

# TISSUE COMPONENT AND ORGAN MASS CHANGES ASSOCIATED WITH DECLINES IN BODY MASS IN THREE SEABIRD SPECIES RECEIVED FOR REHABILITATION IN CALIFORNIA

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## SUMMARY

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Critical care for aquatic birds undergoing rehabilitation as a result of oil spills currently proceeds with minimal information about the nutritional status and needs of the affected animals, and lack of such information may substantially affect the survival of birds through the rehabilitation process and after their release. To objectively evaluate the nutritional condition of aquatic birds undergoing rehabilitation, we performed proximate analyses on carcasses of adult Common Murres *Uria aalge* (COMU), Western Grebes *Aechmophorus occidentalis* (WEGR) and Surf Scoters *Melanitta perspicillata* (SUSC) that died or were euthanized during rehabilitation. Carcasses were selected to capture the widest available range of body conditions. A regression analysis of protein, fat, ash mass and liver and leg muscle mass against total carcass mass examined changes related to presumed loss of body mass. WEGR and COMU were found to have a nadir of fat that illustrates the boundary between phase II and III starvation, at which fat stores have been exhausted. The bird mass at this boundary was approximately 900 g for male WEGR and 675 g for both female WEGR and COMU, which corresponds to 63.0%, 56.3% and 66.0% of their wild mean masses, respectively. For comparison purposes, the mean body mass of WEGR and COMU received for care during the *S.S. Jacob Luckenbach* oil spill were 70.9% and 70.5% of the wild mean mass, respectively. Protein mass showed a linear decline as carcass mass declined in all comparisons, and the slope of the relationship did not change at a critical point, as would be expected from species that conserve protein while mobilizing fat stores. This suggests the phases of starvation in these species, or in faunivorous birds in general, may vary from that in better-studied omnivorous species. This work shows that birds commonly affected by oil spills in California may not be healthy animals in need of washing, but a large proportion may be presented for care in extreme catabolic states. Rapid capture of oiled animals is advisable to initiate treatment before excessive tissue catabolism results in severe nutritional depletion.

Keywords: carcass composition, Common Murre, *Uria aalge*, oiled wildlife, seabird, starvation, Surf Scoter, *Melanitta perspicillata*, Western Grebe, *Aechmophorus occidentalis*

## INTRODUCTION

Wildlife response agencies may be presented with hundreds or even thousands of live-captured wild birds in need of care after petroleum products are discharged into the aquatic environment. Public concern for animal welfare provides a stimulus for oil companies and governmental agencies to provide ethical and humane care for oiled wild animals during such events, but published studies regarding the provision of optimal medical and rehabilitative care of typically affected species are scarce. The medical status of animals collected for rehabilitation depends on numerous factors, such as each individual's health status and life stage before exposure, the length of time from exposure to capture and initiation of care and bodily resources consumed during this interval, environmental conditions during and after the event and characteristics of the spilled product. Historically, oiling events in California have affected aquatic birds of the nearshore environment such as Common Murres *Uria aalge* (COMU), Western Grebes *Aechmophorus occidentalis* (WEGR) and Surf Scoters *Melanitta perspicillata* (SUSC). When collected for rehabilitation, these birds have typically exhibited hypothermia, emaciation, anemia, hypoproteinemia and chemical burns from exposure to caustic substances, among other medical problems (Mazet *et al.* 2002).

Treatment protocols have been based on the natural history of affected species, extrapolation from domestic species and the subjective and practical collective experiences of veterinary and rehabilitative caregivers (OWCN 2000). Several commonly oiled Californian aquatic species, such as Western, Clark's *A. clarkii*, Eared *Podiceps nigricollis* and Horned Grebes *P. auritus*, are rarely kept in captivity even when healthy because they are well known to be difficult to manage even for short periods (Stoskopf 2003).

Contamination of plumage may result in an immediate loss of its functional attributes, requiring increases in energy expenditure to compensate for impaired thermoregulation, buoyancy and hydrodynamics. Plumage-contaminated birds may also experience inflammatory responses to contaminants and an impaired ability to swim and fly at the same time they may need to travel long distances to get out of the water to avoid drowning. The metabolic rates of Common Eiders *Somateria mollissima* increased up to 400% when these birds were oiled and forced to swim in cold water (Jennsen & Ecker 1991). In Mallards *Anas platyrhynchos*, colder environmental conditions reduced the amount of body mass loss tolerated before death (Jordan 1953). This study also showed that Mallards deprived of food died at approximately 55%–60% of their normal body mass when ambient temperatures were cold

(-1.2 °C [29.8°F]) but at 45%–50% of their normal body mass when ambient temperatures were warm (21.9 °C [71.5°F]). Sea surface temperatures range annually from 10 °C to 15.6 °C (50°F to 60°F) in the northern part of California to 12.8 °C to 21.1 °C (55°F to 70°F) in the southern part of the state (NOAA 2013). However, even without thermoregulatory challenges or extreme energetic expenditures necessary to avoid drowning, many avian species lose body mass quickly simply because of food deprivation. American Kestrels *Falco sparverius* that fasted for 72 h lost 16.8%–19.6% of their body mass while inactive, under thermoneutral conditions and presumably normally hydrated (Shapiro & Weathers 1981); Herring Gull *Larus argentatus* chicks that fasted for four days lost 18% of their body mass (Jeffrey *et al.* 1985); and young COMU housed in a vivarium lost 21.5% of their body mass during a three-day fast (Herzberg *et al.* 1988).

