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Reproductive Impacts of Elevated Selenium Levels

By

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INTRODUCTION

Selenium is bioaccumulated in organisms at each successive level of the detrital food chain in connected backwater lakes along the lower Colorado River (Radtke et al. 1988, Lusk 1993). Elevated selenium levels first occur in aufwuchs and the organic layer at the surface of the sediment on the pond bottom (Lusk 1993, Villegas 1997). Selenium bioaccumulates in organisms that feed on aufwuchs and detritus (benthic insects, crayfish, and clams) and reaches still higher levels in fish that feed on these organisms. The highest levels are found in fish eating birds (Rusk 1991, Lusk 1993, Martinez 1994, Welsh and Maughan 1994). In contrast, levels are only slightly elevated in plants and aquatic plant eating birds, such as coots. Based on these data, researchers initially concluded that only organisms that feed on aquatic insects, crayfish, clams or fish are in danger of having elevated levels of selenium in their tissues (Rusk 1991, Lusk 1993, Martinez 1994, Welsh and Maughan 1994). Birds, other than those that eat fish, were not initially considered to be at risk of accumulating selenium. However, Martinez (1994) analyzed selenium levels in a single nest of nighthawk eggs and found levels were similar to those in the eggs of fish eating birds.

Prior to the initiation of this study, much consideration was given to how the selenium levels in the nighthawk eggs sampled by Martinez (1994) had become elevated. The most logical source of the selenium in the eggs appeared to be the benthic insects from the backwaters that had metamorphosed from the aquatic to the aerial phase of their life cycle. Birds that fed on these insects might accumulate selenium in their tissues and pass it on into their eggs. Therefore, any bird feeding on aquatic insects emerging from the backwaters and becoming aerial would be at risk for accumulating selenium. Thus, we decided to study selenium levels in five passerine bird species along the lower Colorado River.

The objectives of the study were to:

1. Evaluate selenium levels in Neotropical migrants and riparian obligate birds on Imperial National Wildlife Refuge (Imperial NWR).

2. Evaluate selenium-induced reproductive impacts on birds along the lower Colorado River.

METHODS

Sites Sampled and Species Collected

Samples were collected from three sites on Imperial NWR and one site near Mittry Lake Wildlife Management Area (Mittry Lake). Species were collected based on availability across sites. We collected 10 adult individuals from four passerine species at Imperial NWR and one from Mittry. Samples of eggs from the same species were also collected. Species collected included red-winged blackbird (Agelaius phoeniceus), western kingbird (Tyranus verticalis), cliff swallow (Hirundo pyrrhonota), song sparrow (Melospiza melodia), and verdin (Auriparus flaviceps). Inadvertantly a few bank swallows (Riparia riparia) and tree swallows (Iridoprocne bicolor) that were flocking with cliff swallows were also collected.

Sample Collection and Preparation

Birds were collected throughout the breeding season, April to August 1995. Therefore, we are confident that the levels we measured represent levels acquired on the breeding grounds. One of three collection methods was used; a 20-gauge shotgun loaded with steel shot, a BB gun, or hand capture. Nests from which birds were hand captured were located during the day and then revisited at night and the birds captured on the nest. Specimens were kept on wet ice, for no more than six hours, until dissection. Whole birds (feathers, bill, feet and GI tract removed) were weighed. Livers were removed with a stainless steel scalpel, individually weighed, and individually wrapped in aluminum foil. Between dissections, all instruments were washed with distilled, deionized water and rinsed with hexane to prevent cross contamination. Eggs were measured, the contents removed and placed in chemically cleaned jars, and frozen in accordance with the guidelines of the Patuxent Analytical Control Facility (PACF). Egg shells were not analyzed. Most eggs were collected from nests on Imperial NWR but two song sparrow eggs were obtained from Havasu NWR. Samples were stored in a freezer until analysis at the Research Triangle Institute. Egg collection was completed in 1996.

Overall nest success and incidence of embryo malformations in a colony of cliff swallows was monitored from March to June 1997 at Mittry Lake. We carefully examined all chick mortalities from this population for indications of abnormalities. We also attempted to evaluate nest success and incidence of embryonic malformations for the other four species during March to June 1998. However, nesting was limited during that year and we could not locate sufficient nests for evaluation.

Selenium Analysis

Selenium analyses were conducted by Research Triangle Institute (RTI), Research Triangle Park, North Carolina; a contract laboratory of the PACF. Selenium was analyzed using Graphite Furnace Atomic Absorption (GFAA). Tissue samples were homogenized using a food processor. A portion of the tissue sample was freeze-dried for determination of moisture content and ground to 100 mesh. Digestion for GFAA measurement was conducted using a CEM microwave oven. From 0.25 to 0.5 g of freeze-dried sample was heated in a capped 120 ml Teflon vessel in the presence of 5 ml of Baker Instra-Analyzed nitric acid for three minutes at 120 watts, three minutes at 300 watts, and fifteen minutes at 450 watts. The residue was then diluted to 50 ml with laboratory pure water. GFAA measurements were made using a Perkin-Elmer Zeeman 3030 or 4100ZL atomic absorption spectrometer.

Quality Assurance/Quality Control

Quality assurance used by RTI for the chemical determinations included the analysis of duplicate samples, procedural blanks, analysis of standard reference materials (i.e., dogfish liver {NRCC DOLT-2} and lobster hepatopancreas {NRCC TORT-1}, and spike recoveries. PACF monitored and approved all quality assurance/quality control methodology.

Statistical Analysis

Range and standard error of the arithmetic mean are reported as measures of dispersion. Bartlett's test was used to determine if variances were equivalent. Selenium levels in the whole body tissues of the five principle species were analyzed with analysis of variance (ANOVA) and then by multiple comparisons of means with the Tukey-Kramer procedure (Sokal and Rolf 1981). Selenium levels in the livers (cliff swallow, redwing blackbird and western kingbird) and eggs (verdin, cliff swallow, redwing blackbird, and western kingbird) were analyzed with ANOVA and then by multiple comparisons of means with the Tukey-Kramer procedure. The levels of selenium among tissues (whole body, liver and egg) of each passerine species were analyzed with a t-test (two tissues) or ANOVA followed by multiple comparisons of the means with the Tukey-Kramer procedure (three tissues). All statistical tests were considered significant at $P \leq 0.05$.

STUDY AREA

The Lower Colorado River includes approximately 453 km of the Colorado River from Davis Dam to the United States-Mexico international border. Average annual precipitation along the Lower Colorado River is 10-13 cm. Average temperatures ranges from 21.1° C to 4.4°C in the winter and 40.6° C to 22.7° C the summer.

Imperial NWR is one of four national wildlife refuges located along the lower Colorado River. This refuge is located approximately 105 km north of the international boundary and includes the lower Colorado River along the Arizona-California border (Martinez 1994). Imperial NWR encompasses a total of 10,706 ha. Wetlands, lakes and marshes comprise 4,324 hectares, 5,630 ha are associated with desert and mountains, and 96 ha are dedicated to croplands (Lusk 1993). The Refuge was established in 1941 to mitigate the effects of channelization and to manage wildlife attracted to the backwaters formed after the construction of Imperial Dam (Rosenberg et al. 1991). This refuge protects two unique types of environment: the desert and a variety of wetland and riparian environments associated with the lower Colorado River. The desert areas are characterized by vegetation typical of the Lower Colorado River Valley Sonoran Desert including Velvet Mesquite (Prosopis velutina), Blue Paloverde (Cercidium floridum), Desert Ironwood (Olneya tesota), Creosote (Larrea tridentata), Salt Bush (Atriplex canescens), Graythorn (Ziziphus obtusifolia), Bur Sage (Ambrosia deltoidea), Big Galleta (Hillaria rigida), Beavertail Cactus (Opuntia basilaris), Ocotillo (Fouguieria splendens), Teddybear Cholla (Opuntia bigelovii), Hedgehog Cactus (Echinocerus engelmannii), and Desert Agave (Agave deserti).

