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INVESTIGATION OF A LARGE-SCALE EARED GREBE (*PODICEPS NIGRICOLLIS*) DIE-OFF AT THE SALTON SEA, CALIFORNIA, IN 1992

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Abstract. An estimated 150,000 Eared Grebes (*Podiceps nigricollis*) died at the Salton Sea between 16 December 1991 and 21 April 1992. This represented the largest documented mortality event of Eared Grebes at the time and approximately 6% of the North American population. During the die-off, grebes exhibited several uncharacteristic behaviors, such as congregating at freshwater tributaries, repeatedly gulping freshwater, preening excessively, moving onto land, and allowing close approach and/or capture. Avian cholera was diagnosed in Eared Grebes collected along the north and west shoreline of the Sea late in the die-off but not from the majority of the Eared Grebes dying along the south shore. Gross and histological examinations and diagnostic testing for viruses, bacteria, and parasites did not identify the cause of mortality in the majority of Eared Grebes examined from the south shore of the Sea. Liver concentrations of arsenic, chromium, DDE, mercury, selenium, and zinc were elevated in some Eared Grebes, but none of these contaminants exceeded known thresholds for independent lethality. Poisoning by heavy metals, organochlorine, organophosphorus, or carbamate pesticides, avian botulism, and salt were ruled out as the cause of mortality. Hypotheses for the die-off are interactive effects of contaminants, immunosuppression, a yet unidentified biotoxin or pathogen present in the Salton Sea, impairment of feather waterproofing leading to hypothermia, or a unique manifestation of avian cholera that evades laboratory detection.

Key Words: avian cholera; biotoxin; contaminant; Eared Grebe; mortality; *Podiceps nigricollis*; Salton Sea.

INVESTIGACIÓN SOBRE UNA MORTANDAD A GRAN ESCALA DEL ZAMBULLIDOR OREJUDO (*PODICEPS NIGRICOLLIS*) EN EL MAR SALTON, CALIFORNIA EN 1992

Resumen. Un estimado de 150,000 Zambullidores Orejudos (*Podiceps nigricollis*) murieron en el Mar Salton entre el 16 de Diciembre de 1991 y el 21 de Abril de 1992. Esto representó el evento de mortandad más grande que se haya documentado para el Zambullidor Orejudo en nuestro tiempo y aproximadamente 6% de la población de Norteamérica. Durante el evento los zambullidores exhibieron un comportamiento fuera de lo común, tal como congregarse en los tributarios de agua dulce, tomar agua dulce repetidamente, limpiarse excesivamente las plumas, moverse hacia tierra y permitir la proximidad e incluso la captura. Se les diagnosticó cólera aviar a los zambullidores colectados a lo largo de la playa norte y oeste del Mar Salton hacia el fin del evento pero no para la mayoría de los zambullidores que murieron a lo largo de la playa sur. Examenes brutos e histológicos y las pruebas para diagnosticar virus, bacterias y parásitos no identificaron la causa de la mortalidad en la mayoría de los zambullidores examinados provenientes de la playa sur del Mar Salton. Las concentraciones de Arsénico, Cromo, DDE, Mercurio, Selenio y Zinc en el hígado de los zambullidores fueron elevadas en algunos individuos, pero ninguno de esos contaminantes excedió los niveles conocidos como letales. El envenenamiento por metales pesados, organoclorados, organofosforados o pesticidas, botulismo aviar y la salinidad fueron descartados como la causa de la mortandad. Las hipótesis para explicar la mortandad fue que hubo una interacción entre el efecto de los contaminantes, inmunosupresión en las aves, una biotóxina o algún agente patógeno aun no identificado presente en el Mar Salton, el deterioro en la impermeabilidad de las plumas lo que llevó a hipotermia, o una manifestación singular de cólera aviar que no fue detectada por las pruebas de laboratorio.

Palabras claves: cólera aviar; biotoxina; contaminante; Mar Salton; mortandad; *Podiceps nigricollis*; Zambullidor Orejudo.

From December 1991 through April 1992, thousands of dead Eared Grebes (*Podiceps nigricollis*) accumulated along the shoreline of the Salton Sea, California. During this die-off, many grebes and fewer Ruddy Ducks (*Oxyura jamaicensis*) exhibited uncharacteristic behaviors, such as congregating in large numbers at fresh water tributaries, repeatedly gulping freshwater, preening excessively, and moving onto land allowing close approach and/or capture. Sick birds became increasingly lethargic and then died without evidence of paralysis or other neurologic impairment. The magnitude of the die-off attracted extensive media and scientific interest and much speculation as to cause.

The majority of North American Eared Grebes migrate through the Salton Sea area, numbering between the hundreds of thousands

to over one million in several years (Jehl 1996, Jehl and McKernan 2002). The grebes arrive at 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 overwinter at the Salton Sea (numbers vary greatly annually), whereas others continue south to winter in the Gulf of California, Mexico. The Salton Sea population of grebes normally reaches a peak during February when birds return from Mexico and congregate before departing in March or April for northern breeding grounds. Wintering grebes at Salton Sea consume vast quantities of aquatic invertebrates, primarily pileworms (*Nianthes succinea*), water boatmen (Corixidae), and amphipods (Jehl and McKernan 2002).