Starvation is generally considered to have three phases. Phase I encompasses the time span from when the last meal has been absorbed until glycogen stores are exhausted. This phase may be extremely brief in birds, as glycogen stores are typically small relative to those in mammals. During phase II, the animal catabolizes primarily lipid stores for energy while conserving proteins. Phase III commences once adipose stores have been exhausted and the animal must catabolize proteins, which results in loss of organ function and eventually, death (Wang *et al.* 2006). Carnivorous birds maintain blood glucose through obligate gluconeogenesis primarily from amino acid precursors, either from food or from skeletal muscle during fasting. Thus, in carnivorous birds, blood glucose is correspondingly unaffected by fasting (Migliorini *et al.* 1973, Myers & Klasing 1999). The length of time a bird remains in each stage of increasingly severe nutritional depletion depends on its initial energy stores and metabolic demands, ranging from several months in penguin chicks to only a few days in species with small fat stores (Klasing 1999). Marine and aquatic birds generally have prominent energy stores in subcutaneous and mesenteric fat deposits (Jeffrey *et al.* 1985). Eared Grebes were found to have up to 47% of dry carcass mass as lipid (Jehl 1997). It is presumed that, as with other species of birds, seabirds under nutritional stress preferentially use lipid energy reserves rather than skeletal muscle (Jacobs *et al.* 2011), but changes in body composition associated with acute, extreme catabolism, such as that following plumage contamination, are largely unknown. As a consequence of elevated energetic expenditures and lack of food intake, birds oiled at sea and unable to forage may be expected to lose mass rapidly, and may catabolize enough mass to become extremely emaciated relatively quickly after oiling. Previous work has shown that the survival of oiled Common Murres through rehabilitation is closely correlated to their body mass at admission, and birds with the lowest body mass fare the worst (Duerr 2008).

With few exceptions, marine avian species in California are obligate faunivores (i.e. carnivores, piscivores, zooplanktonivores, molluscivores, etc.). Nutritional support is a cornerstone of therapy for animals in critical care for petroleum product exposure, but nutritional literature on faunivorous birds is sparse, and studies have primarily used healthy raptors (Migliorini *et al.* 1973, Shapiro & Weathers 1981, Myers & Klasing 1999). It is known that the nutritional needs of dogs and cats change when in a debilitated state (Delaney *et al.* 2006, Goy-Thollot & Elliott 2008). Hence, it is reasonable to expect that the debilitated states of faunivorous birds admitted for care during oil spills may pose a distinct challenge to successful rehabilitation. Feeding affected animals as though they

are healthy wild animals may be suboptimal. In mammals, severe catabolism may change nutritional requirements from what the animal would normally obtain from foods ordinarily eaten when healthy. For example, emaciated mammals may experience a life-threatening metabolic disturbance known as “refeeding syndrome” due to compartmental shifts of electrolytes when normal foods are reintroduced (Remillard *et al.* 2000). It is not known whether this or a similar syndrome occurs in birds during recovery from extreme catabolic states. In some avian species, the intestine and related digestive organs atrophy during fasting to a greater degree than the body as a whole, and recovery to normalized digestive function and food intake after a significant episode of fasting requires several days (Lee *et al.* 2002, Karasov *et al.* 2004). Gastrointestinal atrophy in oiled COMU was found to be reversible during refeeding over 24 h–72 h (Gieger *et al.* 2009).

In an effort to increase our knowledge regarding the medical and nutritional condition of species commonly admitted for rehabilitative care during oil spills in California, this study had the following primary goals: 1) to evaluate changes in protein, fat, and ash mass associated with declines in body mass in commonly oiled species, 2) to identify the body mass indicative of the transition to phase III starvation and 3) to compare the mass of the chemically analyzed carcasses to both published values of healthy non-oiled wild conspecifics as well as to the body mass of conspecifics admitted for care during the *S. S. Jacob Luckenbach* (hereafter, SSJL) oil spill in 2001/2002. (The SSJL was a ship that sank in the Gulf of the Farallones off San Francisco, CA, in 1953. It was the source of numerous “mystery spills” until a salvage operation removed much of the remaining oil in 2002.) Secondary goals included: 4) examination of relative changes in liver and leg muscle mass associated with declines in body mass, and 5) examination of relative changes in liver mass associated with presence of inflammatory lesions.