Description of wetland areas

Backwaters on Imperial NWR have a detrital-silt substrate underlain by sand and gravel and surrounded by dense, monotypic stands of cattail (Typha latifolia.) bulrush (Scirupus validus.) or giant reed (Arundo donaxi) depending on water depth and slope. The predominant submergant is spiny naiad (Najas marina) but sago pondweed (Potamogeton pectinatus), water milfoil (Myriophyllum spp.), macroalgae (Chara spp.) and coontail (Ceratophyllum spp.) are present along the edges of the wetlands. True seeps support no submerged aquatic macrophytes and are lined almost exclusively by cattails.

Almost all underwater surfaces are covered with a layer of yellowish-brown slimy aufwuchs, i.e., the combined assemblage of epipelic, epiphytic, and epizoic periphyton and suctorian cilieates (Cole 1994). During the summer, floating mats of fiilamentous algae (mostly cyanophyta) (Kennedy 1979) are present in many of the backwaters. Seep lakes contain benthic algae and freshwater sponges that may be unique to these wetlands (Prieto 1998).

Mittry Lake is an ox-bow lake located between Imperial and Laguna Dams that receives water by way of a diversion from Imperial Reservoir. The lake is located approximately 29 km north of Yuma, Arizona. The Arizona Game and Fish Department, the United States Bureau of Reclamation, and the United States Bureau of Land Management manage Mittry Lake. Mesquite, Arrowweed (Pulchea sercea), Cottonwood (Populus fremontii), and saltcedar (Tamarix pentandra) dominate the riparian vegetation surrounding the lake. Cattails, bulrushes, and saltcedar dominate bank vegetation (Schluesner 1997).

RESULTS

Whole body samples were collected from each bird species. However, due to minimum one-gram weight requirements for sample analysis, livers were only collected from cliff swallows, red-winged blackbirds, and western kingbirds. Livers in verdins and song sparrows did not meet the one-gram minimum. Selenium levels are reported on a dry weight basis unless otherwise stated.

Each species accumulated selenium at different rates in individual tissues and eggs. For example, western kingbirds, had the highest levels of selenium in the eggs, while on average they were third in whole body levels and second in liver levels. Red-winged blackbirds had the second highest level in their eggs but had the highest levels in whole bodies and livers (Table 1).

Table 1. Selenium levels in whole bodies and livers of five bird species from Imperial National Wildlife Refuge.

			Se Dry Wt. (ppm)					
Species	Collection Site	Date (1996)	Body	(n)	Range	Liver	(n)	Range
Cliff Swallow	Backwater	7/18 – 8/31	2.07	9	1.15 – 3.33	3.86	9	2.65 – 6.02
Blackbird	Seep Lake	6/2 – 6/12	4.67	10	1.19-4.82	12.30	10	5.05 - 19.2
Song Sparrow	Seep Lake	5/23 – 6/28	3.81	10	2.56-4.81	N/A	10	N/A
Verdin	Desert Upland	5/22 – 6/28	2.32	10	0.94 – 3.66	N/A	10	N/A
Kingbird	Farm Fields	5/22 –7/26	2.81	10	1.19 – 4.82	6.86	10	3.9 – 10.44

Selenium levels were not significantly different between eggs and whole bodies of verdins (t-test, df=15, P=0.144) and song sparrows (t-test, df=12, P=0878). However, selenium levels were significantly higher in livers than in whole bodies in tree swallows (t-test, df=4, P=0.0002) and bank swallows (t-test, df=2, P=0.372). Selenium levels were also significantly different between livers, whole bodies, and eggs in cliff swallows (ANOVA, P=<0.0002), red-winged blackbirds (ANOVA, P=<0.0001) and between livers and whole bodies in western kingbirds (ANOVA, P=<0.0002).

Selenium levels were generally higher in whole bodies and livers of females than in whole bodies and livers of males (Table 2). Exceptions were in western kingbirds where both levels were higher in males than females and in red-winged blackbirds where whole body levels were higher in males than females.

Table 2. Arithmetic means of selenium concentrations (ppm, dry weight) in adult tissues by species and gender (n = number of birds analyzed, F=females, M=males and ?=birds of unknown sex).

				Whole Body			Liver		
Species	F(n)	M(n)	? (n)	F	M	?	F	M	?
Cliff Swallow	2	4	3	2.46	1.72	2.27	3.83	3.71	4.1
Red-Winged Blackbird	3	7	0	4.08	4.41	0	12.53	11.92	0
Song Sparrow	3	7	0	3.91	3.77	0	N/A	N/A	N/A
Verdin	2	3	5	3.15	1.92	2.23	N/A	N/A	N/A
Western Kingbird	2	8	0	2.54	3.9	0	6.02	6.94	0

Selenium levels in livers were always higher than those in whole bodies within a given species. Selenium levels in livers of red-winged blackbirds were about three times those in whole bodies.

Among Species Comparisons

Selenium levels in whole bodies of bank swallows and tree swallows did not differ from those in cliff swallows (t-test, df=9, p=0.306 and t-test, df=9, p=0.456), nor did they differ in livers of cliff swallows and tree swallows (t-test, df=10, P=0.407). Selenium levels were significantly higher in the whole bodies of red-winged blackbirds than those in the other species (ANOVA, P=0.0002). Levels were lowest in cliff swallows but differences were only significantly lower than those in red-winged blackbirds and song sparrows.

Selenium levels in the livers of cliff swallows, red-winged blackbirds, western kingbirds were significantly different (ANOVA, P<0.0001). Levels were significantly higher in red-winged blackbirds than the other two species, but levels between the other two species were not significantly different from one another.

Eggs

A total of 30 eggs were collected during the early breeding season. Seven eggs were collected from cliff swallows, red-winged blackbirds, verdins, and western kingbirds. No song sparrow nests that contained eggs could be found on Imperial National Wildlife Refuge, but two eggs were obtained from Havasu NWR. All the eggs were near or at the level for chronic toxicity (Table 3).

Table 3. Selenium levels is eggs of five bird species from Imperial National Wildlife Refuge and Havasu National Wildlife Refuge.

	Imperial National Wildlife Refuge			Amt. Se	Havasu National Wildlife Refuge			Amt. Se
Species	Location	Date	(n)	Eggs	Location	Date	(n)	
Cliff Swallow	Backwater	4/12/96	7	2.56	N/A	N/A	N/A	N/A
Red-Winged	Seep Lake	5/1/96	7	4.54	N/A	N/A	N/A	N/A
Song Sparrow	N/A	N/A	N/A	N/A	Seep Lake	6/21/97	2	3.72
Verdin	Desert	4/5/96	7	2.9	N/A	N/A	N/A	N/A
Western Kingbird	Farm Fields	6/21/96	7	5.99	N/A	N/A	N/A	N/A

Selenium levels in the eggs were significantly different among the five species (ANOVA, $P<0.001$). Levels were highest in the eggs of western kingbird and redwing blackbird and lowest in cliff swallows.

