BIOLOGICAL EFFECTS OF SELENIUM AND OTHER CONTAMINANTS ASSOCIATED WITH IRRIGATION DRAINAGE IN THE SALTON SEA AREA, CALIFORNIA 1992-1994

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ABSTRACT

The National Irrigation Water Quality Program (NIWQP) investigated contaminants associated with drainwater in the Salton Sea area from 1986-1990 and concluded that Department of the Interior (DOI) trust resources were adversely at risk to contaminants, especially reduced reproductive capacity due to elevated levels of selenium, organochlorine pesticides and possibly boron in the Salton Sea environment. The Salton Sea project advanced to Phase IV, remediation planning, in the NIWQP. Typically, in that phase of work, remediation projects are planned, and/or additional data collection is conducted if needed. In the case of the Salton Sea project, fairly extensive additional data collection was conducted during 1992-1994 to clarify the biological effects of the environmental contaminants documented to occur in the previous investigations.

This report describes the biological effects of environmental contaminants on several important fish and wildlife species in the Salton Sea area that were investigated in 1992-94. Three studies were conducted to obtain information about species either thought to be at particular risk, or those for whom the effects of selenium and DDE (a DDT-metabolite) are fairly well understood. The studies: 1) determined embryotoxicity of colonial waterbirds (fish-eaters); 2) determined the embryotoxicity and nesting proficiency of black-necked stilts (shoreline aquatic invertebrate feeders); and 3) determined contaminant body burdens of sailfin mollies, a surrogate species for the endangered desert pupfish that inhabits agricultural drains. The recent bird die-offs which have occurred at the Salton Sea are being investigated separately because of the need to incorporate disease and biotoxicity studies in addition to contaminants data.

The findings indicated: 1) colonial nesting water birds (great egrets, snowy egrets, and black-crowned night herons) are at risk of reduced reproduction from DDE-induced eggshell thinning and, to a lesser extent, selenium-induced embryo mortality; in addition, egret embryos displayed a high incidence of abnormalities (possibly over 100 times normal) of unknown causes; 2) 95% of black-necked stilt eggs sampled indicated they came from nests with a four-fold increased risk of nest failure due to selenium-induced embryo mortality, and that stilts are experiencing a 4.5% reduction in nesting proficiency due to selenium exposure in the Salton Sea area; and 3) the federal and state endangered desert pupfish inhabiting agricultural drains are at risk of reproductive failure due to selenium concentration in the adult fishes sampled.

Management implications are that the biological effects of selenium in the Salton Sea area are more clearly understood. These effects include reproductive depression in black-necked stilts, hazard to reproduction of the desert pupfish, and selenium levels in fish which make them hazardous food items for fish-eating birds. The apparent high incidence of egret embryo abnormalities would require additional verification to identify management options.

INTRODUCTION

The Salton Sea area is located within a very important agricultural region that includes the Coachella and Imperial Valleys which currently support a $1 billion per year agricultural industry (Steve Knell, Imperial Irrigation District, personal communication, 1995). Because approximately 97% of California's wetlands have been converted to other uses, fish and wildlife now frequently use agricultural waterways as alternative habitat. Therefore, Coachella and Imperial Valley agricultural drains and the Salton Sea are also important fish and wildlife habitat. In addition to its importance as the single major inland nesting and wintering area for hundreds of species of birds, the Salton Sea also supports habitat for four endangered species and is the site of both a federal and state wildlife refuge.

The U.S. Fish and Wildlife Service (USFWS) is concerned that contaminants in the Salton Sea area present a risk to fish and wildlife resources. The area is a critical component of the Pacific Flyway with over 270 species of birds utilizing the Imperial Valley as either permanent residents or seasonal migrants. The area also contains habitats of several endangered species including the Yuma clapper rail *(Rallus longirostris yumanenis)*, brown pelican *(Pelecanus occidentalis)*, peregrine falcon *(Falco peregrinus)*, and the desert pupfish *(Cyprinodon macularius)*. There are also several wildlife management areas in the Imperial Valley including the USFWS Salton Sea National Wildlife Refuge (SSNWR) and the California Department of Fish and Game (CDFG) Imperial Wildlife Management Area.

The NIWQP investigated contaminants associated with drainwater in the Salton Sea area of California in 1986-1990 (Setmire et al. 1990; Schroeder, Rivera et al. 1993; and Setmire et al. 1993). Those studies found irrigation drainwater from various locations in the Imperial Valley contained from 3 to 360 micrograms per liter (µg/l) selenium, with higher values typically found in subsurface drains. The federal water quality criteria for protection of aquatic life is 5 µg/l (USEPA 1987a). Biological sampling in these studies found that drainwater contaminants including selenium, boron and the DDT-metabolite DDE were accumulating in tissues of migratory and resident birds that use food resources in the Imperial Valley and the Salton Sea. Selenium concentrations in fish-eating birds, shorebirds and the endangered Yuma clapper rail were at levels that could affect reproduction. Setmire et al. (1993) also concluded that waterfowl and fish-eating birds may also be experiencing reproductive impairment as a result of DDE contamination in food resources. DDE concentrations measured in biota in that study are some of the highest values documented in California. These NIWQP studies clearly documented that certain drainwater contaminants were elevated in the Imperial Valley and Salton Sea environment. However, the studies had not been designed to quantify the associated biological impacts. Therefore, the Salton Sea NIWQP Study Team decided to conduct additional studies and collect information describing biological effects associated with this contamination in order to determine the need for remediation planning.

Several other studies have indicated persistent contaminants that bioaccumulate are at levels in the environment that could reduce resource productivity in the Salton Sea area. For example, a recent study of the endangered Yuma clapper rail (Roberts 1996) found that eggs collected from the CDFG Wister Wildlife Management Unit when drainwater was being used as the water source for wetlands had maximum selenium concentrations of 7.8 micrograms per gram (µg/g) dry weight (DW), an amount considered hazardous to reproduction as described in detail below.

In addition to selenium, organochlorine compounds are a persistent contaminant problem for the area's wildlife. Exceptionally high concentrations of DDE were found in black-crowned night-heron *(Nycticorax nycticorax)* and great egret eggs collected from the Salton Sea area in 1985 (Ohlendorf and Marois 1990).

The geometric mean DDE concentration in black-crowned night-heron eggs was 8.62 parts per million (ppm) wet weight (WW), and was 24.0 ppm WW in great egrets. The reproductive effects of DDE in night herons becomes significant at residue levels in the eggs of about 8 ppm WW, with effects including eggshell thinning, egg breakage, and reduced clutch size, hatching success and subsequent productivity (Custer et al. 1983, and Henny et al. 1984). Although Ohlendorf and Marois (1990) did not determine reproductive success in their study, based upon exposure-response relationships known for DDE they concluded that heron and egret reproduction at the Salton Sea could almost certainly be impaired.

A dramatic decline in colonial waterbird nesting productivity was documented at Salton Sea nest sites since 1986 (USFWS 1991 and 1992, and Audet et al. 1997). The total number of nesting colonial birds at Salton Sea shoreline colonies declined from 1,950 in 1987 to 231 in 1989, with precipitous reductions in productive nests observed for great blue herons *(Ardea herodias)*, cattle egret *(Bubulcus ibis)*, snowy egret *(Egretta thula)* and great egret *(Casmerodius albus)*. Double crested cormorants *(Phalacrocorax auritus)* and white pelicans *(Pelecanus occidentalis)* apparently no longer attempt to nest at the Sea. Other species of colonial waterbirds, such as the black skimmer *(Rynchops niger)* and gull-billed tern *(Sterna nilotica)* have had relatively stable numbers of active nests, but have experienced low reproductive success for several years (USFWS 1992, and USFWS 1994). It is unknown to what extent contaminant-induced reproductive failure has contributed to the decline in nesting success of fish-eating birds at the Salton Sea shoreline colonies.

There is also concern about the influence of contaminants on fish resources in the Salton Sea. Four species of recreational fish from the Salton Sea were studied in 1985 to determine if certain elemental contaminants were accumulating to unacceptable levels in the fishery (Saiki 1990). In that study whole bodies of bairdiella *(Bairdiella icistia)*, orangemouth corvina *(Cynoscion xanthulus)*, sargo *(Anisotremus davidsoni)* and Mozambique tilapia *(Tilapia mossambica)* contained average selenium residues of 10.3, 9.11, 7.05, and 8.7 ppm DW, respectively. Selenium toxicity thresholds for saltwater fishes are not known (White et al. 1987), but levels of 4 ppm selenium in whole bodies of freshwater fish are known to cause adverse effects (Lemly 1993). Saiki (1990) concluded the excessive accumulations of selenium could seriously affect the fishes and sport fishery of the Salton Sea. In addition, the selenium levels observed in those fish greatly exceed the 3 ppm DW dietary toxicity threshold for other wildlife (Lemly 1993), meaning they should be viewed as toxic to other fish and aquatic birds that consume them.

ACKNOWLEDGMENTS

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OBJECTIVES

This project was designed to more clearly define the biological effects of contaminants associated with drainwater on fish and wildlife resources in the Salton Sea area. Three studies were conducted in 1992-94 to obtain information about species thought to be at particular risk, and for those whom the effects of selenium and DDE (in particular) are fairly well understood. The three studies and their objectives were:

Study 1. Determine the embryotoxicity of colonial waterbirds nesting at the Salton Sea. Fish-eating birds, black-crowned night herons, and snowy and great egrets were found to have among the highest concentrations of contaminants that bioaccumulate including selenium, boron, and organochlorine pesticides (Setmire et al. 1993). In this study those species were examined for eggshell thinning, embryo deformities, and egg contamination.

Study 2. Determine the nesting proficiency and embryotoxicity of black-necked stilts, a resident species at the Salton Sea. Most stilt eggs sampled in the NIWQP Detailed Study contained from 3-35 ppm DW selenium, meaning those eggs were at an increased risk of embryonic death and possibly embryonic deformity (Setmire et al. 1993). In this study, evaluations were made of black-necked stilt nest proficiency, embryotoxicity, and egg contamination.

Study 3. Determine the body burdens of contaminants in a surrogate species for the endangered desert pupfish. This issue was not previously investigated, but was a concern because the pupfish inhabit drains that convey various amounts of contaminants in drainwater to the Salton Sea.

GUIDELINES FOR EVALUATING BIOLOGICAL RISK FROM SELENIUM EXPOSURE

A large amount of information has been generated recently on the biological hazards of selenium to fish and wildlife resources, and general toxicity guidelines have been developed by several researchers. Also, a working group of the Kendrick Project (Wyoming) and Middle Green River Basin (Utah) Interdisciplinary teams of the NIWQP synthesized the scientific information regarding effect thresholds of selenium in various environmental compartments. As a result, general predictive thresholds for biological hazards have been developed for use in hazard assessment relative to selenium [Bureau of Reclamation 1993 Internal Memorandum to NIWQP Manager regarding Predicted Selenium Effect Levels for Kendrick and Middle Green River Remediation (Irrigation Drainage) 4 p.]. These thresholds are summarized in Table 1. A summary of the recent literature regarding selenium hazards to fish and wildlife resources is presented below to provide background for these guidelines, and to aid in the interpretation of results from this study.

