek 2										
2/8/94	0.006	100	0.5	3.0	15	42	6.7	80	147	87
Switzer Creek										
3/24/94	0.010	63	0.5	3.6	15	29	<5.0	120	168	70
Rose Creek 1										
2/4/94	0.001	120	0.8	3.6	28	56	7.6	170	266	32
Yarrow										
1/25/94	0.008	99	0.8	3.0	24	14	10	210	262	31
Chollas Creek										
2/17/94	0.008	120	1.5	4.8	34	110	11	260	421	26

ND = no data
Data adapted from: Kinnetic Laboratories, Inc., 1994

seen most often (dead eggs and abnormal swim bladder inflation in hatchlings) was statistically significant. There was a strong inverse correlation (−0.84) between total metals and LD50s for egg survival (Table 3). Measures of toxicity also corroborated exceedences of regulatory trace metal WQC. Seventy-five percent of the samples that produced significant acute toxicity or impairment of normally developing eggs and larvae also exceeded WQC for at least one of the trace metals shown in Table 3. As many as four trace metals exceeded WQC in a single sample. The number of samples in which toxicity results did not corroborate exceedences in WQC was low; that is, only 12% of the samples were toxic when WQC values were not exceeded and 12% of the samples were not toxic when the WQC of at least one trace metal was exceeded.

Discussion

Collectively, these results illustrate three principal findings concerning urban stormwater effects on fish development. The first is that effluent concentrations as low as 5–10% produced statistically elevated abnormal fish embryo or larval development in the majority of these stormwater samples. Toxic concentrations of stormwater were comparable to those causing chronic toxicity to *Ceriodaphnia* in a prior study of this region (Schiff and Stevenson 1996). Significant daphnid toxicity was observed in that study with 12.5–25% effluent concentrations from Chollas Creek, while significant fish developmental toxicity resulted with exposure to 5–25% effluent concentrations from the same site in the present study. These newer findings indicate that stormwater runoff in this region has some potential for causing adverse impacts on local fish populations. Decreased viability of developing larvae can eventually lead to a reduction in population size. Impacts may be especially significant if stormwater volume and pollutant flow are high into an enclosed area such as a bay or estuary (Katznelson *et al.* 1993). Effluent may not receive much dilution or mixing initially, especially in the vicinity of the inputs to these bodies of water.

Environmental factors and species-specific behaviors can be expected to alter impacts of pollutants in natural settings from what might be predicted from laboratory studies. Dilution of stormwater constituents by the receiving water will generally reduce toxicity and this needs to be examined in future studies; however, even the lower concentrations tested (5–25% effluent) often produced developmental abnormalities in fish and egg lethality, and significant failure to hatch was seen in several samples with EC50s of approximately 30% effluent. Some organisms breeding near stormwater inputs may be at risk, although most euryhaline fish are not spawning during the late fall and winter seasons, when storms are most likely to occur. Other mitigating factors include reduced bioavailability of contaminants in the water column through adsorption or binding to sediments and the tendency of fish to avoid polluted areas when able to escape in the wild.

The use of *Menidia* and medaka eggs instead of indigenous species deserves discussion. Eggs of these species were much more commercially available than those of the Pacific coast topsmelt (*Athernops affinis*), a local fish, at the time these experiments were conducted. Now topsmelt can be cultured year round for toxicity testing (Anderson *et al.* 1994, 1995; Middaugh *et al.* 1992) and are more commercially available than before, so they would provide directly relevant information for future studies of this region. Responses of *Menidia* were considered to be predictive of effects in topsmelt because the two species had shown similar sensitivities to several toxicants (US EPA 1989; Hemmer *et al.* 1992; Anderson *et al.* 1994). Medaka eggs were also readily available; furthermore, its development and genetics have been studied extensively, so it was the primary model used in these experiments. Results of three split sample comparison tests using both *Menidia* and medaka suggested that these two species are also likely to be roughly similar in sensitivity to the adverse effects of storm water. The split samples from Chollas and Switzer Creeks with any appreciable activity in these few assays appeared to be slightly more toxic to *Menidia* than to medaka (Table 2). Different sensitivities to pollutants or assay conditions that were more favorable to medaka are both possible explanations if this difference is real. Even though both species are considered euryhaline, medaka are primarily freshwater and *Menidia* live in a more saline marine environment. Preliminary experiments with diluent ERM water exposures adjusted to different salinities had confirmed that both medaka and *Menidia* controls would hatch without significant abnormalities at 1.2 ppt and other low salinities expected with diluted storm water. In the absence of another stressor, however, the effects of low salinity may not be apparent. Others have shown that *Menidia* eggs and larvae placed in different salinity conditions and exposed to cadmium manifested less toxicity at higher salinities within its normal range (Voyer *et al.* 1979), whereas embryo response to the pesticide terbufos tended to be greater with increasing salinity (Hemmer *et al.* 1990).