A total of 94 cliff swallow nests were monitored weekly. Seventy four of these nests contained eggs at some time during the study period. However, chicks were produced in only seven nests. Many eggs were destroyed before they had the opportunity to hatch (Table 4). In early June many nests were destroyed during causeway construction. Only limited nesting occurred after that disturbance. Prior to that date, mortalities appeared to result from intraspecific interactions.

Table 4. Status of Cliff Swallow Nests on the Mittry Wildlife Management Area during the Spring and Summer of 1997. Numbers refer to numbers of eggs (eg 3e), or fledglings (eg 3f). Additional letters define the special conditions of the eggs or fledglings (eg b=eggs present but broken and d=fledlings present but dead). A zero indicates the nest was empty and a D indicates that the nest that had been at this location during the previous sampling effort had been destroyed during the interval between samples.

Nest	Nest Status							
	3/30	4/5	4/12	4/19	4/27	5/3	5/14	5/22	6/12
2	0	0	3e	4e	0	0	0	0	0
3	0	0	0	3e	0	0	0	0	0
4	0	0	0	3e	0	0	0	0	0
5	0	1e	4e	4e	0	1f	0	0	-
6	0	0	0	3e	0	0	0	0	0
7	0	4e	0	2e	0	1e	0	0	0
8	0	0	1e	0	1e	0	0	0	D
9	4eb	1e	0	1e	1e	0	0	0	D
11	0	0	1e	0	0	0	0	0	D
12	0	3e	0	0	1e	0	0	0	D
14	0	0	2e	0	0	0	0	0	D
15	0	0	1e	0	0	0	0	0	D
16	0	0	3e	0	0	1e	0	0	D
17	0	1e	0	3e	0	0	0	0	D
18	1e	1e	2e	0	0	0	-	D	D
19	3e	0	2e	0	0	0	0	0	D
20	3e	0	1e	0	0	0	0	0	D
21	0	1e	1e	0	0	1e	0	0	D
22	0	0	0	1e	0	0	0	1e	D
23	1e	0	3e	0	0	0	0	0	D

24	0	0	2e	0	0	1e	1e	0	D
25	0	0	4e	1e	0	0	0	0	D
26	1e	3e	3e	0	0	0	0	0	D
27	0	3e	3f	0	0	0	0	0	-
28	0	3e	0	0	-	0	0	0	D
29	0	0	3e	3f	-	0	0	0	D
30	0	0	3e	3e	-	0	-	D	D
31	0	0	0	1e	-	0	0	0	D
32	1e	0	1e	0	-	0	0	0	D
33	3e	0	0	0	-	0	0	1eb	D
34	0	3e	0	3e	-	0	0	0	D
35	0	3e	0	0	-	1e	0	0	D
36	0	0	1e	0	-	0	0	0	D
37	3e	1e	3f	0	-	0	0	0	D
38	0	0	4e	0	-	0	0	0	D
39	0	1e	3e	0	-	0	0	0	D
40	0	0	0	0	-	0	0	2eb	D
41	0	0	0	0	-	0	2e	0	D
43	1e	0	0	0	-	0	0	0	D
44	1e	2e	2e	3e	-	1e	0	0	D
45	2e	4e	4e	3f	-	0	0	0	D
47	0	2e	2e	4e	-	0	0	0	0
48	0	0	0	3e	-	0	0	0	-
49	0	0	0	3e	-	0	0	0	-
50	3e	3e	2e	0	-	1f	0	0	0
51	0	0	1e	1e	-	0	0	0	-
52	0	0	1e	0	-	0	0	0	0
53	1e	1e	3e	0	-	0	0	0	0
55	0	2e	0	3e	-	0	0	0	0
56	0	3e	3e	1e2f	-	0	0	0	0
57	0	2e	0	0	-	1e	0	0	0

58	0	0	0	0	-	0	2e	2e	2e
59	0	4e	4e	2f	-	0	0	0	0
60	0	3e	0	3e	-	0	0	0	0
61	0	4e	0	4e	-	0	0	0	0
63	0	0	0	1e	-	0	0	0	0
64	0	0	1e	0	-	0	0	0	0
65	0	0	0	0	-	0	0	4eb	0
66	1e	0	0	0	-	0	1e	0	1e
67	0	0	1e	1e	-	0	0	0	0
68	3e	0	1e	1e	-	0	0	0	0
69	0	0	3e	3e	-	0	0	0	0
70	4e	0	1e	0	-	0	0	0	0
70b	-	-	-	-	-	0	0	1e	0
71	0	0	2e	0	-	0	0	0	0
74	0	0	1e	0	-	1e	0	0	-
77	0	0	1e	1e	-	0	0	0	-
79	0	3e	0	3e	-	0	0	1e	0
81	0	3e	0	0	-	0	0	0	0
83	3e	0	4e	0	-	0	0	0	-
85a	-	-	-	-	-	-	0	1e	0
87	-	-	0	-	-	0	0	1e	0
88	-	-	0	-	-	0	0	1e	0

DISCUSSION

Selenium was elevated to levels of concern in the tissues and eggs of all five bird species. Previous data has shown elevated selenium levels in fish eating birds and nighthawks. Our data now add red-winged blackbirds, song sparrows, western kingbirds, verdins, and cliff swallows to the list of potential species at risk. The fact that selenium levels were elevated in all of the birds studied, suggest most passerine birds along the lower Colorado River face some risk of accumulating selenium.

Why are we concerned about the levels of selenium in tissues and eggs of these birds? Selenium levels of 3 ug/g dry weight in bird eggs is considered to be the threshold of concern for teratogenesis and levels in the livers of adults of >10 ug/g dry weight have been associated with adverse biological effects (Lemly 1993). Selenium levels in the livers of the birds we studied ranged from 2.7-10.4 ug/g dry weight and levels in their eggs ranged from 2.6-6.0 ug/g dry weight. Martinez (1994) had previously reported that levels in eggs and egg masses (unlaid eggs taken from the female reproductive tract) of several species of fish eating birds averaged from 8.7 to 10.3 ppm dry weight. It appears from these data that levels of selenium in the eggs of both passerine and fish eating species exceed those where there is risk of teratogenesis.

We had hypothesized that birds that were primarily insectivorous would have higher selenium levels in their tissues and eggs than those that generally feed upon seeds. This hypotheses was based on the assumption that passerine birds in the riparian corridor obtained selenium by feeding on the aerial stage in the life cycle of aquatic insects. Although the pathway through the aerial stage of aquatic insects is still the most plausible route for the entry of selenium into the diet of riparian birds, the hypothesis that birds that were primarily insectivorous would have the highest levels, was not substantiated.

There are several possible reasons why this hypothesis was not supported by the data. First, the source of the selenium could be other than that obtained through the ingestion of aquatic insects. While it is possible that there is a source of selenium, other than insects, there is no obvious alternative source. Soils in the study area are not seleniferous and irrigation return flows do not contain elevated selenium levels (Welsh and Maughan 1994, Villegas 1997). Selenium levels in the water in the river and plants in the backwater are low and obtaining selenium directly from these sources would not result in the observed elevated levels in the birds. Therefore, it seems probable that aquatic insects are the source from which riparian birds obtain selenium.

Another possibility is that all of the bird species studied may have been primarily insectivorous at the time of the study. The study took place during the breeding season, when there are major energy and protein demands. During the period of egg laying, at least female birds take larger numbers of insects than during other periods because of the need for extra protein (Rosenberg et al. 1991). Since all the species studied were nesting during the study period, they may have been primarily feeding on insects.

