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An experimental soft-release of oil-spill rehabilitated American coots (*Fulica americana*): II. Effects on health and blood parameters

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"Capsule": *Blood analysis, coupled with post-release survival data, may help explain increased mortality of oiled and rehabilitated birds.*

Abstract

The Unocal–Metrolink oil spill of 21 February 1995 resulted in approximately 7800 barrels of San Joaquin crude oil being deposited into the San Gabriel River in Huntington Beach, CA, USA. In order to determine long-term pathological effects of oil exposure and rehabilitation, hematological and serum biochemical parameters for both rehabilitated (RHB) American coots (*Fulica americana*) and reference (REF) coots were examined every 3–4 weeks (56, 81, 108 and 140 days post oil exposure) after birds were cleaned, rehabilitated and soft-released. Most significant differences in monthly comparisons between RHB and REF birds occurred 56 days following oil exposure. Total white blood cell (WBC) count, albumin:globulin (A:G) ratio and calcium concentration were higher in RHB birds compared to REF birds 56 days post oil exposure. In addition, mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase and creatine kinase activities, and creatinine, total protein (TP) and globulin concentrations were lower in RHB birds. Blood results from 56 days post oil exposure for RHB coots which subsequently died were compared to blood results from days 108 and 140 for REF coots which survived. Oiled and rehabilitated birds which died had significantly higher WBCs, packed cell volume, TP and globulin concentrations, and lower A:G ratio, MCH, MCHC, glucose and sodium concentrations compared to REF birds which survived. Blood result differences detected at 3–4-week intervals between RHB and REF survivors, and differences detected between RHB coots which died and REF coots which survived, suggested that RHB coots developed an inflammatory response (infectious or non-septic) and, concurrently, may have experienced decreased immune responsiveness. Additionally, RHB coots experienced either an iron (Fe) utilization or Fe metabolism problem. These pathophysiological mechanisms were consistent with increased hemosiderin (stored Fe) present in the liver, spleen and kidney of necropsied RHB birds, and may have contributed to RHB coot mortality. When blood parameter differences were examined for their impact on survival time, it was determined that RHB coots had shorter survival times if they had very high cholesterol ($\geqslant$449 mg/dl) or chloride ($\geqslant$110 MEQ/l) concentrations on day 56 post oil exposure. Interestingly, the lack of differences between RHB and REF coots from day 81 through day 140 suggested that, from a hematologic and clinical chemistry perspective, coots which were oiled, rehabilitated, released and survived at least 3.5 months could not be differentiated from wild (REF) coots. From these findings it appears that blood analysis, coupled with post-release survival data, may help discern reasons for increased mortality of oiled and rehabilitated birds, compared to non-oiled reference birds.

Keywords: Oil spill; Rehabilitation; Post-release survival; Blood parameters; Hematology; Serum biochemistry; American coots, *Fulica americana*

1. Introduction

Oil spills pose a significant threat to marine wildlife worldwide (King et al., 1979; Piatt and Lensink, 1989; Piatt et al., 1990; Leighton, 1991; Folsom, 1994: Jessup

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and Leighton, 1996). As more experience is gained by responding to oil spills, it is apparent that there are differing species susceptibilities to petroleum toxicosis and impacts of rehabilitation. Other variables which likely influence survivorship of oiled and rehabilitated birds include petroleum type, environmental factors associated with the geographical locations of spills, the season, the pre-existing condition of birds, rapidity with which oiled birds are recovered from the wild, facilities and trained personnel available to care for oiled wildlife and available biomedical techniques. To further assess the efficacy of oiled wildlife care, post-release survival studies are also needed for a variety of aquatic bird species and response situations.

Plasma or serum biochemical analyses provide information about internal organs (liver, kidney), electrolytes [sodium (Na), chloride (Cl), potassium (K), calcium (Ca), phosphorus (P)], proteins (globulins, albumin) and nutritional or metabolic parameters (cholesterol, triglycerides, glucose) (Franson et al., 1985; Jain, 1986; Allen, 1988; Duncan et al., 1994). Hematological analyses, which include red blood cell (RBC) counts, white blood cell (WBC) counts and differential WBC counts, provide information about the hematopoietic system and immunological response. These blood tests can serve as diagnostic adjuncts in the development of a presumptive or definitive diagnosis (Duncan et al., 1994; Campbell, 1995). With the establishment of reference values for blood parameters for a variety of marine bird species (Kocan, 1972; Balasch et al., 1974; Bradley and Threlfall, 1974; Wolf et al., 1985; Melrose and Nicol, 1992; Rosa et al., 1993; Work, 1996; Newman, 1998; Newman et al., 1997), it is now possible to better monitor the health status of oiled birds upon intake, prior to cleaning, and prior to release (Newman, 1995).