The second main observation in these studies is that the presence of significant adverse effects in these bioassays did not correlate significantly with concentrations of any individual pollutants that were measured in stormwater, but did correspond with total toxic metal pollutants in the samples and frequency of exceedences of toxicity-based water quality criteria for metals. Lack of correlation between individual stormwater pollutant levels and toxicity is consistent with previous studies (Gadbois 1992; Ingersoll *et al.* 1992). Our experimental approach could not prove causality, but explored associations and correlations that can serve as hypotheses for future studies. For example, it is well established that heavy metals, especially copper, lead, and zinc, are by far the most common priority pollutants in urban runoff (US EPA 1994). The types of abnormalities observed were similar to those reported in previous studies of fish exposed to cadmium, chromium, copper, lead, nickel, and zinc (Klapow and Lewis 1979; Weis and Weis 1983, 1989; Hiraoka and Okuda 1983). Many factors and interactions are responsible for the toxicity observed with complex mixtures like storm water. A number of recent studies demonstrate that mixtures of metals may act additively, synergistically, or antagonistically in fish (Hickie *et al.* 1993; Pelgrom *et al.* 1994, 1997; Abdelghani *et al.* 1995; Roy and Campbell 1995).

Alternative explanations for toxicity of these stormwater samples must also be considered. Ammonia does not seem to be an important factor in this case. Un-ionized ammonia ranging from 0.001–0.1 mg/L was generally below the NOECs for recently hatched or larval stages of most fish, reported to be in the range of 0.1 to 0.4 mg/L (Thurston *et al.* 1986; Bader and Grizzle 1992; Diamond *et al.* 1993). A level of 0.061 mg/L (at pH 8.0, 25°C, 31 g/kg salinity) has been reported specifically for *M. beryllina* larvae (Miller *et al.* 1990). An NPDES permit specified analytes to be measured, and not all toxicants that could cause adverse effects in fish were analyzed. For example, environmentally stable organochlorine and cyclodiene pesticides were measured in these samples, but no cholinesterase-inhibitor organophosphates and carbamates, pyrethroid pesticides, or herbicides. These classes of pesticides are less persistent, but as a consequence are used in large quantities in landscaping and agriculture in San Diego County and are more toxic to aquatic life. The volatile and semivolatile organics analyzed in these samples were mostly below detection limits of 0.02–1 µg/L (ppb). These detection limits should be low enough to detect levels causing toxicity to fish larvae based on other studies we reviewed (Solomon and Weis 1979; Hose *et al.* 1981; Wisk and Cooper 1990; Marty *et al.* 1990b). Methyl mercury is highly developmentally toxic to fish larvae (Hiraoka and Okuda 1983), but was not analyzed in these samples. Further exploration into the high correspondence between fish developmental toxicity and expectations based on metal contaminant levels seems warranted and also more of a focus on understanding the potential impacts of residential and agricultural pesticides in stormwater runoff in this region.

A Toxicity Identification Evaluation (TIE) approach is used to isolate specific classes of agents causing toxicity. One experiment reported here using an initial step in that approach was a comparison of medaka developmental responses to filtered and unfiltered aliquots of two different samples (Table 2). The results of this were inconclusive. One sample (Park) turned out to be relatively nontoxic even before filtration; the other (Chollas Creek) seemed to become more toxic after removal of particulates. More extensive testing using complete phase I and II TIEs is necessary to clearly establish the identities of the toxic agents in samples from these sites.