A third possibility may be that some of the birds studied may have fed on insects that had lower selenium levels than those that emerged from the connected backwaters. For example, selenium levels in cliff swallows were lower in whole bodies, eggs, and livers than in any other species, even though cliff swallows are primarily insectivorous and were nesting adjacent to an area where the aquatic biota were known to have elevated selenium levels in their tissues (Schleusner 1997, Villegas 1997). We believe these low levels occurred because swallows foraged away from the location where they nested. In the morning, swallows left the nesting area by following a irrigation canal away from the river. In the evening they returned to the nesting area, from across the desert. These observations suggest that the birds from this particular nesting colony did not feed over the backwater areas adjacent to the nesting area and thus, may have minimized their exposure to the elevated selenium levels in emerging insects.

We had also hypothesized that selenium levels would be highest in birds nesting beside backwater lakes, where there was more potential for exposure to insects that are known to have higher selenium levels in their tissues than those that emerge from seep lakes (Lusk 1993, Prieto 1997). Again the data did not support the hypothesis. Red-winged blackbirds that nested beside a seep lake consistently had higher selenium levels in their tissues than cliff swallows that nested beside a connected backwater lake. We believe these lack of differences in selenium levels result from a combination of the feeding location hypothesis presented for cliff swallows earlier and the fact the insects that emerge from the connected backwater disperse beyond the immediate location of the waters from which they emerge.

We had also hypothesized that birds that nested further from the river would have lower selenium levels in their tissues than those that nested adjacent to the river or the associated backwaters. Again the data did not support the hypothesis. Western kingbirds that nested the fartherest from the river had the highest selenium levels in their eggs. The failure of the data to support the hypothesis suggest either that insects disperse widely after emerging from the backwaters or that all of the birds studied foraged in the riparian corridor even though they nested outside that corridor. It appears that all of the bird species studied were exposed to selenium regardless of where they nested or their primary food source.

Selenium levels in most organisms are generally higher in liver than they are in eggs and higher in eggs than they are in whole bodies (Fairbrother and Fowles 1990, Heinz et al. 1990, Martinez 1994). We also saw this trend in the birds that we studied. Levels varied among species but they were always highest in livers and lowest in whole bodies. Levels in eggs were generally more comparable to those in livers than those in whole bodies. Selenium levels in tissues generally reflect the rate of metabolic activity (Beilstein and Whagner 1987). The liver is an organ of active metabolism and excretion and thus selenium levels in the liver are generally higher than they are in most other tissues. The high metabolic activity that is required to produce eggs accounts for the high levels of selenium in eggs. Metabolic rates vary greatly in the several tissues of the body. Therefore, selenium levels in the whole bodies are generally lower than they are in the metabolically active tissues such as liver and eggs.

In the five species studied, females generally had higher selenium levels in whole bodies than did males, and in two of the three species where livers were taken, liver levels were also higher in females. However, female red-winged blackbirds had lower whole body selenium levels than did males, but levels in livers were higher. These differences may indicate some differences in foraging pattern or metabolism between the sexes. No doubt there are different metabolic requirements for males and females associated with reproduction. In general, one would expect metabolic requirements to be higher for females and thus one would expect selenium levels to also be higher in females.

Despite elevated selenium levels in the eggs, we failed to see any evidence of selenium induced reproductive failure or abnormality in any of the species studied. It is probable that our sample size was too small to document abnormalities even if they occurred. However, it is also possible that there were no abnormalities even though selenium levels were high. Selenium in the lower Colorado River originates from cretaceous shale in the upper basin. Origin at this source makes it probable that selenium levels in the lower Colorado River have been chronically elevated perhaps over geological time. Birds nesting in the lower Colorado River could have been exposed to these elevated selenium levels over many generations and may have developed some resistance to the impacts of these levels that are not present in birds from other areas.

We saw no malformed chicks in the cliff swallow colony, but there was very high egg mortality. Destruction of eggs and chicks by conspecifics is a common phenomenon in cliff swallow colonies were there is intraspecific competition for resources (Rosenberg et al. 1991). In addition, virtually all nests were destroyed when causeway construction began in June. However, mortalities that occurred prior to June were not associated with construction.

This study would have been more conclusive if we could have taken more individuals of each species and taken representatives from each species from each habitat type. However, the permitting agencies – the Arizona Game and Fish Department and the U. S. Fish and Wildlife Service – would not permit us to take a larger sample size. We could not compare across habitat types because individual species nested in different habitats and thus we could not take representatives of each species from each habitat. We collected eggs and birds from the same nesting area so that we could directly compare levels in adults and eggs.

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APPENDIX A. STATISICAL ANALYSIS OF SELENIUM LEVELS AMONG TISSUES AND SPECIES

Se Dry Weight By Bird

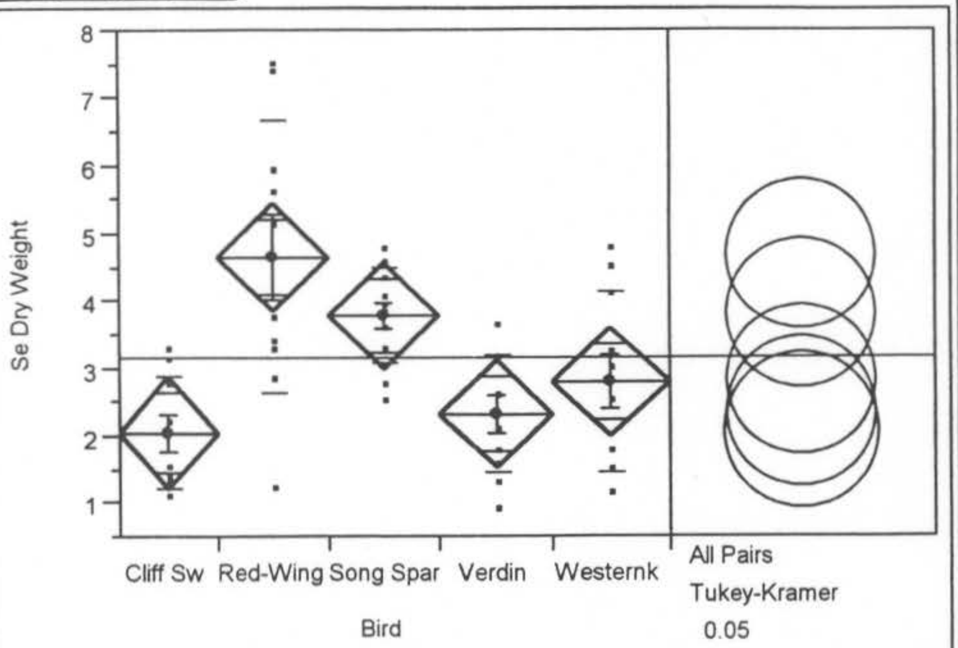


Table 1 - Statistical analysis of whole body selenium levels among species.