## METHODS

### Proximate analysis

Non-oiled animals received for routine rehabilitation during 2009 and 2010 were used in this study as proxies for oiled conspecifics in the tissue component analyses because oiled animals may be needed for evidence in criminal proceedings against perpetrators and because the chaotic environment of wildlife care centers during oil spills makes detailed data collection improbable. Carcasses of adult COMU, WEGR and SUSC admitted alive for rehabilitation at the San Francisco Bay Oiled Wildlife Care and Education Center, managed by International Bird Rescue, were frozen following death or euthanasia. These species were selected because of their taxonomic diversity and frequency of admittance into rehabilitation, and because they were the species most affected by the 2007 *M/V Cosco Busan* oil spill in San Francisco Bay. Carcasses for compositional analysis were selected opportunistically to obtain samples of a wide range of carcass conditions. Low numbers of SUSC entering rehabilitation during the time span of carcass collection resulted in fewer carcasses of this species available for analysis. These three species typically enter rehabilitation because of fishing hook or line injuries, boat strikes, predator attacks, starvation, adverse weather events, heavy parasite loads, plumage damage or contamination.

Carcasses were frozen at -28.9 °C until processing, then were scalded in 82 °C water for 2 min, plucked, blotted dry and allowed

to thaw briefly at room temperature until malleable enough for necropsy. Carcass mass was recorded before freezing, before processing and after plucking. The following morphometric data were collected: exposed culmen (CUL), skull length (SKU), bill length from distal margin of nare to tip (DNT), bill width at distal end of nare (DNW), bill depth at distal end of nare (DND), bill depth at proximal end of nare (PND), bill circumference at distal end of nares (DNC), elbow to carpus length (ELW), tarsometatarsus length (TMTL), tarsometatarsus circumference midshaft (TMTC) and length of right digit #3 from dorsal crease at foot fold to base of nail (TOE) (see Duerr 2013 for full discussion of morphometric results). Full necropsies were performed, with all lesions described in standard gross necropsy reports. Carcasses were categorized dichotomously as being with or without gross evidence of inflammatory lesions on necropsy to determine whether death was related to apparent simple starvation (i.e. no lesions) or to a more complex disease process (e.g. fungal plaques, granulomatous lesions, tissue necrosis). Sex and gonad measures were recorded for each bird. Age was assessed through plumage characteristics, the presence/absence of a bursa of Fabricius and development of the supra-orbital ridge in COMU (Nevins & Carter 2003). The liver and *cranial tibial* and *extensor digitus longis* muscles were finely dissected, weighed and collected separately. These muscles were selected because their proximal and distal tendons facilitate quantitative removal. The gastrointestinal (GI) tract was removed from the body, and the contents were manually expressed and weighed. For grebes, the ventricular feather balls were collected and weighed separately. Carcasses were manually cut into small pieces and ground to coarse consistency using a meat grinder. Samples were weighed, freeze-dried and re-weighed to assess the percentage of dry

matter. Livers and leg muscles were processed in the same manner as the rest of the carcass. The two leg muscles were combined before analysis due to small sample sizes.

Freeze-dried subsamples were further ground through a 1 mm mesh screen. Aliquots were removed for subsequent proximate analysis (Analytical Laboratory, College of Agricultural and Environmental Sciences, University of California, Davis). Nitrogen was measured by standard methods (AOAC International 2006b) and multiplied by 6.25 to estimate crude protein. Crude fat was measured by Randall modification of the standard Soxhlet ether extraction (AOAC International 2006a). Ash content was obtained by heating at 550 °C for 3 h to burn off organic materials (AOAC International 2005). Proximate analysis values were used to calculate grams of protein, fat and ash mass for each tissue sampled (liver, muscles, rest of carcass), and values were summed to acquire mass values for each whole carcass. Carcasses with lesions suspicious for potentially zoonotic pathogens were discarded. Full composition data were obtained for 23 male WEGR, 19 female WEGR, 31 COMU (sexes not differentiated), 7 male SUSC and 1 female SUSC. “N” values greater than these include data from discarded carcasses.

COMU males and females were considered together because this species is not sexually dimorphic; WEGR and SUSC males and females were assessed separately as they are sexually dimorphic (Pyle 2008). The initial carcass mass of birds was standardized as mass of each carcass when removed from the freezer minus GI contents in order to approximate an unfed state. WEGRs normally maintain a feather ball within the ventriculus; hence, feather ball mass was included in grebe mass.

