

EARED GREBE MORTALITY IN IMPERIAL COUNTY, CALIFORNIA, 1991-93

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Abstract: Between January 9, 1992 and April 7, 1992, 46,040 dead eared grebes (*Podiceps nigricollis*) were salvaged at the Salton Sea, near Brawley, California. An estimated 150,000 eared grebes died at Salton Sea between December 16, 1991 and April 21, 1992, based on shoreline surveys and aerial surveys. This represented the largest documented mortality event of eared grebes and approximately 3.5-4.0 percent of the North American population. In 1994, 1,500 eared grebes were confirmed dead with estimates as high as 20,000 for total dead at the Salton Sea. During the die-off, grebes exhibited several uncharacteristic behaviors, such as congregating at freshwater tributaries, repeatedly gulping freshwater, excessive preening, moving out of water onto land, and allowing close approach and/or capture. Bird liver concentrations of arsenic, chromium, DDE, mercury, selenium, and zinc were elevated; however, none of these contaminants exceeded known thresholds for independent lethality. Selenium and zinc liver concentrations were significantly higher than reference samples, and selenium in symptomatic grebes was significantly higher than asymptomatic grebes from the Sea and grebes from several staging areas. Gross and histopathologic examinations disclosed no lesions suggestive of infectious disease common to waterbirds. Diagnostic testing was negative for viral, bacterial, or parasitic agents that could be responsible for the mortality. Avian cholera was isolated from eared grebes and several other species in the latter stages of the die-off, but enhanced culturing supported the conclusion that avian cholera did not play a significant role in the vast majority of grebe mortality. Poisoning by organochlorine, organophosphorus, or carbamate pesticides, avian botulism, domoic acid, and salt were also ruled out as the cause of the mortality event. Potential causes of the mortality are interactive effects of contaminants, immunosuppression, a yet unidentified biotoxin found in the Salton Sea and/or a difficult to isolate manifestation of avian cholera.

INTRODUCTION

The majority of the North American eared grebe population migrates through the Salton Sea area, Imperial County, California. The peak number of eared grebes visiting the Salton Sea averages 250,000 and has exceeded one million in some years (USFWS unpubl. data, R. McKernan, pers. comm.). The grebes arrive at the Salton Sea after food supplies have become depleted at fall molting/staging areas such as Mono Lake and Great Salt Lake (Storer and Jehl 1985, Jehl 1988). Some will overwinter, while others continue south to the Gulf of California, Mexico. The population normally peaks during early March when birds arrive from Mexico and congregate before departing the sea in March or April for northern breeding grounds. Wintering grebes at Salton Sea consume vast quantities of aquatic invertebrates, primarily pileworms (*Neanthes succinea*), water boatmen (Corixidae), and amphipods.

The Salton Sea lies within the Cahuilla Basin, or Salton Sink, 226 feet below sea level within Riverside and Imperial Counties, California. Uncontrolled flooding during 1905 diverted the Colorado River from its former course to the Gulf of California, into the Salton Sink and created the present Salton Sea. Since 1907, water has entered the sea through a system of controlled drains that are influenced by irrigation practices in the Coachella and Imperial Valleys of southeastern California. The sea acts as a sump for irrigation water drainage. The Salton Sea is a closed basin that has seen a tenfold increase in salinity since it was formed in 1907 (Setmire et al. 1990). In 1992, the salinity concentration was 46,000 mg/L. The Salton Sea is an artificial saline sink or an athalassic saline wetland as described by Bayly (1991).

In 1986, the Salton Sea was included in the U.S. Department of the Interior Irrigation Drainwater Program (now called the National Irrigation Water Quality Program) because of concerns about the quality of irrigation drainage and its potential effects on human, fish and wildlife health (Setmire et al. 1990). Elevated levels of DDE, selenium, and boron have been found in numerous species of birds at the Salton Sea (Koranda et al. 1979, Ohlendorf and Miller 1984, Setmire et al. 1990, Ohlendorf and Marois 1990, Setmire et al., 1993). In recent studies eggshell and embryo abnormalities have been documented in colonial waterbirds at the Salton Sea (Bennett, 1998; K. Molina, unpubl. data). It is not unusual for large numbers (>3000) of eared grebes, ruddy ducks (*Oxyura jamaicensis*), and ringed-billed gulls (*Larus delawarensis*), collectively, to succumb to avian cholera each winter at Salton Sea (USFWS, unpubl. data).

On December 16, 1991 and on January 19, 1992 small numbers of dead eared grebes, approximately 20 and 180 individuals respectively, were observed by Salton Sea NWR staff at the south end of the sea. In both cases, there were also observations of live grebes apparently ill and hiding beneath rocks to escape predation of herring gulls (*Larus argentatus*).