Mortality events of Eared Grebes along the Pacific Flyway have been previously documented in Gulf of California, Mexico (Nishikawa et al. 1984, Jehl et al. 2002), Great Salt Lake, Utah (Jensen and Cotter 1976), and Mono Lake and the Salton Sea, California (Jehl 1988, 1996). Although none were of the magnitude that occurred at Salton Sea during the winter of 1991–1992, beached-bird counts for a grebe die-off at Salton Sea from January to March 1989 were extrapolated to 39,600 grebes and may have been much higher (Jehl 1996). Most previous Eared Grebe die-offs involved <10,000 individuals. Jehl (1988) estimates an average mortality of Eared Grebes at Mono Lake at 1370 to 3628 birds/yr. Many die-offs were attributed to severe weather (Jehl and Bond 1983, Ryser 1985, Jehl 1988), avian cholera (National Wildlife Health Center (NWHC), unpubl. data), or food shortages (Jehl 1988, Jehl et al. 2002). Jensen and Cotter (1976) reported 5000 Eared Grebes died of bacterial infection (*Erysipelothrix rhusiopathiae*) in Great Salt Lake, Utah, in 1975. The causes for many mortality events involving grebes remain undiagnosed (Nishikawa et al. 1984, Jehl 1988) or were never investigated (Jehl 1996). This paper reports the size of the grebe die-off in 1991–1992 and the extensive investigation to determine the potential role of various disease agents, contaminants, or biotoxins.

METHODS

Estimation of Number of Dead Eared Grebes

Biologists from the Salton Sea National Wildlife Refuge picked up Eared Grebes along 169 km of Salton Sea shoreline plus 40 km of shoreline associated with freshwater drains and wetlands adjacent to the Sea. Dead birds were collected between 16 December 1991 and 21 April 1992. Biologists felt this encompassed three peaks in mortality: the first began 16 December 1991, the second 19 January 1992, and the more major event that occurred 19 February through April 1992. However, low-level ongoing mortality may have also been occurring without detection during this time. Aerial and shoreline surveys documented that all beaches contained dead birds in approximate proportion to the number of live grebes concentrated offshore. While it was not possible to pick up carcasses from the entire area, carcasses were collected from complete surveys during February and March 1992 that covered 64 km (31%) of the most accessible shoreline (Fig. 1). An estimate of the total Eared Grebe mortality was then calculated by extrapolating the number of carcasses picked up in the area thoroughly surveyed to the total shoreline where the carcasses were documented. Carcasses were either saved for necropsy, frozen for later chemical analysis, or incinerated.

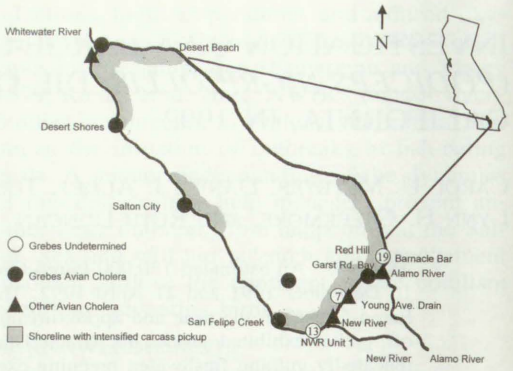

FIGURE 1. Distribution of birds collected and submitted to the National Wildlife Health Center during the 1992 Eared Grebe die-off at the Salton Sea, California. The numbers inside the symbols indicate the number of birds with that diagnosis submitted from that site.

Diagnostic Evaluation

Gross examination

Fifty Eared Grebes, six Ruddy Ducks (*Oxyura jamaicensis*), two Herring Gulls (*Larus argentatus*), and single specimens of the American Widgeon (*Anas americana*), Ross's Goose (*Chen rossii*), Ring-billed Gull (*Larus delawarensis*), American Coot (*Fulica americana*), Short-billed Dowitcher (*Limnodromus griseus*), Western Sandpiper (*Calidris mauri*), and American White Pelican (*Pelecanus erythroryhnchos*) were shipped chilled to the NWHC. Within 48 hrs of collection, 23 carcasses were necropsied and 42 carcasses were frozen. Nineteen birds were euthanized; the others were found dead. Collection date and location were recorded as well as sex, age, physical condition, and body mass (four decapitated grebes were not weighed). Birds were categorized as immature if the gonads were undeveloped and thymus or bursa were detectable at necropsy. Birds classified as emaciated had no obvious subcutaneous, pericardial, or intra-abdominal fat; birds in poor to fair condition had trace body fat; birds in good body condition had moderate to abundant fat. Samples were collected for routine bacterial culture, virus isolation, histology, parasitology, and toxicology.

Histopathology

Twenty-four different tissues were sampled from 44 eared grebes (a total of 420 tissues), and fixed in 10% neutral buffered formalin. Tissues were processed as previously described (Meteyer et al. 1997). No histopathology was performed on other species.

Routine bacteriology

Samples of liver (63) and assorted tissues (24) were cultured for aerobic bacteria and salmonella (12) as previously described (Meteyer et al. 1997). Identity of bacterial isolates was determined using the API-20E system (bioMerieux Vitek, Inc, Hazelwood, MO). *Pasteurella multocida* isolates from seven Eared Grebes and five other species were sent to the National Veterinary Services Laboratories (NVSL) in Ames, Iowa, for serotyping (Rhoades et al. 1989) and DNA analysis (Wilson et al. 1992). The same 12 *P. multocida* isolates were sent to the National Animal Disease Center (NADC) to test for presence of dermonecrotizing toxin using a colony-blot assay described by Magyar and Rimler (1991) and Ackerman et al. (1994). The air sac from one American White Pelican was cultured for fungi using Sabauraud's dextrose agar (Remel, Lenexa, KS). Trachea and lungs from seven grebes were cultured for mycoplasma using culture and identification methods described by Goldberg et al. (1995).

Intensive P. multocida *assays*

In addition to routine culture on blood agar, culture attempts using *P. multocida* selective broth (PMSB) and agar (PMSA; Moore et al. 1994) were performed on heart (8), blood (12), intestine (13), brain (2), lung (8), liver (6) and trachea (3) collected from 26 grebes and frozen after necropsy. Extracts from these same frozen tissues were inoculated intraperitoneally into mice. Livers from mice that subsequently died (22) were cultured for aerobic bacteria and for *P. multocida* using PMSA, and isolates were identified as described above.

