**U. S. DEPARTMENT OF THE INTERIOR**
FISH AND WILDLIFE SERVICE
REGION 1, PORTLAND, OREGON

# Factors Affecting Reproductive Success of the California Clapper Rail (*Rallus longirostris obsoletus*) in San Francisco Bay.

## Final Report, Investigation ID #: 199810005

**By**

**Steven E. Schwarzbach, Joy D. Albertson, and Carmen M. Thomas**

Sacramento, California
July, 2003

BLANK PAGE

U. S. Department of the Interior
Fish and Wildlife Service
Region 1, Portland, Oregon

# Factors Affecting Reproductive Success of the California Clapper Rail (*Rallus longirostris obsoletus*) in San Francisco Bay.

**Final Report, Investigation ID #: 199810005**

**Steven E. Schwarzbach[1], Joy D. Albertson,[2] Carmen M. Thomas [3]**

[1] Current address: U. S. Geological Survey, Western Ecological Research Center, 7801 Folsom Blvd, Suite 101 Sacramento, California 95826.

[2] U. S. Fish and Wildlife Service, San Francisco Bay National Wildlife Refuge Complex, P.O. Box 524, Newark, California 94560.

[3] Current Address: U. S. Fish and Wildlife Service, Snake River Basin Office, 1387 South Vinnell Way, Room 368, Boise, Idaho 83709.

**ABSTRACT.** We assessed the reproductive success of the California clapper rail (*Rallus longirostris obsoletus*), an endangered species restricted to San Francisco Bay, and the relative importance of predation, flooding and contaminants as factors affecting that success. Our study was conducted in six tidal marshes in both the north and south reaches of San Francisco Bay. This assessment, conducted over a period of 4 years (1991, 1992, 1998, 1999), determined that fecundity of clapper rails was much reduced over the natural potential. Only 69 percent of clapper rail eggs whose viability could be assessed were viable. Rail egg hatchability in north and south bay marshes was 65 percent and 70 percent respectively. Only 45 percent of the nests successfully hatched at least one egg. Despite mean clutch sizes of 6.66 and 6.94 in the north and south bays respectively, clapper rails produced only 1.9 and 2.45 young per nesting attempt. Flooding was a minor factor in reducing fecundity, accounting for only 2.3 percent of eggs lost. Predation was a major factor in reducing nest success and accounted for one-third of the eggs lost. Failed clapper rail eggs were collected for embryo examination and chemical analysis of trace elements and organochlorines. Contamination appeared to exert an adverse influence over clapper rail reproductive success as evidenced by observations of deformities, embryo hemorrhaging, embryo malpositions, a depressed rate of hatchability, the exceedance of avian embryotoxic threshold concentrations for mercury, barium and chromium in some eggs, and the correlation of deformities with elevated concentrations of trace elements in fail-to-hatch rail eggs. While all marshes had impaired hatchability, the marshes with the lowest rate of hatchability were those adjacent to potential contaminant sources. Mercury was the only significant contaminant common to all marshes. Mercury concentrations exceeded the LOAEC of 0.5 ug/g on a fresh wet weight basis in at least some failed eggs in every marsh sampled. Mercury concentrations in failed clapper rail eggs in the south and north bays were 0.54 ug/g and 0.36 ug/g, respectively, and ranged from 0.17 to 2.52 ug/g in the south and from 0.11 to 0.87 ug/g in the north bay.

# INTRODUCTION

The California clapper rail (*Rallus longirostris obsoletus*) is an obligate salt marsh dweller of the tidal marshes of San Francisco Bay. Formerly found in tidal marshes along the California coastline from San Luis Obispo County to Humboldt County, the range of this sub-species has greatly contracted over the last century and the only breeding population of *R. l. obsoletus* is now located solely in the inter-tidal margins of San Francisco Bay. *R. l. obsoletus* was one of the first species to be listed in the United States as endangered under the Federal Endangered Species Act (U. S. Fish and Wildlife Service, 1970 and 1973). At its nadir, the rail population in 1991 was estimated to have declined to about 700 individuals (Harding *et al.*, 1998). The most recent population surveys estimate between 1040 and 1264 individual rails remain in all of the San Francisco Bay area (Harding *et al.*, 1998; Collins *et al.*, 1994 ) with roughly 60 percent of the remaining rails located in the south bay.

Predation by introduced predators, including man, and habitat loss are the proximate causes for the historic decline of the rail. Present day tidal marsh habitat in San Francisco Bay is about 15 percent of historic (1850s) acreage (Dedrick, 1989). Remaining rail habitat in San Francisco Bay is extremely fragmented (Gill, 1979; Collins *et al.*, 1994) with the central bay and north bay having less habitat and more isolated fragments than the south bay (U. S. Fish and Wildlife Service, 2000). Collins et al., (1994) found much ostensibly suitable habitat in the north and central bay was unoccupied by rails. These authors hypothesized that in a fragmented habitat landscape, such as tidal wetlands in San Francisco Bay, a particularly low fecundity could be one explanation for the apparent absence of rails.

Clapper rails, however, are a species with potentially high fecundity. *R. l. obsoletus* produces large clutches (5-14 eggs (DeGroot, 1927; Gill, 1972), and has a prolonged breeding season that may sometimes include a second brood (Wilbur and Tomlinson, 1976). Under optimal circumstances clapper rails have shown good hatching success (percent eggs hatched of those laid) and hatchability (percent of eggs incubated to term which hatch). Kozicky and Schmidt (1949) observed a northern clapper rail (*Rallus longirostris crepitans*) egg hatching success of 87.3 percent and a hatchability rate of 91.6 percent in New Jersey in the mid-1940s. Johnson observed a hatchability rate of 92.3 percent in northern clapper rails in 98 nests in Nassau County, New York in the late 1960s (Johnson, 1973). Zembal and Massey (unpubl. report) found hatching success of light-footed clapper rails (*Rallus longirostris levipes*) in southern California at upper Newport Bay and Anaheim Bay in 1979 and 1980 to be 73.6 percent, while hatchability was 90.5 percent. By contrast, hatching success of *R. l. obsoletus* in three south San Francisco Bay marshes (Dumbarton, Mowry, and Ideal) was reported as only 37.6 percent (Harvey, 1988) in 1980 and an even lower 18.7 percent at two of these same three south bay marshes (Dumbarton and Mowry) in 1989 (Foerster et al., 1990).

Reasons for low hatching success of California clapper rails which have been cited by previous investigators primarily include nest flooding and predation by rats (Harvey, 1988; Zucca, 1954). Previous investigations of contamination in the rail have shown elevated egg concentrations of mercury, selenium and polychlorinated biphenyls (PCBs) in some eggs (Lonzarich et al., 1992; Schwarzbach et al., 2001). The hatchability of eggs of the California clapper rail has not, however, been previously reported. Mercury, PCBs and selenium are contaminants of

particular concern because they are known to accumulate in avian eggs and to adversely impact birds by directly reducing the hatchability of eggs, as well as reducing growth and post hatch survival of juveniles exposed in ovo (Hoffman et al.,1996; Thompson, 1996; Heinz, 1996). Other metals and trace elements, such as chromium and lead, have also been shown to accumulate in bird eggs in other urbanized estuaries (Gochfeld and Burger, 1998). San Francisco Bay has been listed under section 303(d) of the Clean Water Act as an impaired waterbody for selenium, mercury, and PCBs (http://www.swrcb.ca.gov/tmdl/303d_lists.html). Smith et al., (1986) concluded the south bay was a major reservoir of mercury pollution within San Francisco Bay. Ohlendorf et al., (1986) found elevated concentrations of mercury and selenium in livers of diving ducks in south San Francisco Bay. When comparing diving ducks with other species the higher concentrations of selenium were found in benthic foragers.

We report here on the hatching success and hatchability of clapper rail eggs in four south bay marshes in 1992 and two central bay marshes in 1998-99. We assess the relative contributions of nest flooding, nest predation, and contamination of rail eggs to inhibiting rail egg hatching success.

## METHODS

### Nest Monitoring

*Study areas.* Clapper rail nest success was evaluated in six intertidal salt marshes in San Francisco Bay. In this paper we refer to marshes located north of the Golden Gate Bridge (Corte Madera and Wildcat) as north bay marshes and to those south of the San Mateo Bridge (Greco Island, Mowry, Laumeister and Faber) as south bay marshes. Faber Marsh was assessed in 1991 and 1992. Other south bay marshes were assessed only in 1992. North bay marshes were assessed in 1998 and 1999.