These and similar studies of storm water from different locations also shed light on major pollutant sources and activities. Although industrial areas and freeways are often regarded as main contributors of pollutants in urban runoff (Hoffman *et al.* 1984), commercial and residential areas can have potentially equally deleterious impacts on fish populations. Here automobiles; use of pesticides, fertilizers, and other lawn and garden chemicals; and construction activities can all contribute pollutants. The proportion of total developed land cover was usually a good predictor of relative toxicity of storm water draining from a given watershed in this study, although there were some exceptions. These fish development bioassays identified Chollas Creek as the most toxic of the six mass loading sites tested in this area. The LD50 for medaka egg lethality was the lowest of all tested (26%); the LOAECs for developmental effects were also consistently among the lowest (5 to 25%); and concentrations of cadmium, chromium, copper, lead, and zinc at this sampling site were the highest of the mass loading sites evaluated. Commercial and industrial operations in this watershed, together comprising 17% of the total land cover, were undoubtedly contributing to the pollutant load, but with 63% of this watershed classified as residential, it is also likely that automobiles and lawn and garden chemicals are equally important sources. Additional sampling and TIE efforts focused on the most toxicologically active sampling sites can identify the major toxic species and help isolate probable sources in this watershed.

Likewise, this study also demonstrated that the pollutant load entering surface waters from the San Diego River watershed is less likely to cause harm to the environment, even though a total of approximately 42% is developed urban land use and the percentage of industrial land use is actually greater here than in the Chollas Creek watershed (4% versus 2%). Even full strength (100%) San Diego River stormwater exposures caused no elevated egg lethality or failure to hatch and very few morphological defects, which is consistent with the observation that this sample had the lowest heavy metal concentrations and also the lowest ammonia levels and highest hardness. In addition, it was interesting that the Rose Creek watershed with the most open space (approximately 60% of total acreage) was the source of one of the more acutely toxic samples during the first of two storms sampled there and was one of the least toxic in the second storm 4 days later. Clearly, more extensive sampling efforts beyond this screening study would be needed to positively identify major sources of contaminants in this region. More sampling is also required to differentiate variability observed between the sites sampled from the variability inherent in both the sampling method and that associated with different storm events.

Depending on land use within a given watershed drainage, stormwater runoff can contain agricultural chemicals in both urban and farming areas; metals, oil grease, and other chemicals from urban areas and energy production; sediments from improperly managed construction sites, pollutants from crop and forest lands and eroding stream banks; salts from irrigation and acid drainage from abandoned mines; and atmospheric deposition (US EPA 1996b). Even though stormwater composition varies by location, data from our study might be used to develop hypotheses about the potential for stormwater runoff in other areas to adversely impact fish populations. Concentrations of metals in these San Diego coastal area samples are low compared with median values for other areas of the United States reported by the National Urban Runoff Program (NURP) monitoring program (Schiff and Stevenson 1996). They are also at or below the low end of the distribution of the majority of metal pollutant levels reported in fish developmental toxicity studies (Klapow and Lewis 1979; Hiraoka and Okuda 1983; Weis and Weis 1989). If one assumes that heavy metals are usually among the most significant contributors to stormwater toxicity, then greater toxicity might be predicted elsewhere where metal levels are higher than in this region. Differences in water hardness and other factors that influence bioavailability of metals and other pollutants should also be considered. Ultimately site-specific information is needed to fully characterize impacts and identify sources of pollutants for regulatory purposes.

The relevance of using individual chemical standards, such as WQC, for regulating complex effluents like urban stormwater runoff has been questioned (Cooke and Lee 1993). Currently there are no numerical WQC for discharges of stormwater to the inland, estuarine, or oceanic waters of California (US EPA 1996a). The WQCs developed by the state of California still represent the best available guidance for assessing impacts of local stormwater runoff. The U.S. EPA has recognized the importance of testing for toxicity of the whole effluent, rather than monitoring individual contaminants, by promulgating guidance on whole effluent toxicity (WET) testing in the WET Control Policy and training programs (US EPA 1994). Fish developmental toxicity endpoints should play an important role in setting standards for stormwater runoff and other non–point source pollution and also in measuring the success of efforts to reduce pollutant levels. A clear advantage of this developmental bioassay approach to characterizing stormwater effects on ecosystems is that it relates effluent concentration to a sensitive toxicological endpoint with clear ecological significance.