Oneway Anova

Summary of Fit

RSquare	0.385024
RSquare Adj	0.329117
Root Mean Square Error	1.280268
Mean of Response	3.15551
Observations (or Sum Wgts)	49

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	45.15265	11.2882	6.8869
Error	44	72.11976	1.6391	Prob>F
C Total	48	117.27241	2.4432	0.0002

Means for Oneway Anova

Level	Number	Mean	Std Error
Cliff Sw	9	2.07333	0.42676
Red-Wing	10	4.64700	0.40486
Song Spar	10	3.81100	0.40486
Verdin	10	2.32500	0.40486
Westernk	10	2.81300	0.40486

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
Cliff Sw	9	2.07333	0.83531	0.27844
Red-Wing	10	4.64700	2.04185	0.64569
Song Spar	10	3.81100	0.74173	0.23456
Verdin	10	2.32500	0.89509	0.28305
Westernk	10	2.81300	1.36843	0.43274

Table 1 - Continuation

Means Comparisons

Dif=Mean[i]-Mean[j]	Red-Wing	Song Spar	Westernk	Verdin	Cliff Sw
Red-Wing	0.00000	0.83600	1.83400	2.32200	2.57367
Song Spar	-0.83600	0.00000	0.99800	1.48600	1.73767
Westernk	-1.83400	-0.99800	0.00000	0.48800	0.73967
Verdin	-2.32200	-1.48600	-0.48800	0.00000	0.25167
Cliff Sw	-2.57367	-1.73767	-0.73967	-0.25167	0.00000

Alpha= 0.05

Comparisons for all pairs using Tukey-Kramer HSD

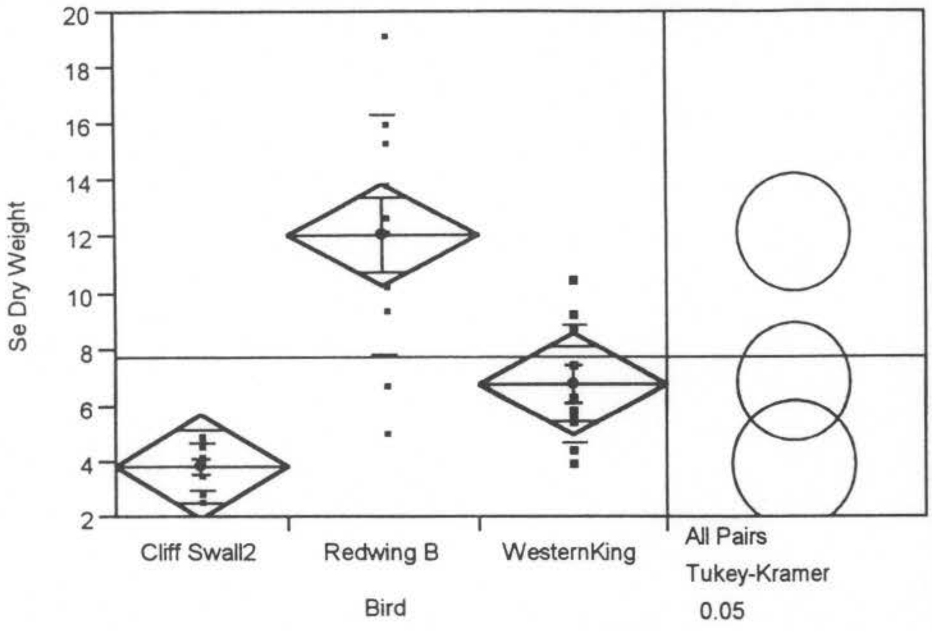
q*
2.84411

Abs(Dif)-LSD	Red-Wing	Song Spar	Westernk	Verdin	Cliff Sw
Red-Wing	-1.62840	-0.79240	0.20560	0.69360	0.90064
Song Spar	-0.79240	-1.62840	-0.63040	-0.14240	0.06464
Westernk	0.20560	-0.63040	-1.62840	-1.14040	-0.93336
Verdin	0.69360	-0.14240	-1.14040	-1.62840	-1.42136
Cliff Sw	0.90064	0.06464	-0.93336	-1.42136	-1.71649

Positive values show pairs of means that are significantly different.

Table 2 - Statistical analysis of selenium levels in livers among species.

Se Dry Weight By Bird



Oneway Anova

Summary of Fit

RSquare	0.607013
RSquare Adj	0.576784
Root Mean Square Error	2.889157
Mean of Response	7.706207
Observations (or Sum Wgts)	29

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	335.22472	167.612	20.0800
Error	26	217.02797	8.347	Prob>F
C Total	28	552.25268	19.723	<.0001

Means for Oneway Anova

Level	Number	Mean	Std Error
Cliff Swall2	9	3.8678	0.96305
Redwing B	10	12.1070	0.91363
WesternKing	10	6.7600	0.91363

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
Cliff Swall2	9	3.8678	0.88350	0.2945
Redwing B	10	12.1070	4.33430	1.3706
WesternKing	10	6.7600	2.15273	0.6808

Means Comparisons

Dif=Mean[i]-Mean[j]	Redwing B	WesternKing	Cliff Swall2
Redwing B	0.00000	5.34700	8.23922
WesternKing	-5.34700	0.00000	2.89222
Cliff Swall2	-8.23922	-2.89222	0.00000

Alpha= 0.05

Comparisons for all pairs using Tukey-Kramer HSD

q*

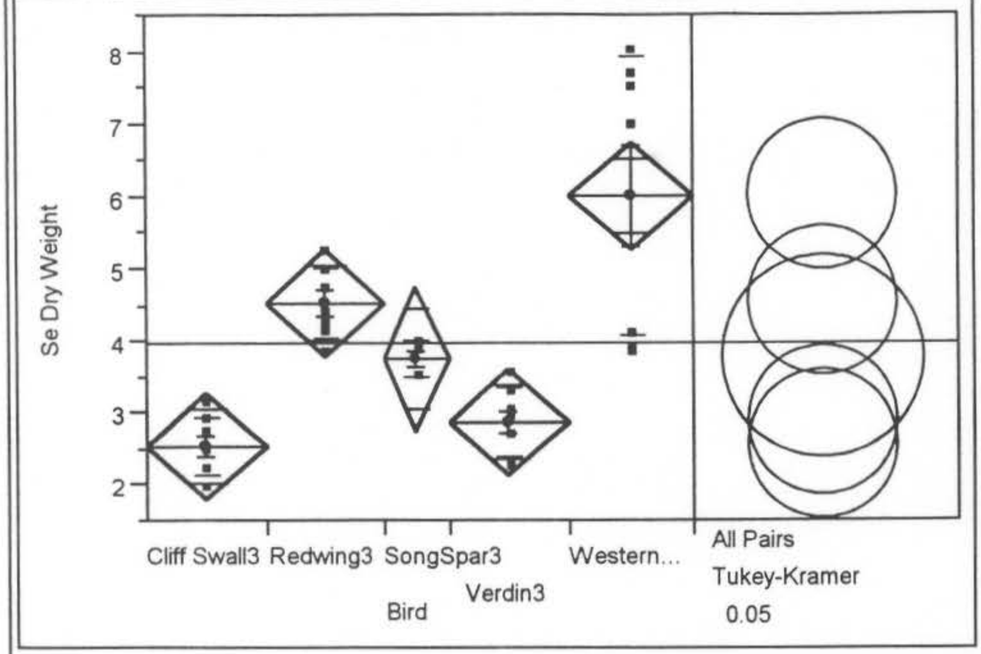
2.48489

Abs(Dif)-LSD	Redwing B	WesternKing	Cliff Swall2
Redwing B	-3.21066	2.13634	4.94058
WesternKing	2.13634	-3.21066	-0.40642
Cliff Swall2	4.94058	-0.40642	-3.38433

Positive values show pairs of means that are significantly different.