Tests for Clostridium botulinum *types C and E toxins*

Thirty-four grebes were tested for types C and E avian botulism. Several positive or suspect samples for type C toxin were further tested to determine if type C-1 or C-2 toxin was present. The tests were performed on centrifuged heart blood collected at necropsy or serum from blood collected prior to euthanasia using the mouse protection test (Quortrup and Sudheimer 1943) and antitoxins specific for *botulinum* neurotoxin types C-1, C-1 and C-2 combined, and E. The mice were observed for clinical signs for five to seven consecutive days.

Assay for cyanobacterial toxins

Liver samples from five grebes and the contents of gizzard and intestines from five more grebes were analyzed for cyanobacterial microcystin toxins using enzyme-linked immunosorbent assay (Chu et al. 1989 as modified in An and Charmichael 1994) and a protein phosphatase 2A inhibition assay (Lee 1992).

Virology

Virus isolation was attempted on 91 tissues from 33 grebes, two Ruddy Ducks, and one American Coot. Tissue homogenates were centrifuged and inoculated into duck embryo fibroblast cell culture or the allantoic cavity of 10-day-old chick embryos as previously described by Docherty and Slota (1988) and Senne (1989).

Parasitology

Standard parasitological procedures were used to examine the small intestine from ten grebes and one Short-billed Dowitcher, and the gizzard from two grebes. The mucosa and serosa of intestinal sections were examined grossly; the mucosa was scraped, and the scraped material was sedimented using a standard sedimentation glass. Wet mounts of the sediment were examined under a dissecting microscope. Sections of skin from the back, neck, and wing area of five grebes were also examined for parasites using a dissecting scope. In addition, feathers pulled from these skin sections were dissected, and the feather and feather sheaths were examined directly using a dissecting microscope. The remainder of the parasitological information was obtained from examination of histologic sections of intestinal tract, skin, adrenal glands, liver, and skeletal muscle.

TISSUE AND ENVIRONMENTAL TOXICOLOGY

Tests and methodology are summarized in Table 1. Briefly, liver was analyzed for lead, and brain was measured for cholinesterase (ChE) activity to assess potential exposure to organophosphate or carbamate. Brain was also analyzed for sodium to rule out the possibility of sodium poisoning in the highly saline Sea.

An additional 38 Eared Grebes (five dead, five dying, five healthy; nine recently dead from the north and nine from the south of Salton Sea; six reference Eared Grebes from Camp Pendleton Marine Corps Base, San Diego, CA) were collected by gunshot, euthanized by cervical dislocation, or picked-up within 24 hrs of death to analyze liver for contaminants and heavy metals; further diagnostic information was not collected from these grebes. Ten Ruddy Ducks co-mingling with Eared Grebes at the Salton Sea and showing clinical signs similar to the sick grebes, as described above, were subsequently found dead, and liver from these birds was also collected for analysis. In addition, liver samples from Eared Grebes were collected in 1993 from staging or stopover areas in the Great Basin region (Iron Mountain, Mono Lake, Snow Summit, Great Salt Lake) for similar contaminant analyses (Rattner and Jehl 1997) to provide further comparison to Eared Grebes sampled at the Salton Sea in 1992. After removal from carcasses, all liver samples were immediately frozen in 8-oz chemically clean jars and sent to USFWS's Patuxent Analytical Control Facility (PACF) in Laurel, Maryland, where they were analyzed for heavy metals and organochlorine compounds (Table 1).

Sediment sampling

On 27–28 February 1992, sediment samples were collected in chemically clean jars for organochlorine, heavy metal, and Microtox testing using a petit ponar dredge from areas where grebes were congregating. Surface water samples were also collected for Micro-

TABLE 1. ANALYTICAL TESTS AND METHODS CONDUCTED ON SAMPLES COLLECTED FROM THE SALTON SEA, CALIFORNIA

Test	Analytical technique	Material tested	Reference	Species tested
Sodium	Emission spectrometry	Brain	Windingstad et al. 1987	Grebe (6), Ruddy Duck (2)
Selenium arsenic	Graphite furnace atomic absorption	Sediment, liver, invertebrates	Krynitsky 1987	
Mercury	Cold vapor atomic absorption spectrophotometry	Sediment, liver, invertebrates	Monk 1961	
Lead	Atomic absorption spectrometry	Liver	Locke et al. 1991	Grebe (1), Herring Gull (1), Ruddy Duck (2)
Other metals	Inductively coupled plasma emission spectrometry	Sediment, liver, invertebrates	Haseltine et al. 1981	Grebe (1), Herring Gull (1), Ruddy Duck (2)
Organochlorine pesticides	Gas chromatography/mass spectrophotometry	Sediment, liver, invertebrates	Cromartie et al. 1975	
Organophosphate, carbamate	Gas chromatography	Invertebrates	Belisle and Swineford 1988	
Cholinesterase activity	Visible spectrometry	Brain	Hill and Fleming 1982	Grebe (11), Ruddy Duck (2)
Microbe toxicity (Microtox)	Microbe Model 500 Microtox Unit	Water	Microbics Corporation 1991	

tox testing in 50-ml polypropylene conical centrifuge tubes from three major inflows to the Sea from 27–28 February 1992 and 3–13 March 1992 to evaluate potential effects of local rain events. Water samples were centrifuged for 10 min in preparation for testing. Sediment samples were collected and immediately put on ice. Only sediments not in contact with the dredge surface were analyzed. Thirty samples of pileworms, amphipods, and corixids were collected with light traps or hand-held nets and composited by species into 10-g samples. These invertebrate samples represented food items of the birds and were analyzed for concentrations of metals, 17 organochlorines, 12 organophosphates and carbamates, and 40 additional agricultural pesticides.