Two dead birds collected during the first event were sent to the National Wildlife Health Center (NWHC) for diagnostic evaluation. The final diagnosis was undetermined, based on the absence of gross and histopathologic lesions and negative bacterial culture and virus isolation attempts. Testing for avian botulism also was negative and brain cholinesterase levels appeared normal. During the second mortality event, eight sick grebes were euthanized by cervical dislocation and sent to NWHC for diagnosis. Four of these birds were necropsied. Again, the diagnosis was undetermined based on negative test results and the event seemed to have passed.

Much larger numbers were noted along the Salton Sea NWR shoreline between February 19-26, 1992, when thousands of dead grebes washed ashore. Extrapolation of initial carcass surveys at the north end of the Salton Sea tallied an estimated 79,000 to 135,000 dead eared grebes (J. Jehl, Jr. and R. McKernan, unpubl. data). During this die-off, grebes were observed exhibiting several uncharacteristic behaviors such as congregating at freshwater tributaries, repeatedly gulping freshwater, excessive preening, moving out of water onto land, and allowing close approach and/or capture. The USFWS responded to this mortality event by immediately sending wildlife health specialists from the NWHC and environmental contaminant specialists from the Carlsbad (San Diego County) Field Office to coordinate an onsite investigation with Salton Sea NWR staff. The die-off continued into April 1992 with an estimated 150,000 eared grebes dead. In the 1994 die-off event, over 1,500 eared grebes were confirmed dead. Estimates ranged as high as 20,000 for the total dead in that event.

Mortality events of eared grebes along the Pacific Flyway have been previously documented in Baja California, Mexico (Nishikawa et al. 1984), southern Utah (B. Waddell, pers. comm.), Great Salt Lake, Utah (Jensen and Cotter 1976), Mono Lake, California (Jehl 1988), and the Salton Sea (Jehl 1996; USFWS, unpubl. data). These die-offs typically represented <10,000 birds, although Jehl (1996) reported a die off of grebes of approximately 40,000 in 1989. Jehl (1988) estimates an average mortality of eared grebes at Mono Lake at 1370 to 3628 a year. Many die-offs were concluded to be the result of severe weather (Jehl and Bond 1983, Ryser 1985, Jehl 1988 and 1996, B. Waddell, pers. comm.), avian cholera (W. Radke, pers. comm.), or food shortages (Jehl 1988 and 1996). Jehl (1988) suggests that large-scale losses seem most likely when food remains abundant in late fall, encouraging grebes to migrate later during winter storms. Jensen and Cotter (1976) reported 5000 eared grebes died of the bacterial infection erysipelas in Great Salt Lake, Utah in 1975. However, many grebe die-off events were not clearly understood (Nishikawa et al. 1984, Jehl 1988) or were never investigated.

This paper presents the results of an intensive USFWS investigation to determine the cause of the 1992 and subsequent 1994 Salton Sea eared grebe die-offs. Numerous individuals from the USFWS, were involved in various aspects of this investigation including staff from the Salton Sea NWR, NWHC, Carlsbad Field Office, Patuxent Wildlife Research Center (PWRC), Ashland Forensics Laboratory; and Stillwater, San Francisco, Sacramento, Kern, Modoc, Sheldon, and Tijuana Slough NWRs. Other agencies involved were California Department of Fish and Game, Hubbs-Sea World Research Institute, San Bernardino County Museum, California Regional Water Quality Control Board, U.S. Geological Survey, Camp Pendleton Marine Corps Base, and

the U.S. Department of Agriculture Animal Damage Control. Individuals of special recognition include Robert McKernan, Joseph Jehl, Jr., Joe Skorupa, John Moore, and Ken Voget (USFWS). We appreciate the assistance of all of these agencies and individuals. Comments on the manuscript during various stages were made by Joe Skorupa, Joe Jehl, Jr., Gary Heinz, and Jewel Bennett.

METHODS

Estimation of number of dead eared grebes. Dead birds were collected between 3 March - 21 April 1992 with an emphasis in areas of high waterfowl and shorebird use, endangered species habitat, near communities, marinas, resorts, and other points of human concentration at the Sea. All carcasses salvaged were saved for necropsy and various chemical analysis or incinerated. This effort preempted potential outbreaks of avian cholera and botulism and provided a means for estimating the total number of birds which died during the mortality event.

During the mortality event, carcasses were found along 105 miles of Salton Sea shoreline plus an additional 25 miles of shoreline associated with freshwater drains and wetlands adjacent to the Sea. An aerial survey was conducted on February 24 to determine eared grebe distribution on the Salton Sea at that time. Live grebes were concentrated at the freshwater inflows, but dead birds were observed over the entire Sea with a generally even distribution along the shoreline. While it was physically impossible to pick up carcasses from the entire combined shoreline area, 40 miles (30.77%) of the most accessible shoreline were completely surveyed and carcasses collected. An estimate of the total number of dead birds was calculated based on the number of dead birds retrieved in 40 miles and extrapolated to include the 130 miles of total affected shoreline.