Acknowledgments. The authors would like to thank the following individuals for advice and other valuable assistance: Dr. David Hinton, University of California, Davis; Dr. Alex Villalobos, Michigan State University; Brian Anderson and colleagues at the University of California, Santa Cruz, Granite Canyon Marine Pollution Studies Laboratory; and Dr. Sue Barnes, EPA Gulf Coast Research Laboratory.

References

Abdelghani AA, Pramar YV, Mandal TK, Tchounwou PB, Heyer L (1995) Levels and toxicities of selected inorganic and organic contaminants in a swamp environment. J Environ Sci Health (Part B) 30:717–731

Anderson BS, Hunt JW, McNulty HR, Turpen SL, Martin M (1994) Off-season spawning and factors influencing toxicity test development with topsmelt *Atherinops affinis.* Environ Tox Chem 13:479–485

Anderson BS, Hunt JW, Piekarski WJ, Phillips BM, Englund MA, Tjeerdema RS, Goetzl JD (1995) Influence of salinity on copper and azide toxicity to larval topsmelt *Atherinops affinis.* Arch Environ Contam Toxicol 29:366–372

Bader JA, Grizzle JM (1992) Effects of ammonia on growth and survival of recently hatched channel catfish. J Aquat Anim Health 4:17–23

Benoit DA, Holcombe GW, Spehar RL (1991) Guidelines for conducting early life stage toxicity tests with Japanese medaka (*Oryzias latipes*). EPA/600/3-91/063, US Environmental Protection Agency, Cincinnati, OH

Borthwick PW, Patrick JM, Middaugh DP (1985) Comparative acute sensitivities of early life stages of atherinid fishes to chlorpyrifos and thiobencarb. Arch Environ Contam Toxicol 14:465–473

Conway JB, Gilb RA (1990) San Diego Bay storm sewer study: final report on the San Diego Bay drainage sampling program. SDRWQCB Contract #45881647, San Diego Regional Water Quality Control Board, San Diego, CA

Cooke TD, Lee CC (1993) Toxicity identification evaluations in San Francisco (CA) Bay area urban stormwater runoff. Proceedings, 66th Annual Water Environment Federation Conference and Exposition, Anaheim, CA (October 3–7, 1983) 7:369–378

Cross JN, Hose JE (1988) Evidence for impaired reproduction in white croaker (*Genyonemus lineatus*) from contaminated areas off Southern California. Marine Environ Res 24:185–188

Cross JN, Schiff KC, Schaeffer H (1992) Contaminant mass emissions from surface runoff to the Southern California Bight. In: Cross JN (ed), Southern California Coastal Water Research Project annual report 1990–91 and 1991–92. Long Beach, CA, pp 19–28

Dial NA (1978) Methylmercury: some effects on embryogenesis in the Japanese medaka, *Oryzias latipes.* Teratol 17:83–92

Diamond JM, Mackler DG, Rasnake WJ, Gruber D (1993) Derivation of site-specific ammonia criteria for an effluent-dominated headwater stream. Environ Toxicol Chem 12:649–658

Gadbois LE (1992) Reconnaissance sampling of stormwater runoff pollution at an arid industrial Navy base. NRaD Technical Report #1497, Naval Command, Control and Ocean Surveillance Center, RDT&E Division, San Diego, CA

Hampson BL (1977) Relationship between total ammonia and free ammonia in terrestrial and ocean waters. Cons Int Explor Mer 37:117–122

Hemmer MJ, Middaugh DP, Comparetta V (1992) Comparative acute sensitivity of larval topsmelt, *Atherinops affinis*, and inland silverside, *Menidia beryllina*, to 11 chemicals. Environ Toxicol Chem 11:401–408

Hemmer MJ, Middaugh DP, Moore JC (1990) Effects of temperature and salinity on *Menidia beryllina* embryos exposed to terbufos. Dis Aquatic Org 8:127–136

Hickie BE, Hutchinson NJ, Dixon DG, Hodson PV (1993) Toxicity of trace metal mixtures to alevin rainbow trout (*Oncorhyncus mykiss*) and larval fathead minnow (*Pimephales promelas*) in soft, acidic water. Can J Fish Aquatic Sci 50:1348–1355

Hiraoka Y, Okuda H (1983) Characteristics of vertebral abnormalities of medaka as a water pollution indicator. Hiroshima J Med Sci 32:261