**TABLE 1**  
**Carcass mass of COMU, WEGR and SUSC and comparison values from birds oiled during the S.S. *Jacob Luckenbach* oil spill and wild conspecifics**

Species	Adult carcass mass, <sup>a</sup> g								
	Study carcasses			<i>S.S. Jacob Luckenbach</i>			Wild <sup>b</sup>		
	n	Mean ± SD (% wild mean ± SD)	Median (range)	n	Mean ± SD (% wild mean ± SD)	Median (range)	n	Mean ± SD (SE)	Median (range)
COMU	31	722 ± 124.0 (70.6 ± 12.1)	700 (532–959)	475	720 ± 76.2 (70.5 ± 7.5)	718 (520–1 084)	40	1 022 ± 111	– (805–1 175)
WEGR males	23	1 020 ± 213.1 (71.4 ± 14.9)	962 (693–1423)	–	–	–	41	1 429 ± 163	– (1 137–1 826)
WEGR females	19	860 ± 218.4 (71.7 ± 18.2)	850 (492–1157)	–	–	–	21	1 199 ± 209	– (808–1 753)
WEGR all	42	948 ± 227.6 (71.5 ± 17.2)	956 (492–1423)	59	939 ± 143.9 (70.9 ± 10.9)	930 (588–1 206)	62	1 325 ± 184 <sup>c</sup>	– (808–1 826)
SUSC males	7	804 ± 88.4 (70.0 ± 7.7)	831 (642–890)	–	–	–	221	1 148 ± (7)	–
SUSC females	1	780	780	–	–	–	21	1 047 ± (22)	–
		–	–		–	–			–

<sup>a</sup> Study carcass mass without gastrointestinal contents mass but with grebe feather ball mass. The SSJL data reflect live birds entering care in a presumably unfed state. Wild mass includes unknown mass of ingesta in wild-caught individuals.

<sup>b</sup> Wild reference data: COMU (Newman 1998); WEGR (Storer & Nuechterlein 1992); SUSC (Savard *et al.* 1998).

<sup>c</sup> Value extrapolated from wild mass by sex ratio of study carcasses.

Medical records housed at the Wildlife Health Center at University of California, Davis, of 457 after-hatch-year (AHY) COMU and 59 AHY WEGR oiled by the 2001/2002 SSJL oil spill were examined to determine the admission body mass of actual oiled conspecifics admitted for care in California.