A Microtox solid phase test was performed on sediment, and a 100% Microtox test was performed on undiluted water samples using a Microbics Model 500 Microtox (R) unit with procedures and EC50 calculations by Microbic Manual and Software, Microbics, Inc. (1991).

Statistics

Contaminant concentrations in tissues were compared between grebes collected from the north (SSN92) and south ends of the Sea (SSS92). These data sets were initially separated because previous results (Setmire et al. 1993) suggested substantial differences in baseline contaminant levels in sediment and aquatic life between these areas. The SSN92 and SSS92 data sets were also compared to data from Ruddy Ducks collected simultaneously at the Sea (RD92), pre-die-off data collected from Salton Sea grebes in 1989 (SS89; Setmire et al. 1993), and grebes collected from Camp Pendleton in 1992 (CP92). Geometric means were calculated for all data sets with <50% 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 so non-parametric statistical tests were used. The Mann-Whitney test was used to determine when data sets could be combined for specific contaminants and to test between reference and Salton Sea data sets.

Contaminant concentrations were also compared between Eared Grebes collected at the Salton Sea and Camp Pendleton in 1992, and those collected at other stopover or staging locations in 1993 (Rattner and Jehl 1997). These samples were grouped by locations regardless of date of collection. Salton Sea birds were separated into two groups, those that were dead or clinically ill (symptomatic) and those that were apparently healthy (asymptomatic). The Kruskal-Wallis test was used to look for significant differences between groups. To identify pair-wise significant differences, an ANOVA was conducted followed by a Tukey multiple comparison test. Results were considered only if the results of the ANOVA agreed with the results of the Kruskal-Wallis test. All statistical tests were run on STATGRAPHICS Version 5.0 (Manugistics, Inc. 1994) and the P-value was set at <0.05/number of comparisons.

RESULTS

ESTIMATIONS OF MORTALITY IN EARED GREBES

A total of 46,040 dead birds were picked up over 64 km of shoreline. These represented 47

different species, including 42,587 Eared Grebes. By extrapolation to the 209 km of shoreline on which carcasses were deposited, we estimated that about 150,000 Eared Grebes died at the Salton Sea during the mortality event of 1991–1992.

Diagnostic Evaluation

Necropsy findings

Necropsy findings were variable in the 50 Eared Grebes examined (25 female, 24 male, sex undetermined in one; nine immature, 41 adult). Wet and disheveled feathers were a common observation made by field biologists and present in 7/36 Eared Grebes submitted to the NWHC; all those were from the south end of the Sea without a diagnosed cause of death. Grebes varied in body condition: 19 were emaciated (mean 212 g, range 151–290 g); 12 were poor to fair (mean 265 g, range 230–320 g), and 19 were good (mean 321 g, range 230–450 g). The mean mass of Eared Grebes dying from avian cholera was 298 g versus 242 g for those dying from an undetermined cause. Gulls attacked weakened grebes, which appears to explain the bruising over the back of the head, neck, and shoulders seen in 17 Eared Grebes dying from both avian cholera and an undiagnosed cause. No external lesions were seen in 26 grebes. Urate material was seen on the feathers around the vent of 11 grebes but no significant changes were seen in the kidneys microscopically. Livers were swollen and congested in 21 grebes, one grebe had liver fracture with hemorrhage, and two grebes had livers with white spots (one was ultimately diagnosed with avian cholera). Lungs were congested and firm in 31 grebes; 13 of these sank in formalin, suggesting severe edema with possible hemorrhage. Spleens were enlarged and friable in four grebes. Of the ten bone marrows evaluated, three were very pale. The ventriculus of normal grebes contains a large, moist, loose feather pellet; in ten grebes from the Salton Sea, this feather pellet was very dry and compact, suggesting dehydration. Results from microscopic examination of tissues were inconsistent and inconclusive in the grebes that died without a diagnosis. Kupffer cells in the liver and phagocytic cells in the spleen contained structures that resembled red cell fragments in seven grebes and moderate hemosiderin was common in both organs (23 grebes). Small vacuoles were often seen in hepatocytes (32 grebes). All of these are nonspecific changes and their significance remains unknown. Eared Grebes (15) and other species (14) with *P. multocida* isolated had both gross and microscopic lesions consistent with avian cholera (Friend 1999), although the lesions were more subtle in the grebes.

Pulmonary edema was present in 18 grebes. Bacteria were seen in sections of lung in ten of these grebes and *P. multocida* was isolated from nine of them. Microscopic inflammation was seen in the skin of seven grebes, four of which had trauma (gull predation) noted at necropsy. Inflammation was not associated with mites seen in the keratin layer of five grebes. The four grebes with bacteria in the subcutaneous tissue died of avian cholera. Schistosomes were seen in the venous channels of adrenal glands from 15 of 28 grebes, often associated with amyloid. Although mild lymphocytic inflammation was seen along the adrenal periphery of four grebes, this inflammation did not seem directly associated with schistosomes or amyloid and the significance of the adrenal changes is unknown.

The American White Pelican was emaciated, had tan nodules in the lung and severe airsacculitis with sheets of fruiting fungal organisms consistent with aspergillosis.