Table 1. Predicted selenium effect levels (Bureau of Reclamation 1993).

Water	No Effect[1]	Level of Concern[2]	Toxicity Threshold[3]
Water (ppb, total recoverable)	<1	1-3	>3
Sediment (ppm dry wt.)	<2	2-4	>4
Dietary[4] (ppm dry wt.)	<2	2-6	>6
Waterbird eggs[5] (ppm dry wt.)	<3	3-8	>8
Warmwater fish (ppm dry wt., whole body)	<3	3-6	>6
Coldwater fish (ppm dry wt., whole body)	<2	2-5	>5

1 Selenium concentrations below this level in various media do not appear to be related to any discernable adverse effects on fish and wildlife and are typical of background levels in environments not impacted by selenium.

2 Selenium concentrations at this level in various media rarely appear to be related to any discernable adverse effects on fish and wildlife, but are elevated above typical background levels.

3 Selenium concentrations exceeding this level in various media do appear related to adverse effects on some fish and wildlife species, such as increased risk of teratogenesis and embryo mortality.

4 Dietary criteria are on an average daily exposure basis.

5 Waterbird criteria are based on population means.

Selenium in the Environment

Freshwater ecosystems that are not heavily influenced by agriculturally or industrially mobilized sources of selenium usually contain less than 0.5 parts per billion (ppb) total recoverable selenium in the water. Inland saline sinks surveyed in Oregon, California, Nevada, and Utah typically contained less than 1 ppb total recoverable selenium. Chemically, selenium behaves similarly to sulfur and therefore is biologically active in that it can enter into the metabolism of plants and animals. Aquatic plant and insect tissues in selenium-normal environments usually contain on average less than 2 ppm DW total recoverable selenium. Bird's eggs, one of the best wildlife tissues for monitoring exposure to selenium, usually have concentrations that average less than 3 ppm DW. Whole body concentrations in freshwater fishes are usually less than 2 ppm DW. Plants take up selenium from water and concentrate it in their tissues to varying degrees depending upon the species of plant. Fish and wildlife bioaccumulate selenium in their tissues principally by eating contaminated plants, insects, and other animals. Selenium has a uniquely narrow separation between dietary exposures that are nutritionally beneficial and those that are toxic (Wilber 1980, Hodson and Hilton 1983). Experiments with less than 1 ppb seleno-amino acids in the water have led to tissue concentrations of selenium in aquatic insects that would be toxic to fish and wildlife eating those insects. As a very general rule, environmental concentrations just 10-times or more above normal background concentrations are sufficient to cause biological hazards (Skorupa 1994).

Selenium Risk to Birds

Avian embryos are very sensitive to the toxic effects of selenium (Ohlendorf 1989). Hatchability of fertile eggs is considered the most sensitive measure of selenium toxicity. The hatchability of chicken eggs is reduced when concentrations of selenium are 6 to 9 ppm in the egg. Fertility is not affected, but excess selenium causes unusually high rates of embryo mortality and developmental abnormalities (teratogenicity) in birds. Malformations of embryos caused by selenium apparently result from tissue necrosis of the brain and spinal cord, optic cups and lens vesicles, mesenchyme of the limb buds, and sometimes the tail region (Gruenwald 1958). These malformations include microphthalmia, anomalies of the extremities, and microcephaly.

Extensive field studies of avian response to elevated environmental selenium have been conducted in the San Joaquin River Basin and near the Tulare Lakebed in California, and, to a lesser degree, in other regions of the western United States. These studies have provided a large data base for classic dose-response and epidemiological statistical analyses of avian response to selenium exposure that have proven to be taxonomically and geographically robust (Skorupa et al. 1992, and USFWS 1995). It has been determined, on a bird population basis, that when the mean selenium concentration in the eggs are below 3 ppm DW there is a low risk of reproductive failure due to selenium. When bird egg concentrations are above 20 ppm selenium DW, the population is considered to be at high risk to selenium toxicity and is essentially certain to experience reproductive failure. Therefore, the low and high risk ends of the toxicity response for birds, with respect to reproductive performance, is clearly defined and is relatively narrow. When egg selenium concentrations fall within 3-20 ppm DW that bird population is at some intermediate amount of risk and direct studies of reproductive performance are generally necessary for more precise risk assessment (Skorupa, J. P., S.P. Morman, J.S. Sefchick-Edwards 1996, Internal Memorandum to the NIWQP regarding Guidelines for Interpreting Selenium Exposures of Biota Associated with Nonmarine Aquatic Habitats, 74 p.). Those assessments have specifically determined that nests with black-necked stilt eggs containing as little as 4.2-9.7 ppm selenium DW are about four times more likely to be reproductively impaired than nests with sample eggs containing < 4.2 ppm selenium (Ohlendorf et al. 1993, Skorupa 1994).

Selenium Risk to Fish

Lemly (1993) provided guidelines for evaluating the risk posed to fish by selenium in aquatic environments. Biological effect thresholds for the health and reproductive success of freshwater and anadromous fish are 4 ppm DW selenium for whole body, 8 ppm DW for skeletal muscle, 12 ppm DW for liver, and 10 ppm DW for ovaries and eggs. Further, the chronic dietary selenium threshold for fish and wildlife is considered 3 ppm DW, food organisms containing that level of selenium would supply a toxic dose of selenium while being unaffected themselves. Taxa of fish differ in their sensitivity to selenium, with cold water fishes, such as salmonids, more sensitive than warmwater fishes. In laboratory studies, rainbow trout fry (Oncorhynchus mykiss) experienced significant mortality when whole-body residues exceeded 4 ppm DW (Hunn et al. 1987).

Juvenile rainbow trout exposed to waterborne and dietary sodium selenite exhibited significant changes in blood chemistry when whole-body tissue residues reached about 3 ppm DW selenium; survival was reduced when whole body residues reached 5 ppm DW (Hodson et al. 1980, and Hilton et al. 1980). Hamilton et al. (1986, 1989, and 1990) studied juvenile chinook salmon *(Oncorhynchus tshawytscha)* exposed to waterborne and dietary selenium and observed growth was impaired at whole body residue levels of only 2 to 3 ppm DW selenium, and mortality occurred when concentrations exceeded 10 ppm.

Lemly (1993) studied the teratogenic effects of selenium in natural populations of centrarchids and other warm-water fish species. Whole-body selenium concentrations of 15 ppm DW were associated with a 10-fold higher incidence of defects in centrarchid populations in a contaminated lake. The relationship between tissue selenium residues and the prevalence of malformations approximated an exponential function over the range of 1-80 ppm DW selenium and 0-70% deformities (R^2=0.881, P<0.01). Lemly concluded that this relationship could be used to predict the role of teratogenic defects in warm-water fish populations suspected of having selenium-reproductive failure. Hermanutz et al. (1992) observed pronounced mortality and deformities in larval bluegill *(Lepomis macrochirus)* when breeding adults were experimentally exposed to 10 µg/l DW selenium (as sodium selenite) in the water for 40 weeks prior to egg-laying. Statistically significant differences ($p \leq 0.05$) between the control and 10 µg/l treatment were observed in mortality (69.7 and 28.8 percent larvae survival, respectively), larvae with edema or body swelling (0.1 and 80.0 percent, respectively), larvae with hemorrhaging (0.1 and 28.5 percent, respectively), and larvae with lordosis or crooked spine (1.8 and 11.6 percent, respectively, $p \leq 0.10$). The whole body residues of selenium in adult bluegills at the end of 365 days in the 10 µg/l water averaged 4.6 ppm WW (approximately 18.4 ppm converted to a DW basis).

Juvenile fathead minnows *(Pimephales promelas)* had growth inhibition at whole body tissue levels of 6 to 8 ppm DW selenium and above (Bennett et al. 1986, and Ogle and Knight 1989). In outdoor experimental streams dosed with sodium selenite, Hermanutz et al. (1992) observed that reproductive success of fathead minnows (ability of fry to swim-up) was impaired when the ovarian tissue of spawning females contained about 15 ppm and resultant fry contained about 8 ppm DW selenium on a whole body basis. A significant ($p \leq 0.05$) increase in edema and lordosis was also observed in larval fathead minnows when adults were exposed to 10 µg/l selenium in the water (Schultz and Hermanutz 1990). Control and selenium-exposed fathead minnow larvae had a 0.9 and 24.6 percent occurrence of edema, respectively, and a 5.6 and 23.4 percent occurrence of lordosis, respectively. Selenium residues in the fathead minnow embryos spawned in the selenium-treated stream averaged 3.9 ppm WW (about 15.64 ppm when converted to DW, assuming 80% moisture). Depending upon the specific tissue sampled, concentrations of selenium in fish from control groups or habitats with low ambient selenium levels usually ranged from 1 to 8 ppm DW (whole body would have lower levels than liver or ovary tissues) (Lemly 1993). However, tissue damage in major organs, reproductive impairment, and mortality were observed when levels reached 4 to 16 ppm DW. This extremely narrow margin between "normal" background and toxic levels, along with the propensity of selenium to bioaccumulate in aquatic food chains, underscores the biological importance of even slight increases in environmental selenium.

Lemly (1993) also recommended that 4 ppm DW selenium in whole bodies, 8 ppm in skeletal muscle, 10 ppm in ovary and eggs, and 12 ppm in liver be considered toxic effect thresholds for the overall health and reproductive vigor of freshwater and andronomous fish. Laboratory and field studies indicate that reproductive success is the most sensitive indicator of selenium impacts on both fathead minnow (Pyron and Beitinger 1989, Schultz and Hermanutz 1990) and centrarchid populations (Cumbie and Van Horn 1978, Gillespie and Baumann 1986, Woock et al. 1987, Hermanutz et al. 1992, and Coyle et al. 1993).

STUDY AREA AND METHODS

Study 1. Colonial Waterbird Eggshell Thickness and Embryotoxicity

Egg collections for this study in 1992 and 1993 focused on nesting colonies of black-crowned night-herons, and great and snowy egrets that have historically been located at both the north and south ends of the Salton Sea. The colonies were assigned names based on nearby roads and waterways and are indicated in Figure 1. Most nest sites were on snags, dikes or islands surrounded by 0.1 to 0.2 meters of water and were approached by kayak or small boat with an outboard motor. Eggs were placed in an egg carton and chilled on ice until returned to the SSNWR where they were refrigerated until transfer to the Carlsbad Field Office of the USFWS for processing.