Hoffman EJ, Mills GL, Latimer JS, Quinn JG (1984) Urban runoff as a source of polycyclic aromatic hydrocarbons to coastal waters. Env Sci Technol 18:80–87

Hose JE, Hannah JB, Landolt ML, Miller BS, Felton SP, Iwaoka WT (1981) Uptake of benzo(a)pyrene by gonadal tissue of flatfish (family *Pleuronectidae*) and its effects on subsequent egg development. J Toxicol Environ Health 7:991–1000

Ingersoll CG, Dwyer FJ, Burch SA, Nelson MK, Buckler DR, Hunn JB (1992) The use of freshwater and saltwater animals to distinguish between the toxic effects of salinity and contaminants in irrigation drain water. Environ Toxicol Chem 11:503–511

Katznelson R, Jewell WT, Anderson SL (1993) Spatial and temporal variations in toxicity in a marsh receiving urban runoff. Contract #0-137-120-0/Calif SWRCB, Energy and Environment Division, Lawrence Berkeley Laboratory, UC Berkeley, Berkeley, CA, 51 pp

Kinnetic Laboratories, Inc. (KLI) (1994) 1993–1994 City of San Diego and co-permittee NPDES stormwater monitoring program: annual report. Kinnetic Laboratories, Carlsbad, CA

Kirchen RV, West WR (1976) The Japanese medaka—its care and development. Carolina Biological Supply

Lagler KF, Bardach JE, Miller RR, Passino DR (1977) Icthyology, 2d ed. John Wiley and Sons, New York, NY 506 pp

Klapow LA, Lewis RH (1979) Analysis of toxicity data for California marine water quality standards. J Water Poll Control Fed 51:2954–2070

Marsh JG (1993) Assessment of nonpoint source pollution in stormwater runoff in Louisville (Jefferson County), Kentucky, USA. Arch Environ Contam Toxicol 25:446–455

Marty GD, Cech JJ, Hinton DE (1990a) Effect of incubation temperature on oxygen consumption and ammonia production by Japanese medaka, *Oryzias latipes,* eggs and newly hatched larvae. Environ Toxicol Chem 9:1397–1403

Marty GD, Nunez JM, Lauren DJ, Hinton DE (1990b) Age-dependent changes in toxicity of N-nitroso compounds to Japanese medaka (*Oryzias latipes*). Aquatic Toxicol 17:45–62

Marty GD, Wetzlich S, Nunez JM, Craigmill A, Hinton DE (1991) Fish-based biomonitoring to determine toxic characteristics of complex chemical mixtures: documentation of bioremediation at a pesticide disposal site. Aquatic Toxicol 19:329–340

McCain BB, Chan S, Krahn MM, Brown DW, Myers MS, Landahl JT, Pierce S, Clark RC, Varanasi U (1992) Chemical contamination and associated fish diseases in San Diego Bay. Environ Sci Technol 26:725–733

McKim JM (1977) Evaluation of tests with early life stages of fish for predicting long-term toxicity. J Fish Res Board Can 34:1148–1154

Middaugh DP, Hemmer MJ, Lamadrid-Rose Y (1986) Laboratory spawning cues in *Menidia beryllina* and *M. peninsulae* (Pisces, *Atherinidae*) with notes on survival and growth of larvae at different salinities. Env Biol Fishes 15:107–117

Middaugh DP, Hemmer MJ, Goodman LR (1987) Methods for spawning, culturing, and conducting toxicity tests with early life stages of four Atherinid fishes: the inland silverside, *Menidia beryllina,* Atlantic silverside, *M. menidia,* tidewater silverside, *M. peninsulae,* and California grunion, *Leuroesthes tenuis.* EPA/600/8-87-004, Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC

Middaugh DP, Hemmer MJ, Lores EM (1988) Teratological effects of 2,4-dinitrophenol, ‘‘produced’’ water and naphthalene on embryos of the inland silverside *Menidia beryllina.* Dis Aquat Organ 4:53–66

Middaugh DP, Anderson BS, Hemmer MJ (1992) Laboratory spawning of topsmelt, *Atherinops affinis,* with notes on culture and growth of larvae. Env Toxicol Chem 11:393–399

Middaugh DP, Resnick SM, Lantz SE, Heard CS, Mueller JG (1993) Toxicological assessment of biodegraded pentachlorophenol using Microtox and fish embryos. Arch Environ Contam Toxicol 24:165–172