*Field methods*. Nest monitoring of clapper rails was done with appropriate state and federal permits and conducted per U. S. Fish and Wildlife Service and California Department of Fish and Game protocols. Nest searches were conducted by teams on foot. Rope drags were not permitted. Nests were flagged at discrete distances and investigators minimized evidence of human disturbance of vegetation near the nest. Time at the nest was kept to the minimum. Nests were visited at least twice. Eggs were candled at the first visit to assess embryo development and to project a hatch date. Eggs with clearly dead embryos were removed from the nest to prevent their loss to potential predators. The second visit corresponded to the day after the projected hatch date and the fate of the nest and individual eggs was recorded. Any eggs with dead embryos were collected at the second visit. The collected eggs were opened in the laboratory under clean conditions.

Embryos of all eggs were examined for stage of development and the presence of gross deformities. Late stage embryos were also examined for malpositions. A late stage embryo which is correctly positioned to hatch will have the head in the blunt end of the egg near the air cell with the head tucked under the right wing. This position is necessary as the embryo must begin pulmonary respiration in the air cell prior to pipping through the shell. Any avian embryo not in this position prior to hatch will fail to hatch successfully and is said to be malpositioned

(Romanoff and Romanoff, 1972).

Analytical Chemistry

Analytical chemistry for trace element concentrations was performed by the Environmental Trace Substance Research Center in Columbia Missouri under a contract to the U.S. Fish and Wildlife Service. Percent moisture was determined by placing a weighed aliquot of the sample in a Fisher isotemp oven, drying and re-weighing the dried sample. For samples too small for oven dried moisture determination, the percent moisture was calculated from the moisture loss during freeze drying in the Labcono freeze-dryer. Digestion of samples for mercury analysis was accomplished with a nitric acid reflux. Mercury concentrations were determined by cold vapor atomic absorption using a Perkin-Elmer Model 403 AA. Aliquots for selenium and arsenic determinations underwent a nitric-perchloric digestion. Determination of selenium and arsenic concentration was via hydride generation with a Perkin-Elmer Model 603 AA or model 3030 (B) AA. Silver concentrations were determined in aliquots subjected to a nitric digestion by graphite furnace AA with either a Perkin-Elmer Model 3030B with Model HGA-500 graphite furnace, or the Perkin-Elmer Model 5100 with Model HGA-600 graphite furnace. Aluminum (Al), boron (B), barium (Ba), cadmium (Cd), chromium (Cr), molybdenum (Mo), lead (Pb) and strontium (Sr) were determined by inductively coupled plasma emission spectroscopy (ICP) using a Jarrell-Ash Model 1100 Mark III spectrophotometer after a pre-concentration step and nitric-perchloric digestion.

Analyses of organochlorines in rail eggs were conducted by Mississippi State University Chemical Laboratory under contract to the U. S. Fish and Wildlife Service. The analysis included 22 organochlorine analytes: α-BHC, β-BHC, γ-BHC, δ-BHC, α-chlordane, γ-chlordane, cis-nonachlor, dieldrin, endrin, HCB, heptachlor epoxide, mirex, o, p'-DDD, o, p'-DDE, o, p'-DDT, p, p'-DDD, p, p'-DDE, p, p'-DDT, oxychlordane, toxaphene, trans-nonachlor, and total PCBs. Sub-samples of the homogenate to be analyzed for organochlorines were digested with sodium sulfate, and Soxhlet extracted with hexane. Aliquots were concentrated via rotary evaporation and cleaned up using florisil columns. A silicic acid chromatographic column was used to separate PCBs from other organochlorines. All organochlorines were determined using a Varian 3400 gas chromatograph equipped with an electron capture detector. Method wet weight detection limits were 0.01 ug/g for organochlorines and 0.05 ug/g for PCBs. Confirmation of DDE, PCBs, trans-nonachlor and oxychlordane was done with mass spectrometry for a subset of eggs. Total PCBs were calculated as the sum of two PCB mixture concentrations using Aroclors 1254 and 1260 as individual standards. Aroclors 1242 and 1248 standards were also used to compare with egg PCB mixtures but egg PCB mixtures corresponded more closely to the more heavily chlorinated 1254 and 1260 Aroclors.

Data Analysis

*Contaminant concentrations*. Statistical analysis of the data was done with Statistica™, a computerized statistics package by StatSoft Inc. Normality of data was assessed with a Shapiro-Wilk W test. When contaminant concentration data were non-normal, as in the case of egg mercury concentrations, statistical comparisons of the population of failed rail eggs between marshes and regions of the bay were based on transformed data and geometric means.

Individual marsh comparisons with one way ANOVA were done using log transformed clutch means. For assessing the importance of a contaminant to embryo toxicity, comparisons to toxicological thresholds were made using distribution of contaminant concentrations in the population of all failed eggs without accounting for nest effects, as the egg concentration not the average clutch concentration is the meaningful unit of toxicological interest. As all clapper rail eggs were fail-to-hatch eggs and not randomly collected, generalizations can not be made on concentrations in the population of all eggs.

All concentrations for trace elements in eggs, are presented in ug/g dry weight (dw), with the exception of mercury. Mercury concentrations are adjusted via the method of Stickel et al., (1973) to a fresh wet weight (fww) to facilitate toxicological comparisons with laboratory studies of mercury. On average the fresh wet weight adjustment lowered egg concentrations by 27 percent. To make the volume based adjustment of Stickel et al., (1973), volume of rail eggs was measured by weight of water displaced by the whole egg, or if the shell was not intact, volume was estimated from a formula derived from the intact eggs based upon length and breadth of the egg:

Volume = 0.439(length) + 1.78(width) - 53.3.

Organochlorine data in eggs is also presented as fresh wet weight (fww). Percent moisture in rail eggs was 75.6 percent ($\pm$ a standard error of 0.4 percent).

## RESULTS

### Nest Monitoring

*Hatchability.* Clapper rail egg hatchability was depressed below the normal hatchability rate of >90 percent (Johnson, 1973; Kozicky and Schmidt, 1949; Zembal and Massey, 1983) in all marshes studied. Only 69 percent of clapper rail eggs whose viability could be assessed were viable. Rail egg hatchability in north and south bay marshes was 65 percent and 70 percent respectively. Nest fate and hatchability are summarized for each marsh in Table 1.

*Clutch size.* In four south bay marshes monitored in 1992, we assessed the fate of 377 eggs in 62 nests. In 1991, only Faber Marsh was monitored and the fates of 53 eggs in nine nests were followed. In two north bay marshes in the 1998 and 1999 breeding seasons we assessed the fates of 98 eggs in 18 nests. In south bay nests we found a mean clutch size of 6.94 eggs. In the north bay marshes mean clutch size was estimated to be slightly lower, at 6.66 eggs per nest. Clutch size means, however, were not statistically distinguishable between north and south bay (t-test result, $p = 0.47$) or between marshes (one way ANOVA, $p = 0.32$). Clutch sizes in this study were also not statistically different from the 7.22 found by Zucca (1954) in the south bay. We found a bimodal distribution around peaks of six and eight eggs per nest found in the south bay, a finding similar to the observations of Zucca (1954).

*Nest and hatching success.* Nest success, defined as the successful hatching of at least one egg from a nest, was 27 percent at Corte Madera Marsh and 57 percent at Wildcat Marsh in the north bay. Of the 18 active nests monitored in the north bay marshes, only seven were successful.

Hatching success, the proportion of eggs laid which hatched, was also low in the two north bay marshes for 1998-99. In north bay marshes only 34 of 98 eggs hatched, a hatching success of just under 35 percent. Wildcat Marsh produced 2.2 eggs hatched per nesting attempt and Corte Madera Marsh only 1.6 eggs. In the two north bay marshes together, combined production was 1.9 young per nesting attempt.

In the four south bay marshes, nest success varied from 30.8 percent at Laumeister Marsh to 60 percent at both Faber and Greco marshes. Mowry Marsh had 54 percent of nests classified as successful. Young production was especially low at Laumeister Marsh with only 1.3 eggs hatched per active nest. Greco, Faber and Mowry Marshes produced 3.1, 3.2 and 3.8 young per nest. Overall 29 of 62 monitored nests in the south bay were successful in 1992. These nests hatched 152 of 378 laid eggs, a hatching success of just over 40 percent, and produced 2.45 young per nesting attempt.

Only 45 percent of the 89 monitored rail nests in the north and south bay combined were successful. A mean clutch size of 6.89 produced 2.33 young per active nest with an egg hatching success of 39 percent. Table 1 provides the data on egg fates for each marsh.