Study 2. Black-Necked Stilt Nesting Proficiency

The study area for the black-necked stilt nesting study was five locations around the Salton Sea (Figure 1). The Johnson Drain site consisted of a small island east of Whitewater River Delta near where Johnson Drain empties into the Sea. The Elmore Ranch site is a detached levee that functions as a small island in the south-west portion of the Sea. The Davis Road site consisted of plowed roadsides along the main road that runs north-south through the Wister Unit of the CDFG Imperial Wildlife Area. The Hazard Tract site is on land leased from CDFG and managed by SSNWR. This included nesting areas in the dry pond bed of the Hazard 1 Unit and those on the earthen dike at the north end of the Hazard 10 pond. The Garst Road Barnacle Bar nesting site was along the south dike of a small pond adjacent to Garst Road and immediately west of Hazard 4 pond (north of the Elmore Geothermal plant where Garst Road runs adjacent to the Sea).

Black-necked stilt nests monitored in this study were located in May and June of 1993. Nests were located along dike roads by sighting incubating adult black-necked stilts from a vehicle. Nests were indirectly marked with a numbered piece of flagging attached to a flat metal washer placed 20 meters (m) south of nest bowls. Eggs were immersed in water to determine the sequence in which eggs were laid. For consistency, the first egg laid was collected for contaminant analysis and all remaining eggs were returned to the nest bowl. When possible, egg handling took place without the observer leaving the vehicle, to minimize the possibility of leaving a scent trail for predators. Nests were monitored at weekly intervals. When incubating adults were no longer present, nests were checked for hatching. Nest bowls with signs of predation of eggs or no sign of eggshell fragments were considered nests which had been preyed upon and eliminated from further hatchability analysis. Unhatched eggs were also collected for contaminant analysis.

Study 3. Desert Pupfish Exposure Evaluation using Sailfin Mollies as Surrogates

The desert pupfish was listed as a state endangered species by the CDFG in 1980, and as a federally endangered species by the USFWS in 1986. The species' current distribution is limited to shoreline pools of the Salton Sea, two natural streams, eight artificial ponds, and several irrigation drains discharging into the Sea. Because of the extremely limited range of this species, degradation of habitat that could limit its reproductive capacity is an important issue. However, because the pupfish is a state and federal endangered species it could not be extensively sampled for this study. Therefore, a surrogate species was sampled instead of desert pupfish. Sailfin mollies *(Poecilia latipinna)* were collected to represent the chemical body burdens of the endangered desert pupfish. Of the fish species inhabiting irrigation drains, the sailfin molly was thought to be a reasonable surrogate for the pupfish. Sailfin mollies, native to the Southeastern U.S. and Mexico, are primarily vegetarians that feed on a variety of algae and detritus. These fish have been introduced to the Salton Sea area where they are thought to compete with desert pupfish due to similarities in feeding habits (McGinnis 1984).

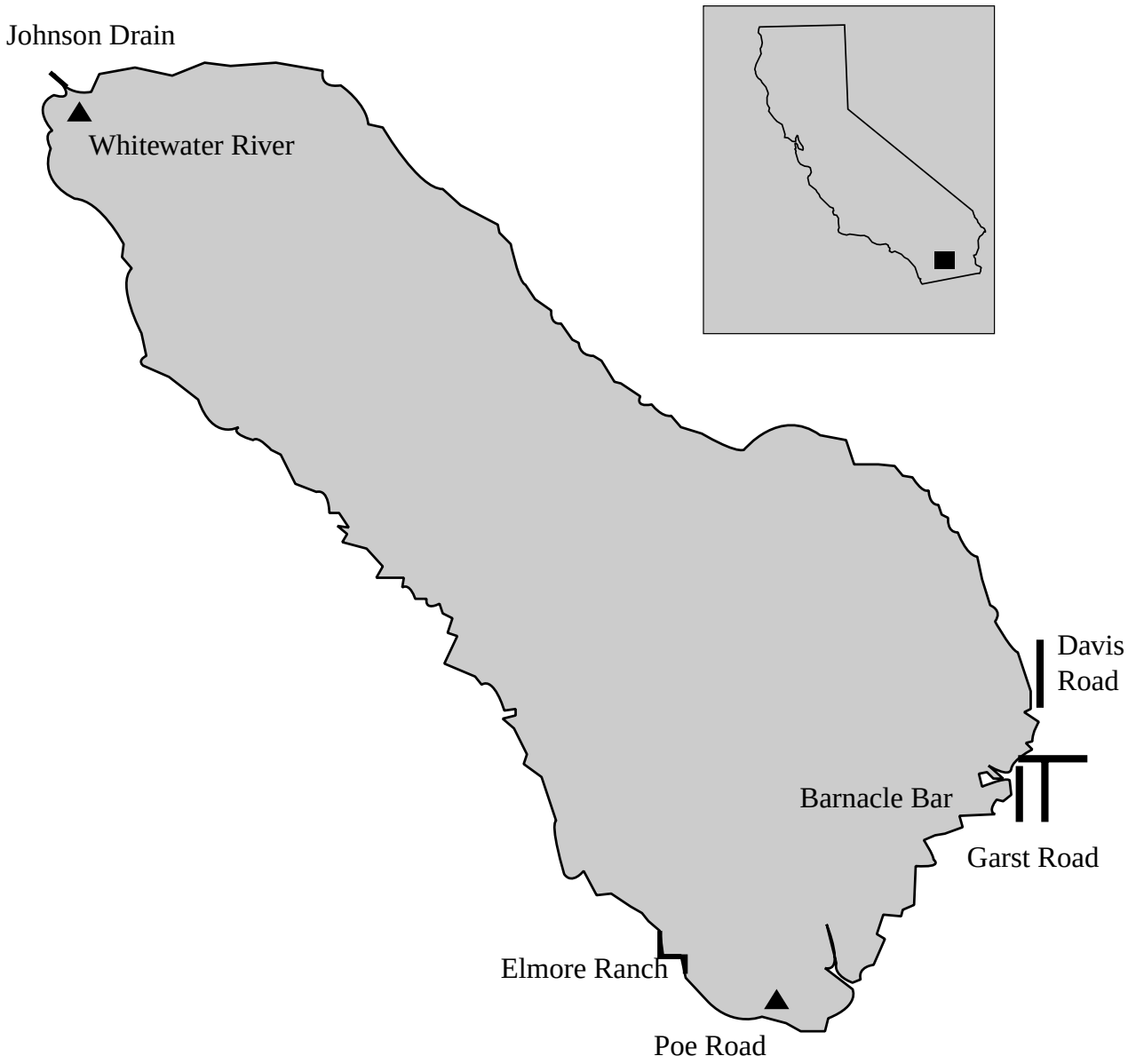

Figure 1. The locations of snowy egret and great egret nesting colonies are indicated by ▲. Locations of the black-necked stilt survey are indicated by the solid purple lines.

Sailfin mollies sampled in this study were primarily collected during a desert pupfish survey conducted in 1994 by the Imperial Irrigation District (IID). Some additional fish collections were conducted by USFWS biologists using methods similar to those employed in the IID pupfish survey (Remington and Hess 1993). Fish collections were conducted from July 20 to August 12, 1994. Sailfin mollies were collected from 13 drains (Figure 2). Wire mesh minnow traps were set at locations along the last 2-5 km of a drain before it entered the Salton Sea. Traps were placed in slow-moving areas of water at depths of about 6 centimeters to 1 meter, and baited with cat food in perforated, sealed plastic bags. The traps were left in place for approximately 4 to 24 hours, then retrieved and their contents recorded. Up to seven traps were placed along the lower portion of a drain for purposes of the IID desert pupfish survey, but for this study, mollies were collected from only three traps generally located at upstream, midpoint, and downstream locations of the trapline. In most cases, approximately 20 to 60 grams of whole body mollies (several fish) were taken from a trap and composited into a chemically clean glass jar and frozen until analysis. In one instance (at a trap on Trifolium Drain 13) only a single fish was collected, so in that case only a metal scan analysis was conducted on that sample. All other samples received both a metal scan analysis and an organochlorine scan analysis. Chemical concentrations in the mollies from each of the three trap locations along a drain were averaged to determine the geometric mean chemical content of mollies in that drain. In one drain (W Drain) there was unexplained mortality of desert pupfish in two traps. That situation presented the opportunity to conduct contaminant analysis on desert pupfish to compare with mollies collected at the same location.

Analytical chemistry methods for the bird and fish tissues are detailed in Appendix 1.

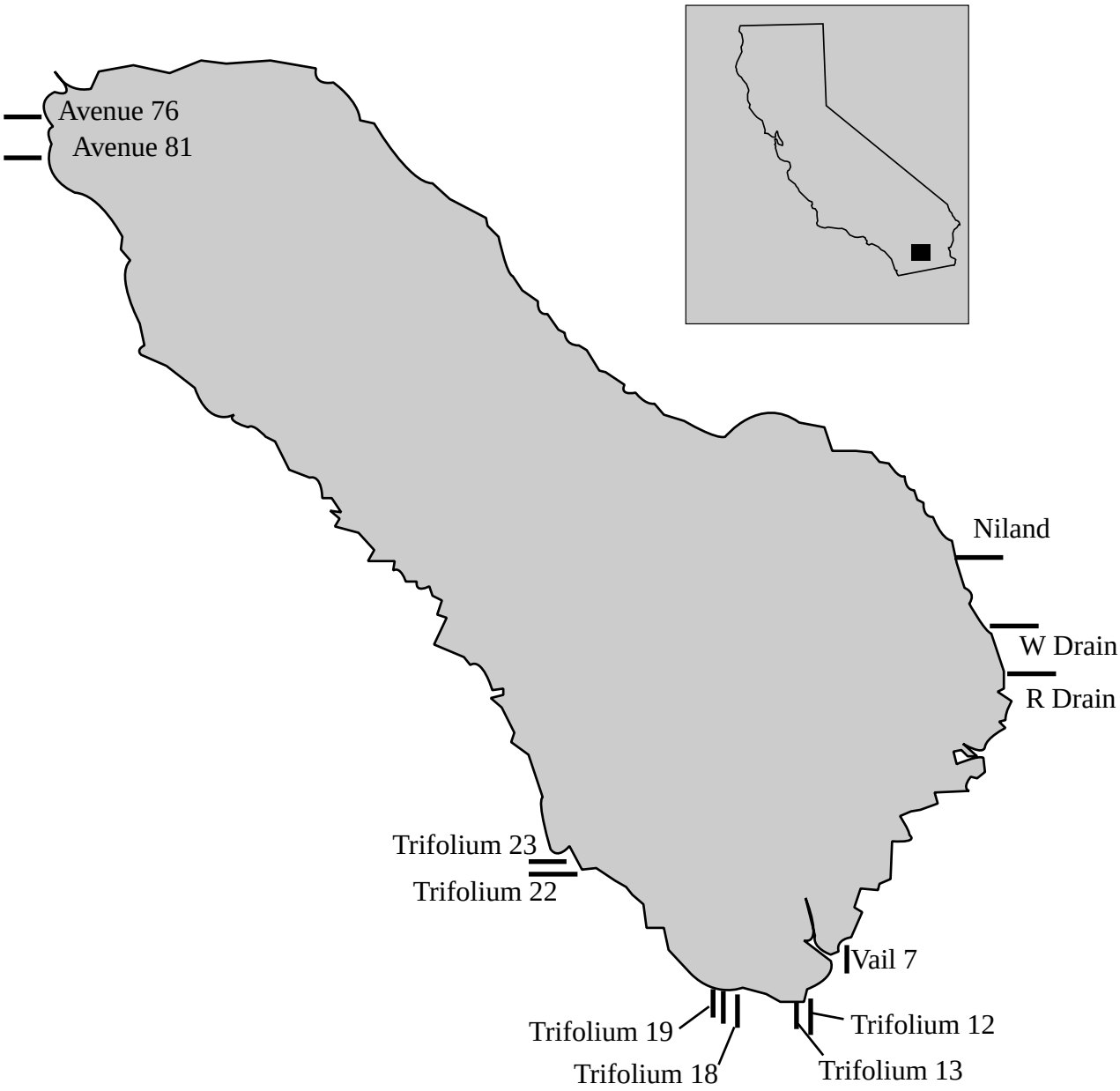

Figure 2. The locations of drains from which sailfin mollies were collected.