To date, there have been relatively few post-release survival studies, especially compared with the number of large-scale oil spills that have occurred. These studies have been performed using different techniques, including band or ring returns, and radio-telemetry techniques.

In most cases, different petroleum products were spilled, different environmental conditions existed, different species were impacted and different quality rehabilitation was provided. These variables may have contributed to dissimilar survival rates (15–100%) for different species (Underhill et al., 1994; Williams, 1994; Sharp, 1996; Harris and Wanless, 1997; Kees et al., 1997; Wernham et al., 1997; Golightly et al., 1999). Furthermore, some avian species never returned to breeding colonies or demonstrated atypical breeding behavior (Anderson et al., 1996), while other studies documented successful breeding and chick fledging (Morant et al., 1981; Crawford et al., 1998; Goldsworthy et al., 1998). Although it is acknowledged that oiled wildlife care does not result in survivability comparable to that of non-oiled, non-rehabilitated marine birds, reasons for higher mortality have yet to be determined. Recovery of dead, radio-tagged individuals has proven challenging, and necropsy results of animals dead for more than a few days are often non-diagnostic.

In this study, we used blood parameters as health indicators to evaluate long-term impacts of oil exposure and rehabilitation on American coots (*Fulica americana*, hereafter referred to as coots) in a soft-release situation. We hypothesized that: (1) oiled and rehabilitated (RHB) coots would have lower survival than non-oiled reference (REF) coots; (2) RHB and REF coots would have different blood results at 3–4-week intervals following oil exposure and rehabilitation; and (3) RHB and REF coots which eventually died, and RHB and REF coots which survived would have different blood results. Additionally, we expected that blood result, coupled with necropsy findings, would help provide an understanding of pathophysiological mechanisms which contributed to RHB coot mortality.

2. Materials and methods

2.1. Bird intake and processing

On 21 February 1995, a Unocal pipeline was ruptured during construction on the Los Angeles railway system (Metrolink). An estimated 7800 barrels of San Joaquin crude oil was spilled into the San Gabriel River in Huntington Beach, CA. Ninety-six live birds (mostly coots) were oil contaminated and recovered during search and collection.

Birds were stabilized by the California Department of Fish and Game, Office of Oil Spill Prevention and Response veterinary services and transported to the International Bird Rescue Research Center (IBRRC, Berkeley, CA) for cleaning, rehabilitation, and continued veterinary care. After rehabilitation, coots were determined to be healthy and releasable from IBRRC if the following criteria were met: no external oil was present; birds were waterproof; birds exhibited normal buoyancy in recovery pools; birds appeared to behave and feed normally; packed cell volume (PCV) $>35\%$; total protein (TP) concentration between 3.5 and 6.0 g/dl; body weight within 50 g of intake body weight; and physical exam findings were normal (Williams, 1985). Forty-seven RHB coots were transported to the Wildlife Health Center at the University of California, Davis (UCD) between 24 March and 7 April 1995 despite approximately 50% of the birds having developed various levels of pododermatitis due to housing constraints and delayed release from rehabilitation associated with logistical research delays.

Free ranging, healthy REF coots were captured from Gray Lodge Wildlife Management Area, CA (39° 19.7′ N, 121° 49.8′ W) by rocket net on 9 April 1995 and

transported to UCD for immediate entry into the study. Due to the large number of RHB and REF birds, processing was performed on either 10 or 18 April 1995. For details on bird processing, refer to Anderson et al. (1999).

Every 3–4 weeks, RHB and REF birds from a small marsh enclosure were herded, recaptured, re-examined, weighed and bled. On 13 May (81 days post oil exposure), seven RHB and 12 REF coots were recaptured. On 9 June (108 days post oil exposure), nine RHB and 14 REF coots were recaptured and on 11 July (140 days post oil exposure), six RHB and 17 REF coots were recaptured. Due to the large size of the experimental ecosystem (Anderson et al., 1999), heavy vegetation and the evasive qualities of coots, it was not possible to recapture all birds on each sampling date.