In 1994, a small number of birds congregating at the freshwater outflows which showed obvious symptoms (coming up on land and preening excessively) were euthanized and collected for diagnosis along with carcasses that were in adequate condition for diagnostic purposes. A few birds which were apparently healthy based on their behavior were also collected for diagnostic comparison with sick birds.

Diagnostic testing procedures. Necropsies were performed in the field by NWHC wildlife disease specialists and in the laboratory by a NWHC pathologist according to procedures described in Wobeser (1981). Body condition based on fat reserves and pectoral muscle development and the presence of traumatic injuries or lesions of tissues or organ systems was recorded. Tissues were collected for histopathologic examination, bacterial culture, virus isolation, avian botulism testing, determination of brain cholinesterase activity and sodium levels, and parasitology. All tests were performed on some birds while various subset combinations of tests were performed on others. Collection and shipping procedures were broadly based on Friend (1987).

Contaminant procedures and analysis. Collection areas (Salton Sea, Camp Pendleton, staging and stopover points) for grebe samples are presented in Figure 1.

Sediment chemistry. Sediment samples were collected from the Salton Sea near the river deltas (Figure 1) using a petit ponar dredge and only sediments not in contact with the dredge surface were analyzed. Samples were collected in 125 ml chemically clean jars and immediately put on ice. Sediments were analyzed for organochlorines and metals at USFWS's Patuxent Analytical Control Facility (PACF) in Laurel, Maryland. Samples were analyzed for selenium and arsenic by graphite furnace atomic absorption according to methods described in Krynitsky (1987) with a detection limit of 0.1 ppm (wet wt). Mercury determination was performed by cold vapor atomic absorption spectrophotometry as described by Monk (1961) with a lower limit of detection of 0.05 ppm (wet wt). All other metal analyses were performed by inductively coupled plasma emission spectrometry using the dry ash procedure described by Haseltine et al. (1981). Organochlorine analysis generally followed Cromartie et al. (1975) with the lower limit of detection of 0.01 ppm for pesticides and 0.05 ppm for PCBs based on a 10 g aliquot wet weight.

Bird tissues. An initial collection of grebe livers from dead, dying, and apparently healthy grebes (n=5 for each category) was conducted. Apparently healthy grebes were distinguished by observations of behavior reported as normal (Jehl 1988). Birds were collected by shotgun using steel shot. Dying grebes were defined as grebes found on shore excessively preening and required little effort for capture. Dying birds were euthanized by cervical dislocation. Due to the warm temperatures conditions of the Salton Sea area, dead birds used for chemical analysis were collected within 24 hours of death. Liver samples were removed from 15 birds and composited based on bird category (apparently healthy, dying, dead) and placed into 250 ml chemically clean jars and frozen. The liver samples were sent to PACF for metal and organochlorine analysis using methods previously described.

Individual analysis of liver from 17 eared grebes was conducted soon after the preliminary screen. This included 9 collected from the north and 8 from the south end of the Salton Sea. In addition, six eared grebes were collected by shotgun or rifle at Camp Pendleton Marine Corps Base, north of San Diego, California to serve as a reference site. Ruddy ducks found dead (n=10) at the Salton Sea were also collected during February 1992 for liver analysis. The second set of grebe liver samples collected in 1992 during the die-off and 1993 from staging areas used by this species on its northward migration and submitted for analysis in 1993 included: 29 dead or dying grebes from the Salton Sea (symptomatic), 26 apparently healthy grebes from the Salton Sea (asymptomatic), 4 grebes from Camp Pendleton on the coast, 15 from Great Salt Lake, 5 from Iron Mountain, 18 from Mono Lake, and 3 from Snow Summit. All underwent analysis for trace elements, and a subset of the samples was analyzed for organochlorine compounds.

Aquatic invertebrates. Food items of the eared grebe were collected from the Salton Sea and chemically analyzed for organochlorine pesticides and metals following previously described methods. In addition, an organophosphate/carbamate pesticide scan was used for 3 water boatmen and 4 pileworm composite samples. Gas chromatography using a flame photometric detector for organophosphate determinations and a nitrogen phosphorus detector for carbamate determinations was used in the analysis using standard PACF procedures.

