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Dramatic Fluctuations in Liver Mass and Metal Content of Eared Grebes *(Podiceps nigricollis)* During Autumnal Migration

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Among vertebrate classes, birds exhibit some of the most pronounced fluctuations in body mass and composition associated with the reproductive and migratory components of their annual cycle. The eared grebe *(Podiceps nigricollis)* is a common waterbird in western North America that is remarkable in this context. Following reproduction at freshwater lakes in the northern U.S. and southern Canada (May-August), most of the population migrates to hypersaline lakes in the Great Basin (late July-October) where they molt and become flightless for several months (Storer and Jehl 1985; Jehl 1988). At staging lakes, they double their body mass by adding enormous fat deposits and increasing the size of digestive organs, while allowing pectoral muscles to decrease below the size needed for flight. When their food supply (brine shrimp) becomes exhausted, grebes rebuild breast muscle while uniquely undergoing a major loss in body mass (fat depots, liver, stomach and intestine) *prior to* their nonstop migration (November-January) to wintering grounds (Jehl 1997). At wintering areas in the Salton Sea and Gulf of California (November-March), grebes feed upon aquatic invertebrates (pileworms, water boatmen, amphipods), and return to breeding grounds in spring (April-May).

Variation in the digestive organs of grebes during their annual cycle is impressive, with the liver exhibiting up to a sixfold range in mass (Jehl 1997). This fluctuation is significant in that hepatic elemental determination is commonly employed to assess environmental contaminant exposure. The interpretation of such data is often based on tacit assumptions that concentrations reflect local environmental contamination and are independent of organ mass. While these assumptions may sometimes be true, there are physiological reasons to doubt their validity in all cases. Free-ranging grebes present a unique opportunity to test these assumptions because the decrease in their liver mass occurs during a period of fasting, when grebes have not changed their environment but merely stopped feeding. Thus, we may ask: How does the relative concentration of an element in a fat hyperphagic bird change after several weeks of fasting? Do all elements respond in a similar manner to changes in liver mass, or are some sequestered while others are redistributed or rapidly excreted? Herein we report findings of an investigation describing concentrations of various liver elements of eared grebes collected at staging and wintering areas along the Pacific flyway when changes in body and organ mass are most extreme.

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MATERIALS AND METHODS

Adult eared grebes were collected in California by shotgun or timely salvage of carcasses of birds downed and traumatized by a storm while migrating from staging to wintering areas in 1992-93. Newly arrived migrants (late July-September) and staging birds (late October-November) were collected at Mono Lake (38°1' N, 119°0'W). Grebes that were killed during migration (January) were obtained at Iron Mountain (40°40' N, 122°31'W) and Big Bear (34°15' N, 116°50'W), while others were collected (January) at the Salton Sea (33°19' N, 115°48' W). Distribution of sexes among collection periods was approximately equal (60-77% males), and gender does not appear to affect the general pattern of body and organ mass change during these sample collection times (Jehl, 1997).

After collection, total body and liver mass were determined. The liver was wrapped in aluminum foil and frozen for subsequent analysis. Moisture content of the liver was determined by weight difference following drying (93°C) and desiccation. Concentrations of Al, B, Ba, Be, Cd, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Pb, Sn, Sr, V, and Zn were quantified by inductively coupled argon plasma spectrometry using a dry ash procedure (Haseltine et al. 1981). Recovery from spiked samples exceeded 85%, with the exception of B (67%) and Sn (69%). Samples were analyzed for As and Se using graphite furnace atomic absorption spectroscopy (recovery >90%) (Kryntisky 1987), and for Hg using cold vapor atomic absorption spectroscopy (recovery 101 %)(Monk 1961).

Body and liver mass, percent moisture, and elemental concentrations and total burdens (elemental concentration/g liver dry wt x total liver dry wt) were tested for homogeneity of variance using the F_{max} test. Elemental concentrations and total burdens were $\log_{10}$ transformed to stabilize variances. A value of one-half the lower limit of detection was assigned to samples with undetectable concentrations. Differences in these parameters among sample collection times were compared using analysis of variance and Tukey's HSD method of multiple comparison (Kirk, 1968).