2.2. Blood collection and analyses

On each blood collection date, 2.0 ml of blood was obtained from either the metatarsal (leg) vein, basilic (wing) vein or right jugular (neck) vein after cleaning the venipuncture site with an alcohol swab. Blood was collected using a 23-gauge hypodermic or butterfly needle attached to a 3-ml syringe. Blood was immediately transferred into Microtainer™ serum separator and EDTA tubes (Becton-Dickinson and Co., Franklin Lakes, NJ). Blood smears were prepared at the time of blood collection, air dried and stored at room temperature until stained at a veterinary diagnostic laboratory (CVD Inc., West Sacramento, CA).

Hematologic evaluation was performed within 24 h of sample collection. PCV was determined by microhematocrit centrifugation (Jain, 1986) and RBC was performed using the RBC™ Unopette (Becton-Dickinson & Co., Franklin Lakes, NJ) technique (Campbell, 1995). Hemoglobin (Hb) concentration was determined by a cyanmethemoglobin method following centrifugation of the lysate (Zinkl, 1986). Mean cell volume, mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) were calculated (Duncan et al., 1994). WBC counts were performed using the modified Natt-Herrick's technique (Zinkl, 1986).

Wright-Giemsa-stained blood smears were examined for RBC morphology, parasites, thrombocytes and differential WBC counts (heterophils, lymphocytes, monocytes, eosinophils, basophils).

Blood in serum separator tubes was refrigerated for up to 6 h and centrifuged for 15 min at 3500 rpm (Triac Centrifuge™, Clay Adams, Sparks, MD). Disposable polyethylene pipettes were used to pipette sera into plastic 1.5-ml microcryovials (Out Patient Services, Petaluma, CA). Serum was analyzed for alkaline phosphatase (Alk Phos), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), gamma glutamyltransferase (GGT) and lactate dehydrogenase (LDH) activities, and concentrations of albumin, TP, total bilirubin (TBili), direct bilirubin, creatinine, cholesterol, blood urea nitrogen, glucose, Ca, P, bicarbonate (HCO_3), Cl, K, Na and uric acid (UA). The albumin:globulin (A:G) ratio and globulin concentration were also calculated.

2.3. Statistical analyses

Descriptive statistics were calculated for RHB and REF birds at monthly intervals using BMDP™ Statistical Software (Los Angeles, CA). Mann–Whitney rank sum tests were used to identify statistically significant differences ($p \leqslant 0.05$) between RHB and REF groups. Kruskal–Wallis analysis of variance (KWANOVA), modified Mann–Whitney rank sum test (Hollander and Wolfe, 1973) and a Bonferroni–Dunn multiple comparison type test (Dunn, 1964) were used to compare blood results among 14 RHB and seven REF coots which died and seven RHB and 18 REF coots which survived. Blood results from 56 days post oil exposure were used for RHB and REF coots which died with the exception of one RHB coot blood sample collected 81 days post oil exposure. Blood results from 140 days post oil exposure were used for RHB and REF coots which survived, with the exception of two RHB and four REF coot blood samples collected 108 days post oil exposure. KWANOVA and a Bonferroni–Dunn multiple comparison-type test was also used to identify within-group differences in blood results over the study duration (140 days post oil exposure).

For survival analysis, continuous data were categorized into the following groups (based on the descriptive data presented above): very high ($\geqslant$mean + 90% confidence interval [CI]); high (from mean to mean + 90% CI); low (from mean to mean − 90% CI); and very low ($\leqslant$mean − 90% CI). Using these categories, Mantel-Cox and Tarone-Ware survival analyses (Dixon, 1992) were calculated to determine blood parameter influence on survivorship.

3. Results and discussion

3.1. Monthly comparisons between RHB and REF coots

Significant differences detected in blood values (WBC count, MCH, MCHC, Alk Phos, ALT, AST and CK activities; creatinine, TP, globulin and Ca concentrations and A:G ratio) between RHB and REF birds 56 days post oil exposure (Table 1) suggested that RHB birds underwent biological changes attributable to oil exposure, rehabilitation, prolonged captivity, stress or a combination of these factors. With the exception of CK activity, every significant difference between RHB and REF coots was identified on the April sampling date, 56

Table 1
Mean ± standard deviation of hematological and serum biochemical results which differed significantly ($p \leqslant 0.05$) between oiled and rehabilitated (RHB) American coots (*Fulica americana*) and non-oiled reference (REF) coots on given blood collection dates [a]