Aquatic invertebrates collected included water boatmen, pileworms, and amphipods. Water boatmen and amphipods were collected with light traps and hand held nets. Pileworms were collected by sieving through sediments and pelagic stages were caught with hand held nets. As part of an ongoing study by the USFWS, pileworms and water boatmen were being collected on a monthly and bimonthly basis, respectively, at established stations at the north and south ends of the Salton Sea near the river deltas (Figure 1). Data from these stations are included in this report. Samples collected represented composited samples of >10 g.

Statistics. The 1992 die-off data used for statistical analysis included grebe tissues collected from the north end of the Sea (SSN92) and the south end of the Sea (SSS92). The reference data included grebe tissue collected from the Salton Sea prior to the die-off in 1989 (SS89; from Schroeder et al. 1993) and from Camp Pendleton Marine Corps Base in 1992 (CP92). Grebe liver samples collected from different portions of the sea during the die-off were initially treated as separate data sets because previous data (Setmire et al. 1993) reported substantial differences in baseline contaminant levels in sediment and aquatic life from the north and south portions of the Sea. Geometric means were calculated for all data sets with less than 50 percent non-detect results. A value of one half the detection limit was used in the calculation for non-detect values.

The data were not normally distributed, calling for non-parametric statistical tests. The Mann Whitney test was used to determine when data sets could be combined for specific contaminants and to test between reference and die-off data sets. The P value for rejection of H₀ was set at P < .05/number of comparisons.

Liver samples from grebes collected in 1993 from several staging or stopover areas were analyzed to provide a comparison to birds sampled at the Salton Sea. Archived liver samples collected from the Salton Sea (including dead, dying and healthy birds) in 1992 were also submitted for analysis at that time. The samples from the various staging or stopover points were grouped for statistical analysis based on the location of collection in order to achieve adequate sample size for the analysis. This was considered acceptable because the birds using the site were considered to constitute a functional unit of the population regardless of the specific collection date. The Salton Sea birds were separated into two groups based on whether they had exhibited symptoms of or succumbed to the die-off (symptomatic or dying and dead) or not (asymptomatic or healthy) regardless of collection date. Significant differences between the groups for each analyte were identified by means of the Kruskal-Wallis test. In order to identify pairwise significant differences, an ANOVA was conducted followed by a Tukey multiple comparison test. Results were only considered if the results of the ANOVA agreed with the results of the Kruskal-Wallis test. The P value for rejection of H₀ was set at P < .05/number of comparisons. (Please note that the program used reports very low P values in the ANOVA test as P=0.0000.)

Aquatic invertebrate data sets included samples assumed to be baseline or reference samples collected in 1988-89 (CRXD8-9 and PWRM8-9). This data was previously published in Schroeder et al. (1993). Water boatmen (CRXD1-2) and pileworms (PWRM1-2) collected just

prior to the 1992 die-off were compared to these baseline samples. As before, Mann Whitney was used to determine significant differences between data sets. All statistical tests were run on STATGRAPHICS v5.0.

RESULTS

Estimate of die-off. A total of 46,040 dead eared grebes were salvaged over 40 miles of shoreline, averaging 1,151 birds/mile from 3 March to 21 April, 1992. Assuming the carcasses were uniformly distributed over the 105 miles of Salton Sea shoreline and 25 miles of associated drains and wetlands, an estimated 150,000 eared grebes died at the Salton Sea during this mortality event. The die-off represented about 3.5-4.0 percent of the North American population of this species, which is estimated as 4 million birds (J. Jehl, written communication). The estimated total number of grebes that succumbed should be viewed as conservative. Many died and became waterlogged and sunk before ever reaching any shoreline. Other carcasses were buried by sand or crushed barnacles. Predation of sick and dead grebes was also widespread, with coyotes and gulls observed feeding on carcasses.

Diagnostic evaluation. A total of 64 necropsies was performed on birds collected during February 1992. Forty-six of the birds were eared grebes and 18 were other species of waterbirds including ruddy duck (n=7), herring gull (n=2), and individuals of northern shoveler (*Anas clypeata*), northern pintail (*Anas acuta*), Ross' goose (*Chen rossii*), American coot (*Fulica americana*), ring-billed gull, American avocet (*Recurvirostra americana*), short-billed dowitcher (*Limnodromus griseus*), and western sandpiper (*Calidris mauri*). Six eared grebes collected by gunshot from the Camp Pendleton area were necropsied, representing baseline conditions.

Of 18 birds of other species necropsied, 14 had gross lesions suggestive of avian cholera and in 13 of the 14 birds, *Pasteurella multocida* was isolated by bacterial culture. Three birds (ruddy duck, northern pintail, and northern shoveler) were too autolyzed to assess gross lesions or attempt bacterial culture. The American avocet had serosal hemorrhages, but no liver necrosis; bacterial culture was not attempted. Virus isolation attempts on multiple tissues from three of these birds were negative. Brain sodium levels measured in two ruddy ducks were normal.