RESULTS AND DISCUSSION

Study 1. Colonial Waterbird Eggshell Thickness, Embryotoxicity and Egg Contamination

Relatively low numbers of colonial waterbirds were nesting at the Salton Sea in 1993 and eggs for this portion of the study were collected opportunistically and included failed-to-hatch eggs (William Radke, personal communication, 1993). In some cases, two eggs were collected from a nest. A total of 35 great egret eggs were collected from 29 nests, 24 snowy egret eggs from 21 nests, and 17 black-crowned night-heron eggs from 13 nests. Fifteen great egret eggs were collected from locations at the southern end of the Salton Sea (Poe and Lack/Lindsey Roads), but all other egret and heron eggs were collected from the northern end of the Sea at the Whitewater River colonies. All eggs were measured and the embryos removed and observed for gross deformities. The contents from 10 great egret and 10 snowy egret eggs were submitted for further analysis including a detailed examination for malformed embryos and chemical analysis.

In calculating average eggshell thickness measurements for each species, averages were first calculated for sibling eggs from the same nest, and then the nest averages were combined for the species average. The average eggshell thickness measurements for the snowy egret was 0.207±0.024 millimeter (mm) (n=21); 0.282±0.024 mm (n=29) for great egrets; and 0.247 mm±0.019 (n=13) for night-herons.

Little information is available regarding "normal" shell thickness of these species from clean environments, but Ohlendorf and Marois (1990) report mean shell thickness for night-heron eggs collected before 1947 (i.e., pre-DDT) was 0.280±0.011 mm at San Francisco Bay and 0.266±0.15 mm at the San Joaquin Valley. In the present Salton Sea study, the night-heron egg shells were 7-12% thinner than the pre-DDT era night-heron eggs from those sites. The night-heron shell thinning observed in this study approaches the amount observed in Lake Ontario night-herons in 1972-1973 (14-17%) when hatching success was 36-39% of normal (Price 1977). Davis (1993) presented a summary of pre- and post-DDT era eggshell thickness for black-crowned night-herons from a variety of studies in North America. The mean for the former was 0.284 ± 0.014 mm. The mean thickness found for black-crowned night-herons in this study was among the lowest of post-DDT era means and was lower than all pre-DDT era means provided in that summary. In the present study, great egret eggs were 13% thicker than the mean shell thickness of 0.244±0.016 mm reported for 1985 Salton Sea great egret eggs that contained a geometric mean concentration of 24 ppm WW DDE (Ohlendorf and Marois 1990).

Seventeen egret eggs contained embryos that could be examined for embryonic malformations. Of those, five embryos had some sign of deformity and three embryos exhibited some signs of embryo toxicity but did not show defects. The malformations observed in the egret embryos are detailed in Table 2. Although the observed beak defects could be in keeping with selenium terata, other chemical agents are also capable of causing them, and, in general, the embryos examined did not present the multiple deformities involving the eyes, bill and limbs that are typical of classic selenium-induced teratogenicity (Dr. David Hoffman, NBS, Patuxent Environmental Research Center, Laurel, MD; and Dr. Joseph Skorupa, USFWS, Sacramento, CA; personal communications, 1995). A single egret embryo (Great Egret 11) did have multiple malformations and was a co-joined twin (two embryos) joined at the body cavity with a single head. However, the twinning malformation is not typical of selenium terata (Dr. Joseph Skorupa, personal communication, 1995). The phenomenon of twinning ("duplicity") has previously been described in common tern *(Sterna hirundo)* embryos and in that case was attributed to organochlorine contamination (Hays and Risebrough 1972). Therefore, organochlorine contamination (including, but not limited to, DDE) may be related to the unusual malformation observed in the single egret embryo.

Table 2. Description of malformations observed in egret eggs collected in the Salton Sea area in 1993.

Embryo Identification	Nest Location	Embryo Development and Malformation
Great Egret 3	Poe Road	1/2 incubation stage; twisted upper beak and shorter lower beak (could be due to deterioration of embryo)
Great Egret 10	Whitewater River	3/4 incubation stage; some signs of embryotoxicity including mild to moderate swelling of the neck and slight hemorrhaging of the body.
Great Egret 11	Whitewater River	2/3 incubation stage; cojoined twins (two embryos) sharing the same head, misshapen toes with left foot exhibiting an extra toe, left eye absent (anophthalmia), cranium open, lower beak showed little development relative to the upper.
Snowy Egret 2	Whitewater River	2/3 incubation stage; signs of embryotoxicity included slight swelling of throat.
Snowy Egret 4	Whitewater River	3/4 incubation stage; swelling of throat, lower beak appeared slightly shorter than upper (may be due to deterioration).
Snowy Egret 18	Whitewater River	3/4 incubation stage; swelling of throat, left ventricular wall of heart appeared thinner than expected (may be due to deterioration).
Snowy Egret 19	Whitewater River	1/3 incubation stage, signs of embryotoxicity included hemorrhaging.
Snowy Egret 21	Whitewater River	1/3 incubation stage; lower beak appeared shorter than upper.

The apparent high incidence of abnormal embryos in fish-eating birds nesting at the Salton Sea is a situation that merits additional investigation. The combined rate of embryo deformity observed in this study in great and snowy egret eggs was 29 percent (5 of the 17 egret eggs containing embryos that were of adequate condition and stage of development for examination). If this incidence of abnormal embryos is actually representative of the Salton Sea population of fish-eating birds, then the rate is approximately 100 times higher than the typical incidence of abnormal embryos (Dr. Joseph Skorupa, USFWS, personal communication, 1995). It would be desirable to conduct additional field work on egrets nesting at the Salton Sea to determine if rates of embryonic malformations are truly that high, or were a function of the small number of embryos examined in this study, and to compare these rates with egrets nesting at other wetland locations in the Imperial Valley. If the situation described in this study is real, a possible management solution would be to encourage egrets to nest at alternative, less hazardous locations, if they exist.

Geometric mean concentrations of the principal contaminants in the snowy and great egret eggs collected at the Salton Sea in 1993 are presented in Table 3. The average moisture and lipid content in the snowy egret eggs was 80.2 and 5.5%, respectively, and the average moisture and lipid content of the great egret eggs was

76.6 and 6.2 %, respectively. The geometric mean selenium concentration was 4.97 µg/g DW (range 3.51 to 8.32) in the snowy egret eggs and 7.14 µg/g DW (range 6.1 to 9.9) in the great egret eggs, which places those populations at the Level of Concern (3-8 ppm) for selenium toxicity [Bureau of Reclamation 1993 Internal Memorandum to NIWQP Manager regarding Predicted Selenium Effect Levels for Kendrick and Middle Green River Remediation (Irrigation Drainage) 4 p.]. In 1985, 10 black-crowned night-herons and 10 great egret eggs were collected from the Salton Sea area (Ohlendorf and Marois 1990), and (when converted to DW) they contained geometric mean selenium concentrations of 4.4 (range 3.7-5.6) and 2.6 µg/g (2.2-3.3), respectively. In this study great egret eggs on average contained over 2.7 times the amount of selenium than eggs observed in 1985. On a individual egg basis, birds are at increased risk of reproductive failure when selenium concentrations in the egg is 5.1-20 ppm DW (Skorupa et al. 1992). Therefore, 30% of the snowy egret eggs and 100% of the great egret eggs were in that reproductive depression range due to their selenium concentrations.

Table 3. Principal contaminants detected in snowy and great egret eggs collected at the Salton Sea in 1993. In calculating the geometric mean a value of one half the detection limit was used for samples in which no measurable quantity was detected. Geometric means are presented if ≥ 50% of samples contained measurable quantities of a given contaminant. No mean is presented if < 50% of samples contained measurable quantities of a given contaminant.

Species	Location	Egg Id.	Boron µg/gdw	Selenium µg/gdw	Dieldrin µg/g ww	p'-DDEp µg/g ww	PCB µg/g ww	Toxaphene µg/g ww
Snowy Egret	Whitewater	2	<.503	4.8	0.06	4.3	0.14	<.05
		3	<.996	4.6	0.03	2.6	<.05	<.05
		4	<.501	4.7	<.01	13	<.05	<.05
		9	<.504	5.3	0.19	2.1	<.05	<.05
		15	<.502	4.6	0.02	2.1	<.05	<.05
		16	<.505	8.3	0.2	41	0.35	1.3
		18	<.502	4.1	0.17	14	0.07	<.05
		19	<.504	4.1	<.01	27	0.05	0.62
		20	<.506	3.5	0.05	6.1	<.05	<.05
		21	0.77	7.4	0.02	1.7	<.05	<.05
	Mean			4.97	0.04	6.33		
	Minimum			3.51	<.01	1.7		
	Maximum			8.32	0.2	41		
	Whitewater	1	<.51	6.1	0.26	23	0.42	1.3
		6	<502	6.6	0.08	4.5	0.55	<.05
Great Egret		8	<.504	6.6	0.25	50	0.75	.063
		10	2.1	6.4	0.47	6.4	1.1	0.76
		11	0.63	7.9	0.26	7.2	0.47	1.4
	Poe Road	1	<.5	7.8	0.41	17	0.8	2.2
		2	0.57	6.9	0.42	16	0.7	2.2
		3	1.28	6.8	0.37	14	0.6	2.1
		5	0.78	9.9	0.45	15	2.5	0.77
	Poe Road Delta	3	0.90	7.1	0.16	11	4.6	1.3
	Mean		0.55	7.14	.28	13.11	0.90	0.68
	Minimum		ND	6.1	0.08	4.5	0.42	ND
	Maximum		2.1	9.9	0.45	50	4.6	2.2