Analyte	Days post oil exposure [b]	RHB (*n*)	REF (*n*)	RHB	REF
WBC (/μl)	56	42	42	14,448 ± 8033	9264 ± 5507
MCH (pg)	56	26	29	33.2 ± 9.6	42.6 ± 13.4
MCHC (g/dl)	56	26	29	23.5 ± 2.7	25.9 ± 1.7
Alk Phos (IU/l)	56	39	39	515 ± 314	807 ± 445
ALT (IU/l)	56	39	39	34 ± 56	78 ± 62
AST (IU/l)	56	42	40	536 ± 621	744 ± 567
CK (IU/l)	56	40	38	1137 ± 375	4602 ± 4156
CK (IU/l)	140	5	16	1981 ± 1411	669 ± 987
Creatinine (mg/dl)	56	39	39	0.6 ± 0.1	0.7 ± 0.1
TP (g/dl)	56	39	40	5.3 ± 0.8	5.7 ± 1.0
Globulin (g/dl)	56	39	40	3.7 ± 0.6	4.2 ± 0.9
A:G ratio	56	39	40	0.44 ± 0.10	0.37 ± 0.10
Ca (mg/dl)	56	39	40	10.4 ± 1.2	9.3 ± 0.8

[a] WBC, white blood cell; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; Alk Phos, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase, TP, total protein; A:G, albumin:globulin; Ca, calcium.

[b] Fifty-six days post oil exposure correlates with blood sampling on 18 April 1995. One-hundred and forty days post oil exposure correlates with blood sampling on 11 July 1995. REF coots were not exposed to oil.

days after oil contamination. In May and June (81 and 108 days post oil exposure), no significant differences were found between RHB and REF coots (Tables 1, 2 and 3). These data indicated that blood result differences persisted for no longer than 2 months after birds became oiled. After that time, blood results could no longer be used to differentiate RHB and REF coots. The higher initial (56 days post oil exposure) WBC count of RHB birds may partially be attributed to foot and hock lesions (edema, swelling, inflammation, infection) which developed secondary to housing constraints and delayed release from rehabilitation. Foot lesions began to heal once birds were placed in an outdoor pen and became 'free ranging'. Later blood sample results (81, 108 and 140 days post oil exposure) revealed no differences between RHB and REF bird WBC counts. In addition, physical examinations confirmed that coot pododermatitis lesions had healed.

Although it has been shown that crude oil can cause oxidative damage to Hb and RBC resulting in Heinz body anemia (Leighton et al., 1983; Leighton, 1985), this is not a consistent finding (Pattee and Franson, 1982; Fry and Lowenstine, 1985; Newman et al., 1999). Fifty-six days after petroleum exposure, RHB birds had lower MCH and MCHC (Table 1), but concurrent anemia was not present (Tables 1 and 2b). Although there was no evidence for iron (Fe) deficiency anemia, the low MCH and MCHC together with hemosiderin deposition in several organs of necropsied RHB coots (Table 4) suggested that either macrophages sequestered Fe in association with infection and inflammation, or some abnormality in Fe utilization or metabolism existed. Once birds were released from the indoor rehabilitation facility and allowed to feed on wild vegetation and invertebrates both the MCH and MCHC increased. These changes may reflect dietary change, release of sequestered Fe, decreased oxygen demand secondary to being flightless for months or stress associated with handling at 3–4-week intervals. Further research is necessary to determine the relationships between oil toxicosis, captivity and Fe metabolism in birds.

It is likely that REF birds had higher CK and AST activities than RHB coots (Table 1) upon initial blood sampling (56 days post oil exposure, April) because REF coots had recently been captured using rocket nets (Franson, 1982; Franson et al., 1985; Bollinger et al., 1989). Although it is possible that this CK activity could be associated with long-term muscle damage or myopathy, without reference intervals for CK activity in coots, it is difficult to interpret the higher CK activities. By July (140 days post petroleum exposure), RHB coot CK and AST activities may have been higher than REF birds because RHB coots were significantly more active than REF coots during daily behavioral observations (Anderson et al., 1999).

Both TP and globulin concentrations were lower in RHB birds compared to REF birds on initial (April) blood sampling (Table 1). Between April and July (56–140 days post oil exposure) all birds had declines in WBC, heterophil and lymphocyte counts (Tables 2b and 3b). This suggested that decreased immune stimulation or decreased immunoresponsiveness in coots was secondary to 'captivity', periodic handling or stress, as opposed to oil exposure (Rocke et al., 1984; Briggs et al., 1996).