Contaminant analysis

Sediment chemistry. The only organochlorine found above detection limits was p,p'-DDE with all values below 0.1 g/g (dry wt). The highest concentration (0.098 g/g) was from the Alamo River outlet and was higher than previously reported concentrations (0.064 g/g) from the same location (Setmire et al. 1990) The median p,p'-DDE concentration (0.04 g/g) was also higher than previous calculated median concentrations (0.014 g/g) for the Salton Sea (Setmire et al. 1990).

Cadmium, molybdenum, tin, and beryllium were found below detection limits for all locations. The median concentrations of arsenic, barium, chromium, copper, nickel, lead, selenium, vanadium, and zinc were lower than previously reported values for the Salton Sea in Setmire et

al. (1990). Selenium concentrations from the Whitewater and Alamo River outlets were very similar to previous levels, while the New River outlet sample was below previous levels reported in Setmire et al. (1990). However, all selenium data still was considered elevated compared to national levels. The analytical techniques were similar between the two studies, with the exception of arsenic and selenium. Setmire et al. (1990) used hydride-generation atomic absorption for these two elements.

Bird tissue analysis. Concentrations of selected contaminants in livers from dead, dying, and apparently healthy grebes collected during the initial phase of the 1992 die-off are presented in Table 1. These data were collected as a preliminary screen to determine if contaminants were elevated and potentially implicated in the cause of the die-off. Elevated levels of arsenic (Eisler 1988), chromium (Eisler 1986), mercury (Eisler 1987, G. Heinz, pers. comm.), selenium (Eisler 1985, Ohlendorf et al. 1988), and zinc (Eisler 1993, Skorupa, pers. comm.) in the livers justified further investigation. Dead and dying grebes had higher concentrations of arsenic, cadmium, chromium, zinc, and DDE than grebes assumed to be healthy.

The results of later analysis of individual liver samples from eared grebes and ruddy ducks are presented in Table 2. There were no significant differences between the north (SSN92) and south (SSS92) eared grebe data sets for arsenic, cadmium, chromium, mercury, selenium or zinc. Therefore, data for these two sets were combined to make one data set (SSNS), representing the 1992 Salton Sea eared grebe die-off data set. There were no significant differences between the 1989 Salton Sea (SS89) and Camp Pendleton (CP92) data sets for cadmium, mercury, or selenium, so those data sets were combined to increase sample size making one reference data set (SSCP). Arsenic and chromium did not have high enough detection frequencies to be included in this analysis.

There were no significant differences between the reference data set (SSCP) and the die-off data set (SSNS) for cadmium or mercury. However, selenium concentrations were significantly higher ($P=.047$, $Z=1.9842$, $n=27$) in grebe liver tissues collected during the die-off (SSNS) versus the reference data set (SSCP).

Zinc concentrations were significantly higher ($P=.022$, $Z=-2.2978$, $n=10$) in grebe tissues collected at SS89 versus CP92, but sample size was low. Multiple comparisons of concentrations of zinc in eared grebe tissues indicated significant differences between SSNS versus CP92 ($P=.002$, $Z=-3.1543$, $n=22$) and SSNS versus SS89 ($P=.006$, $Z=-2.76$, $n=22$), but not SS89 versus CP92 (Table 2). Thus concentrations of zinc in the eared grebe liver tissues were significantly higher in birds collected in the eared grebe die-off versus either reference set.

Ruddy ducks had selenium liver concentrations somewhat lower than the reference eared grebes, and much lower than the die-off eared grebes (Table 2). The zinc liver concentrations showed the opposite pattern with the ruddy duck livers having concentrations very similar to the die-off eared grebes and higher than the reference eared grebes. Selenium concentrations were similar to those reported for Salton Sea ruddy ducks collected in 1988-89 ($x=11.7$ g/g) reported in Setmire et al. (1993). The mean selenium concentration was lower than ruddy ducks collected

earlier from the Salton Sea in 1986-87 ($x=15.3$ g/g) reported in Setmire et al. (1990) and 1975-76 ($x=39.1$ g/g) reported in Koranda et al. (1979). The zinc concentrations were slightly higher than those provided in Schroeder et al. (1993) for ruddy ducks from 1988-89 ($x=134$ g/g).

The results of the eared grebe liver analyses including additional samples from 1992 and 1993 grebe livers collected from birds at staging and stopover points are presented in Table 3. No significant differences were found between the various groups in terms of their cadmium and p,p DDE concentrations. Both the Kruskal-Wallis test and the ANOVA identified significant differences in terms of the chromium concentrations in the livers ($H=15$ and $P=0.02$, $F=2.36$ and $P=0.037$), but the Tukey test did not identify any significant pairwise comparisons. This was reasonable as all groups had mean concentrations within the same order of magnitude.