Bacteriology

No bacteria were isolated from the liver of 27 of 48 Eared Grebes. *P. multocida* serotype 1, the causative agent of avian cholera, was isolated from 15 Eared Grebes, 14 grebes using standard culturing procedures. Of the 15 grebes with isolation of *P. multocida,* 13 were collected dead and nine were from the west or north shores of the Sea. Avian cholera usually kills quickly without chronic loss of mass, which is consistent with our findings. The mean mass of the grebes dying of avian cholera was 298 g whereas the mean mass of grebes without *P. multocida* isolated was 242 g. Analyses of 12 *P. multocida* isolates for unique DNA or potential dermonecrotizing toxin production was negative. The following additional bacteria were isolated from only 1–3 grebes and were not considered significant to the mortality: *Staphylococcus* spp., *Vibrio parahemolyticus, Vibrio alginolyticus, Aeromonas hydrophila, Escherichia coli, Pseudomonas putrifaciens, Pseudomonas* sp., *Enterococcus feacalis, Serratia marcescens, Proteus* sp., and *Hafnia alveii.*

Pasteurella multocida was isolated from liver by routine culture in 14 of 15 non-grebe species submitted. Of these, the only one without *P. multocida* was the American White Pelican, which had gross and microscopic lesions of severe aspergillosis in the lungs and air sacs from which *Aspergillus fumigatus* was isolated.

Intensive P. multocida *assays*

Culture attempts using selective media (PMSB and PMSA) did not yield additional iso-

TABLE 2. PRELIMINARY SCREEN OF LIVER CONCENTRATIONS (μG/G, GEOMETRIC MEAN) FROM COMPOSITED DEAD, DYING AND HEALTHY EARED GREBES COLLECTED FROM THE SALTON SEA, CALIFORNIA, IN 1992

	Arsenic	Cadmium	Chromium	Mercury	Nickel	Lead	Selenium	Zinc	p,p′-DDE
Dead (N = 5)	1.4	2.3	6.7	16	1.8	1.6	47	140	5.7
Dying (N = 5)	2.1	2.1	1.9	7.7	<0.64	<0.64	34	160	1.5
Healthy (N = 5)	0.26	1.9	1.7	14	0.8	<0.77	44	97	<0.019

Note: Concentrations are in dry mass for trace elements and wet mass for DDE.

lations of *P. multocida* from Eared Grebes. Of 49 tissues cultured from 26 grebes, *P. multocida* was only isolated from tissues of three grebes (lung, blood, and intestine), all of which had been previously confirmed by routine culture. Inoculation of mice was slightly more successful. One mouse inoculated with blood from a grebe without isolation of *P. multocida* died one day after inoculation, and *P. multocida* was isolated from the liver of this mouse.

Tests for Clostridium botulinum *type C and E toxin*

The test results from only 24 of 34 grebes blood samples tested for avian botulinum toxins could be interpreted. Of these, two samples were positive for botulism C-1 toxin but these were from grebes that were moderately autolyzed, and the presence of C-1 toxin likely represents post-mortem toxin formation. The other ten samples were from grebes that died from avian cholera, and it was determined that the inoculated mice died from *P. multocida* infection that was subsequently isolated from their livers. No *Clostridium* toxin type C-2 or E was detected.

Virology

An enveloped RNA virus was isolated from the lung of one Eared Grebe and from the trachea of another using Muscovy duck embryo fibroblast cell culture. These viruses were not embryo-lethal and were not further characterized.

Mycoplasma *cultures*

A *Mycoplasma* sp. was isolated from the lung of one of seven grebes, but was untypable using conventional methods and was considered an incidental finding.

Parasitology

Cestodes were identified in the intestine of nine of ten grebes. *Acuaria* sp. was identified in both of the gizzards submitted for parasitologic examination. Demoglyphic quill mites (*Paralges* sp.) were found within the sheaths of new emerging contour feathers of the neck and/or wing of four of six grebes and in histologic sections of skin from four others. Mites were not associated with inflammation microscopically and they were not considered to be the cause of the excessive preening in the grebes. Lice (*Pseudomenopon insolens*) were seen in four grebes. The intestine of the Short-billed Dowitcher contained flukes (*Galactosomum* sp.), and nematodes (*Contracaecum* sp. and *Capillaria* sp.).

TISSUE AND ENVIRONMENTAL TOXICOLOGY

Tissue analysis

Lead was not detected in livers of birds tested. No inhibition of brain cholinesterase activity was detected in the 11 Eared Grebes and two Ruddy Ducks tested (median 17.4 ± 0.6 micromoles/min/g and 13.7 ± 0.2 micromoles/min/g respectively). Brain sodium levels in six Eared Grebes and two Ruddy Ducks were within the normal range with respective means of 1675 and 1295 ppm wet mass.

Concentrations of selected contaminants in livers from dead, dying, and apparently healthy grebes collected late February 2002 are presented in Table 2. These data were collected as a preliminary screen to determine if contaminants were potentially implicated in the cause of the die-off. Based on elevated levels of arsenic, chromium, mercury, selenium, and zinc (Ohlendorf et al. 1988; Eisler 2000a–e) in tissue samples, further sampling was justified. Although zinc levels in dead and dying birds were about two-fold higher than in healthy birds and the p,p′-DDE concentrations in liver suggested a trend of increase in DDE from healthy to sick to dead grebes, the concentrations were below lethal thresholds known for birds (Stickel et al. 1970, Blus 1996, Eisler 2000c).