RESULTS AND DISCUSSION

Dramatic differences in mean wet wt body mass (1.8-fold), liver mass (2.7-fold), and liver to body mass ratio (1.6-fold) were observed (Table 1) among newly arrived migrants, staging, and immediate post-migratory adult eared grebes. Among individual birds, body mass varied 2.5-fold, whereas liver mass varied up to 3.6-fold, and may even vary up to 6-fold (Jehl 1997). Moisture content of liver samples did not differ among collection periods ($p > 0.25$; range 55.8 to 69.3%).

Twelve elements were detected in more than half of the samples (Table 1). There were no differences in dry wt concentrations of B, Cd, Cr, Cu, Fe, Hg, Mg, Mn, and Ni among newly arrived migrants, staging, and immediate post-migratory grebes,

Table 1. Body and liver mass, and hepatic concentrations of elements in eared grebes[a]

	Newly Arrived Migrants (July-Sept.)	Staging (Oct.-Nov.)	Immediate Post-migration (January)
Body mass (g wet wt)	354[B] (280-414)	619[A] (565-687)	346[B] (294-406)
Liver mass (g wet wt)	12.9[B] (9.0-18.8)	29.9[A] (25.6-32.7)	10.9[B] (7.2-13.7)
Liver:body weight x 100 (g/100 g wet wt)	3.66[B] (2.16-5.06)	4.87[A] (3.91-5.78)	3.13[B] (2.44-3.63)
Aluminum (*u*g/g dry wt)	10.27[A] (4.07-26.86)	6.26[A,B] (1.54-10.98)	3.48[B] (1.30-8.96)
Boron (*u*g/g dry wt)	21.42 (2.71-56.61)	27.87 (17.11-46.12)	11.46 (1.43-40.18)
Cadmium (*u*g/g dry wt)	1.57 (0.83-2.33)	1.23 (0.77-2.17)	1.82 (0.78-4.09)
Chromium (*u*g/g dry wt)	4.66 (2.13-12.83)	7.81 (4.32-10.42)	5.07 (1.30-9.15)
Copper (*u*g/g dry wt)	12.28 (3.27-25.16)	8.60 (6.38-10.19)	9.15 (6.61-13.04)
Iron (*u*g/g dry wt)	1327 (1056-2127)	620 (243-951)	1272 (291-5299)
Mercury (*u*g/g dry wt)	6.47 (1.50-17.53)	5.07 (3.96-8.03)	10.70 (5.79-17.23)
Magnesium (*u*g/g dry wt)	559 (436-856)	542 (434-616)	598 (433-728)
Manganese (*u*g/g dry wt)	10.26 (4.90-15.53)	8.39 (6.91-10.70)	7.63 (4.33-11.94)
Nickel (*u*g/g dry wt)	2.81 (1.33-9.08)	4.49 (1.90-9.25)	4.34 (1.35-9.78)
Selenium (*u*g/g dry wt)	10.37[B] (6.42-22.09)	15.11[A,B] (9.03-25.06)	21.14[A] (12.26-35.65)
Zinc (*u*g/g dry wt)	82.4[B] (53.0-124.2)	76.2[B] (63.9-91.2)	122.4[A] (61.6-191.7)

[a]Body and liver mass are arithmetic means (range), and elemental concentrations are geometric mean (range). Means with similar capital letter superscripts are not significantly different by Tukey's HSD method of multiple comparison ($p < 0.05$).

despite marked changes in body and liver mass. Following the decline in organ mass, most of which is incurred before migration (Jehl 1997), Al values decreased, while Se and Zn concentration increased, in the immediate post-migration period.

Total liver burdens of elements tended to be greatest in staging grebes compared to other collection periods (Table 2), and paralleled changes in body and liver mass. Total burdens of Cu and Mn were lowest ($p < 0.05$) in immediate post-migratory

Table 2. Total hepatic burdens of elements in eared grebes[a]