The geometric mean DDE concentration in snowy egret eggs was 6.33 µg/g WW (range 1.7 to 41) and the geometric mean in great egret eggs was 13.11 µg/g WW (range 1.7 to 41) (Table 3). This mean concentration is below that found in 1985 by Ohlendorf and Marois (1990) for great egrets (24.0 µg/g WW), but the maximum in this study was slightly higher than the maximum of 48 µg/g WW measured in that study. The maximums from both snowy egrets and great egrets measured in this study exceeded the maximum of 20 µg/g WW measured in black-crowned night-herons by Ohlendorf and Marois (1990). The mean for black-crowned night-herons in that study was 8.62 µg/g WW, between the two means for the species examined here. The geometric mean DDE contamination in Salton Sea snowy egrets approaches and in great egrets exceeds the 8.0 µg/g WW level associated with reduced reproductive success in night-herons (Custer et al. 1983, and Henny et al. 1984). Nearly half of the egret eggs contained from 1.5 to 6 times the amount of DDE associated with reproductive effects in night-herons (reproductive effects associated with DDE include eggshell thinning, egg breakage, and reduced clutch size, hatching success and subsequent productivity). In addition, this study observed exceptionally high concentrations of DDE in some egret eggs, with one snowy egret egg containing 41 µg/g WW DDE and a great egret egg containing 50 µg/g. This was twice the amount of DDE observed in a great egret egg considered remarkable by Ohlendorf and Marois (1990) because it contained 24 µg/g WW DDE in 1985. Three other snowy egret egg samples and six other great egret egg samples individually exceeded the 8.0 µg/g WW level described by Custer et al. (1983) and Henny et al. (1984).

The geometric mean boron concentration in great egret eggs was 0.55 µg/g DW and the single snowy egret with measurable quantities of boron contained 0.77 µg/g DW (Table 3). Because these amounts are below the 3 µg/g DW level associated with reduced weight gain in ducklings (Smith and Anders 1989), egrets do not seem to be at risk to boron toxicity.

Toxaphene is a persistent organochlorine pesticide that was present in most great egret eggs (Table 3). The geometric mean toxaphene concentration in great egrets eggs was 0.68 µg/g WW. This value was nearly 5 times higher than the geometric mean concentration of toxaphene (0.140 µg/g WW, range ND-0.33) observed in egret eggs from the Salton Sea in 1985 (Ohlendorf and Marois 1990). Toxaphene was banned for use in 1986 (USEPA 1982), but has a half life in soil of up to 11 years (Eisler and Jacknow 1985). Toxaphene has been shown to cause severe embryonic effects including dislocated joints and poor growth in mallard ducklings when used at application rates in excess of 1.12 kilograms per hectare (Hoffman and Eastin 1982). Dieldrin and PCBs are two other persistent contaminants that were also found at measurable, but non-hazardous, quantities in many of the egret eggs, and those results are also presented in Table 3.

Study 2. Black-Necked Stilt Embryotoxicity, Nesting Proficiency, and Egg Contamination

In 1992, 38 black-necked stilt eggs were collected from the Salton Sea area for embryonic and chemical analysis, but nesting was not followed that year. Twenty of those eggs contained embryos that could be examined in detail for malformations and all of the 38 stilt eggs collected in 1992 were submitted for chemical analysis. In 1993, 40 stilt eggs were collected from several locations around the Salton Sea. Twenty-eight of the stilt eggs collected contained embryos that could be examined in detail and all 40 eggs collected in 1993 were submitted for chemical analysis. Each of these 1993 nests was observed for nest proficiency (defined as producing a full clutch of viable eggs) and those results are presented below.

The principal contaminants observed in each of the stilt eggs collected in 1992 and 1993 are presented in Tables 4 and 5, respectively. On a population basis, the 1992 stilt eggs contained a geometric mean of 6.60 µg/g DW selenium (range 3.74 to 14.2) and the 1993 stilt eggs contained a geometric mean of 5.82 µg/g DW selenium (range 3.67 to 8.96). These levels indicate the Salton Sea black-necked stilt populations are, on average, at the Level of Concern for bird hazard [Bureau of Reclamation 1993 Internal Memorandum to NIWQP Manager regarding Predicted Selenium Effect Levels for Kendrick and Middle Green River Remediation (Irrigation Drainage) 4 pp.].

Table 4. Principal contaminants in black-necked stilt eggs collected around the Salton Sea in 1992. In calculating geometric mean a value of one half the detection limit was used for samples in which no measurable quantity was detected. Geometric means are presented if ≥ 50% of samples contained measurable quantities of a given contaminant. No mean is presented if < 50% of samples contained measurable quantities of a given contaminant.

Black-necked Stilt Eggs Collected in 1992							
Egg. Id	Percent Moisture	Percent Lipid	Boron µg/g dw	Selenium µg/g dw	Dieldrin µg/g ww	p,p'-DDE µg/g ww	Toxaphene µg/g ww
1	73	14	0.7	8.1	0.05	1.9	<0.05
2	72	13	<0.5	4.0	0.1	3.5	<0.05
3	74	14	<0.5	5.7	<0.01	3.6	<0.05
4	74	12	0.64	5.9	<0.01	2.3	<0.05
5	75	12	1.29	4.7	<0.01	0.62	<0.05
6	73	14	0.51	5.1	0.05	1.9	<0.05
7	73	10	<0.5	8.3	0.04	1	<0.05
8	76	10	<0.5	6.1	<0.01	3.4	<0.05
9	76	8	<0.5	12.4	0.12	4.7	<0.05
10	70	17	0.76	6.29	0.03	1.4	<0.05
11	76	12	0.52	8.3	0.03	1.1	<0.05
12	68	17	<0.5	3.7	0.07	2.2	<0.05
13	73	16	<0.5	6.5	0.01	0.69	<0.05
14	74	15	<0.5	5.1	0.06	3.8	<0.05
15	72	14	<0.5	6.3	0.04	1.2	<0.05
16	75	6	0.9	4.8	<0.01	0.36	<0.05
17	76	12	0.93	7.3	<0.01	1.7	<0.05
18	73	12	0.7	5.7	0.04	0.92	<0.05
19	70	18	<0.5	7.9	0.06	2.4	0.9

Black-necked Stilt Eggs Collected in 1992							
Egg. Id	Percent Moisture	Percent Lipid	Boron µg/g dw	Selenium µg/g dw	Dieldrin µg/g ww	p,p'-DDE µg/g ww	Toxaphene µg/g ww
20	74	14	0.76	7.9	<0.01	5.7	<0.05
21	72	6	0.94	5.5	0.02	1.4	<0.05
22	74	15	<0.5	12.9	0.05	0.8	<0.05
23	74	10	<0.5	6.8	<0.01	0.9	<0.05
24	73	14	0.69	5.7	0.04	2.2	<0.05
25	74	12	<0.5	7	0.03	1	<0.05
26	75	9	<0.5	13.6	0.04	2.7	<0.05
27	72	12	1.17	6.2	0.04	2	<0.05
28	70	13	<0.5	11.3	0.04	1.8	<0.05
30	75	12	<0.5	4.6	0.06	2.6	<0.05
31	75	12	2	6.9	0.07	2.7	<0.05
32	69	14	0.6	6	0.04	2.6	<0.05
33	72	13	1.3	5.2	0.06	2.2	<0.05
34	69	14	0.5	6.1	0.05	3.1	<0.05
35	66	13	0.7	6.1	0.02	1.1	<0.05
36	63	14	0.7	7.3	0.06	5.8	3.2
37	5	39	<0.5	14.2	0.05	6	<0.05
38	26	32	<0.5	4.3	0.09	5.9	<0.05
39	0.1	36	1.2	5.6	0.01	7.5	<0.05
Mean			0.47	6.60	0.03	2.02	
Minimum			<0.05	3.74	<0.01	0.36	
Maximum			2.0	14.2	0.12	7.5	

Table 5. Principal contaminants in black-necked stilt eggs collected around the Salton Sea in 1993. In calculating geometric mean, a value of one half the detection limit was used for samples in which no measurable quantity was detected. Geometric means are presented if ≥ 50% of samples contained measurable quantities of a given contaminant. No mean is presented if < 50% of samples contained measurable quantities of a given contaminant.

Black-necked Stilt Eggs Collected in 1993								
Egg Id	Location	Percent Moisture	Percent Lipid	Boron µg/g dw	Selenium µg/g dw	Dieldrin µg/g ww	p,p'-DDE µg/g ww	Toxaphene µg/g ww
1	Davis Road	73	15	2.26	9.0	<0.01	5.9	<0.05
2	Davis Road	70	14	<0.5	5.9	0.06	3.1	<0.05
3	Davis Road	70	8	2.19	5.7	0.01	0.96	<0.05
4	Davis Road	71	15	1.77	7.1	0.04	8.7	0.32
5	Davis Road	71	14	1.85	6.5	0.05	2.9	<0.05
6	Davis Road	70	13	2.89	7.1	0.03	2.3	<0.05
7	Davis Road	72	12	1.69	7.2	<0.01	0.65	<0.05
8	Davis Road	69	16	1.77	6.7	0.01	2.4	<0.05
9	Davis Road	70	15	1.91	6.3	0.03	2.9	<0.05
10	Davis Road	69	18	1.17	6.5	0.02	1.7	<0.05
11	Davis Road	68	17	0.96	5.4	0.03	2.6	<0.05
12	Hazard Tract	71	15	1.4	4.7	0.03	1	<0.05
13	Hazard Tract	71	15	0.95	4.6	0.17	9.2	<0.05
14	Hazard Tract	71	14	<0.5	5.7	0.07	3.1	<0.05
15	Hazard Tract	72	12	1.4	5.6	0.04	1.1	<0.05
16	Hazard Tract	68	15	1.08	4.4	0.04	1.9	<0.05
17	Hazard Tract	67	16	1.96	7.4	0.07	1.6	<0.05
18	Hazard Tract	70	17	0.58	4.3	0.03	1.4	<0.05
19	Hazard Tract	69	14	1.97	4.9	0.06	3	<0.05
20	Hazard Tract	70	17	1.1	4.3	0.06	2.1	<0.05
21	Hazard Tract	70	15	<0.5	4.2	0.1	2.8	<0.05
22	Hazard Tract	64	19	<0.5	6.0	0.03	8.2	<0.05
23	Hazard Tract	70	14	<0.5	3.7	0.02	1.1	<0.05