The results of further analysis of Eared Grebes and Ruddy Ducks were not in the lethal range for any metal or contaminant (Table 3). There were no significant differences between results for the north and south 1992 Salton Sea Eared Grebes for arsenic, cadmium, chromium, mercury, selenium or zinc. There were no significant differences between the Salton Sea 1989 and Camp Pendelton 1992 reference sets for cadmium, mercury, or selenium, and there were no significant differences between these reference data and the results for cadmium, chromium, or mercury in birds dying at Salton Sea in

TABLE 3. Comparison of Geometric Mean (Range) of Liver Contaminant Concentrations (μG/G[a]) in Liver from Eared Grebes, Ruddy Ducks, and Food Items of Eared Grebes from the Salton Sea and Liver from Reference Eared Grebes Collected from Staging or Stopover Locations

Location	Species	Collection year	N	Arsenic	Cadmium	Chromium	Mercury	Selenium	Zinc	p,p'-DDE
North Salton Sea	Eared Grebe[b]	1992	9	1.0 (<0.29–37)	2.2 (1.3–12)	1.6 (<1.1–11)	6.1 (0.9–17)	27 (21–46)	150 (110–170)	
South Salton Sea	Eared Grebe[b]	1992	8	0.98 (<0.29–2.6)	2.5 (0.89–11)	1.3 (<0.59–6.1)	8.9 (4.2–15)	30 (17–53)	140 (74–170)	
Salton Sea	Eared Grebe[c,f]	1989	5	nd[d] (<0.1–0.1)	2.5 (1.4–6.7)	nd (<1–1)	5.1 (1.0–13)	13 (2.7–35)	97 (75.6–116)	
Camp Pendleton	Eared Grebe[c]	1992	4	nd (<0.295)	nd (<0.221–1.44)	nd (2.64–5.41)	3.15 (0.933–5.48)	7.01 (3.65–12.1)	64.3 (49.9–72.2)	0.0814 (0.0157–382)
Great Salt Lake	Eared Grebe[c,e]	1988	15	nd (<0.230–2.22)	1.20 (<0.327–3.20)	10.4 (3.08–48.6)	4.47 (0.757–9.34)	6.42 (2.61–11.0)	77.9 (53.8–124)	0.0192 (<0.01–0.100)
Iron Mountain	Eared Grebe[c,e]	1988	5	nd (<0.305)	1.31 (<0.783–3.81)	3.48 (1.30–7.29)	8.57 (5.80–11.3)	17.2 (12.3–21.3)	96.4 (61.6–140)	0.0460 (<0.01–0.296)
Mono Lake	Eared Grebe[c,e]	1988	18	0.376 (<0.187–958)	1.59 (0.778–4.36)	6.35 (2.14–18.7)	6.21 (1.50–17.5)	12.8 (6.42–35.7)	85.7 (53.0–198)	0.0211 (<0.01–0.263)
Snow Summit	Eared Grebe[c,e]	1988	3	nd (<0.302)	3.33 (2.53–4.09)	7.64 (6.33–9.15)	14.3 (11.8–17.2)	27.7 (20.5–35.7)	138 (111–190)	
Salton Sea	Eared Grebe[b]	1992	29	1.04 (<0.297–10.4)	1.63 (0.831–4.23)	10.7 (1.90–39.0)	7.27 (0.184–19.8)	29.0 (17.1–56.2)	133 (86.5–210)	0.0761 (<0.01–2.31)
Salton Sea	Eared Grebe[c]	1992	26	1.56 (<0.297–6.41)	1.44 (0.819–11.3)	7.77 (1.27–23.5)	7.57 (1.26–17.4)	22.9 (12.0–38.1)	137 (80.9–204)	0.0870 (<0.01–1.93)
Salton Sea	Ruddy Duck	1992	10	nd (<0.31–0.48)	0.91 (<0.36–2.0)	2.8 (<0.70–10)	0.22 (<0.08–1.0)	12 (9.2–24)	150 (100–230)	
Salton Sea	Water boatmen[f]	1988–1989	4	0.80 (0.30–2.0)		1.0 (<0.40–2.47)	nd (<0.10–0.12)	2.2 (1.4–3.3)	105 (90.4–121)	
Salton Sea	Water boatmen	1991–1992	14	0.49 (0.16–3.3)		nd (<2.5–7.5)	nd (<0.72)	2.9 (1.2–11)	127 (61–253)	0.028 (0.01–0.09)
Salton Sea	Pile-worms[f]	1988–1989	6	5.1 (2.9–22)		7.5 (1.7–27)	nd (<0.38)	3.1 (0.82–12.1)	58 (32–164)	
Salton Sea	Pile-worms	1991–1992	6	3.9 (1.9–5.1)		3.2 (2.6–5.9)	nd (<0.46)	6.6 (4.7–12)	85 (47–122)	0.13 (0.05–0.31)

[a] Concentrations are in dry mass for trace elements and wet mass for DDE.
[b] Symptomatic (sick/dead birds).
[c] Asymptomatic (healthy birds).
[d] nd = ≥50% of samples had non-detectable concentration.
[e] Data from Rattner and Jehl (1997).
[f] Data from Setmire et al. (1993).

1992. Selenium concentrations were significantly higher ($Z = 1.9842$, $N = 27$, $P = 0.047$) in grebe liver tissues collected during the die-off versus the reference data set, but were lower than selenium values in Ruddy Ducks collected earlier from the Salton Sea (Koranda et al. 1979, Setmire et al. 1990).

Zinc concentrations in the Eared Grebe liver tissues were significantly higher in Eared Grebes collected during the die-off in 1992 versus either reference or pre die-off results but were comparable to levels of selenium in normal Eared Grebes at various stages of their migration/staging (6.42–35.65 ug/g; Rattner and Jehl 1997). These levels are not in a range that would be considered toxic (Eisler 2000c).

Contaminant concentrations in grebes varied among additional samples from asymptomatic grebes in 1992 and from birds at staging (Mono Lake and Salton Sea, California) and stopover points (Iron Mountain and Snow Summit, California) in 1993 (Table 3). No significant differences were found among the various groups in cadmium and p,p′-DDE concentrations. Arsenic values were significantly different among groups in both the Kruskal-Wallis test ($H = 25.9$, $P < 0.001$) and ANOVA ($F = 3.88$, $P = 0.012$). In the pairwise comparison, the Mono Lake group had significantly lower arsenic concentrations than each of the Salton Sea groups. The other groups lacked adequate data for comparison.