	Newly Arrived Migrants (July-Sept.)	Staging (Oct.-Nov.)	Immediate Post-migration (January)
N	9	6	10
Aluminum	48.18^{A} (17.40-115.6)	65.62^{A} (16.32-108.0)	13.03^{B} (5.98-33.86)
Boron	100.5^{A,B} (11.59-281.2)	292.0^{A} (191.0-462.5)	42.95^{B} (5.29-166.8)
Cadmium	7.39 (4.66-11.76)	12.90 (7.72-20.75)	6.81 (2.15-18.88)
Chromium	22.65^{B} (7.70-81.74)	81.82^{A} (43.32-121.0)	19.00^{B} (5.97-42.24)
Copper	57.60^{B} (22.48-84.96)	90.10^{A} (79.56-99.60)	34.31^{C} (20.03-44.36)
Iron	6227 (3567-9880)	6492 (2584-9540)	4769 (1163-24466)
Mercury	30.34 (6.79-63.34)	53.18 (41.97-78.96)	40.09 (18.12-44.13)
Magnesium	2622^{B} (1868-4368)	5680^{A} (4984-6537)	2243^{B} (1503-2692)
Manganese	48.12^{B} (33.69-64.73)	87.89^{A} (69.30-105.2)	28.58^{C} (18.01-44.13)
Nickel	13.65^{B} (5.69-50.82)	47.02^{A} (19.05-98.03)	16.28^{B} (6.21-32.83)
Selenium	48.67^{C} (24.68-112.7)	158.32^{A} (95.07-266.2)	79.25^{B} (53.25-134.4)
Zinc	387.0^{B} (296.7-513.1)	797.9^{A} (752.2-871.1)	458.9^{B} (322.2-878.5)

[a]Values (µg/liver) are geometric mean (range). Means with similar capital letter superscripts are not significantly different by Tukey's HSD method of multiple comparison ($p < 0.05$).

grebes, and several other elements (Al, B, Cr, Mg) appeared to follow this trend. Liver burdens of Hg and Cd were relatively well conserved among newly arrived migrants, staging, and immediate post-migratory grebes.

In the present study, hepatic concentrations of elements known to be toxic to free-ranging birds (e.g., Cd, Hg, Pb, Se) were well below known effect thresholds. During the period between arrival and staging at Mono Lake, eared grebes undergo molt and an impressive increase in body and liver mass; yet, liver concentrations of several micronutrients and minerals (Al, Cu, Fe, Mg, Mn, Ni, Se, and Zn), and heavy metals (Cd and Hg), were relatively unaffected. Nevertheless, total liver burdens of most of these elements do increase in hyperphagic staging grebes (Table 2), presumably due to dietary uptake. Upon exhaustion of their food supply (Jehl 1988, 1997), grebes are forced to fast for several weeks, as evidenced by loss of body and liver mass. During this period (reflected in immediate post-migration samples), the hepatic concentration of Al, and total hepatic burdens of Al, B, Cr, Mg, Ni, Se, and Zn, decrease relative to values of staging birds. The loss of minerals and micronutrients, and the depuration of potentially toxic metals, are presumed to be a normal component of the annual cycle of eared grebes. The increase in Se and Zn concentrations, despite reduction in their total liver burdens (Table 1 versus 2), may reflect a redistribution or sequestering process associated with loss of body mass.

Although seasonal variation in hepatic metal content has been studied in several avian species (e.g., sparrow, *Passer domesticus,* Haarakangas et al. 1974; starlings, *Sturnus vulgaris,* Osborn 1979; blue-winged teal, *Anas discors,* Warren et al. 1990; guillemots, *Uria aalge,* Stewart et al. 1994) surprisingly few have concurrently examined liver mass, metal concentration, and total burdens. In one such study in starlings, whose liver mass fluctuates nearly twofold, it was concluded that total metal burden was of preferential diagnostic value to metal concentration for exposure assessments (Osborn 1979). As liver mass of adult eared grebes varies up to 6-fold during its annual cycle (Jehl 1997), greatly exceeding that of starlings, use of total burdens may be more prudent. However, for those species whose liver masses exhibit only modest seasonal variation (e.g., guillemots), it has been suggested that concentration changes equate changes in total burdens (Stewart et al. 1994). The need for temporally- and physiologically-matched reference birds, and at least knowledge of circannual organ mass fluctuations, appears to be a requirement for ecotoxicological exposure assessments in species such as the eared grebe. Furthermore, generation of additional avian toxicity data from controlled dosing studies during potentially vulnerable phases of their annual cycle (e.g., molt, post-migration) seems warranted.