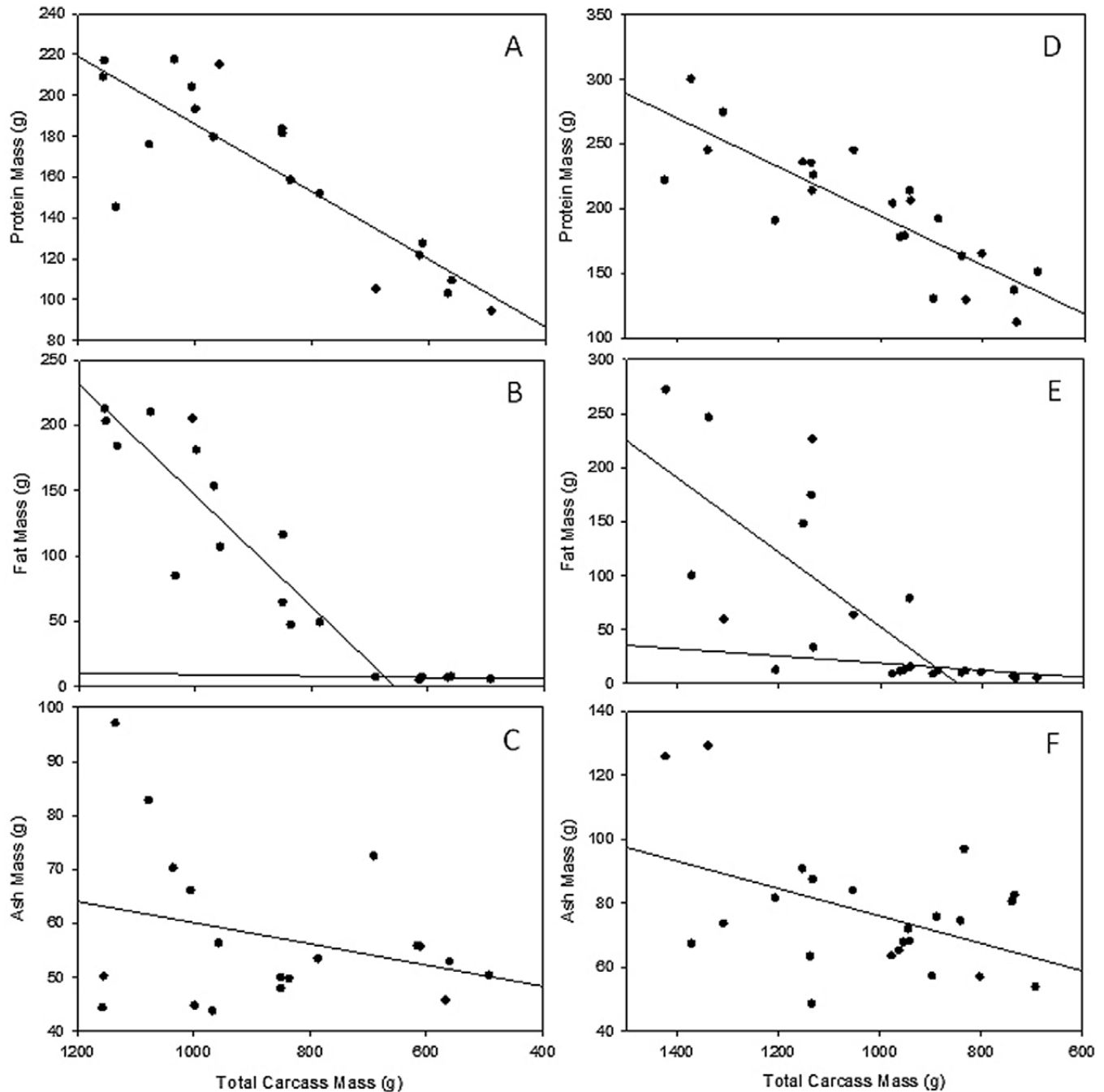
### Data analysis

Descriptive statistics (including mean, SD, median and range) were computed using Microsoft Excel 2010 (14.0.6126.5003) (Microsoft Corporation, Redmond, WA, USA). Ordinary least squares regressions were used to compare protein, fat and ash mass, liver and muscle

mass, and whole carcass mass. Normality assessment with Shapiro-Wilk tests, Student's *t*-tests and one-way ANOVAs comparing amino acid content against dichotomous or ordinal data were performed using JMP version 10 statistical software (JMP, Cary, NC, USA).

### RESULTS

Table 1 displays the mean, median, and range of mass from the study carcasses, and includes comparison admission mass values of conspecifics affected by the SSJL oil spill and previously published mean mass of wild birds. Masses of COMU, male and female WEGR and male SUCS were normally distributed.



**Fig. 1.** Changes in tissue components protein, fat and ash with decreasing carcass mass in adult female (A-C) and male (D-F) Western Grebes (WEGR). WEGR females:  $n = 19$ , A and B ( $P < 0.001$ ), C ( $P = 0.212$ ); WEGR males:  $n = 23$ , C and D ( $P < 0.001$ ), E ( $P = 0.029$ ). Note that the x-axis on each graph has been reversed to illustrate declining body mass.



Liver mass was positively related to whole carcass mass in male WEGR ( $n = 29$ ,  $P < 0.001$ ,  $R^2 = 0.646$ ,  $y = 0.0714x - 25.137$ ), female WEGR ( $n = 20$ ,  $P < 0.001$ ,  $R^2 = 0.838$ ,  $y = 0.089x - 32.097$ ) and COMU ( $n = 43$ ,  $P < 0.001$ ,  $R^2 = 0.409$ ,  $y = 0.0764x - 23.457$ ), but it was not significantly different in male SUSC ( $n = 11$ ,  $P = 0.662$ ). Liver mass also increased as a fraction of whole carcass mass in male WEGR ( $n = 29$ ,  $P = 0.035$ ,  $R^2 = 0.154$ ,  $y = 2.299e^{-5}x + 0.0224$ ), female WEGR ( $n = 20$ ,  $P < 0.001$ ,  $R^2 = 0.605$ ,  $y = 5.065e^{-5}x + 0.00519$ ) and COMU ( $n = 43$ ,  $P = 0.025$ ,  $R^2 = 0.123$ ,  $y = 4.596e^{-5}x + 0.00987$ ), but not in SUSC ( $P = 0.329$ ). Gross evidence of tissue inflammation was identified in 11 of 42 (26.2%) COMU, 13 of 29 (44.8%) male WEGR, eight