Selenium concentrations also were significantly different among sample groups (Kruskal-Wallis test $H = 70$, $P < 0.001$; ANOVA $F = 27$, $P < 0.001$). Selenium values of the Camp Pendleton, Great Salt Lake, and Mono Lake samples were all significantly lower than those of the Snow Summit, Salton Sea symptomatic, and Salton Sea asymptomatic groups. Samples for Iron Mountain and the Salton Sea asymptomatic group were each significantly lower in selenium than that of the Salton Sea symptomatic group; however, there was no significant difference in selenium concentration between Salton Sea symptomatic group and Snow Summit samples.

Zinc concentrations also were significantly different among sample groups for both the Kruskal-Wallis test ($H = 53$, $P < 0.001$) and ANOVA ($F = 14$, $P < 0.001$). The Camp Pendleton, Great Salt Lake, and Mono Lake samples were significantly lower in zinc than those of the Salton Sea symptomatic and Salton Sea asymptomatic groups. However, there was no significant difference in zinc concentration between the Salton Sea and Snow Summit samples.

Microtox assays

Multiple repetitions of the 100% Microtox test for water were inconclusive with an indication of high nutrient input or low level toxicity (B. Walburn and M. Henry, pers. comm.) based on an increase in light output (enhancement) of the test bacteria for all locations. No EC50 could be determined with increased light output.

Sediment chemical analysis

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

Cadmium, molybdenum, tin, and beryllium were below detection limits for all locations. Concentrations of arsenic, barium, boron, chromium, copper, manganese, nickel, lead, vanadium, and zinc were all well within the baseline range for soils in the western United States (Shacklette and Boerngen 1984, Severson et al. 1987). Concentrations of arsenic, barium, chromium, copper, nickel, lead, selenium, vanadium, and zinc were lower than previously reported values for the Salton Sea (Setmire et al. 1990). Selenium concentrations from the Whitewater and Alamo river outlets were very similar to previous levels, whereas the New River outlet sample was well below previous levels (Setmire et al. 1990).

Aquatic invertebrate analysis

There were no 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 corixid samples collected in 1988–1989 and those in 1991–1992 collected 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, and mean values for pileworms and corixids collected during the 1992 die-off were higher than ones collected in 1989 (Table 3).

Organochlorine, p,p′-DDE, was found in trace amounts in all invertebrate samples ($N = 7$), and 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 estimated 150,000 Eared Grebes that died at the Salton Sea in 1992 represented about 6% of the North American population of approximately 2.5 million birds (Jehl 1996). We speculate that this mortality estimate is conservative. Low-level aerial surveys of the Salton Sea throughout the die-off documented many dead grebes floating at sea. Some of these carcasses would likely have become waterlogged and sunk before reaching the shoreline. Some carcasses that reached the shore were destroyed by wave action along the rocky shore and others were likely buried by sand or crushed barnacles. Predation and scavenging of sick and dead grebes was also widespread, with coyotes and gulls observed feeding on carcasses.

Gaps in the data make it difficult to estimate the percent of Eared Grebes that were killed by avian cholera or died from an undetermined cause. Biologists did not collect Eared Grebes from the north end of the Sea during the smaller die-offs in December 1991 and January 1992. When grebes were collected from the north and west shores of the Sea in March 1992 toward the end of the die-off, they were diagnosed with avian cholera. Although biologists felt that symptoms in grebes from the north were similar to those from the south and that the same undiagnosed syndrome was occurring throughout the Sea from December through April, data were unavailable to confirm this hypothesis. From the last week in February through April 1992 the cause of mortality in the majority of grebes (26/32) from the southern end of the Salton Sea continued to be undetermined even though other species picked up from similar southern and northern locations were positive for avian cholera (Fig. 1).

Large epornitics in waterbirds caused by *P. multocida* have been summarized by Friend (1999). For example, during an avian cholera die-off in Chesapeake Bay 31,295 carcasses were picked up. The carcasses were estimated to represent 10–80% of actual mortality and consisted primarily of Long-tailed Ducks (*Clangula hyemalis*), but also included Horned Grebes (*Podiceps auritus*; Montgomery et al. 1979). An estimated 32,000 from a population of 150,000 Eared Grebes died from avian cholera at the Great Salt Lake in 1998 (NWHC, unpubl. data). Most avian cholera mortality events, including the grebe die-offs at both the Salton Sea in 1992 and Great Salt Lake in 1998, are due to *Pasteurella multocida* serotype 1 (Wilson et al. 1995). A notable difference in the Chesapeake Bay event was the isolation of *P. multocida* serotype 3 as the cause of mortality, which is unusual in waterfowl.

Avian cholera was not the cause of all Eared Grebe mortality at the Salton Sea in 1992, and, despite the use of selective and enrichment media and mouse bioassays, *P. multocida* was not isolated from 35 grebes collected at the south end. Furthermore, the isolates of *P. multocida* from this event were not serotypically or genetically unique (all were serotype 1), and assays for dermonecrotoxins, produced by some of the other pathogenic serotypes of *P. multocida,* were not detected, suggesting that the *P. multocida* isolates were not uniquely pathogenic. For 35 birds, no cause of death could be determined.

Salt toxicosis was considered a potential cause of the illness because grebes were congregating and appeared to be drinking at the freshwater inflows. Brain sodium levels, however, did not confirm toxicity. Furthermore, Eared Grebes have been found to tolerate high salinity levels at Mono Lake, California (Mahoney and Jehl 1985), as well as salinity as high as 160,000 mg/L at Great Salt Lake, Utah (Jehl 1988), which is four times greater than the salinity of the Salton Sea during the 1992 mortality.