Black-necked Stilt Eggs Collected in 1993								
Egg. Id	Location	Percent Moisture	Percent Lipid	Boron µg/g dw	Selenium µg/g dw	Dieldrin µg/g ww	p,p'-DDE µg/g ww	Toxaphene µg/g ww
24	Hazard Tract	72	13	<0.5	7.7	0.02	1.5	<0.05
25	Hazard Tract	72	13	1.3	5.4	0.03	3	<0.05
26	Davis Road	70	14	1.7	4.0	0.06	3.8	<0.05
28	Davis Road	72	13	2.87	6.9	<0.01	2.6	<0.05
29	Davis Road	70	15	2.71	4.7	0.06	2	<0.05
30	Davis Road	72	13	1.89	7.5	<0.01	23	<0.05
31	Hazard Tract	71	13	<0.5	6.9	0.3	7.2	1.6
32	Hazard Tract	73	11	1.65	7.9	0.04	1.3	<0.05
33	Hazard Tract	59	25	1.57	4.4	0.04	2.3	<0.05
34	Hazard Tract	62	19	1.5	3.9	0.04	1.9	<0.05
35	Hazard Tract	71	18	0.85	6.6	0.37	8.7	2.4
36	Johnson Drain	70	14	1.9	4.8	0.02	0.7	<0.05
37	Johnson Drain	70	13	2.2	5.9	0.02	1.1	<0.05
38	Barth Road	71	10	1.5	7.7	0.15	2.1	<0.05
39	Barth Road	69	15	0.95	6.7	0.02	1.4	<0.05
40	Barth Road	74	10	2	6.8	0.05	1.5	<0.05
41	Hazard Tract	71	11	1.87	8.0	0.05	1.5	<0.05
42	Hazard Tract	70	14	2.48	6.4	0.08	1.9	<0.05
43	Hazard Tract	64	17	<0.5	6.6	0.39	6.3	1.6
44	Hazard Tract	39	32	1.29	4.5	0.06	2.5	0.41
45	Hazard Tract	65	14	<0.5	5.8	0.31	5	<0.05
Mean				1.09	5.82	0.040	2.48	
Minimum				0.25	3.67	<0.01	0.65	
Maximum				2.89	8.96	0.39	23	

The number of stilt eggs collected from nests in 1992 and 1993 considered at increased risk to hatching failure because of selenium toxicity is presented in Figure 3. In both years of the study, 95% of the stilt eggs sampled contained ≥4.2 ppm selenium, meaning the nests those eggs came from were four times more likely to fail than if the eggs contained less than 4.2 ppm DW selenium (Ohlendorf et al. 1993, Skorupa 1994).

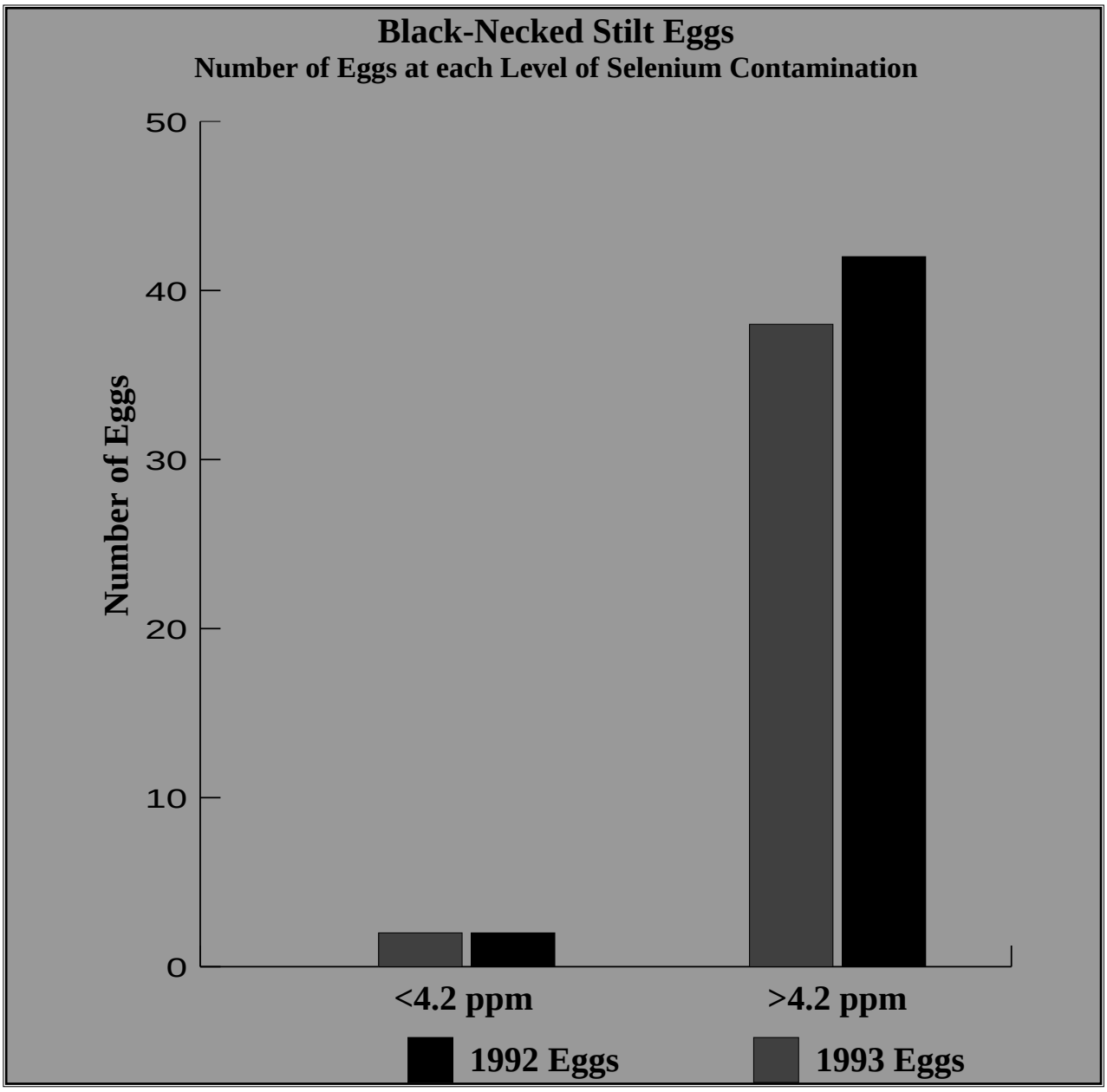

Figure 3. Number of black -necked stilt eggs collected in 1992 and 1993 at different levels of reproductive rsk relative to selenium concentrations in the egg. Eggs containing 4.2 ppm or more selenium are about four times more likely to be from reproductively impaired nests than eggs with less than 4.2 ppm selenium (Ohlendorf et al. 1993, Skorupa 1994).

The stilt eggs contained geometric mean concentrations of 0.47 µg/g DW boron in 1992 and 1.09 µg/g DW boron in 1993 (Tables 4 and 5). Both geometric mean concentrations are below the 3 µg/g DW threshold value for boron affects to duckling growth (Smith and Anders 1989).

The stilt eggs also contained geometric mean concentrations of 2.02 µg/g WW DDE in 1992 and 2.48 DDE in 1993. In 1993, when 40 stilt eggs were sampled, a single stilt egg was found to contain 23 µg/g WW DDE, a value twice as high as the 12.0 µg/g WW maximum observed when 84 stilt eggs were analyzed in the 1988-90 NIWQP irrigation drainwater study of the Salton Sea area (Setmire et al. 1993). Adverse effects occur in many fish-eating birds at <10 ppm DDE, but the level of DDE associated with reproductive failures in black-necked stilts is not specifically known.

One stilt egg collected in 1992 (Egg ID 11) had signs of embryotoxicity that included hemorrhaging of the throat and had a selenium content of 8.3 µg/g DW (Table 4). One stilt egg collected in 1993 at Johnson Drain at the north end of the Salton Sea (Egg ID Johnson Drain 36) had a lower mandible slightly smaller relative to the upper, and had a selenium content of 5.9 µg/g DW (Table 5). Neither of these malformations by themselves are considered selenium terata, as selenium induced deformities typically include multiple malformations of the eyes, bills and limbs.

The fate of the black-necked stilt nests monitored at the Salton Sea in 1993 is presented in Table 6. The black-necked stilt nest proficiency data set was analyzed to determine the proportion of nests with reproductive impairment by having ≥1 eggs that failed to hatch. Of the 37 nests observed, 27 were full term nests that were not preyed upon, abandoned or destroyed. However, four of those full term nests were excluded from further analysis because their fate was ambiguous in that at least one egg from each nest was known to have hatched, but the fate of the remaining eggs could not be determined (these four nests are indicated in Table 6 as having hatched 1+ eggs). One full term nest (Elmore Ranch 1) was observed to initially contain a full clutch of eggs and, although the fate of two eggs in that nest was unknown, the nest did contain one egg that failed to hatch. Two other nests each contained one failed-to-hatch egg (Davis Road 10 and Hazard Tract 18). Therefore, three of the 23 full term nests had ≥1 fail-to-hatch eggs, or 13% of the black-necked stilts nests studied were affected by hatching failure.

Table 6. Fate of black-necked stilts nests (and associated egg contamination) located around the Salton Sea in 1993. Shaded selenium values indicate the selenium concentration in that egg was in the hatchability depression range (5.1-20 ppm) for individual recurverostrid eggs (Skorupa et al. 1992).

Nest Fate and Egg Contaminant Information of Black-Necked Stilts Nesting Around Salton Sea, 1993							
Nest Location	Nest Id.	Clutch Size[1]	Number Hatched	Number Fail to Hatch	Number Young Dead	Nest Fate	Selenium µg/gdw2
Johnson Drain	2	3	1+	0	0	Hatched	4.8
	3	3	1+	0	0	Hatched	5.9
Elmore Ranch	1	3	0	1	0	Unknown	7.7
	2	3	1+	0	0	Hatched	6.7
	3	3	1+	0	1	Hatched	6.8
Davis Road	1	3	3	0	1	Hatched	9.0
	2	3	3	0	0	Hatched	5.9
	3	3	3	0	0	Hatched	5.7
	4	3	3	0	0	Hatched	7.1
	5	3	0	0	0	Predated	6.5
	6	3	3	0	0	Hatched	7.1
	7	3	3	0	0	Hatched	7.2
	8	3	3	0	0	Hatched	6.7
	9	3	3	0	0	Hatched	6.3
	10	3	2	1	0	Hatched	6.5
	11	3	3	0	0	Hatched	5.4
	26	3	0	0	0	Predated	4.0
	27	3	0	0	0	Predated	-
	28	3	3	0	0	Hatched	6.9
	29	3	3	0	0	Hatched	4.7
	30	3	3	0	0	Hatched	7.5
Hazard Tract	12	3	0	2	0	Abandoned	4.7
	13	3	3	0	0	Hatched	4.6