Could changes in liver mass during the course of the annual cycle in eared grebes mobilize or concentrate metals to toxic levels ? It has long been recognized that nutritional deprivation, and other stressors, can cause mobilization, redistribution, and concentration of lipid soluble contaminants, that can ultimately result in mortality (e.g., DDT in cowbirds, *Molothrus ater,* Van Velzen et al. 1972; Furness

1993). Nutritional deprivation, or even emaciation, have not been causally-linked to metal intoxication in free-ranging adult birds, although mobilization and transfer of a small fraction of Hg body burden into the eggs of some species is well documented (reviewed by Furness 1993).

Although it is commonly accepted that hepatic metal concentrations are principally affected by contaminant exposure, we have shown differential alterations related to the stage of the annual cycle. Major changes in liver mass are not unusual in birds. Although contaminant exposure and disease states are often associated with liver enlargement or atrophy, significant changes can occur in healthy birds that have not moved from their immediate environment, but merely undergone fast or deposition of mass in preparation for migration (Raveling 1979; Thompson and Drobney 1996). Thus, proper interpretation of liver composition requires sufficient information on the normal biology and natural history of the species in question. Our findings may have broad significance for species that undergo dramatic variations in organ weight. The implications of these findings may also be useful in re-evaluating the role of contaminants in mortality events involving such species, such as the case in which 150,000 eared grebes (perhaps 7% of the North American population) died at their wintering grounds at the Salton Sea in 1991-1992 (Jehl 1996).

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REFERENCES

Furness RW (1993) Birds as monitors of pollutants. In: Furness RW and Greenwood JJD (eds) Birds as Monitors of Environmental Change, Chapman and Hall, London, pp 86-143

Haseltine SD, Heinz GH, Reichel WL, Moore JF (1981) Organochlorine and metal residues in eggs of waterfowl nesting on islands in Lake Michigan off Door County, Wisconsin. Pestic Monit J 15:90-97

Haarakangas H, Hyvarinen H, Ojanen M (1974) Seasonal variations and the effects of nesting and moulting on liver mineral content in the house sparrow *(Passer domesticus* L.). Comp Biochem Physiol 47A:153-163

Jehl Jr JR (1988) Biology of the eared grebe and Wilson’s phalarope in the nonbreeding season: a study of adaptation to saline lakes. Stud Avian Biol No.12

Jehl Jr JR (1996) Mass mortality of eared grebes in North America. J Field Ornithol 67:471-476

Jehl Jr JR (1997) Cyclical changes in body composition in the annual cycle and migration of the Eared Grebe *Podiceps nigricollis.* J Avian Biol 28: (In Press)

Kirk RE (1968) Experimental design: Procedures for the behavioral sciences. Brooks/Cole, Belmont, California

Krynitsky AJ (1987) Preparation of biological tissue for determination of arsenic and selenium by graphite furnace atomic absorption spectrometry. Anal Chem 59:1884-1886

Monk HE (1961) Recommended methods of analysis of pesticide residues in food stuffs. Reports by the Joint Mercury Residue Panel. Analyst 82:608-614

Osborn D (1979) Seasonal changes in the fat, protein and metal content of the liver of the starling *Sturnus vulgaris.* Environ Pollut 19:145-155

Raveling DG (1979) The annual cycle of body composition of Canada Geese with special reference to the control of migration. Auk 96:232-252

Stewart FM, Thompson DR, Fumess RW, Harrison N (1994) Seasonal variation in heavy metal levels in tissues of common guillemots, *Uria aalge* from northwest Scotland. Arch Environ Contam Toxicol 27:168-175

Storer RW, Jehl Jr JR (1985) Moult patterns and moult migration in the black-necked grebe *Podiceps nigricollis.* Ornis Scand 16:253-260

Thompson JE, Drobney RD (1996) Nutritional implications of molt in male canvasbacks: variation in nutrient reserves and digestive tract morphology. Condor 98:512-526

Warren RJ, Wallace BM, Bush, PB (1990) Trace elements in migrating blue-winged teal: Seasonal-, sex- and age-class variations. Environ Toxicol Chem 9:521-528

Van Velzen AC, Stiles WB, Stickel LF (1972) Lethal mobilization of DDT by cowbirds. J Wildl Manage 36:733-739