Evaluation of the data for arsenic indicated a significant difference between groups in both the Kruskal-Wallis test and ANOVA ($H=25.9$ and $P=9 \times 10^{-6}$, $F=3.88$ and $P=0.012$). In the pairwise comparison the Mono Lake group had significantly lower arsenic concentrations as compared to each of the Salton Sea sample groups. The other groups lacked adequate data for comparison.

Mercury was found to have significant differences between groups in both the Kruskal-Wallis test ($H=25.7$ and $P=2.6 \times 10^{-4}$) and ANOVA ($F=4.5$ and $P=5 \times 10^{-4}$). Camp Pendleton, Great Salt Lake and Mono Lake were all found to be significantly lower than Snow Summit in pairwise comparisons. Great Salt Lake was significantly lower than Salton Sea symptomatic samples.

Significant differences between selenium concentrations in the group were also found by the Kruskal-Wallis test ($H=70$ and $P=4.7 \times 10^{-13}$) and ANOVA ($F=27$ and $P=0.0000$). The Camp Pendleton, Great Salt Lake and Mono Lake groups were all significantly lower than Snow Summit, Salton Sea symptomatic and Salton Sea asymptomatic groups. The Great Salt Lake group was significantly lower than the Iron Mountain group. The Iron Mountain group and the Salton Sea asymptomatic group were each significantly lower than the Salton Sea symptomatic group.

Significant differences were also found for zinc concentrations in both the Kruskal-Wallis test ($H=53$ and $P=1.4 \times 10^{-9}$) and ANOVA ($F=14$ and $P=0.0000$). The Camp Pendleton and Great Salt Lake groups were significantly lower than the Snow Summit, Salton Sea symptomatic and Salton Sea asymptomatic groups. The Mono Lake group was significantly lower than each of the Salton Sea groups.

Aquatic invertebrate analysis. Geometric mean and range contaminant concentrations in aquatic invertebrates are given in Table 4. There were no significant differences in arsenic ($Z=0.7882$, $P=0.431$), selenium ($Z=-0.1214$, $P=0.903$), or zinc ($Z=-1.173$, $P=0.203$) between water boatmen samples collected in 1988-89 (CRXD8-9) versus 1991-92 (CRXD1-2) during the eared grebe die-off. Similarly, there were no significant differences in the pileworm data sets for chromium ($Z=1.1726$, $P=0.241$), selenium ($Z=-1.8412$, $P=0.066$), or zinc ($Z=0.0001$, $P=0.999$). However, sample sizes were small and somewhat variable in both cases. Mean selenium and zinc values

for pileworms and water boatmen collected during the die-off were higher than those from samples collected in 1989, although the opposite pattern was seen for arsenic and chromium.

The only organochlorine found in all invertebrate samples tested for this group of compounds (n=7) was p,p'-DDE. Trace amounts of all parent and metabolite compounds of DDT were found in one pileworm sample collected at the Alamo River delta. No other organochlorine, organophosphate, or carbamate pesticide compounds were detected.

DISCUSSION

The eared grebe die-off was documented at several locations throughout the 130 miles of shoreline and numerous inflows (rivers, creeks, drains) of the Salton Sea. The origin of the die-off may be associated with a source encountered by grebes prior to arrival at Salton Sea in December 1991, while at Salton Sea, or from areas where grebes wintered prior to returning to Salton Sea in February 1992. Most carcasses were found in February and March 1992, after the arrival of an estimated 1.5 million eared grebes from the Gulf of California. However, grebes salvaged during earlier stages of the mortality event in December 1991 and January 1992 had similar diagnostic results as the larger part of die-off in February-March 1992, suggesting the Salton Sea as the origin of the die-off.

Eared grebe distribution was not uniform, but concentrated near the mouths of freshwater inflows where freshwater was available. During the major part of the die-off in February-March 1992, the eared grebes were observed to be flightless as described in Jehl (1988). Intermixing of flocks 10-20 miles apart was assumed not to occur and carcasses were found in numerous locations at the Salton Sea and nearby drains and wetlands. Therefore, if the source of the die-off was associated with the Salton Sea, the causative agent or substance should have been present throughout the sea.

For a particular contaminant(s) or toxin(s) to be implicated, presence of such a substance in water or food items of the grebe would need to be established, a significant increase in the substance should be found when compared to baseline conditions, and levels of the contaminant or toxin should be above hazardous thresholds. Contaminant(s) or toxin(s) found in water or food items of grebes at levels of concern included arsenic, chromium, DDE, salt, selenium, and zinc. Pileworms and water boatmen collected from the Salton Sea area just prior to and during the die-off had contaminant concentrations similar to those previously reported for the Sea. Selenium concentrations in grebe livers indicated at the time the grebes were present at the Salton Sea, they had higher liver concentrations than most other sites and higher maximum concentrations than previously reported for the Sea. However, no data was available for livers from grebes wintering south of the Sea.