*Predation losses.* Predation was a significant factor in reducing hatching success but this importance varied significantly by marsh in both north and south bay marshes. Predation losses are summarized by marsh in Table 1. In the north bay in 1998-99, we observed that 52.3 percent of rail eggs in Corte Madera Marsh were eaten by predators while at Wildcat Marsh only 12.9 percent of the eggs were lost to predation. In the south bay, Laumeister Marsh had the most severe predation with 61 percent of all eggs laid taken by predators. Greco, Mowry and Faber Marshes experienced egg predation losses of 39.3 percent, 24.7 percent and 14 percent respectively. Overall 41.5 percent of the eggs in the south bay study marshes and 38.8 percent of the eggs in the north bay study marshes were taken by nest predators.

Predators did not always take an entire clutch of eggs, nor did they always consume eggs at the nest. In some cases, eggs would be removed from the nest one at a time. At Laumeister Marsh, we observed a rat carrying a rail egg in its mouth. Predators took at least one egg from 50 percent of the nests we monitored.

Pacific gopher snakes (*Pituophis melanoleucus catenifer*) were another documented predator upon rail nests and were observed in both Laumeister and in Corte Madera Marshes. A gopher snake observed in Corte Madera Marsh in 1998 was palpated to produce a rail egg. We also observed a raven take a rail egg and consume it.

*Tidal flooding losses.* Losses due to flooding did occur but were minimal. Flooding that resulted in eggs being lost from the nest occurred in one nest each in Faber and Corte Madera Marshes. Overall, flooding appeared to have destroyed only 2.5 percent of the monitored nests. High spring tides of 6.7 and 6.8 feet preceded our discovery of the flooded nests.
*Embryo observations in fail-to-hatch eggs.* Three of fourteen late stage embryos which we examined were malpositioned; the head was not correctly placed at the air cell pole of the egg and/or not correctly tucked beneath the right wing. One of these embryos was from Wildcat Marsh in 1998 and two were found in the same nest in Mowry Marsh in 1992.

Deformed embryos were found only in the north bay at Wildcat Marsh in 1998 where four failed eggs were collected. Three of these were late-stage embryos with abnormal development. One of these embryos was polydactylus with an extra digit on each foot. The same embryo also had edema in the neck region. Two other embryos had abnormally stunted toes and reduced wings. One of the embryos with shortened toes was also malpositioned (noted above). General body hemorrhaging was observed in three dead embryos from the south bay. One died at a late stage and two at the mid stage of incubation.

Trace Element Concentrations in Eggs.

*Aluminum*. Of 38 rail eggs analyzed for aluminum, in the south bay, only three were above the 1992 detection limit of 3 ug/g dw. These three eggs had reported concentrations of 4, 5, and 24 ug/g dw. The detection limit for aluminum in 1998 eggs was 0.5 ug/g dw. Aluminum was detected in all nine eggs collected in1998 from Wildcat and Corte Madera Marshes. Wildcat Marsh eggs had unusually high concentrations of aluminum with a mean concentration of 20 ug/g, dw and a maximum egg aluminum concentration of 36 ug/g dw. Mean concentrations at Corte Madera Marsh in 1998 were 4.5 ug/g dw where concentrations ranged from 2.9 to 6.8 ug/g dw. Aluminum concentrations in eggs with deformed embryos ranged from 8.7 to 36.0 ug/g dw.

*Arsenic*. Arsenic was at or below the detection limit of 0.1 ug/g dw in nearly all south bay rail eggs. Two of thirty-seven eggs analyzed for arsenic in the south bay had concentrations of 0.2 ug/g dw. In north bay eggs arsenic was generally below the detection limit of 0.4 ug/g dw with the exception of two eggs from Wildcat Marsh which had concentrations of 0.5 ug/g dw and 0.6 ug/g dw. The egg with 0.5 ug/g dw was a deformed embryo and the egg with 0.6 ug/g dw was dead at an early stage.

*Barium*. Barium was detected in 36 of 38 eggs from the south bay in 1992 with a mean concentration of 0.34 ug/g dw. The maximum barium concentration found in the south bay was 0.59 ug/g dw. The barium detection limit for 1992 eggs was 0.1 ug/g dw. Barium in north bay eggs was found above the 1998 detection limit of 0.2 ug/g dw in eight of nine eggs. Concentrations in these eight eggs ranged from 0.45 to 4.13 ug/g dw.

Barium, which is chemically related to strontium and calcium, is heavier and more toxic than these elements. When injected into chicken eggs at 3.3 micromoles per egg deformities of the feet, including polydactyly, were produced in 50 percent of the embryos tested (Ridgway and Karnofsky, 1952). The three rail embryos with deformities of the feet had barium concentrations ranging from 2.2 to 4.1 ug/g dw. Barium concentrations were significantly higher in late-stage eggs and highest in the three embryos with deformities of the feet. Higher barium was also somewhat associated with malpositioning of late-stage embryos. Mean barium concentrations of 1.28 ug/g and 0.51 ug/g dw were found in malpositioned and normal late-stage embryos respectively, though this difference was not statistically significant at an alpha of 0.05 ($P = 0.1$). The egg with the maximum barium concentration of 4.1 ug/g dw was both deformed and malpositioned.

*Boron.* The detection limit for boron in north bay eggs was 0.5 ug/g dw. Boron was undetected in eggs from Corte Madera Marsh and found in all eggs at Wildcat Marsh at concentrations ranging from 0.52 to 10.2 ug/g dw. The detection limit for boron in eggs from the south bay was 2 ug/g dw. Boron concentrations ranging between 2 ug/g and 6 ug/g dw were found in 7 of 37 eggs from the south bay. Boron is a known avian terratogen at high

concentrations (Romanoff and Romanoff, 1972). Smith and Anders (1989) reported reduced embryo weight in ducklings associated with an egg boron concentration of 13 ug/g dw. Most boron concentrations in both north and south bay eggs, however, were below known avian embryotoxic thresholds (Smith and Anders, 1989). Only the one egg from Wildcat Marsh had an elevated boron concentration.

*Cadmium*. Cadmium was undetected in 36 of 38 eggs from the south bay at a detection limit of 0.06 ug/g dw. The two eggs with detectable cadmium had concentrations of 0.07 ug/g and 0.31 ug/g dw. Cadmium was undetected in eggs from the north bay in 1998 at a detection limit of 0.02 ug/g dw. Cadmium concentrations in avian eggs less than 0.5 ug/g dw are considered non-toxic (Ohlendorf, 1993).

*Chromium.* Chromium was detected in only 7 of 38 eggs from the south bay in 1992. Chromium detection limits for 1992 eggs varied from 0.5 to 0.6 ug/g dw and reported concentrations varied from 0.5 to 0.7 ug/g dw. The detection limits for chromium in 1998 eggs varied from 0.28 to 0.30 ug/g dw. Chromium was detected in only 3 of 9 eggs from the north bay in 1998 at concentrations of 0.46 ug/g, 0.41 ug/g, and 2.06 ug/g dw. The maximum chromium concentration occurred in an embryo with reduced toes and wings from Wildcat Marsh.

Embryo toxicity of chromium is dependent upon the valence state with hexavalent chromium ($Cr^{+6}$) being much more toxic than trivalent $Cr^{+3}$ (Asmatulah and Shakoori, 1998). Hexavalent chromium has been shown to be terratogenic when injected at 60 ug/egg into chicken eggs producing among other things achondroplasia (shortened bones) and microopthalmia (Ridgeway and Karnofsky, 1952). Asmatulah and Shakoori (1998) found $Cr^{+6}$ to be terratogenic at 25 ug/egg when injected into the yolk of chicken eggs. Assuming an average chicken egg weight of 55 grams this would roughly correspond to a whole egg concentration of 0.45 ug/g wet weight or nearly 2 ug/g dw. The valence state of chromium in our clapper rail eggs is not known.

The maximum chromium concentration we observed in an egg collected in 1998 from Wildcat Marsh (2.06 ug/g dw) is an order of magnitude higher than the maximum chromium concentration usually seen in bird eggs (Burger, 1994) and is possibly high enough to be embryotoxic if present in the hexavalent form. Hui et al., (2002) reported elevated chromium in addled eggs of light-footed clapper rails (*Rallus longirostris levipes*) from Seal Beach and Tijuana Slough with concentrations as high as 3.85 ug/g dw, but the species of chromium and toxicological significance was unknown.