Table 3 - Statistical analysis of selenium levels in eggs among species.

Se Dry Weight By Bird



Oneway Anova

Summary of Fit

RSquare	0.668501
RSquare Adj	0.61939
Root Mean Square Error	0.996762
Mean of Response	3.975938
Observations (or Sum Wgts)	32

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	54.096115	13.5240	13.6120
Error	27	26.825457	0.9935	Prob>F
C Total	31	80.921572	2.6104	<.0001

Means for Oneway Anova

Level	Number	Mean	Std Error
Cliff Swall3	7	2.56000	0.37674
Redwing3	7	4.54429	0.37674
SongSpar3	4	3.75000	0.49838
Verdin3	7	2.90000	0.37674
WesternKing3	7	6.02857	0.37674

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
Cliff Swall3	7	2.56000	0.42230	0.15961
Redwing3	7	4.54429	0.49729	0.18796
SongSpar3	4	3.75000	0.25871	0.12936
Verdin3	7	2.90000	0.48090	0.18176
WesternKing3	7	6.02857	1.94436	0.73490

Table 3 - Continuation

Means Comparisons

Dif=Mean[i]-Mean[j]	WesternKing3	Redwing3	SongSpar3	Verdin3	Cliff Swall3
WesternKing3	0.00000	1.48429	2.27857	3.12857	3.46857
Redwing3	-1.48429	0.00000	0.79429	1.64429	1.98429
SongSpar3	-2.27857	-0.79429	0.00000	0.85000	1.19000
Verdin3	-3.12857	-1.64429	-0.85000	0.00000	0.34000
Cliff Swall3	-3.46857	-1.98429	-1.19000	-0.34000	0.00000

Alpha= 0.05

Comparisons for all pairs using Tukey-Kramer HSD

q*

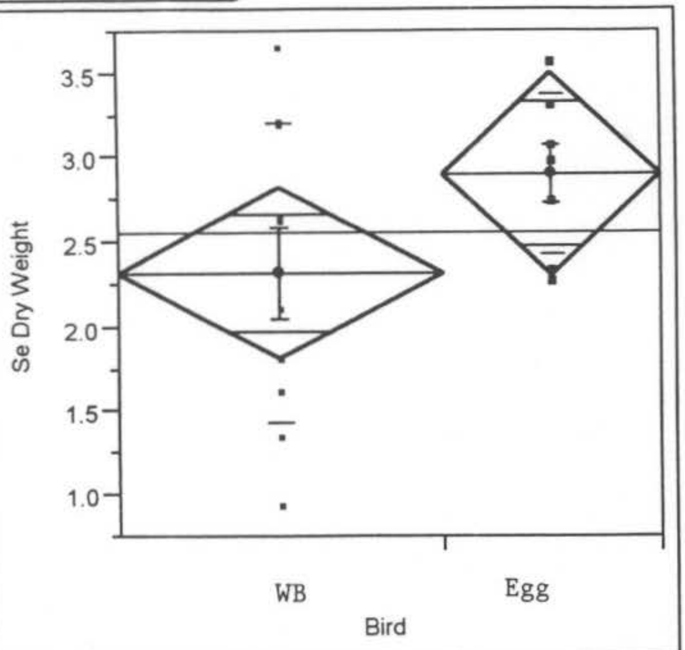
2.92068

Abs(Dif)-LSD	WesternKing3	Redwing3	SongSpar3	Verdin3	Cliff Swall3
WesternKing3	-1.55612	-0.07183	0.45386	1.57245	1.91245
Redwing3	-0.07183	-1.55612	-1.03042	0.08817	0.42817
SongSpar3	0.45386	-1.03042	-2.05855	-0.97471	-0.63471
Verdin3	1.57245	0.08817	-0.97471	-1.55612	-1.21612
Cliff Swall3	1.91245	0.42817	-0.63471	-1.21612	-1.55612

Positive values show pairs of means that are significantly different.

Table 4 - Statistical analysis of selenium levels between tissues in verdins.

Se Dry Weight By Bird



Oneway Anova

Summary of Fit

RSquare	0.136691
RSquare Adj	0.079137
Root Mean Square Error	0.757111
Mean of Response	2.561765
Observations (or Sum Wgts)	17

t-Test

	Difference	t-Test	DF	Prob>|t|
Estimate	-0.57500	-1.541	15	0.1441
Std Error	0.37311			
Lower 95%	-1.37026			
Upper 95%	0.22026			

Assuming equal variances

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	1.3613971	1.36140	2.3750
Error	15	8.5982500	0.57322	Prob>F
C Total	16	9.9596471	0.62248	0.1441

Means for Oneway Anova

Level	Number	Mean	Std Error
WB	10	2.32500	0.23942
Egg	7	2.90000	0.28616

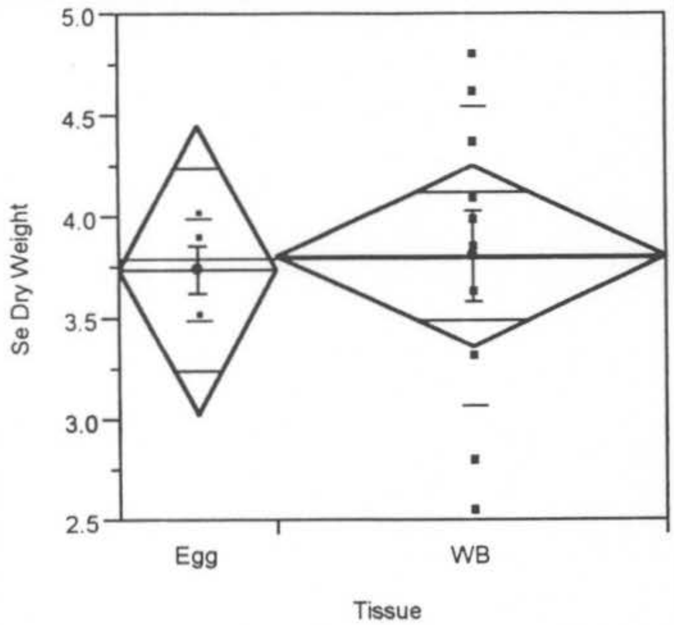
Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
WB	10	2.32500	0.895088	0.28305
Egg	7	2.90000	0.480902	0.18176

Table 5 - Statistical analysis of selenium levels between tissues in song sparrows.

Se Dry Weight By Tissue



Oneway Anova

Summary of Fit

RSquare	0.002059
RSquare Adj	-0.0811
Root Mean Square Error	0.655254
Mean of Response	3.793571
Observations (or Sum Wgts)	14

t-Test

	Difference	t-Test	DF	Prob>|t|
Estimate	-0.061	-0.157	12	0.8776
Std Error	0.387653			
Lower 95%	-0.90563			
Upper 95%	0.783625			

Assuming equal variances

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.0106314	0.010631	0.0248
Error	12	5.1522900	0.429357	Prob>F
C Total	13	5.1629214	0.397148	0.8776