Middaugh DP, Whiting DD (1995) Responses of embryonic and larval silversides, *Menidia beryllina,* to No. 2 fuel oil and oil dispersants in seawater. Arch Environ Contam Toxicol 29:535–539

Miller DC, Poucher S, Cardin JA, Hansen D (1990) The acute and chronic toxicity of ammonia to marine fish and a mysid. Arch Environ Contam Toxicol 19:40–48

Pelgrom SM, Lamers LP, Garritsen JA, Pels BM, Lock RA, Balm PH, Bonga SE (1994) Interactions between copper and cadmium during single and combined exposure in juvenile tilapia *Oreochromis mossambicus:* influence of feeding condition on whole body metal accumulation and the effect of the metals on tissue water and ion content. Aquatic Toxicol 30:117–135

Pelgrom SM, Lock RA, Balm PH, Bonga SE (1997) Calcium fluxes in juvenile tilapia, *Oreochromis mossambicus,* exposed to sublethal waterborne Cd, Cu or mixtures of these metals. Environ Toxicol Chem 16:770–774

Roy R, Campbell PGC (1995) Survival time modeling of exposure of juvenile Atlantic salmon (*Salmo salar*) to mixtures of aluminum and zinc in soft water at low pH. Aquatic Toxicol 33:155–176

Schiff K, Stevenson M (1996) San Diego regional storm water monitoring program: contaminant inputs to coastal wetlands and bays. Bull Southern Calif Acad Sci 95:7–16

Shi M, Faustman WM (1989) Development and characterization of a morphological scoring system for medaka (*Oryzias latipes*) embryo culture. Aquatic Toxicol 15:127–140

Solomon HM, Weis JS (1979) Abnormal circulatory development in medaka caused by the insecticides carbaryl, malathion and parathion. Teratol 19:51–62

Spies RB, Rice DW (1988) Effects of organic contaminants on reproduction of the starry flounder, *Platichthys stellatus,* in San Francisco Bay. Marine Biol 98:191–200

SPSS (1994) SPSS/PC+ for the IBM PC/XT/AT. Chicago, IL

State Water Resources Control Board (SWRCB) (1991) California Inland Surface Waters Plan. California State Water Resources Control Board, Sacramento, CA

Thurston RV, Russo RC, Meyn EL, Zajdel RK, Smith CE (1986) Chronic toxicity of ammonia to fathead minnows (*Pimephales promelas*). Trans Amer Fish Soci 115:196–207

US EPA (1988) Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms. EPA/600/4-87-028, US Environmental Protection Agency, Cincinnati, OH

US EPA (1989) Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms, 2d ed. EPA/600/4-89-001, U.S. Environmental Protection Agency, Cincinnati, OH

US EPA (1992) Overview of the stormwater program. US Environmental Protection Agency, Office of Waste Water Enforcement and Compliance, Washington DC

US EPA (1994) National water quality inventory: 1992 report to Congress. EPA-8 41-R-94-001, US Environmental Protection Agency, Office of Water, Washington, DC

US EPA (1996a) Interim permitting approach for water quality-based effluent limitations in storm water permits. EPA-833-D-96-001, US Environmental Protection Agency, Office of Water, Washington, DC

US EPA (1996b) Nonpoint pointers series. EPA-841-F-96-004, US Environmental Protection Agency, Water Planning Division, Washington, DC

Voyer RA, Heltsche JF, Kraus RA (1979) Hatching success and larval mortality in an estuarine teleost, *Menidia menidia* (Linnaeus), esposed to cadmium in constant and fluctuating salinity regimes. Bull Environ Contam Toxicol 23:475–481

Weis JS, Weis P (1987) Pollutants as developmental toxicants in aquatic organisms. Environ Health Perspect 71:77–85

Weis JS, Weis P (1989) Effects of environmental pollutants on early fish development. Rev Aquatic Sci 1:45–73

Wisk JD, Cooper KR (1990) The stage specific toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in embryos of the Japanese medaka (*Oryzias latipes*). Environ Toxicol Chem 9:1159–1169

Yamagami K (1981) Mechanisms of hatching in fish: secretion of hatching enzyme and enzymatic choriolysis. Amer Zool 21:459–471