*Lead.* The detection limit for lead in 1992 eggs was 1 ug/g dw. Lead was detected in only one egg from the south bay at a concentration of 2 ug/g dw. This was a late-stage embryo from Faber Marsh near East Palo Alto, California. Although detection limits for lead were 10-fold lower in eggs collected in 1998 (0.1 ug/g dw), lead was detected in only one egg, a deformed embryo from Wildcat Marsh with a lead concentration of 0.25 ug/g dw. Concentrations of lead in wild bird eggs are usually less than 1 ug/g dw from non-lead-contaminated environments (Gochfield and Burger, 1998). Where lead is elevated in estuarine environments it has accumulated in bird eggs to mean concentrations of 2.3 to 2.5 ug/g dw (Burger, 1994). Our results seem to indicate that lead is not an important contaminant for California clapper rails in San Francisco Bay marshes.

*Mercury*. Mercury was detected in all rail eggs. South bay mercury concentrations (n = 42) ranged from 0.18 to 2.51 ug/g fww. North bay mercury concentrations in rail eggs (n = 22) ranged from 0.11 to 0.87 ug/g fww. Mercury concentrations varied more among clutches than between clutches. On average, the coefficient of variation within the ten clutches where we had multiple eggs analyzed was 30.5 percent. Among all clutches, the coefficient of variation was 51.7 percent. Between marsh comparisons of mercury concentrations on a clutch-wise basis indicated failed eggs from south bay marshes were generally more contaminated with mercury than north bay marshes. Corte Madera Marsh, located in the northwest portion of the north bay, was the marsh with lowest geometric mean mercury in clutches of failed eggs among the marshes we assessed (Table 2). Mowry and Greco Marshes in the south bay had the greatest geometric mean mercury concentrations of the assessed marshes. Regional comparisons show the geometric mean mercury concentration in south bay clutches of failed eggs was not different from mercury concentrations found in random eggs collected from south bay marshes in the mid-1980s. Mercury concentrations in north bay failed eggs, however, were half those found in the mid-1980s. North bay and south bay rail eggs from all time periods had mercury concentrations at least an order of magnitude greater than those found in light-footed clapper rail *(Rallus longirostris livepes*) eggs from southern California coastal marshes collected in 1991 (Table 3).

Methylmercury is the most toxic form of mercury, and avian toxicological benchmarks for mercury are based upon studies in which methylmercury was the form fed to birds and accumulated in eggs. To assess whether a significant fraction of mercury in rail eggs was not the more toxic methylmercury form, methylmercury concentrations were determined for a subset of nine north bay and eight south bay eggs. While two different labs were used in different years for the different regions, results showed no statistical difference between labs or regions in the ratio of methylmercury to total mercury ($P = 0.12$). Methylmercury was on average 95 percent of the total mercury concentration found in rail eggs with a 95 percent confidence interval between 89 and 100 percent. Methylmercury concentrations in rail eggs were strongly correlated with total mercury concentrations in rail eggs ($r^2 = 0.81$, $p < 0.000001$).

Fimreite observed hatchability declines in pheasants when egg concentrations of methylmercury were between 0.5 to 1.5 ug/g fww (Fimreite, 1971). Fifty percent of all of the fail-to-hatch rail eggs from the south bay were above 0.5 ug/g concentration, whereas only 20 percent of failed eggs in the north bay were above this concentration. Heinz (1979) established a threshold of about 0.8 ug/g mercury in mallard eggs. More recent work by Heinz and Hoffman (2003) appears to confirm that concentrations slightly less than 1 ug/g in mallard eggs are harmful to at least some individual mallard embryos. Twenty-five percent of south bay fail-to-hatch rail eggs analyzed for mercury were above the 0.8 ug/g mallard threshold, whereas in the north bay only 7 percent were above this concentration. The use of the mallard or even a pheasant threshold assumes clapper rail embryos will respond to mercury in a quantitatively similar manner and this may not be the case. Recent work by Heinz using carefully developed techniques for injection of methylmercury into the eggs of many different wild species suggests that avian species vary in the sensitivity of their embryos to methylmercury and also suggests the clapper rail embryo may be much more sensitive to methylmercury than the mallard (Heinz pers. comm.).

*Molybdenum*. Molybdenum was undetected in rail eggs from either the north or south bay.

Detection limits for the south bay were 1 ug/g dw and 0.26 ug/g dw for the north bay. In domestic chicken eggs normal egg viability has been observed up to a concentration of 23 ug/g dw. The EC 50 for adverse effects upon hatchability in chickens is 33 ug/g (Lepore and Miller, 1965). While molybdenum is present in soils and groundwater of the Central Valley (Deverel and Millard, 1986) and transported to the San Joaquin River by sub-surface irrigation drainage, it appears from our results that molybdenum is unlikely to be a contaminant of any importance to California clapper rails in San Francisco Bay.

*Selenium.* Mean selenium concentrations in fail-to-hatch rail eggs at all marshes varied between 1.89 and 2.22 ug/g dw. No differences were observed in selenium concentrations between marshes. Concentrations in individual eggs ranged between 1.12 and 3.2 ug/g dw. The bay-wide mean for selenium in rail eggs was 2.1 ug/g dw. There is a narrow window of normal selenium concentrations in avian eggs between 1 and 3 ug/g dw (Skorupa and Ohlendorf, 1991). Concentrations of selenium in rail eggs from both the north bay in 1998 and 1999 and the south bay in 1991 and 1992 were all within the normal range. Selenium concentrations in rail eggs in the north bay also appear to have declined by about 50 percent since the mid 1980s (Table 3.) when selenium was elevated in Wildcat Marsh in particular.

*Silver.* Silver was analyzed only in south bay eggs from 1992. The detection limit was 0.01 ug/g dw. Silver was detected in 35 of the 38 eggs collected in 1992. The mean dry weight concentration of silver was 0.02 ug/g and the maximum concentration was 0.04 ug/g. Very little information exists on the occurrence of silver in avian eggs and the effects of silver upon avian embryos. The U. S. Department of the Interior's National Irrigation Water Quality Program analyzed 1,137 avian eggs in the western United States for silver in 8 different investigations over the last 15 years with detection limits between 0.8 and 2 ug/g dw (Seiler and Skorupa, 2001; Seiler et al., 2003). Only two eggs in this data set had detectable concentrations of silver (2.4 and 2.0 ug/g dw). The U. S. Fish and Wildlife Service has collected and analyzed 160 eggs from 15 sites at 8 locations in the San Joaquin Valley with silver detection limits of 0.1 ug/g dw and has not found silver in any of these eggs (Skorupa, unpub. data). From all of the above data it would appear that silver can, but does not usually, bioconcentrate in eggs and the concentrations we found in rail eggs were not exceptionally high.

*Strontium.* Detection limits for strontium were 0.02 and 0.1 ug/g dw for the north and south bay eggs, respectively. Strontium was detectable in all eggs and ranged from 11.1 to 176 ug/g dw. The mean strontium concentration in north and south bay eggs was 62 ug/g and 44 ug/g dw, respectively, though these means were not quite statistically different at an alpha of 0.05 ($p = 0.053$). The highest strontium concentrations were also associated with embryo deformity, but this may be related to the increase of strontium in egg contents of late-stage embryos due to mobilization of minerals from the shell (Table 4). The biological function and physiological action of strontium resembles that of calcium. The chemical toxicity of non-radioactive strontium, however, is considered nil (Doull et al., 1980). Strontium concentrations were in general similar, though slightly lower, than the range of concentrations observed in addled eggs of light-footed rail eggs at Seal Beach and Tijuana Slough in southern California (Hui et al., 2002)

*Statistical associations among trace elements.* Molar concentrations (nanomoles/g) of mercury and selenium in rail eggs were weakly correlated ($r^2 = 0.394$) but this correlation was statistically

quite significant ( $p = 0.00003$). Thus we can have confidence in the trend of increasing mercury with increasing selenium but could not with confidence predict the concentration of one element based on the observed concentration of the other. The mean molar ratio of mercury to selenium was 0.64. This ratio varied from 0.3 to 2.0 with most of the variability due to the more highly variable mercury concentrations.

The avian eggshell consists largely of the calcium and magnesium carbonates and tricalcium phosphate, with little variation in major mineral content between species (Romanoff and Romanoff, 1949). The eggshell is the major source of calcium for the developing avian embryo (Tuan, 1987). Burger (1994) has reported varying levels of metals in the eggshells of different species of aquatic birds. With the exception of cadmium and manganese in herring gulls, these trace elements were always higher in egg contents. Burger (1994), however, did not assess trace elements associated with calcium and phosphate metabolism such as aluminum, strontium and barium, nor did she report the association of trace element concentrations associated with stage of embryo development.