In April (56 days post oil exposure), RHB coots also had a higher Ca concentration than REF coots. Because electrolyte physiology is influenced by multiple factors (endocrine, renal and gastrointestinal tract function, sex, season), small, clinically irrelevant differ-

Table 2
Mean ± standard deviation of (a) serum biochemical and (b) hematological results for oiled and rehabilitated (RHB) American roots (*Fulica americana*) for each sampling date[a]

Analyte	56 days post oil exposure[b] 18 April 1995	81 days post oil exposure 13 May 1995	108 days post oil exposure 9 June 1995	140 days post oil exposure 11 July 1995
(a) *Serum biochemical results*				
Alk Phos[c] (IU/l)	515 ± 314 A	364 ± 111 AB	454 ± 79 AB	227 ± 79 B
ALT (IU/l)	34 ± 56	28 ± 8	62 ± 48	32 ± 10
AST (IU/l)	536 ± 621	307 ± 198[c]	522 ± 480	514 ± 421
CK (IU/l)	1137 ± 375	600 ± 428	631 ± 508	1981 ± 1411
LDH (IU/l)	No data	No data	No data	377 ± 161
BUN (mg/dl)	No data	No data	No data	2.2 ± 0.5
GGT (IU/l)	5.0 ± 5.6	4.6 ± 1.3	6.0 ± 3.7	No data
Albumin (g/dl)	1.6 ± 0.3	1.5 ± 0.1	1.4 ± 0.3	1.4 ± 0.2
TP (g/dl)	5.3 ± 0.1 A	4.3 ± 0.4 B	4.5 ± 0.8 B	4.2 ± 0.5 B
Globulin[c] (g/dl)	3.7 ± 0.6 A	2.9 ± 0.3 B	3.0 ± 0.7 AB	2.8 ± 0.4 B
Total bilirubin (mg/dl)	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	No data
Direct bilirubin (mg/dl)	0.0 ± 0.1	0.0 ± 0.1	0.1 ± 0.1	No data
Creatinine (mg/dl)	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	No data
Cholesterol[c] (mg/dl)	290 ± 56 A	206 ± 34 B	260 ± 48 AB	264 ± 45 AB
Glucose (mg/dl)	178 ± 47	209 ± 30	185 ± 24	200 ± 21
Ca (mg/dl)	10.4 ± 1.2	10.0 ± 0.4	10.2 ± 0.9	9.4 ± 0.9
PO_4 (mg/dl)	4.2 ± 1.9	2.7 ± 0.6	4.6 ± 1.8	3.1 ± 0.7
HCO_3 (MEQ/l)	18 ± 4	16 ± 2	13 ± 5	No data
Cl (MEQ/l)	106 ± 5	107 ± 2	101 ± 5	No data
K (MEQ/l)	2.9 ± 1.2	2.5 ± 0.5	2.9 ± 1.0	3.4 ± 0.4
Na (MEQ/l)	147 ± 6	149 ± 4	150 ± 7	152 ± 5
A:G ratio	0.44 ± 0.10	0.51 ± 0.07	0.50 ± 0.11	0.50 ± 0.07
Uric acid[c] (md/dl)	5.4 ± 2.1 A	5.4 ± 2.7 A	10.2 ± 3.3 B	6.9 ± 2.6 AB
(b) Hematological results				
WBC[c] (/μl)	14,448 ± 8033 A	8429 ± 2299 AB	11,078 ± 4827 AB	6283 ± 2905 B
Heterophils (/μl)	4818 ± 6624	3487 ± 3761	2116 ± 2535	3116 ± 3098
Lymphocytes (/μl)	2154 ± 2473	1596 ± 1690	2575 ± 2854	1039 ± 912
Monocytes[c] (/μl)	0 ± 0 A	0 ± 0 A	460 ± 1362 B	0 ± 0 A
Eosinophils (/μl)	112 ± 256	135 ± 169	155 ± 243	414 ± 616
Basophils (/μl)	124 ± 205	87 ± 144	106 ± 181	49 ± 55
RBC (10^6 /μl)	3.66 ± 1.12	No data	2.92 ± 0.11	2.62 ± 0.25
Hb (g/dl)	11.3 ± 2.0	No data	11.9 ± 1.19	11.5 ± 0.5
PCV[c] (%)	49 ± 7 A	No data	46 ± 3 A	39 ± 2 B
MCV (fl)	137 ± 39	No data	159 ± 5	153 ± 12
MCH (pg)	33.2 ± 9.6	No data	40.8 ± 4.3	44.1 ± 5.0
MCHC[c] (g/dl)	23.5 ± 2.7 A	No data	25.7 ± 2.3 AB	28.7 ± 1.2 B

[a] Alk Phos, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase; LDH, lactate dehydrogenase; BUN, blood urea nitrogen; GGT, gamma glutamyltransferase; TP, total protein; A:G, albumin: globulin; WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin; PCV, packed cell volume; MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration.