In the first round of statistical analyses, significantly higher concentrations of selenium and zinc were observed in the die-off grebe livers, and significantly higher concentrations of selenium were found for die-off grebe livers in the second round of analyses. However, recent water (K.

Coulter, R. Schroeder, pers. comm.), sediment, and invertebrate samples from Salton Sea did not have significantly higher concentrations of either of these inorganic constituents. While not significant, chromium and arsenic concentrations in die-off grebe livers also had higher concentrations compared to 1988-89 grebe data, but the water and invertebrate samples did not have higher concentrations of either of these elements. This may suggest that arsenic, chromium, selenium, and zinc concentrations in grebe livers may not be completely associated with an exposure or pathway at Salton Sea, but it does not eliminate these contaminants as a secondary or synergistic component of the die-off.

Ohlendorf et al. (1986,1990) found eared grebes to have the greatest level of selenium bioaccumulation compared to many other waterbirds. Many selenium toxic thresholds have been established and compiled for waterbirds (Eisler 1985; Skorupa et al. 1998), and eared grebe embryotoxicity associated with elevated selenium has been documented (Ohlendorf et al. 1986, Ohlendorf et al. 1989). The mean selenium level for grebe livers found during the die-off was within the high risk adverse effect threshold described in Skorupa et al. (1998). However, this threshold was meant to be used only during the egg-laying season. Laboratory and field studies have determined dietary, egg, and liver concentrations associated with impaired waterfowl reproduction (Ohlendorf et al. 1986, Heinz et al. 1989, Heinz and Fitzgerald 1993b, Skorupa et al.1998), but reliable guidelines for assessing risk outside the breeding season are not well documented (Heinz and Fitzgerald 1993a, Skorupa et al. 1998). Both laboratory and field investigations have shown liver selenium as a reliable bioindicator of recent exposure to selenium; however, liver selenium has not been found to have any diagnostic value for verifying death due to selenosis at the level of individual birds (Ohlendorf et al. 1988, Heinz et al. 1987, 1988). Heinz et al. (1987,1988) did find treatment groups that exhibit mortality contain higher average concentrations of selenium in livers than treatment groups that do not exhibit mortality. Grebes associated with the die-off had significantly higher selenium levels than birds collected at the same time of year from the Camp Pendleton reference area, the Salton Sea in 1989, and asymptomatic grebes from the Salton Sea in 1992.

Diagnostic observations of selenosis in overwintering mallard experiments included emaciation, empty gizzard, mottling of the liver, and presence of yellow fluid surrounding some organs (Heinz and Fitzgerald 1993a). Not all of these observations were documented in grebes examined, and the examinations did not indicate selenosis as described for wild birds in Hoffman et al. (1988) and Ohlendorf et al. (1988). Selenium is known to impair immune function (Fairbrother and Fowles 1990, Fairbrother et al. 1994). While the selenium concentrations found would not necessarily have been manifested in symptoms of selenosis, immune function could have been impacted making those birds with higher selenium concentrations more susceptible to some other disease agent.

Zinc dietary concentrations were within acceptable ranges and below any known mortality threshold (>2000 mg/kg) for birds as summarized in Eisler (1993). Stahl et al. (1989) found 178 mg Zn/kg feed fed to chickens caused immunosuppression. Dietary laboratory studies have found diagnostic characteristics such as reduction of sexual organs associated with high levels of zinc (Gasaway and Buss 1972). There were no obvious effects on reproductive organs of the

eared grebes examined to indicate any type of chronic impacts, and dietary concentrations were below 178 ppm. Morris et al. (1986) and Hudson et al. (1984) report excessive drinking in birds as an observed sign of zinc toxicosis. While this was observed in Salton Sea grebes, zinc concentrations in livers of grebes from the Sea and reference areas were similar. Eisler (1993) suggests that zinc concentrations in field collected samples are highly variable and difficult to interpret, due to interactions of zinc with many chemicals (including cadmium, chromium, mercury, and selenium) that alter patterns of accumulation, metabolism and toxicity.

The liver p,p-DDE levels found in the initial screen showed an ascending trend from healthy to sick to dead birds. However, levels found in dead grebes were well below any toxic thresholds for DDE (Stickel et al. 1970, H. Ohlendorf, pers. comm.). The highest concentration found in this study was well below reference levels (healthy birds) in common murrelets (*Uria aalge*) analyzed during a die-off investigation where DDE was suspected as an indirect cause of mortality associated with environmental stress (Scott et al. 1975). The levels found in the additional 1992 samples were much lower than those found in the dead and dying birds in the initial screen suggesting high variability existed in DDE concentrations in grebes.