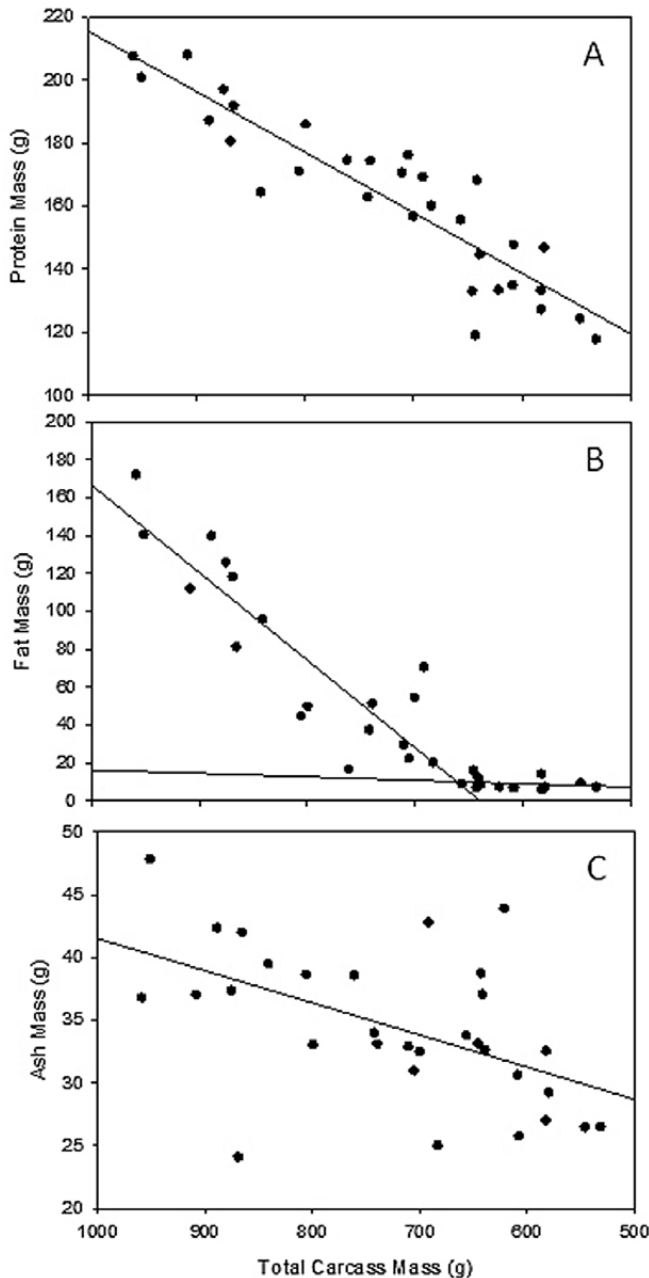
of 20 (40.0%) female WEGR, five of 11 (45.4%) male SUSC and one of two (50%) female SUSC carcasses. Liver mass of birds with gross evidence of tissue inflammation was not significantly different from those without such evidence in COMU ( $P = 0.930$ ), male SUSC ( $P = 0.304$ ) or female WEGR ( $P = 0.606$ ). Male WEGR livers were significantly heavier ( $P = 0.028$ ) in birds with gross inflammation ( $5.0 \pm 1.0\%$  of carcass mass) than in birds without ( $4.1 \pm 1.1\%$ ). The gonads of all carcasses were in an inactive reproductive state.

*Cranial tibial* and *extensor digitus longis* combined muscle mass varied positively with overall carcass mass in male WEGR ( $n = 29$ ,  $P < 0.001$ ,  $R^2 = 0.562$ ,  $y = 7.77e^{-3} + 0.593$ ), female WEGR ( $n = 21$ ,  $P < 0.001$ ,  $R^2 = 0.821$ ,  $y = 7.51e^{-3} + 0.442$ ) and COMU ( $n = 43$ ,  $P < 0.001$ ,  $R^2 = 0.630$ ,  $y = 4.07e^{-3} - 0.932$ ). When considered as a fraction of total carcass mass, male WEGR muscles did not differ significantly from females by *t*-test ( $P = 0.450$ ), and these muscles averaged  $0.8 \pm 0.1\%$  ( $n = 50$ ) of carcass mass throughout the range of carcass masses examined. The muscle mass of male SUSC, expressed as a percentage of carcass mass, increased as carcass mass decreased ( $n = 11$ ,  $P = 0.022$ ). In male SUSC, muscles averaged  $0.4\% \pm 0.09\%$  of the carcass mass. Conversely, the muscle mass of COMU, expressed as percentage of carcass mass, tended ( $P = 0.051$ ) to decrease as carcass mass decreased, but this relationship was not significant. In COMU, these muscles represented  $0.28\% \pm 0.05\%$  of total carcass mass.

#### Proximate analysis

Figure 1 shows the relationships between total tissue protein, fat and ash mass and carcass mass in adult male and female WEGR; Figure 2 shows these relationships in COMU. The protein and fat masses were highly correlated with declining carcass mass in male WEGR ( $n = 23$ , protein:  $P < 0.001$ ,  $R^2 = 0.704$ ,  $y = 0.19x + 3.728$ ; fat:  $P < 0.001$ ,  $R^2 = 0.523$ ,  $y = 0.291x - 230.219$ ), female WEGR ( $n = 19$ , protein:  $P < 0.001$ ,  $R^2 = 0.726$ ,  $y = 0.166x + 20.221$ ; fat:  $P < 0.001$ ,  $R^2 = 0.846$ ,  $y = 0.347x - 200.712$ ) and COMU ( $n = 31$ , protein:  $P < 0.001$ ,  $R^2 = 0.827$ ,  $y = 0.192x + 23.555$ ; fat:  $P < 0.001$ ,  $R^2 = 0.831$ ,  $y = 0.366x - 215.0$ ). Although male SUSC showed similar trends in tissue component changes with declining carcass mass, none was significant ( $n = 7$ , protein: median = 185.7 g, range 146.6–218.2 g; fat: median = 40.4 g, range 8.9–85.8 g; ash: median = 43.6 g, range 27.6–55.5 g). Data were available for only one female SUSC: carcass mass = 780 g, protein = 160.1 g, fat = 51.5 g and ash = 28.8 g.