In avian eggs strontium is deposited mainly in the eggshell (Romanoff and Romanoff, 1949). We observed that concentrations of strontium within pip-stage embryos were greater, by a factor of three, than concentrations in early-stage embryos of clapper rails (Table 4). Strontium concentrations in nine north bay eggs (the only eggs in which calcium was determined) was highly correlated with calcium concentration ($r^2 = 0.99$, $p < 0.0001$). Barium, strontium and calcium are chemically related elements. Barium was also correlated with calcium ($r^2 = 0.79$, $p = 0.02$) though not as strongly as strontium. Aluminum is known to interact with phosphorous metabolism. Aluminum was also correlated with calcium contents of eggs ($r^2 = 0.77$, $p < 0.002$) Barium was only weakly correlated with strontium from which we infer not all barium within the egg contents were derived from the shell.

The biological function of strontium strongly resembles that of calcium.. Increases in strontium concentration with stage of incubation paralleled those of calcium. Barium is the heaviest of the three elements and also the most embryotoxic (Ridgeway and Karnofsky, 1952; Spangenberg and Cherr, 1996). Barium was correlated with calcium ( $r^2 = 0.62$ , $p = 0.02$) but not as strongly as strontium and calcium.

Very high aluminum and very high mercury concentrations tended to occur in the same eggs. In south bay eggs the maximum aluminum concentration (24 ug/g dw) and mercury concentration (15 ug/g dw) were found in the same egg. Among the north bay eggs analyzed for aluminum in 1998, the egg with the maximum concentration of aluminum (36 ug/g dw) was the egg with the second highest mercury concentration (3.5 ug/g dw). Maximum aluminum concentrations were found in late-stage embryos. The maximum lead and cadmium concentrations were found in the same egg from the south bay in 1992, a late-stage, fully feathered, embryo near the pipping stage from Faber Marsh. The highest concentrations of aluminum, barium, boron and chromium were found in the same egg. Late-stage embryos consistently had the highest concentrations of aluminum, strontium and barium.

Organochlorines in Eggs

We have previously reported on organochlorine (OC) concentrations in the 1992 eggs collected from south bay marshes (Schwarzbach et al., 2001) and here report on OC concentrations in an

additional 9 eggs from 1998 collections in Corte Madera and Wildcat Marshes in the north bay. We detected a greater number of OCs in the north bay eggs than in south bay eggs from 1992, albeit at quite low concentrations. In addition to detectable concentrations of dieldrin, heptachlor epoxide, oxychlordane, trans-nonachlor, p, p'-DDE, and PCBs found in all the south bay rail eggs, we found in the north bay eggs traces of toxaphene (all < 0.1 ug/g fww), the p, p' isomers of DDT (≤ 0 .02 ug/g fww) and DDD and the o, p' isomers of DDT (all < 0.01 ug/g fww), DDD, and mirex (all < 0.001 ug/g fww). Organochlorines not detected in any of the rail eggs from the north bay included ; endrin; the o, p' isomer of DDE; cis-nonachlor, alpha-chlordane, gamma-chlordane, and mirex. The magnitude of organochlorine concentrations in north bay rail eggs followed the pattern where PCBs > p, p' DDE > trans-nonachlor > toxaphene > dieldrin > oxychlordane > heptachlor epoxide. This was similar to the pattern found in the south bay eggs (Schwarzbach et al., 2001) except for the presence of toxaphene and relatively more dieldrin in north bay eggs. Concentrations of all organochlorines in rail eggs were greater at Corte Madera Marsh than at Wildcat Marsh. Mean concentrations from each north bay marsh are compared with south bay mean concentrations in Table 5.

As we previously reported for failed rail eggs from the south bay, PCBs were the dominant organochlorine in failed rail eggs from the north bay. The south bay rail eggs, however, had significantly greater concentrations of PCBs than did north bay eggs ( t-test, $p = 0.005$). Wildcat Marsh in the north bay had the lowest mean PCB concentration in rail eggs of any marsh observed (Anova, and Tukey HSD, $p < 0.002$). Mean concentrations of DDE were significantly greater in the north bay eggs than in the south bay eggs (t-test, $p = 0.00013$). The relative concentration of p, p' DDE in proportion to the concentration of PCB in north bay eggs in 1998 was also greater than in south bay eggs in 1992. The PCB/DDE ratio in south bay eggs in 1992 was 12.5 while in the north bay the ratio was 2.0. This was due both to greater concentrations of DDE in the north bay and higher concentrations of PCBs in the south bay.

## DISCUSSION

Our observations of low hatching success, low hatchability, and an overall low fecundity of the California clapper rail in both the north and south San Francisco Bay confirm the previous and more limited observations in the south bay of Harvey (1988) and Forester et al., (1990) of poor reproductive success in California clapper rails. The findings are potentially ominous for the future recovery of the rail. An understanding of the causes of what appears to be bay-wide, low fecundity are important to support efforts underway intended to accomplish recovery of the California clapper rail.

### Flooding

Flooding was not as significant to the impairment of rail reproduction as in some earlier studies but the impact of flooding may vary by marsh and year. Collins et al., (1994) reported all clapper rail nests they observed were constructed entirely above mean higher high water with rims of the nests closely corresponding to the maximum elevation of tides during the breeding season. It is possible however that rails may be deceived about proper nest heights and that restored marshes which are below grade may have more nests lost to spring flooding (Collins, personal communication).

Flooding occurred only at the two marshes which have had elevations modified: Faber Marsh, recreated through dredge spoils, and Corte Madera Marsh, subsided and subsequently reopened to tidal influence. The flooded nest in Corte Madera Marsh was located on a tertiary slough in the outer marsh, a more vulnerable location than more landward locations on primary or secondary sloughs (Josh Collins, pers. com.). Zucca (1954) found San Francisco Bay tides in excess of 6.7 feet were particularly problematic for clapper rail nests located in Grindelia bushes with eggs being lost from all such nests. The two nests we observed to be lost to flooding were also in Grindellia bushes and were lost after tides in excess of 6.7 feet.

Predation

Predation was a major contributing factor in the low hatching success of rail eggs and rodents were the principal culprits, a finding which others have reported as well (Degroot, 1927; Applegarth, 1938; Harvey, 1988). Striplen (1992), as part of our south bay study in 1992, evaluated predation at wild nests in comparison with videotaped predation in the laboratory to better categorize the nature of predation observations in wild rail nests based upon nest and shell remains. His analysis indicated rodents accounted for 90 percent of the eggs lost to predation. Only one rail nest (Mowry Marsh) was lost to the red fox, first reported as predator upon rails by Foerster et al., (1990).

Egg loss via predation was greatest in marshes that lacked significant buffers between adjacent residential areas (Corte Madera and Laumeister). At Corte Madera Marsh houses on stilts abut the marsh. Corte Madera Marsh, perhaps not coincidentally, had the lowest nest success of all marshes we evaluated. Corte Madera and Laumeister Marshes both lost over half their rail nests to predation. Unfortunately, these two marshes had the highest density of rail nests we observed, so the high percentage loss of nests in these marshes was disproportionately devastating. Overall, about a third of all clapper rail eggs were lost to predators.

Contamination

Contamination also appeared to exert an adverse influence over clapper rail reproductive success as exhibited by observations of deformities, embyro hemorrhaging, embryo malpositions; a depressed rate of hatchability; the exceedance of some toxic threshold concentrations; and the correlation of deformities with elevated concentrations of trace elements in fail-to-hatch rail eggs (Table 6). While all marshes had impaired hatchability, the marshes with the lowest hatchability were also adjacent to potential contaminant sources– a hazardous waste site near Laumeister and an oil refinery near Wildcat.

*Trace Elements and Hatchability*. Trace elements found in eggs at potentially toxic concentrations to rails included boron, chromium, lead, barium and mercury. Among the six marshes we investigated, elevated concentrations of boron, chromium, lead and barium were restricted to Wildcat Marsh in the north bay. Toxic or elevated selenium concentrations were not found in any clapper rail eggs. Selenium in all fail-to-hatch clapper rail eggs was below known toxic thresholds in the most sensitive avian species tested (mallards).