Means for Oneway Anova

Level	Number	Mean	Std Error
Egg	4	3.75000	0.32763
WB	10	3.81100	0.20721

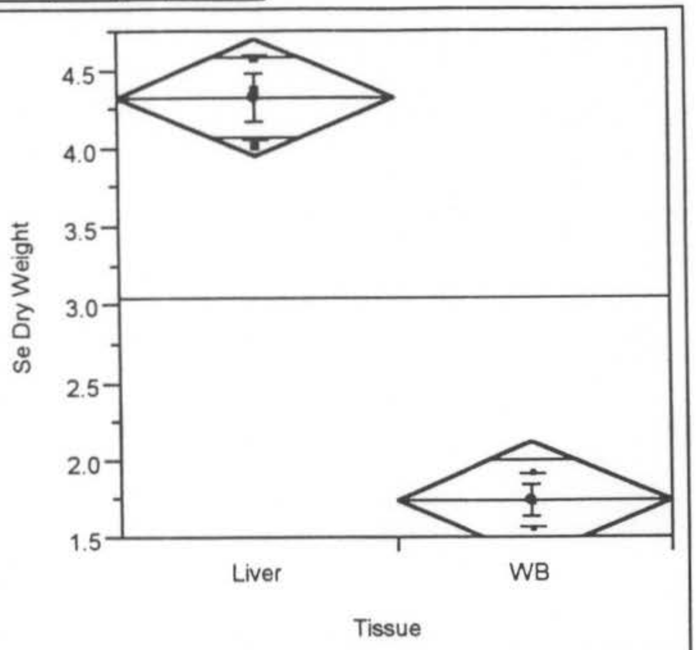
Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
Egg	4	3.75000	0.258715	0.12936
WB	10	3.81100	0.741731	0.23456

Table 6 - Statistical analysis of selenium levels between tissues in tree swallows.

Se Dry Weight By Tissue



Oneway Anova

Summary of Fit

RSquare	0.977362
RSquare Adj	0.971703
Root Mean Square Error	0.240139
Mean of Response	3.041667
Observations (or Sum Wgts)	6

t-Test

	Difference	t-Test	DF	Prob>|t|
Estimate	2.57667	13.141	4	0.0002
Std Error	0.19607			
Lower 95%	2.03229			
Upper 95%	3.12104			

Assuming equal variances

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	9.958817	9.95882	172.6962
Error	4	0.230667	0.05767	Prob>F
C Total	5	10.189483	2.03790	0.0002

Means for Oneway Anova

Level	Number	Mean	Std Error
Liver	3	4.33000	0.13864
WB	3	1.75333	0.13864

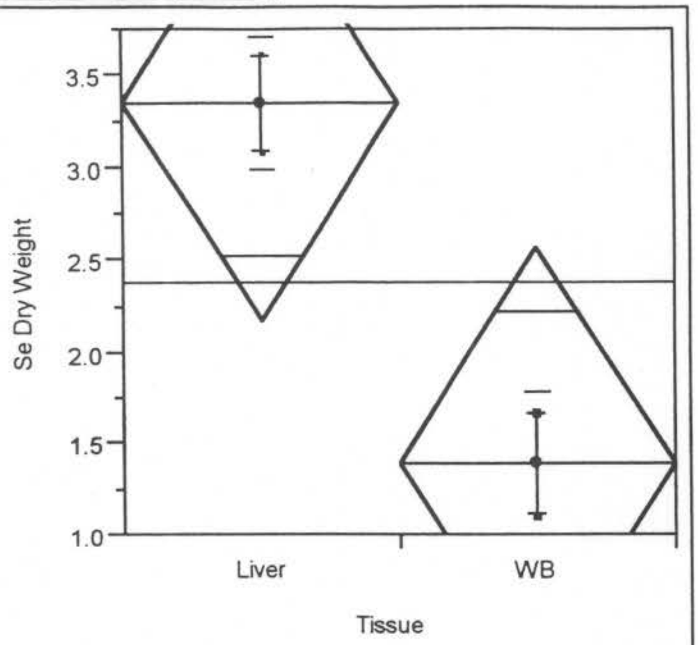
Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
Liver	3	4.33000	0.284781	0.16442
WB	3	1.75333	0.185023	0.10682

Table 7 - Statistical analysis of selenium levels between tissues in bank swallows.

Se Dry Weight By Tissue



Oneway Anova

Summary of Fit

RSquare	0.926915
RSquare Adj	0.890373
Root Mean Square Error	0.389166
Mean of Response	2.375
Observations (or Sum Wgts)	4

t-Test

	Difference	t-Test	DF	Prob>|t|
Estimate	1.96000	5.036	2	0.0372
Std Error	0.38917			
Lower 95%	0.28554			
Upper 95%	3.63446			

Assuming equal variances

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	3.8416000	3.84160	25.3655
Error	2	0.3029000	0.15145	Prob>F
C Total	3	4.1445000	1.38150	0.0372

Means for Oneway Anova

Level	Number	Mean	Std Error
Liver	2	3.35500	0.27518
WB	2	1.39500	0.27518

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
Liver	2	3.35500	0.374767	0.26500
WB	2	1.39500	0.403051	0.28500

Se Dry Weight By Tissue

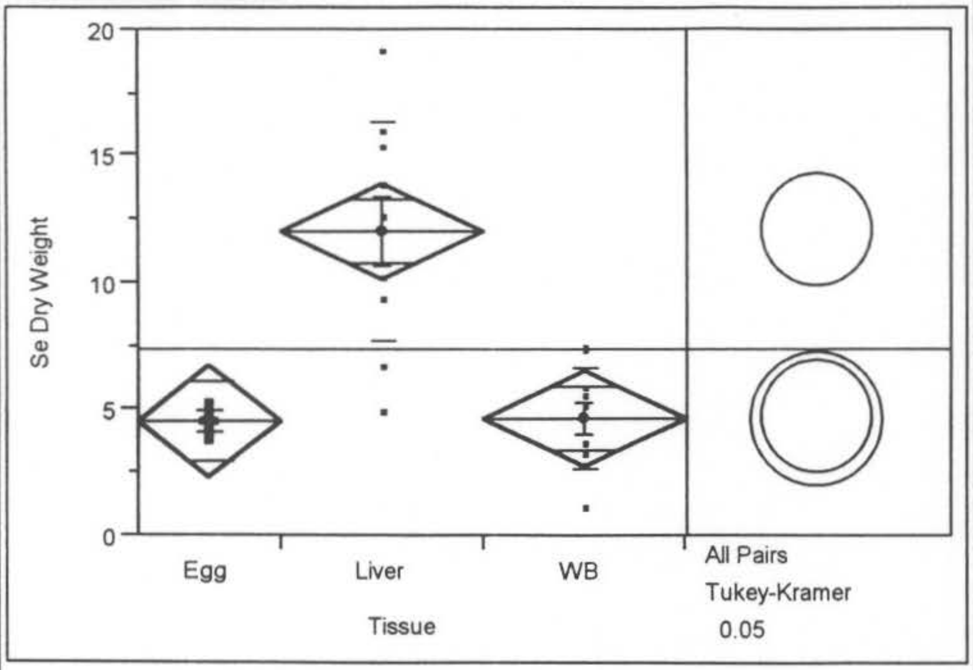


Table 8 - Statistical analysis of selenium levels among tissues in redwinged blackbirds.