[b] Days post oil exposure refers to the number of days since the RHB coots were exposed to petroleum.

[c] For these parameters, differences exist ($p \leqslant 0.05$, Kruskal–Wallis analysis of variance) between monthly means. Means across rows with letters in common are not statistically different.

ences are difficult to interpret. Furthermore, stress-induced corticosterone production may also influence osmoregulation (Holmes et al., 1981; Harvey and Phillips, 1982) which further complicates interpretation of this result.

3.2. Comparisons between RHB or REF coots which either survived or died

Blood samples collected 56 days post oil exposure from RHB birds which subsequently died showed significantly higher WBC, PCV, TP and globulin concentrations, and lower MCH, MCHC, glucose and Na concentrations, and A:G ratio (Table 5) compared with blood results collected 108–140 days post oil exposure from REF birds which survived. The elevated WBC count, and TP and globulin concentrations of RHB coots which died suggested infectious or non-septic inflammation. However, if REF coot blood data had not been available for comparative purposes, the TP and globulin levels of RHB coots which died may have been misinterpreted as 'within reference values'.

Table 3
Mean ± standard deviation of (a) serum biochemical and (b) hematological results for non-oiled reference (REF) American coots (*Fulica americana*) for each sampling date[a]

Analyte	56 days post oil exposure[b] 18 April 1995	81 days post oil exposure 13 May 1995	108 days post oil exposure 9 June 1995	140 days post oil exposure 11 July 1995
(a) *Serum biochemical results*				
Alk Phos[c] (IU/l)	807 ± 445 A	375 ± 125 AB	607 ± 439 AB	252 ± 84 B
ALT[c] (IU/l)	78 ± 62 A	27 ± 7 B	43 ± 35 AB	35 ± 15 B
AST[c] (IU/l)	744 ± 567 A	260 ± 35 B	364 ± 270 B	298 ± 145 B
CK[c] (IU/l)	4602 ± 4156 A	818 ± 639 B	1390 ± 2039 B	669 ± 987 B
LDH (IU/l)	No data	No data	No data	352 ± 196
BUN (mg/dl)	No data	No data	No data	2.2 ± 0.9
GGT (IU/l)	4.3 ± 3.0	4.3 ± 1.4	5.5 ± 2.2	No data
Albumin (g/dl)	1.5 ± 0.2	1.5 ± 0.1	1.5 ± 0.3	1.6 ± 0.2
TP[c] (g/dl)	5.7 ± 1.0 A	4.5 ± 0.4 B	4.5 ± 0.8 B	4.6 ± 0.7 B
Globulin[c] (g/dl)	4.2 ± 0.9 A	3.0 ± 0.4 B	3.1 ± 0.6 B	3.2 ± 0.6 B
Total bilirubin (mg/dl)	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	No data
Direct bilirubin (mg/dl)	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	No data
Creatinine (mg/dl)	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	No data
Cholesterol (mg/dl)	299 ± 92	244 ± 37	256 ± 54	259 ± 69
Glucose[c] (mg/dl)	173 ± 24 A	189 ± 27 AB	192 ± 15 AB	211 ± 20 B
Calcium (mg/dl)	9.3 ± 0.8	9.9 ± 0.6	9.9 ± 0.8	9.9 ± 0.6
PO_4[c] (mg/dl)	3.9 ± 1.5 A	3.1 ± 0.8 ABC	4.3 ± 2.7 AB	2.4 ± 1.1 C
HCO_3[c] (MEQ/l)	18 ± 3 A	15 ± 2 B	14 ± 4 B	No data
Cl[c] (MEQ/l)	102 ± 18 A	106 ± 6 A	101 ± 3 B	No data
K (MEQ/l)	2.6 ± 0.9	2.3 ± 0.9	3.5 ± 0.9	3.0 ± 1.0
Na[c] (MEQ/l)	147 ± 3 A	147 ± 6 A	148 ± 4 A	152 ± 3 B
A:G ratio[c]	0.37 ± 0.10	0.50 ± 0.06	0.49 ± 0.05	0.52 ± 0.09
Uric acid (md/dl)	6.4 ± 2.7	6.5 ± 2.3	9.6 ± 6.5	5.9 ± 3.3
(b) Hematological results				
WBC[c] (/μl)	9264 ± 5507	8417 ± 2843 AB	8377 ± 2917 A	5894 ± 2720 B
Heterophils[c] (/μl)	3043 ± 3414 A	5538 ± 1968 B	3225 ± 2971 ABC	2157 ± 2125 AC
Lymphocytes[c] (/μl)	1483 ± 1792	2513 ± 1029 B	2019 ± 1877 AB	1850 ± 1410 AB
Monocytes (/μl)	0 ± 0	33 ± 116	0 ± 0	4 ± 18
Eosinophils (/μl)	310 ± 519	226 ± 434	141 ± 195	290 ± 479
Basophils (/μl)	70 ± 98	108 ± 150	36 ± 80	93 ± 101
RBC (10^6/μl)	3.09 ± 1.02	No data	2.78 ± 0.54	2.82 ± 0.35
Hb (g/dl)	12.0 ± 1.4	No data	12.4 ± 1.8	11.0 ± 1.0
PCV[c] (%)	47 ± 5 A	No data	46 ± 6 AB	40 ± 5 B
MCV (fl)	165 ± 47	No data	168 ± 27	138 ± 13
MCH (pg)	42.6 ± 13.4	No data	45.3 ± 6.2	39.4 ± 5.0
MCHC[c] (g/dl)	25.9 ± 1.7 A	No data	27.2 ± 1.4 AB	28.5 ± 1.8 A