Mercury, however, occurred in elevated concentrations in at least some eggs from all six

marshes. Hatchability of clapper rails was depressed in all marshes and mercury appears to consistently be the contaminant most likely to produce that low hatchability. The lowest hatchability was in Wildcat Marsh, the only marsh with deformed embryos. Unlike other marshes, however, Wildcat Marsh also had elevated concentrations of other trace elements in addition to mercury. If one assumes a normal hatchability of greater than 90 percent, the magnitude of the impact to hatchability by contamination, principally mercury contamination, among the six marshes evaluated, varies from between 15 to 30 percent.

*Organochlorines and Hatchability*. In 1992, PCBs in the north bay were lower than in the south bay (Schwarzbach et al., 2001). However, DDE and toxaphene were higher in the north bay eggs. This reflects the greater proximity of the north bay marshes to agricultural pesticide inputs to the bay– the north bay has more agricultural land adjacent to the bay and is also the receiving water body for Central Valley agricultural runoff. PCBs were thought to be present at high enough concentrations to exert an effect on hatchability in one clapper rail egg from Laumeister Marsh in 1992 (Schwarzbach et al., 2001).

Eggs from the Corte Madera Marsh had mean trans-nonachlor concentrations nearly three times higher than eggs from Wildcat Marsh. Technical heptachlor contains trans-nonachlor as a small percentage (2.5 percent). Technical chlordane consists of about 45 compounds, including 10 percent heptachlor and 7 percent trans-nonachlor. Oxychlordane is a metabolite of both cis- and trans-chlordane. Uses of chlordane and heptachlor have been greatly reduced since the 1970s when 20 million pounds were used annually. Trans-nonachlor is a component of two organochlorine pesticides, heptachlor and chlordane, commonly used in urban environments for termite control. Use of chlordane on the outside of buildings to kill termites was permitted until 1988. A possible explanation for the elevated trans-nonachlor in Corte Madera eggs is the fact that the marsh at Corte Madera is bordered on the north by houses on stilts with absolutely no buffer. No other marsh examined in this study had human dwellings in that kind of intimate proximity to the wetland.

While trans-nonachlor was probably not present at high enough concentrations to affect hatchability, trans-nonachlor has estrogenic activity in MCF-7 human breast cancer cells (Klotz et al., 1996) and has been shown in experiments with alligator oviduct tissue to effectively bind to estrogen receptors at 0.25 uM as did p, p' DDD. Chlordane and toxaphene did not bind to these receptors, but their presence in combinations increased the binding to estrogen receptors.
*Trace Elements and Deformities*. Because elevated strontium, aluminum and barium were highly associated with the presence of terata, and terata were found only in pip-stage embryos, we assessed whether these elevated concentrations were due to a sampling bias of terata in only the pip-stage embryos. Trace element concentrations in normal and deformed late-stage embryos are presented in Table 4. Among late-stage embryos (or even all eggs) the three deformed eggs from Wildcat Marsh had the highest mean concentrations of aluminum, boron, barium, chromium, and strontium and the lowest mean concentration of selenium. Higher mean concentrations of aluminum, barium, chromium, and strontium were statistically associated with the presence of deformities in the north bay eggs while mean concentrations of lead, boron, and mercury were not associated with deformities. The strongest association between deformities and chemical concentrations was found with barium, which had a mean concentration an order of magnitude greater in deformed embryos. Non-deformed, late-stage California clapper rail embryos had barium and aluminum concentrations consistent with a concentration range

reported for light-footed clapper rails in southern California by Hui et al., (2002).

Concentrations of the trace elements that interact with calcium or phosphate metabolism, such as aluminum, strontium, and barium, were somewhat dependent upon the stage of embryo development, with the later stages having higher concentrations of these elements. Mobilization of these elements from the shell during embryo development seems likely.

The trace elements barium, boron, chromium, mercury, selenium, and lead, when present at elevated concentrations in avian eggs, have been demonstrated to produce very specific developmental abnormalities (Hoffman et al., 1988; Asmatullah and Shakoori, 1998; Ridgway and Karnofsky, 1952). Methylmercury has been shown to produce terrata in avian embryos, including polydactyly and shortened wing and legs, at concentrations $\geq$ 0.93 ug/g in egg contents of mallards fed methylmercury (Heinz and Hoffman, 2003). Mercury produced deformities, however, were only found sporadically in the experiment by Heinz and Hoffman (2003). Selenium, while teratogenic at high concentrations (Hoffman et al., 1988), was actually significantly lower ($p = 0.003$) in the deformed rail embryos (1.61 ug/g dw) than the normal rail embryos (2.45 ug/g dw). These mean selenium concentrations are within concentrations considered normal and non-toxic (Ohlendorf et al.,1986).

Arsenic was elevated in one of the deformed eggs and is known to interact with selenium. Selenium may not have been low enough to produce a deficiency but may not have been high enough to be protective. The arsenic in one of these deformed eggs with 1.7 ug/g dw selenium may have decreased the protective value of selenium.

Strontium is virtually non-toxic and aluminum has not been demonstrated to produce specific terata. Of the elements which produce specific terata only barium and chromium had mean concentrations statistically higher in deformed embryos than in normal late-stage clapper rail embryos. Chromium is known to stunt embryo growth and shorten bones during development, and barium injections have produced shortened toes similar to those observed at Wildcat Marsh in two of three deformed rail embryos. We conclude that elevated chromium and barium were among the most likely candidate trace elements responsible for the reduced feet, polydactyly, and abnormal wings observed in clapper rail embryos at Wildcat Marsh, but given the findings of Heinz and Hoffman (2003) we can not rule out the possibility that mercury was also a potential cause of deformities.

## CONCLUSIONS

Our findings have significant implications for the recovery potential of the California clapper rail, the appropriate design of future rail habitat, and the need for attainment of appropriate sediment and water quality objectives in the bay. While flooding was not a significant source of egg loss in our study, the loss that did occur was related to spring flood tides in relatively newer marshes. New tidal marshes in habitat restoration efforts will need to be designed to achieve appropriate elevations to minimize flooding losses at rail nests. These tidal marsh elevations will need to be achieved with clean sources of sediment at marsh surfaces, particularly with regard to mercury concentrations which are a bay-wide problem, but most severe in the south bay. New marshes will also require significant buffers from residential areas, and may need

active predator control efforts to improve the survival and fecundity of colonizing individuals. Overall, about a third of all rail eggs were lost to predators and about 31 percent of eggs were non-viable. Predation and pollution effects overlap and interact. Contaminated eggs are taken by predators and contamination may also interact with predation at later life stages if contaminants slow growth or impair the ability of young to detect or escape predators

## ACKNOWLEDGEMENTS

This project was partially supported through the refuge contamination assessment funds provided by the U. S. Fish and Wildlife Service. The authors gratefully acknowledge field assistance on this project from John Henderson, Bill Beckon, Terry Adelsbach, Tom Maurer, Cathy Johnson, Courtney Covington, Laurel Schwarzbach, Laura Valoppi, Mike Westphal, Amelia Orton Palmer, Doug Roster, Jean Takekawa, Mike Parker, Stephani Zador, Marianne Piche', Michael Rehberg, Shane Ridge, Dat Mac, Jana Hofius, and Charles Striplen. Thank you also to Kim Taylor for obtaining additional support for the write-up of this work. The authors would also like to acknowledge the helpful comments of the two anonymous reviewers.

## LITERATURE CITED


APPLEGARTH, J. H. 1938. The ecology of the California clapper rail on the South Arm of San Francisco Bay. M.S. Thesis, Stanford Univ., CA. 153 pp.

ASMATULAH, A. R., AND A. R. SHAKOORI. 1998. Embryotoxic and teratogenic effects of hexavalent chromium in developing chicks of *Gallus domesticus*. Bulletin of Environmental Contamination and Toxicology, 61:281-288.

BURGER, J. 1994. Heavy metals in avian eggshells: Another excretion method. Journal of. Toxicology and Environmental Health, 41: 297-220.

COLLINS, J., J. EVENS, AND B. GREWELL. 1994. A synoptic survey of the distribution and abundance of the California clapper rail (*Rallus longirostris obsoletus*) in the northern reaches of the San Francisco Estuary during the 1992 and 1993 breeding season. Draft Technical Report to California Department of Fish and Game, Yountville, CA. 36 pp.

DEDRICK, K.G. 1989. San Francisco Bay tidal marshland acreages: recent and historic values. Published in Proceedings of the Sixth Symposium on Coastal and Ocean Management (CoastalZone '89). Charleston, South Carolina, July, 1989. O. T. Magoon et al., Eds. Amer. Soc. of Civil Engineers, 345 East 47th St., New York, New York 10017. Pages 383-398.