Concentrations of selenium and zinc were significantly higher in livers of dead grebes from 1992 than in healthy grebes collected along the California coast, but water, sediment, and invertebrate samples from the Salton Sea did not have significant concentrations of either of these inorganic constituents, suggesting no biological pathway for exposure at the Sea. Ohlendorf et al. (1988) and Ohlendorf and Marois (1990) found Eared Grebes had the greatest level of selenium bioaccumulation compared to many other waterbirds. Rattner and Jehl (1997) found higher selenium concentrations in livers from normal Eared Grebes collected at Mono Lake compared to Salton Sea, and selenium levels from grebes at Snow Summit were nearly as high as those of grebes that died at the Salton Sea.

Even though the liver concentrations of zinc in dead grebes from the Salton Sea were among the highest reported for grebes (J. Skorupa, pers. comm.), zinc dietary concentrations were within acceptable ranges and below any known mortality threshold (>2000 mg/kg) for birds (Gasaway and Buss 1972, Eisler 2000c), and liver levels were elevated but below the toxic range for domestic poultry (200–700 ppm; Puls 1988). Eisler (2000c) suggested that zinc concentrations in field-collected samples are highly variable and difficult to interpret as interactions of zinc with many chemicals (including cadmium, chromium, mercury, and selenium) may alter patterns of accumulation, metabolism, and tox-

icity. Rattner and Jehl (1997) showed that the body condition of Eared Grebes can greatly influence the size of the liver; birds in poor body condition have higher concentrations of some elements due to liver atrophy. Body condition can fluctuate dramatically in Eared Grebes as part of their normal annual cycle (Jehl 1997). More severe reduction in body condition can occur as a result of decreased food supply (Jehl et al. 2002) or illness. Many of the grebes that were necropsied were emaciated with associated liver atrophy, which might have increased the concentrations of zinc and other elements in this study. Even so, levels of these elements were not in the range known to be toxic. Interestingly, Morris et al. (1986) and Hudson et al. (1984) reported excessive drinking in birds as a sign of zinc toxicosis, and some of the pathology in the Eared Grebes, such as red cell fragments and hemosiderin in the spleen and liver have also been reported in zinc toxicosis (Droual et al. 1991).

The liver p,p′-DDE levels found in the initial screen of grebe tissue had 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, Ohlendorf and Miller 1984, Blus 1996). Median concentrations of arsenic, chromium, and mercury in grebe livers were not at levels known to cause independent lethality (Eisler 2000a,b,d).

The Salton Sea is a nutrient-rich body of water receiving extensive agricultural fertilizers from the Imperial and Coachella valleys and waste inflows from Mexico entering through the New River. These high nutrient loads in association with warm temperatures promote algal blooms with the potential to produce associated biotoxins. Analysis for cyanobacterial toxins in livers and upper gastrointestinal contents of grebes were difficult to interpret because of a lack of clinical and lethal threshold concentrations for microcystins in wild birds. Biotoxins associated with algal blooms in a saline sink such as the Salton Sea are not well documented. Prior to and in the early stages of the mortality event, the Salton Sea area received an abnormally high amount of rain. However, without proper water sampling prior to, during, and following major rain events, pulses of nutrients or other contaminants that might be associated with heavy rains cannot be estimated.

It is interesting to note that the outflow of the New River, which flows north from Mexico, is at the south end of the Salton Sea where the undiagnosed grebe mortality occurred. During a 1963 die-off at a sewage lagoon in Canada (Nero 1968), four species of grebes became wet shortly after landing, presumably from contact with waste detergents, and spent almost all of their time on shore preening. This description of loss of water-proofing and obsessive preening is very similar to the activity seen in grebes at the Salton Sea. Loss of water-proofing can compromise buoyancy and lead to hypothermia (Nero 1968). Obsessive preening and loss of buoyancy might interfere with the grebes' ability to acquire adequate food. Water intake for Eared Grebes is usually via water content in prey (J. Jehl, pers. comm.), and inadequate food consumption could lead to dehydration as well as poor body condition. Unfortunately, deaths associated with hypothermia, poor nutrition, and dehydration are difficult to definitively diagnose, although Eared Grebes that were killed by avian cholera during this die-off (usually a very short clinical course before death) had an average mass of 298 g while the average mass Eared Grebes that died from an undetermined cause was only 242 g.

In summary, avian cholera killed numerous grebes and other waterbirds at Salton Sea in 1992, particularly at the northern end of the Sea, but did not appear to be involved in the death of the majority of the Eared Grebes from the southern end of the Sea. Contaminant concentrations in the livers of Eared Grebes (Tables 2, 3) were below acute mortality (Ohlendorf and Miller 1984; Eisler 2000a–e). Selenium levels measured in Salton Sea grebes were in the range reported to potentially cause reproductive problems (Heinz et al. 1988, 1989; Ohlendorf et al. 1988, 1989; Ohlendorf and Marois 1990) but not mortality (Eisler 2000e). Tests for avian botulism, salmonella, viral infections, and salt toxicosis were also negative. Potential causes of the undiagnosed mortality may have been related to interactive effects of an as yet unidentified biotoxin, contaminants, or contaminant-related immunosuppression (Fairbrother and Fowles 1990, Whiteley and Yuill 1991) that might make the grebes vulnerable to a pathogen at concentrations below detection, a difficult to isolate manifestation of avian cholera, or hypothermia and poor nutrition as a consequence of loss of feather waterproofing. Studies at the Salton Sea continue and have expanded to include investigations of the role of microbial toxins and factors that could lead to hypothermia as potential causes for the die-offs. Until one or more specific etiologies are identified, it is difficult to devise methods to reduce losses in grebes. Although smaller die-offs of Eared Grebes are not unusual in winter, a die-off of the magnitude seen in 1992 has not recurred.

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