[a] Alk Phos, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase; LDH, lactate dehydrogenase; BUN, blood urea nitrogen; GGT, gamma glutamyltransferase; TP, total protein; A:G, albumin: globulin; WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin; PCV, packed cell volume; MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration.

[b] Days post oil exposure refers to the number of days since the RHB coots were exposed to petroleum. Reference (REF) coots were not exposed to oil.

[c] For these parameters differences exist ($p \leqslant 0.05$, Kruskal–Wallis analysis of variance) between monthly means. Means across rows with letters in common are not statistically different.

Despite not detecting any obvious petroleum-induced toxicity to RBC in this study, RHB birds which died had a higher PCV and lower MCHC than REF survivors. Fe deficiency is one cause for low MCH and MCHC. However, anemia, which often accompanies this deficiency, was not observed. Although Fe was not measured, Fe availability may have been decreased from low dietary Fe intake, gastrointestinal malabsorption or sequestering of Fe by the mononuclear phagocytic system (Duncan et al., 1994). Low glucose concentrations of RHB birds which died compared to RHB survivors, also supported the possibility of bacterial infection and sepsis contributing to mortality.

Reference coots which died had a low glucose concentration which approached hypoglycemia < 150 mg/dl (Williams, 1985; OWCN, 1998), and higher AST and CK activity than surviving REF birds. In other avian species, CK, AST and LDH activity have been used to document muscle damage (Franson et al., 1985) and this may have also led to REF coot muscle enzyme elevations.

Table 4
Gross pathological and histological lesions found from 11 oiled and rehabilitated American coots (*Fulica americana*) on post-mortem examination

Organ sytem or tissue	Specific lesion	No. of birds affected
Gastrointestinal tract		
Enteritis	Lymphoplasmacytic	1
	Lymphoplasmacytic/heterophilic	3
Villi infection	Protozoal (Coccidia)	1
Colitis	Necrosuppurative/granulomatous	2
Cloacitis		1
Liver		
Hepatitis	Lymphocytic, plasmacytic, histiocytic	3
Hepatocellular lesions	Vacuolation	1
	Necrosis	2
	Bile stasis	3
	Hemosiderin deposition	4
Spleen		
Enlarged		6
	Degeneration/amyloid deposition	4
	Hemosiderin deposition	1
Respiratory tract		
Bronchi	Pneumoconiotic pigment present	1
Air sacculitis	Pyogranulomatous/aspergillosis	3
Lung tissue	Granulomatous pneumonia	2
	Hemorrhage	2
	Parasitic granuloma	1
Musculoskeletal		
Muscle	Myonecrosis	1
	Heterophilic myositis	1
Pododermatitis	Pyogranulomatous/necrotizing	3
Osteomyelitis	Necrotizing	1
Other		
Thyroid glands	Prominent/enlarged	6
Adrenal glands	Enlarged	1
	Adrenal chromaffin cell vacuolation	1
Kidneys	Renal tubular necrosis	1
Renal epthelium	Hemosiderin deposition	1
Coelom	Granulomatous coelomitis	1

A less likely explanation for increased AST activity is damaged hepatic or renal cells, or erythrocytes (Campbell and Coles, 1986). No differences in blood results were found between RHB and REF coot survivors suggesting that coots which were oiled, rehabilitated, released and survived at least 3.5 months could not be differentiated from wild (REF) coots based on hematological and serum biochemical testing.