DEVEREL, S.J. AND S. P. MILLARD. 1986. Distribution and mobility of selenium and other trace elements in shallow ground water of the western San Joaquin Valley, California. U. S. Geological Survey, Open-File Report 86-538.

DEGROOT, D.S. 1927. The California clapper rail: its nesting habitats, enemies, and habitat. Condor. 29:259-270

DOULL, J, C. D. KLAASSENAND, M. O. AMDUR (EDS.). 1980. Casarett and Doull's - Toxicology: The Basic Science of Poisons: (Second Edition), MacMillan Publishing Co., New York. 1980.

FIMREITE, N. 1971. Effects of dietary methylmercury on ring-necked pheasants. Canadian. Wildlife Service Occasional Paper No. 9. 39 pp.

FOERSTER, K. S., J. E. TAKEKAWA, AND J. D. ALBERTSON. 1990. Breeding density, nesting habitat, and predators of the California clapper rail. Unpubl. Rpt. No. SFBNWR-116400-90-1, prepared for San Francisco Bay National Wildlife Refuge, Newark, CA. 46 pp.

GILL, R., JR. 1972. South San Francisco Bay breeding bird survey, 1971. California Department of Fish and Game, Wildlife Management Branch. Administrative Report 72-6. Sacramento, CA. 69 pp.

GILL, R. 1979. Status and distribution of the California clapper rail (*Rallus longirostris obsoletus*). California Fish and Game, 65: 36-49.

GOCHFELD, M., AND J. BURGER. 1998. Temporal trends in metal levels in eggs of endangered roseate tern (Sterna dougallii) in New York. Environmental Research, 77:36-42.

HARDING, E. K., D. F. DOAK, J. ALBERTSON, AND J. E. TAKEKAWA. 1998. Predator management in San Francisco Bay wetlands: past trends and future strategies. Final Report prepared for U. S. Fish and Wildlife Service, Sacramento, CA.

HARVEY, T. E. 1988. Breeding biology of the California clapper rail in south San Francisco Bay. Transactions of the Western Section of the Wildlife Society, 24:98-104.

Heinz, G. H. 1979. Methylmercury: reproductive and behavioral effects on three generations of mallard ducks. Journal of Wildlife Management. 43(2): 394-401.

HEINZ, G. H. 1996. Selenium in birds. Pages 447-459, *in* Environmental Contaminants in Wildlife; Interpreting Tissue Concentrations. (W. N. Beyer, G. H. Heinz, and A. W. Redmon-Norwood, Eds.). SETAC Special Publications Series, Lewis Publishers, New York, New York.

HEINZ G.H. 2002. U. S. Geological Survey, Patuxent Wildlife Research Center. Personal communication, 2002.

HEINZ, G. H. AND D. J. HOFFMAN. 2003. Embryotoxic thresholds of mercury: estimates from individual mallard eggs. Archives of Environmental Contamination and Toxicology, 44: 257-264.

HOFFMAN, D. J., H. M. OHLENDORF, AND T. W. ALDRICH. 1988. Selenium teratogenesis in natural populations of aquatic birds in central California. Archives of Environmental Contamination and Toxicology, 17(4): 519-525.

HOFFMAN, D. J., G. H. HEINZ, L. J. LECAPTAIN, J.D. EISEMANN, AND G. W. PENDLETON. 1996. Toxicity and oxidative stress of different forms of organic selenium and dietary protein in mallard ducklings. Archives of Environmental Contamination and Toxicology, 31(1): 120-127.

HUI, C. A., S. L. GOODBRED, D. B. LEDIG, AND C. A. ROBERTS. 2002. Inorganic analytes in light-footed clapper rail eggs, in their primary prey, and in sediment from two California salt marsh habitats. Bulletin of Environmental Contamination and Toxicology, 68:870-877.

JOHNSON, R. W. 1973. Observations on the ecology and management of the Northern Clapper Rail, *Rallus longirostris crepitans* Gmelin, in Nassau County, New York. Ph. D. Dissertation, Cornell University.

KOZICKY, E. L., AND F. V. SCHMIDT. 1949. Nesting habits of the clapper rail in New Jersey.

Auk, 66(4): 355-364.

LEPORE, P. D. AND R. F. MILLER. 1965. Embryonic viability as influenced by excess molybdenum in chicken breeder diets. Proceedings of the Society of Experimental Biology and Medicine, 118:155-157.

LONZARICH, D. G., T. E. HARVEY, AND J. E. TAKEKAWA. 1992. Trace element and organochlorine concentrations in California clapper rail (*Rallus longirostris obsoletus*) eggs. Archives of Environmental Contamination and Toxicology, 23:147-153.

OHLENDORF, H. M., R. W. LOWE, P. R. KELLY, AND T. E. HARVEY. 1986. Selenium and heavy metals in San Francisco Bay diving ducks. Journal of Wildlife. Management, 50:64-71.

OHLENDORF, H. M. 1993. Marine birds and trace elements in the temperate North Pacific. In: the status Ecology and Conservation of Marine Birds of theNnorth Pacific, Vermeer K., Briggs, K. T., Morgan, K. H., and Siegael Causey, D. (eds). Canadian Wildlife Service, Ottawa, pp 232-240.

PULS, R. 1988. Mineral levels in animal health - diagnostic data. Sherpa International, Clearbrook, British Columbia, Canada.

RIDGEWAY, L. P. AND D. A. KARNOFSKY. 1952. The effects of metals on the chick embryo: toxicity and production of abnormalities in development. Annals New York Academy of Sciences, 55:203-215.

ROMANOFF, A. L. AND A. J. ROMANOFF. 1949. The Avian Egg. John Wiley and Sons, New York, New York.

ROMANOFF, A. L. AND A. J. ROMANOFF. 1972. Pathogenesis of the avian embryo: An analysis of causes and malformations and prenatal death. John Wiley and Sons, New York, New York.

SCHWARZBACH, S. E., J. D. HENDERSON, C. M. THOMAS, AND J. D. ALBERTSON. 2001. Organochlorine concentrations and eggshell thickness in failed eggs of the California clapper rail from south San Francisco Bay. Condor, 103(3): 620.

SEILER, R. L., AND J. P. SKORUPA. 2001. National Irrigation Water Quality Program Data-Synthesis Data Base. Open-File Report 00-513. U. S. Geological Survey, Carson City, Nevada. 35 pp.

SEILER, R. L., J. P. SKORUPA, D. L. NAFTZ, AND B. T. NOLAN. 2003. Irrigation-induced contamination of water, sediment, and biota in the Western United States– synthesis of data from the National Irrigation Water Quality Program: U. S. Geological Survey Professional Paper 1655, 123 p.

SKORUPA, J. P., and H. M. OHLENDODRF. 1991. Contaminants in drainage water and avian risk

thresholds. Pages 345-368 *in* The Economics and Management of Water and Drainage in Agriculture, Dinar, A. and Zilberman, D. (Eds.),. Kluwer Academic Publishers, Boston, MA.

SMITH, D. R., M. D. STEPHENSON, AND A. R. FLEGAL. 1986. Trace metals in mussels transplanted to San Francisco Bay. Environmental Toxicology and Chemistry, 5: 129-138.

SMITH, G. J. AND V. P. ANDERS. 1989. Toxic effects of boron on mallard reproduction. Environmental Toxicology and Chemistry, 8: 943-950.

SPANGENBERG, J. V. AND G. N. CHERR. 1996. Developmental effects of barium exposure in a marine bivalve (*Mytilus californianus*). Environmental Toxicology and Chemistry, 15:1769-1774.

STICKEL, L. F., S. N. WIEMEYER, AND L. J. BLUS. 1973. Pesticide residues in eggs of wild birds: Adjustment for loss of moisture and lipid. Bulletin of Environmental Contamination & Toxicology, 9:193-196.

STRIPLEN, C. J. 1992. Effects of predation on hatching success of the California clapper rail in the south San Francisco Bay and characterization of nest predation through use of artificial nests and captive animals. Draft report to San Francisco Bay National Wildlife Refuge, November, 1992. 23 pp.

THOMPSON, D. R. 1996. Mercury in birds and terrestrial mammals. Pages 341-356 *in* Environmental Contaminants in Wildlife; Interpreting Tissue Concentrations. (W. N. Beyer, G. H. Heinz, and A. W. Redmon-Norwood Eds.) SETAC Special Publications Series, Lewis Publishers, New York, New York.