Nest Fate and Egg Contaminant Information of Black-Necked Stilts Nesting Around Salton Sea, 1993							
Nest Location	Nest Id.	Clutch Size[1]	Number Hatched	Number Fail to Hatch	Number Young Dead	Nest Fate	Selenium µg/gdw2
	14	3	3	0	0	Hatched	5.7
	15	3	3	0	0	Hatched	5.6
	16	3	0	3	0	Abandoned	4.4
	17	3	3	0	0	Hatched	7.4
	18	3	2	1	0	Hatched	4.3
	19	2	2	0	0	Hatched	4.9
	20	3	0	0	0	Destroyed	4.3
	21	3	0	0	0	Predated	4.2
	22	3	0	3	0	Abandoned	6.0
	23	3	0	0	0	Predated	3.7
	24	3	3	0	0	Hatched	7.7
	25	3	3	0	0	Hatched	5.4
	31	3	3	0	0	Hatched	6.9
Garst Road	32	2	0	2	0	Abandoned	7.9

[1]Clutch size after removal of one egg
[2] Chemical content of single egg removed from clutch

Normally, only 8.9% of stilt nests with less than 4.1 ppm DW selenium in their eggs have ≥1 fail-to-hatch eggs, or, conversely, 91.1% of normal nests are unaffected by fail to hatch eggs (USFWS 1995). When the Salton Sea black-necked stilt productivity (87.0% unaffected nests) is compared to normal productivity, it was calculated the Salton Sea population had 4.5% reproductive depression when compared to stilts in selenium-normal environments [(91.1-87.0)/91.1 =0.045]. Although the sample size was not adequate for statistical evaluation, this amount of reproductive depression in the Salton Sea black-necked stilt population is attributed to the selenium concentrations in the eggs because: 1) the reproductive success calculations were based only on data from full-term nests that did not fail due to predation, disturbance or abandonment, and 2) selenium was the single contaminant measured at known reproduction hazard threshold concentrations in the stilt eggs.

Therefore, there is an apparent small (4.5%) effect in black-neck stilt reproduction that is consistent with known exposure-response data, and there is good reason to consider it biologically real. However, due to the small magnitude of effect, and the low statistical power of the sample size of 23 nests, the possibility that the

result represents pure chance cannot be rejected. For example, a chi-square comparing the null expectation of 2.0 impaired nests (and 21.0 unimpaired, i.e., 8.9% impaired, the background rate) against the observed ratio of 3 impaired nests and 20 unimpaired nests yields a chi-square value of <1.0 (d.f.=1), which does not approach statistical significance at the 5% level. Based on the rate of impairments observed, it has been calculated that a sample size of about 225 full term nests would need to be monitored in order to statistically verify a 4.5% reduction in nesting proficiency. Monitoring that sample size of black-necked stilts is not realistic for any given year at the Salton Sea. However, it could realistically be achieved over several nesting seasons.

The avian reproductive hazard in the Salton Sea area was reviewed in a presentation at the 1994 Salton Sea Symposium (Skorupa 1994). Because there is a close correlation between waterborne selenium and egg selenium in nonmigratory species of water birds, and a close correlation between egg selenium and toxic effects, toxicity thresholds for avian eggs can be used to estimate toxicity thresholds in water and the diet. These relationships and estimates have been independently field validated in two recent studies of avian exposure to selenium (USFWS 1995), therefore, the general toxicological relationships documented for the San Joaquin Valley also appear to apply to data from the Salton Sea. For example, water-to-egg selenium ratios for black-necked stilts documented for the Salton Sea fall on the same regression line as data from Kesterson Reservoir and the Tulare Basin (Dr. Joseph Skorupa, personal communication, 1995). Using that information, it could be predicted that approximately 11.9% (or, when rounded to the nearest whole number of nests for this sample set, three) of Salton Sea stilt nests would be affected by hatching failure. In fact, 13% (i.e., three) of Sea stilt nests were observed to be affected by hatching failure in this study. Therefore, black-necked stilts nesting at the Sea appear to display the same selenium exposure response described for birds in other localities (Skorupa 1994, USFWS 1995). This demonstrates again the taxonomic and geographic robustness of the selenium toxicity thresholds currently established for birds, and that a large amount of predictive information about bird reproductive hazard can be obtained simply by knowing the concentration of selenium in the environment and the egg.

This study demonstrated that black-necked stilts are a good species to monitor for selenium- induced effects at the Salton Sea because: 1) no other contaminant was observed in their eggs at concentrations high enough to be a known reproductive impairment, and 2) the biological response of the stilts to the selenium exposure is apparently consistent with exposure-response data, although the sample size of Salton Sea stilt nest success data is small relative to desirable statistical power. Therefore, it would be desirable to continue monitoring black-necked stilts in the Salton Sea area to describe selenium effects on the area's waterbirds, and more clear conclusions can be reached within a few breeding seasons.

Study 3. Desert Pupfish Study Using Sailfin Molly Surrogates

Sailfin mollies were trapped in 13 agricultural drains. The principal contaminants detected in sailfin mollies and desert pupfish collected in drains around the Salton Sea in 1994 are presented in Table 7. Geometric mean boron concentrations in the fish ranged from 3.3 to 28.0 µg/g DW, with the maximum concentration (32.3 µg/g) observed in mollies from Trifolium 12 Drain. It is not possible to compare these boron results with those of the NIWQP Detailed Study (Setmire et al. 1993) because in that study the boron reporting limit in sailfin molly tissues was 18 µg/g. The results are difficult to evaluate further because there are no boron criteria or effect levels for fish. Geometric mean DDE concentrations in mollies ranged from 0.03 to 0.93 µg/g WW, with the higher DDE concentrations seen in fish collected from the Vail 7 Drain (0.51 µg/g DDE), the R Drain (0.52 µg/g DDE), and the Trifolium 23 Drain (0.93 µg/g DDE). These concentrations of DDE are not hazardous to the fish themselves, but some approach the National Academy of Sciences 1 µg/g organochlorine pesticide threshold for protection of fish-eating birds (NAS 1972). Also, the elevated DDE concentrations probably indicate the relative DDE contamination in a particular drainage system. This information could be used to determine if particular drainages are contributing more bioavailable organochlorines to the ecosystem than others.

Table 7. Geometric mean (range) concentration of principal contaminants in sailfin mollies and desert pupfish from drains around the Salton Sea in 1994. Lightly shaded selenium values indicates the concentrations in fish were in the Level of Concern (3-6 ppm dw), and dark shaded values indicate concentrations in fish were above the Toxicity Threshold (>6 ppm dw) for warmwater fish reproductive hazards (BOR 1993).

Sailfin Mollies Collected in 1994					
Location	Sample Size	Boron µg/g dw	Selenium µg/g dw	Dieldrin µg/g ww	p,p'-DDE µg/g ww
Avenue 76	3	3.7 3.1-4.7	6.4 6.2-6.7	0.022 <.01-0.030	0.37 0.27-0.43
Avenue 81	3	9.3 5.7-11.7	2.4 1.8-3.3	<.01	0.23 0.16-0.33
Niland 1	3	6.0 5.1-7.3	4.8 4.1-5.6	<.01	0.12 0.11-0.14
Poe Road	3	6.1 5.5-7.0	5.0 4.4-5.4	<.01 0.12-0.15	0.13
R Drain	2	4.7 3.4-6.1	3.1 3.1-3.2	<.01 0.44-0.59	0.52
Trifolium 12	3	28.0 24.2-32.3	3.6 3.5-3.8	<.01	0.064 0.06-0.066
Trifolium 13	1	5.4	4.6	No data	No data
Trifolium 18	3	11.3 11.0-11.8	10.2 8.9-11.7	<.01	0.03 0.029-0.031
Trifolium 19	1	4.4	5.3	<.01	0.13
Trifolium 22	3	4.3 4.2-4.6	5.7 5.6-6.3	0.049 0.048-0.051	0.35 0.34-0.37
Trifolium 23	1	3.3	4.2	0.082	0.93
Vail 7	3	16.7 9.5-22.2	5.2 5.0-5.6	0.025 0.015-0.031	0.44 0.31-0.51
W Drain	3	6.2 5.3-7.1	5.6 5.3-5.6	<.01	0.24 0.21-0.26
W Drain Pupfish	2	8.8 8.6-9.1	5.4 5.1-6.1	<.01	0.17 0.14-0.18

In the case of W Drain, where both sailfin mollies and desert pupfish were collected and submitted for analysis, there was general agreement in contaminant levels between the two species, particularly in selenium where mollies contained 5.6 µg/g DW and desert pupfish contained 5.4 µg/g DW. Therefore, it seems that sailfin mollies are reasonably good indicators for desert pupfish selenium contamination loads.

Sailfin mollies from 11 of the 13 drains and the desert pupfish from one of the drains contained geometric mean concentrations between 3 to 6 ppm DW selenium and therefore were at the Level of Concern for warmwater fishes [Bureau of Reclamation 1993 Internal Memorandum to NIWQP Manager regarding Predicted Selenium Effect Levels for Kendrick and Middle Green River Remediation (Irrigation Drainage) 4 p.]. Sailfin mollies in two other drains (Avenue 76 and Trifolium 18) contained geometric mean concentrations of 6.4 and 10.2 µg/g selenium DW, respectively, and so were over the toxicity threshold (>6 ppm) for warmwater fish reproductive hazards. Selenium hazards to desert pupfish are not precisely known, but they may have a sensitivity similar to that of the fathead minnow, another warmwater fish in the same family, Cyprinidae (Dr. Steve Hamilton, National Biological Service, personal communication 1995). Juvenile fathead minnows exhibit growth inhibition at whole body concentrations of 6 to 8 µg/g DW selenium (Bennett et al. 1986, and Ogle and Knight 1989), and there is a significant increase in edema and lordosis (curved spine) in larval fathead minnows when adults were exposed to selenium water at concentrations as low as 10 µg/l. Lemly (1993) concluded that 4 µg/g DW selenium be considered the toxic effect threshold for the overall health of and reproductive vigor of freshwater fish. Therefore, the desert pupfish is apparently at reproductive risk in many of the drains where they are known to occur.

Finally, fish collected from all drains exceeded either the Level of Concern (2-6 ppm DW) or Toxicity Threshold (>6 ppm DW) for dietary criteria, indicating that besides the risk to the fish themselves, the fish also present a risk to organisms that consume them.