Eared grebe liver mass is not constant, and measurements of eared grebe liver mass have shown a 2.7 fold change over the course of the autumnal migration (Rattner and Jehl 1997). It has been found that some contaminants become concentrated in livers of birds with the loss of liver mass during the autumnal migration cycle (Rattner and Jehl 1997). Many liver comparisons discussed in this paper were made among livers collected at the same time of the year, but this may be a consideration in the discussion of selenium and zinc concentrations at the Great Salt Lake and Mono Lake versus the Salton Sea, the stopover points, and Camp Pendleton.

Salt toxicosis was considered a potential cause of the illness with observations of the birds seeking freshwater inflows and continuously gulping freshwater. This behavior is considered abnormal for eared grebes. Mahoney and Jehl (1985) found that grebes do not normally visit freshwater sources to drink or bathe and water requirements are met by consuming prey with high water contents (80-90%). Aquatic invertebrates sampled at the Salton Sea had moisture contents ranging from 72-93%. Mahoney and Jehl (1985) found salt glands of birds at Mono Lake rarely were fully hypertrophied indicating highly saline environments do not pose an osmotic problem for eared grebes. During the die-off, grebe salt glands were microscopically evaluated and showed no evidence of inflammation or necrosis. Eared grebes have been found to tolerate salinity levels of 160,000 mg/L in the Great Salt Lake (Jehl 1988), four times greater than those found at Salton Sea.

The Salton Sea is a nutrient rich body of water due to extensive agricultural fertilizers being used in the Imperial and Coachella Valleys and sewage inflows from Mexico entering through the New River. These high nutrient loads in association with the warm temperatures and lack of water mixing (closed basin) promote algal blooms and the production of associated biotoxins. Nutrient concentrations in the sea can increase with major storm events. Prior to and in the early stages of the die-off, the Salton Sea area received abnormally high amounts of rain. In similar climatic areas, Nishikawa et al. (1984) estimated that 3,000 eared grebes died for undetermined

reasons in Baja California, Mexico with the coincidence of exceptionally strong winter storms. However, without proper water sampling prior to, during, and following major rain events, pulses of nutrients or other contaminants cannot be estimated. Biotoxins associated with algal blooms in an athalassic saline sink such as the Salton Sea are not well documented. In more recent studies microcystins were isolated from Salton Sea water samples (Dr. Anhab Belay, written comm.) and die-off eared grebe liver samples (Dr. Wayne Carmichael, written comm.). Some liver concentrations were at acutely toxic levels, but the sample size was small and an inadequate baseline was available. Additional studies are needed to confirm this as a causative agent in the die-off.

Based on these results, we have concluded that tissue concentrations of organochlorine, organophosphorus, and carbamate pesticides, nickel, lead, and cadmium were well below acute mortality thresholds and were unlikely to be responsible for the die-off. Avian botulism, salmonella, viral infections, domoic acid, and salt toxicosis were also determined not to be the cause of the die-off. Arsenic, chromium, p,p'-DDE, and zinc tissue concentrations were elevated, but not high enough to cause acute mortality. Tissue concentrations of mercury and selenium were high enough to cause reproductive problems, but not death. Potential causes of the mortality are speculative and include interactive effects of contaminants, contaminant-related immunosuppression, biotoxins (possibly microcystin and/or others), or a difficult to isolate manifestation of avian cholera. Data suggest the Salton Sea or areas south of the Sea that were not sampled as the origin of the causative agent or substance responsible for the die-off.

MANAGEMENT IMPLICATIONS

If the cause of the die-off can be determined, it may be possible to develop management measures to prevent such events from occurring in the future. Eared grebe populations need to be monitored at major staging areas to determine if such die-offs are having a significant impact on the population. Hubbs-Sea World Research Institute conducts annual population counts at Mono Lake and other staging areas in the fall. Based on preliminary surveys of those areas in the fall of 1992, the eared grebe population showed a slight increase (J. Jehl, pers. comm.) as compared to previous years.

To better understand the cause of the grebe die-offs at Salton Sea, four areas of investigation are needed:

- 1) additional biotoxin identification at the Salton Sea and determination of baseline conditions.
- 2) additional contaminant and toxin data is needed from overwintering areas of grebes south of the Salton Sea.
- 3) increased sampling of pathways and diagnostic evaluations when die-offs occur.
- 4) controlled experiments to better understand interactive effects of contaminants related to a specialized species like the eared grebe.

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