TUAN, R. 1987. Mechanism and regulation of calcium transport by the chick embryonic chorioallantoic membrane. Journal of Experimental Zoology, Supplement 1:1-13.

U. S. FISH AND WILDLIFE SERVICE. 1970. Appendix D - United States List of Endangered Native Fish and Wildlife; 35 FR 16047 16048

U. S. FISH AND WILDLIFE SERVICE. 1973. Endangered Species Act of 1973. http://endangered.fws.gov/esa.html

U.S. FISH AND WILDLIFE SERVICE. 2000. Species Account: California Clapper Rail (Rallus longirostris obsoletus) http://sacramento.fws.gov/es/animal_spp_acct/clapper_rail.htm

WILBUR, S. R., AND R. E. TOMLINSON. 1976. The literature of the western clapper rails. U. S. Fish and Wildlife Service, Special Scientific Report - Wildlife No. 194. Washington D.C.

ZEMBAL, R. L. AND B. W. MASSEY. Unpublished report. A study of two breeding populations of the light-footed clapper rail in California. 45 pp.

ZEMBAL, R. L. AND B. W. MASSEY. 1983. The light-footed clapper rail: distribution, nesting

strategies, and management. Cal-Nevada Wildlife. Trans., 1983:97-103.

ZUCCA, J. J. 1954. A study of the California clapper rail. Wasmann Journal of Biology, 12:2, 135-155.

**Table 1. Nest fates of California clapper rails from six marshes in San Francisco Bay.**

| South Bay Marshes | # Nests | Mean Clutch Size | (Total Young) Young /Nest Attempt | % Hatch-ability | % Hatch Success | (#) % Nests Predated | (#) % Successful Nests | (# Nests Flooded) % Nest Flooded |
|---|---|---|---|---|---|---|---|---|
| Faber | 14 | 6.75 | (41) 2.9 | 71.9% | 46.6% | (3) 21.4% | (7) 50% | (1) 7.1% |
| Mowry | 11 | 7.60 | (42) 3.8 | 75.0% | 54.5% | (5) 45.5% | (6) 54.5% | 0 |
| Laumeister | 26 | 6.73 | (33) 1.3 | 62.5% | 25.1% | (16) 61.5% | (8) 30.8% | 0 |
| Greco Island | 20 | 6.75 | (61) 3.1 | 75.6% | 45.2% | (10) 50% | (12) 60% | 0 |
| North Bay Marshes | | | | | | | | |
| Wildcat | 7 | 6.25 | (16) 2.2 | 60.0% | 51.6% | (2) 28.6% | (4) 57.1% | 0 |
| Corte Madera | 11 | 6.90 | (18) 1.6 | 69.0% | 26.9% | (6) 54.5% | (3) 27.3% | (1) 9% |
| OVERALL (weighted means) | 89 | 6.89 | (211/89) 2.37 | 69.1% | 38.2% | 47.2% | 44.9% | 2.3% |

Faber, Wildcat and Corte Madera Marshes have two years of data combined.

**Table 2. Geometric mean and range of mercury concentrations in failed California clapper rail eggs on a clutch-wise basis for each of six marshes in San Francisco Bay. Values in ug/g fww.**

| Marsh (years) | Geometric Mean Hg concentration in ug/g fww ( # of clutches) | Hg range in individual egg concentrations in ug/g fww (# of eggs) |
|---|---|---|
| South Bay | | |
| Faber (1991, 1992) | 0.51 (10) | 0.28 to 1.46 (16) |
| Mowry (1992) | 0.79 (4) | 0.44 to 2.51 (7) |
| Laumeister (1992) | 0.47 (9) | 0.18 to 1.44 (14) |
| Greco (1992) | 0.71 (5) | 0.37 to 1.26 (5) |
| North Bay | | |
| Corte Madera (1998, 1999) | 0.27 (4) | 0.12 to 0.64 (13) |
| Wildcat (1998,1999) | 0.45 (5) | 0.24 to 0.87 (9) |

**Table 3. Regional geometric means of mercury and selenium concentrations on a clutch-wise basis in eggs of California clapper rails from south and north San Francisco Bay in the 1980s and 1990s and light-footed clapper rails from southern California in 1991 as compared the Lowest Observed Adverse Effect Concentrations (LOAEC) in avian eggs.**

| Location/Year(s) | Mean Mercury<br>ug/g fww<br>n = Number of clutches<br>(range in all eggs) | Mean Selenium<br>ug/g dw<br>n = Number of clutches<br>(range in all eggs) |
|---|---|---|
| North SF Bay<br>1998-1999 | 0.36<br>n = 11<br>(0.11 - 0.87) | 1.93<br>n = 11<br>(1.12 -3.13) |
| North SF Bay<br>1986-1987[a] | 0.72<br>n = 7<br>(0.45-0.95) | 4.0<br>n =7<br>(1.6 -7.4) |
| South SF Bay<br>1991-1992 | 0.54<br>n = 28<br>(0.17 - 2.52) | 2.08<br>n = 37<br>(1.5 - 3.2) |
| South SF Bay<br>1986-1987[a] | 0.55<br>n = 13<br>(0.38 - 0.87) | 1.63<br>n = 13<br>(1.14 - 2.60) |
| So. Calif.<br>1991[d] | < 0.023<br>(@77% moisture)<br>n = 8<br>(all eggs < 0.1 dw) | not measured |
| LOAEC (concentration in egg) | 0.5 [b] | 6.0 [c] |

[a] From Lonzarich et al., 1992. [b] Fimreite, 1971. [c] Skorupa, 1998. [d] Hui et al., 2002.

**Table 4. Strontium concentrations in contents of failed California clapper rail eggs by stage of incubation.**

| Stage of Incubation (n) | Mean Strontium concentrations ug/g dry weight |
|---|---|
| addled (11) | 34[a] |
| early (14) | 34[a] |
| mid-stage (7) | 39[a] |
| late-stage (7) | 59[a] |
| pip (7) | 97[b] |

Means sharing the same superscript letter are not statistically different. Pipped embryos were highly distinct in their mean strontium concentration ($p < 0.00001$ using Tukey HSD test for unequal sample sizes).

**Table 5. Comparison of geometric mean organochlorine concentrations (ug/g fresh wet weight) in failed California clapper rail eggs from south San Francisco Bay in 1992[a] and central bay eggs in 1998.**

| Organochlorine | South Bay 1992 n = 22 | Corte Madera 1998 n = 5 | Wildcat 1998 n = 4 |
|---|---|---|---|
| total PCB | 1.30 | 0.82 | 0.35 |
| p, p' DDE | 0.11 | 0.36 | 0.20 |
| trans-nonachlor | 0.06 | 0.14 | 0.05 |
| toxaphene | ND | 0.04 | 0.03 |
| dieldrin | 0.02 | 0.04 | 0.02 |
| oxychlordane | 0.03 | 0.03 | 0.02 |
| heptachlor epoxide | 0.01 | 0.01 | 0.004 |

**Table 6. Mean and ranges of trace element concentrations (ug/g dry weight) in normal and deformed late-stage embryos and p values for a two-tailed t-test for differences between means.**

| Element | Normal Embryos (N = 12)[a] | Deformed Embryos (N = 3) | p Value |
|---|---|---|---|
| Strontium | 66.1<br>30.2 - 94.6 | 121.4<br>82.2 - 176 | 0.006 |
| Barium | 0.41<br>0.2 - 0.59 | 3.00<br>2.16 - 4.13 | <0.000001 |
| Mercury [a] | 3.62<br>1.11 - 15.0 | 2.74<br>2.13 - 3.51 | 0.65 |
| Selenium [a] | 2.45<br>1.90 - 3.20 | 1.61<br>1.54 - 1.73 | 0.003 |
| Chromium | 0.3[b]<br><0.5 - 0 .7 | 0.98<br>0.41 - 2.06 | <0.02 |
| Boron | 2.0<br><2.0 - 4.0 | 4.0<br>0.8 - 10.2 | 0.15 |
| Lead | 0.6[b]<br><1.0 - 2.0 | 0.1<br><0.1 - 0.25 | 0.07 |
| Aluminum | 3.86<br><1.5 - 24.0 | 23. 09<br>8.65 - 36.2 | 0.002 |

[a] N = 20 for mercury and selenium analyses of normal, late-stage embryos.
[b] Means are less than detection limits because ½ the detection limit was used as values for non-detects.