Oneway Anova

Summary of Fit

RSquare	0.630083
RSquare Adj	0.599257
Root Mean Square Error	2.944495
Mean of Response	7.383333
Observations (or Sum Wgts)	27

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	354.42681	177.213	20.4397
Error	24	208.08119	8.670	Prob>F
C Total	26	562.50800	21.635	<.0001

Means for Oneway Anova

Level	Number	Mean	Std Error
Egg	7	4.5443	1.1129
Liver	10	12.1070	0.9311
WB	10	4.6470	0.9311

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
Egg	7	4.5443	0.49729	0.1880
Liver	10	12.1070	4.33430	1.3706
WB	10	4.6470	2.04185	0.6457

Means Comparisons

Dif=Mean[i]-Mean[j]	Liver	WB	Egg
Liver	0.00000	7.46000	7.56271
WB	-7.46000	0.00000	0.10271
Egg	-7.56271	-0.10271	0.00000

Alpha= 0.05

Comparisons for all pairs using Tukey-Kramer HSD

q*

2.49729

Abs(Dif)-LSD	Liver	WB	Egg
Liver	-3.28847	4.17153	3.93899
WB	4.17153	-3.28847	-3.52101
Egg	3.93899	-3.52101	-3.93048

Positive values show pairs of means that are significantly different.

Se Dry Weight By Bird

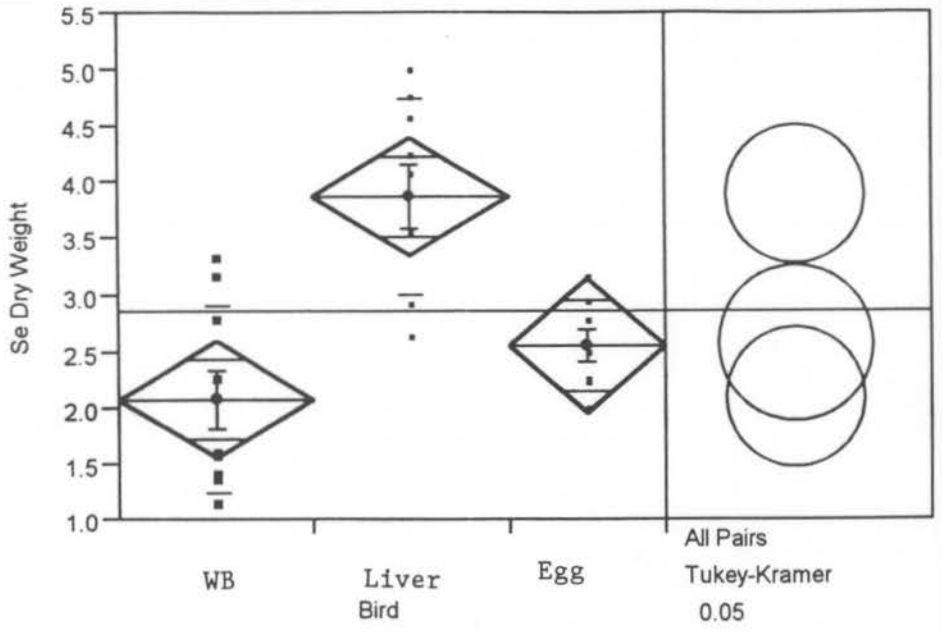


Table 9 - Statistical analysis of selenium levels among tissues in cliff swallows.

Oneway Anova

Summary of Fit

RSquare	0.543262
RSquare Adj	0.50174
Root Mean Square Error	0.765642
Mean of Response	2.8556
Observations (or Sum Wgts)	25

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	15.339660	7.66983	13.0838
Error	22	12.896556	0.58621	Prob>F
C Total	24	28.236216	1.17651	0.0002

Means for Oneway Anova

Level	Number	Mean	Std Error
WB	9	2.07333	0.25521
Liver	9	3.86778	0.25521
Egg	7	2.56000	0.28939

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
WB	9	2.07333	0.835314	0.27844
Liver	9	3.86778	0.883498	0.29450
Egg	7	2.56000	0.422295	0.15961

Means Comparisons

Dif=Mean[i]-Mean[j]	Liver	Egg	WB
Liver	0.00000	1.30778	1.79444
Egg	-1.30778	0.00000	0.48667
WB	-1.79444	-0.48667	0.00000

Alpha= 0.05

Comparisons for all pairs using Tukey-Kramer HSD

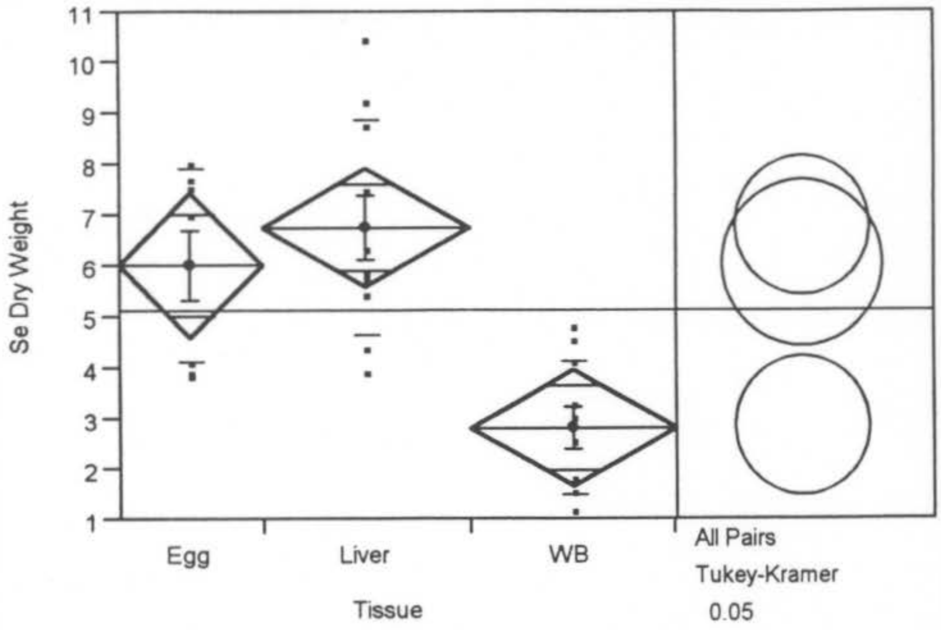
q*
2.51206

Abs(Dif)-LSD	Liver	Egg	WB
Liver	-0.90667	0.33851	0.88777
Egg	0.33851	-1.02807	-0.48261
WB	0.88777	-0.48261	-0.90667

Positive values show pairs of means that are significantly different.

Table 10 - Statistical analysis of selenium levels among tissues in western kingbirds.

Se Dry Weight By Tissue



Oneway Anova

Summary of Fit

RSquare	0.513906
RSquare Adj	0.473398
Root Mean Square Error	1.839892
Mean of Response	5.108519
Observations (or Sum Wgts)	27

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	85.89345	42.9467	12.6866
Error	24	81.24490	3.3852	Prob>F
C Total	26	167.13834	6.4284	0.0002

Means for Oneway Anova

Level	Number	Mean	Std Error
Egg	7	6.02857	0.69541
Liver	10	6.76000	0.58183
WB	10	2.81300	0.58183

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
Egg	7	6.02857	1.94436	0.73490
Liver	10	6.76000	2.15273	0.68075
WB	10	2.81300	1.36843	0.43274

Means Comparisons

Dif=Mean[i]-Mean[j]	Liver	Egg	WB
Liver	0.00000	0.73143	3.94700
Egg	-0.73143	0.00000	3.21557
WB	-3.94700	-3.21557	0.00000

Alpha= 0.05

Comparisons for all pairs using Tukey-Kramer HSD

q*
2.49729

Abs(Dif)-LSD	Liver	Egg	WB
Liver	-2.05483	-1.53288	1.89217
Egg	-1.53288	-2.45599	0.95126
WB	1.89217	0.95126	-2.05483

Positive values show pairs of means that are significantly different.