CONCLUSIONS

The amount of eggshell thinning (up to 12%) observed in black-crowned night herons nesting at the Salton Sea in 1993 indicates that species is likely to be experiencing reproductive depression related to egg failures. Embryonic malformations were observed in 29% of the snowy egret and great egret embryos examined in detail. A variety of defects were observed, including the unusual malformation of a twin embryo joined at the body but with a single head, but none of these malformations were considered typical selenium-induced terata. The deformities observed could be related to the multiple kinds of contaminants observed in the egret eggs, but these kinds of synergistic effects are poorly understood. Chemical analysis of the egret embryos indicated the egg selenium content ranged from 3.5-9.9 µg/g DW -- levels that put the birds at risk to lowered productivity but unlikely to produce observable rates of teratogenicity in the small number of egret eggs examined. Therefore, neither the kinds of deformities observed in the egrets nor the associated levels of selenium in their eggs indicate that selenium-induced terata is occurring in egrets nesting at the Salton Sea area. However, the egret eggs also exhibited high levels of DDE with a geometric mean of 6.33 µg/g WW in the snowy egrets and 13.11 µg/g WW in the great egrets. These levels of DDE approach and exceed the amount (8 µg/g) associated with reduced reproductive success in black-crowned night herons (Custer et al. 1983, and Henny et al. 1984). An additional concern is that some egrets contained surprisingly higher levels of DDE and toxaphene in this study than in another study of contaminants in egrets from the Salton Sea in 1985. Apparently, high levels of these persistent contaminants are still available to some of these birds. Setmire et al. (1993) identified DDE contamination at all trophic levels (including resident species) in the Salton Sea ecosystem. In fact, it was detected in 99% of the samples analyzed, and concentrations in biota were correlated with trophic level. That study concluded that resident species of birds in the Imperial Valley are likely to experience reproductive impairment as a result of the DDE contamination. Other sources of DDE, including possible sources in Mexico, are potentially available to migratory species. However, a recent study by Mora (1997) indicates that there is no clear evidence for increased bioaccumulation of DDE in migratory species while wintering in Mexico. The limited data suggest that bioaccumulation is similar in Mexico and the Southwestern United States. The reported declines in colonial nesting bird success at the Salton Sea is likely to be related to the high levels of multiple contaminants in these fish-eating birds, particularly organochlorines.

The black-necked stilt study indicates that this species is likely to be experiencing selenium-induced reproductive depression. Nesting proficiency of Salton Sea area stilts was 4.5% lower than that reported for stilts with low selenium exposure (USFWS 1995), with 13% of the full term stilt nests affected by having at least one egg that failed to hatch. The geometric mean selenium content of the stilt eggs (6.60 µg/g DW in 1992 and 5.82 µg/g DW in 1993) places the stilts at a four-times greater risk to reproductive depression due to egg mortality than if they had selenium levels below 4.1 ppm (Skorupa 1994). The stilts at Salton Sea did not exhibit selenium-induced terata, but the likelihood of observing embryo deformation at those eggs selenium concentrations and the samples sizes of this study would be very low (Dr. Joseph Skorupa, personal communication, 1995). Therefore, when assessing the hazard of selenium to stilts at the Salton Sea, the most appropriate type of investigation is a nesting proficiency study with chemical analysis of one egg from each nest.

One aspect that needs to be considered when evaluating the data for black-necked stilts is their level of sensitivity to selenium toxicity. Stilts are moderately sensitive to the reproductive effects of selenium toxicity as compared to ducks which are considered sensitive to these effects (Skorupa, J. P., S.P. Morman, J.S. Sefchick-Edwards 1996 Internal Memorandum to the NIWQP regarding Guidelines for Interpreting Selenium Exposures of Biota Associated with Nonmarine Aquatic Habitats, 74 pp.). While the effect measured here was small, an effect was detectable in a moderately sensitive species. This raises concerns about the potential for reproductive impairment in more sensitive species that nest in the Salton Sea ecosystem.

The sailfin mollie study indicated that the endangered desert pupfish is probably also at risk to reproductive hazards from selenium. In addition, the sailfin mollie data indicate that fishes in the agricultural drains are presenting a selenium hazard as a dietary food item.

To summarize the management implications of this study, the reproductive depression in birds due to both selenium and DDE, hazards to the endangered pupfish, and levels of selenium in fish as a dietary food item have emerged as the most serious concerns for fish and wildlife resources in the Salton Sea area. The biological hazards relative to persistent contaminants in the Salton Sea area are now more clearly understood and the information indicates which species and endpoints are most relevant to efforts at improving and monitoring the Salton Sea contaminant issues, with respect to NIWQP responsibilities.

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APPENDIX 1

Analytical Chemistry Methods for Bird Tissues

Chemical analyses for inorganic compounds in bird tissues were performed at Research Triangle Institute (Research Triangle Park, NC). Elements analyzed included arsenic, aluminum, boron, barium, beryllium, cadmium, chromium, copper, iron, magnesium, manganese, mercury, molybdenum, nickel, lead, selenium, strontium, vanadium, and zinc. Tissue samples were prehomogenized using a food processor and a portion of the sample was freeze-dried for determination of moisture content and ground through a 100-mesh screen with a mill. Using a CEM microwave oven, 0.5 g of the freeze-dried samples were heated in a capped vessel in the presence of 5 ml of Baker Instra-Analyzed nitric acid for three minutes at 120 watts, then three minutes at 300 watts, and finally for 35 minutes at 450 watts. Vessel caps were rinsed into the vessel with additional nitric acid and the uncapped vessel was returned to the microwave and heated until the volume remaining was less than 1 ml. Samples were then diluted to 5 ml with laboratory pure water and centrifuged to precipitate the suspended material. Samples then underwent inductively coupled plasma spectroscopy (ICP) analysis (USEPA 1987b, Dahlquist and Knoll 1978) using a Leeman Labs Plasma Spec I sequential or ES2000 simultaneous spectrometer. Samples that underwent graphite furnace or cold vapor atomic absorption were homogenized as described above for ICP analysis. These samples were similarly heated in a microwave, however the duration of microwave heating at 450 watts reduced to 15 minutes. Residues produced were then diluted to 50 ml with laboratory pure water. Graphite furnace atomic absorption measurements for arsenic and selenium (USEPA 1984) were made with a Perkin-Elmer Zeeman 3030 or 4100ZL atomic absorption spectrometer. Cold vapor atomic absorption measurements for mercury (USEPA 1984) were conducted using SnC^{14} as a reducing agent with a Leeman PS200 Hg Analyzer.

Chemical analyses for organic compounds in bird tissues were performed at Mississippi State Chemical Laboratory (Starkville, MS). Organic analytes analyzed included organochlorine pesticides (OCs), polychlorinated biphenyls (PCBs), cis-nonachlor, and delta BHC. Tissue samples were thoroughly mixed with anhydrous sodium sulfate and soxhlet extracted (USEPA 1986) with hexane for seven hours. Extracts were concentrated via rotary evaporation and concentrated todryness for lipid determination. After weighing, samples were dissolved in petroleum ether and extracted four times using acetonitrile saturated with petroleum ether. Residues were partitioned into petroleum ether which was then washed, concentrated, and transferred to a glass chromatographic column containing 20 g of Florisil. The column was eluted with 200-ml 6% diethyl ether and 94% petroleum ether followed by 200-ml 15% diethyl ether and 85% petroleum ether. The second fraction was concentrated to the appropriate volume for quantification by packed or capillary column electron capture gas chromatography using a Varian 6000/6500 or Varian 3600 gas chromatograph. The first fraction was concentrated and transferred to a silicic acid chromatographic column for the additional clean-up required to separate PCB's from the other OC's. Three fractions were eluted from the silicic acid column, and each was concentrated to the appropriate volume for quantification by packed or megabore column electron capture gas chromatography using a Varian 6000/6500 or Varian 3600 gas chromatograph.

Analytical Chemistry Methods for Fish Tissues

Chemical analyses for inorganic elements in fish tissues were performed at Hazleton Laboratories America, Inc. (Madison, WI). Analysis included quantification of the same elements as in the bird tissue analysis described above. Percent moisture was determined by weighing the sample in a tared aluminum dish then drying in an oven at 100 C for 12-18 hours until a constant weight was reached (AOAC 1990). Elemental analysis was conducted via ICP. Digestion was carried out in nitric acid in a microwave digester. Emission intensities were compared to series of identification standards using a Thermo Jarrell Ash ICAP 61E spectrometer, with the spectrometer program correcting for background and interfering elements. Mercury analyses were

conducted using cold vapor atomic absorption spectroscopy (USEPA 1984). Samples were digested using sulfuric and nitric acids, and the mercury was reduced with sodium borohydride for determination. Mercury concentrations were determined at wavelength of 253.7 nm using a Leeman Labs PS200 atomic absorption spectrophotometer with an MHS-20 hydride generation unit, with the signal compared to standard solutions. Arsenic and selenium analyses were conducted using graphite furnace atomic absorption spectroscopy (USEPA 1984) on a Perkin-Elmer Zeeman 5100 PC spectrophotometer. Samples were digested with nitric acid in a microwave digester. Arsenic was determined at 193.7 nm wavelength and selenium was determined at 196.0 nm wavelength. The nickel matrix modification was employed in the analysis, and standard additions were conducted when interferences were indicated. Organic analyses of the fish samples were also performed at Hazelton Laboratories America, Inc. These methods included the determination of OCs and PCBs, but did not include cis-nonachlor and delta BHC. Percent moisture was determined by weighing a 1-10 g of sample in a pre-weighed aluminum pan. Samples were dried in an oven at 105 C for 16 hours and allowed to cool in a desiccator before being weighed again (USEPA 1986). The following equation was used to calculate the percent moisture: [mass (g) pan + wet sample] - [mass (g) pan + dry sample] x 100/grams of sample = % moisture grams of sample. Spiking solutions are added to the samples after the tissues were ground. Pesticide spikes were added to a portion of the sample matrices and control spikes of 2,4,5,6-tetrachloro-m-xylene were used as a blank spike solution for all other samples. Tissue samples were then dried under a hood using anhydrous sodium sulfate. A soxhlet extractor was
used with methylene chloride to extract the desired fractions from the samples (USEPA 1986). The resulting extracts were then concentrated in a Kuderna-Danish apparatus to a volume of 5 ml on a hot water bath, then diluted to 10 ml with methylene chloride. One ml subsamples of this extract were used for lipid determination. Subsamples were placed in a pre-weighed aluminum pan and placed under a hood to evaporate the solvent. Pans were weighed again, and the following equation used to calculate percent lipid: [(weight (g) of pan + lipid) - weight (g) of pan] x 10 ml x 100/grams of sample = % lipid grams extracted. Five ml volumes of the remaining extract were injected on an ABC Laboratories Model 1002B Gel-Permeation Chromatography system for clean-up (USEPA 1990) using a column packed with 70 g of S-X3 Bio-beads with methylene chloride as the carrier solvent. This extract was again concentrated to 5 ml. Then 50 ml of hexane was added to the samples and they were concentrated for a third time to a 5 ml volume. Additional clean-up and separation of PCBs from the OCs was carried out in a silica gel column. The first fraction was eluted with petroleum ether and the second fraction was extracted using a mixture of 1% acetonitrile, 19% hexane and 80% methylene chloride. Both fractions were then concentrated using the Kuderna-Danish apparatus, followed by dilution with hexane and a repeat of the concentration step. The fractions underwent electron capture gas chromatography using a Hewlett-Packard 5890 gas chromatograph for quantification of individual constituents.

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