The above equations for fat describe the linear relationships, but as fat declined with declining total mass, in every case it appeared to reach a nadir with a slope near zero, as seen in Figures 1B, 1E and 2B. Fat mass values were divided into two series based on visual inspection of the graphed data points, and the carcass mass at the intersection of the slopes was chosen as the nadir below which birds likely lack fat reserves and enter phase III starvation. Data limitations precluded the identification of this nadir of fat mass for male SUSC with a significant regression line because of the small number of carcasses. However, according to the non-significant regression line ( $P = 0.243$ ,  $R^2 = 0.260$ ,  $y = 0.134x - 66.931$ ), these birds would reach 10 g of fat mass by the time they weighed 574 g, or 50% of wild mean mass. The ash mass also decreased significantly with declines in carcass mass in male WEGR ( $P = 0.029$ ,  $R^2 = 0.207$ ,  $y = 0.043x + 33.094$ ) and COMU ( $P = 0.002$ ,  $R^2 = 0.277$ ,  $y = 0.026x + 15.869$ ), but it did not



**Fig. 2.** Changes in tissue components protein, fat and ash with decreasing carcass mass in adult Common Murres admitted for rehabilitation in California. A and B ( $P < 0.001$ ), C ( $P = 0.002$ ),  $n = 31$ . Note the x-axis on each graph has been reversed to illustrate declining body mass.

decrease significantly in female WEGR ( $P = 0.212$ ), although this relationship was significant in terms of percentage of dry matter ( $P < 0.001$ ,  $R^2 = 0.655$ ,  $y = -3.23e^{-4}x + 0.486$ ). When considered as a percentage of dry matter, both protein and ash increased markedly relative to fat as overall mass declined, reflecting the loss of fat. With lower amounts of fat, the relative proportion of water increased, and birds with the least fat contained as high as 73% water, while birds with larger fat deposits had as low as 56% water.

The ash mass did not have a significant relationship to any morphometric measurements in male or female WEGR (male: lowest  $P = 0.149$  for TMTC; females: lowest  $P = 0.182$  for DNC). In COMU, there was a positive relationship between ash mass and culmen ( $P = 0.026$ ,  $R^2 = 0.159$ ) as well as skull length ( $P = 0.032$ ,  $R^2 = 0.154$ ). Male SUSC showed significant relationships between ash mass and DNW ( $P = 0.011$ ,  $R^2 = 0.615$ ), protein mass and SKU ( $P = 0.006$ ,  $R^2 = 0.804$ ) and fat mass and ELW ( $P = 0.009$ ,  $R^2 = 0.770$ ). Data relating morphometric measurements to protein and fat mass for COMU and WEGR are presented and discussed in detail by Duerr (2013).

## DISCUSSION

The carcasses used for the composition analysis generally captured the range of body conditions admitted during the SSJL oil spill in 2001/2002. However, the SSJL birds were oiled at the time of weighing, and it is not known how much each of the bird's oil burden contributed to its mass. Therefore, the carcasses selected for compositional analysis may have overestimated the mass of actual oiled birds admitted for care. Both study carcasses and SSJL admission mass data show that birds admitted for rehabilitation commonly have much lower mass than healthy wild conspecifics — approximately 70% of wild mean mass. Although it is possible the carcasses selected for study were structurally smaller than wild conspecifics captured for referenced studies, these data show clear declines in fat and protein mass as well as in total mass, indicating that the selected carcasses were not smaller than conspecifics. Morphometric assessment of these carcasses (Duerr 2013) also does not support a structural size difference from healthy wild birds.

As overall carcass mass declined, fat decreased to a minimal level of approximately 5–10 g/bird, which may represent non-mobilizable phospholipids in cell membranes, while protein continued to decline linearly after fat reached its nadir. These losses in protein and fat may illustrate the division between metabolic phases II and III of starvation. These data indicate that the average bird entering care may be in late phase II starvation and that a substantial proportion of these birds would be in phase III, when adipose stores have been exhausted and proteins are catabolized for energy. It also implies that up to 50% of individual birds of these species entering care during California oil spills may be severely nutritionally depleted. However, in other species, such as rats (Hill & DiGirolamo 1991), body protein is usually conserved during fat mobilization in starvation. If this were the case with these bird species, one might expect to see a shallower slope of protein decline during fat mobilization, which steepens when fat stores are depleted. Instead, our data show that, as carcass mass declines, protein mass declines linearly. Apparently, these species catabolize proteins regardless of fat mass, rather than sparing proteins until fat mass is depleted, indicating that birds in phase II starvation may also experience diminished organ function due to declining protein mass. Our data also suggest that the phases of starvation applied to

omnivorous animals may not be appropriate for these species or to faunivores in general.

The critical thresholds below which WEGR and COMU have depleted fat stores were estimated to be 900 g for male WEGR and 675 g for both female WEGR and both sexes of COMU. These values are 63.0%, 56.3% and 66.0% of the mean wild mass, respectively; the low end of these percentages is comparable to the percentage of mass loss at which Mallards starved to death in the Jordan (1953) study. Presumably, there is a lower limit to loss of protein beyond which declines in organ function result in death. This could explain a portion of the mortality seen early in care when oiled wildlife treatment is undertaken and illustrates the importance of prompt capture of affected birds and initiation of nutritional support before severe tissue catabolism has occurred.

COMU showed significant relationships of two cranial morphometric measurements to ash mass. This suggests that larger COMU contain more mineral ash, which would be expected. However, the WEGR did not show similar relationships to any morphometric measurements evaluated, and male WEGR in particular displayed a distinct decline in mineral mass with declines in carcass mass. There are several possible physiological mechanisms for this mineral mass decline in the absence of, or in addition to, a size-related variation in ash mass. In chickens, the induction of an acute-phase response with the injection of lipopolysaccharides resulted in a loss of calcium and in a decline in tibial breaking strength (Mireles *et al.* 2005). In mammals, responses to inflammation can stimulate loss of minerals from bone (Roux & Orsel 2000), mediated by pro-inflammatory cytokines. In humans, mineral loss and loss of bone strength occur with many pathologic conditions, including starvation, when bone marrow may be replaced with fat despite the fact that fat loss may be occurring in the rest of the body. Several mechanisms have been proposed to explain mineral loss due to starvation, including a reduction in mechanical load bearing, which would lead to mass loss and stimulate a reduction in skeletal mass, or a need to maintain blood levels of minerals liberated from bone tissue (Devlin 2011).

Liver mass was found to be positively related to carcass mass but represented a decreasing fraction of total body mass as body mass declined, which indicates that the liver may be catabolized to a greater extent than other tissues during starvation. This is understandable, given that the liver is known to be an organ with a large functional reserve and the ability to quickly regenerate mass (Myers & McGavin 2007). Liver mass declined to a greater extent than other tissues during starvation in rats (Wagenmakers *et al.* 1984). Catabolic atrophy of the liver during starvation may fuel the maintenance of other tissues, with later reversal of atrophic changes during recovery. Significantly higher liver mass in male WEGR with inflammatory lesions may illustrate another physiological mechanism that drives changes in liver mass: an increased need for liver products such as acute-phase proteins during an inflammatory response may result in increased liver mass (Xie *et al.* 2000, Mireles *et al.* 2005). In our composition study, the effect of inflammatory states on liver mass may have been underestimated, because carcasses with the most severe pathologic abnormalities were rejected to avoid aerosolizing potential zoonotic pathogens. This was the case especially for COMU, in which overt (albeit presumptive) aspergillosis was a common reason for carcass rejection.

In order to forage, each of the species in this study must be able to dive to capture prey. WEGR swim exclusively with the legs,

SUSC swim with both wings and legs and COMU swim with wings only. Leg muscle mass in relation to whole carcass mass may reflect how each species uses its legs for locomotion. Leg muscles accounted for 0.8%, 0.4% and 0.3% of carcass mass, respectively, which corresponds to the relative use of leg muscles during food acquisition. In WEGR, leg muscle mass was maintained in proportion to carcass mass; in male SUSC, fractional leg muscle mass increased as carcass mass declined; while in COMU, leg muscle mass tended to decrease to a greater extent than the whole carcass. This suggests that, during starvation, COMU may catabolize skeletal muscle from legs more than from other muscles, possibly because they are not required for swimming, while male SUSC may preferentially maintain these tissues while others are catabolized. There was no evidence in any species of a threshold where the slope of the relationship between muscle mass and carcass mass changed, as might be expected if skeletal muscle were conserved during mobilization of fat mass.

In conclusion, this study showed that a substantial portion of aquatic birds admitted for rehabilitative care may be near the lower limits of body catabolism at which life persists. Consequently, caregivers should be aware that a large proportion of the animals entering care may be in an extreme state of nutritional depletion, a state that is incompatible with normal organ function. Adult male WEGR under 900 g, female WEGR or COMU under 675 g and male SUSC under 574 g should be considered to be in phase III starvation. Animal care protocols must account for this health status because extreme catabolic states may pose as great a risk to the survival of the animal as exposure to a contaminant. Additional studies of tissue dynamics during starvation and the care of oil-exposed animals are warranted, as increased knowledge may lead to nutritional interventions to improve successful treatment via rapid reversal of catabolic states. Our findings also suggest that rapid capture of oil-affected animals before excessive losses in body mass is advisable, especially in cold environments where energetic expenditures are likely to be extreme once birds become contaminated.

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