For REF coots which died, AST activity was higher than RHB coots which died, globulin concentration was higher than RHB survivors, and glucose concentration was lower and Alk Phos and AST activities higher compared to REF survivors. For RHB coots which died, globulin and TP concentrations were higher compared to RHB survivors.

3.3. Within-group comparisons for RHB coots and REF coots over time

Within-group comparisons of blood results for different sampling dates for RHB birds revealed 13 significant differences for nine analytes. WBC count, PCV, MCHC, monocyte count, TP, globulin, cholesterol and UA concentrations and Alk Phos activity differed once or more during monthly comparisons (Table 2). Within-group comparisons of blood results for REF birds revealed 36 differences for 16 analytes (Table 3) for monthly comparisons. Counts of WBCs, heterophils, and lymphocytes, PCV, MCHC, TP, globulin, glucose, P, HCO_3, Cl and Na concentrations, and activities of Alk Phos, ALT, AST and CK differed once or more during monthly comparisons (Table 3). These results suggested that over 3 months (mid-April through mid-July), the date of blood collection affected blood results of both RHB and REF coots. It is assumed that unavoidable confounding variables (date of sampling, captivity stress, habituation), generally affect all blood samples similarly (Wobeser, 1994).

3.4. Survival analyses

When blood parameter differences were examined between RHB and REF coots as to their impact on survival time, several significant analytes were identified. Coots in the RHB category had shorter survival times if they had a very high Cl ($\geqslant$110 MEQ/l) or cholesterol ($\geqslant$449 mg/dl) concentration in April (56 days post oil exposure). Because multiple factors (endocrine, renal and gastrointestinal tract function, sex, season) can influence electrolyte physiology, the mechanism causing high Cl and its relationship to shorter survival for RHB coots is difficult to determine.

Cholesterol concentrations vary with diet between birds of different families (Lewandowski et al., 1986) and it is possible that diets fed to coots in captivity differed from natural coot diets leading to this difference. In mammalian species, high cholesterol has also been attributed to endocrine (thyroid), hepatic or renal diseases, as well as exogenous corticosteroid administration (Duncan et al., 1994). Previous studies have identified endocrine organs, liver, kidney and adrenal glands as targets of oil toxicosis (Hartung and Hunt, 1966; Szaro et al., 1978; Fry and Lowenstine, 1985; Leighton, 1991); however, these are inconsistent findings and they were not observed from necropsies performed on RHB coots which died (Table 4).

When exposure classes were combined (to determine non-petroleum-exposure survival risk factors), overall survival time was significantly shortened if coots had very high WBC counts ($\geqslant$16,561/μl), RBC counts ($\geqslant 4.82\times10^6$/μl), or concentrations of cholesterol ($\geqslant$449 mg/dl), Cl ($\geqslant$110 MEQ/l), TBili ($\geqslant$0.1 mg/dl) or a very

Table 5
Mean ± standard deviation and sample size of hematological and serum biochemical results that differed significantly among oiled and rehabilitated (RHB) American coots (*Fulica americana*) which survived (RHB live) or died (RHB died), and non-oiled reference (REF) coots which survived (REF live) or died (REF died)[a]

Analyte	RHB died[b]	RHB live[c]	REF died[b]	REF live[c]
WBC (/μl)	15,014 ± 9028 A ($n=14$)	8743 ± 5087 AB ($n=7$)	11,057 ± 7671 AB ($n=7$)	6672 ± 3233 B ($n=18$)
PCV (%)	49 ± 7 A ($n=9$)	42 ± 6 AB ($n=4$)	46 ± 3 A ($n=4$)	39 ± 5 B ($n=14$)
MCHC (g/dl)	23.5 ± 3.0 A ($n=10$)	28.0 ± 0.9 AB ($n=3$)	25.9 ± 1.3 AB ($n=4$)	28.0 ± 1.6 B ($n=13$)
Alk Phos (IU/l)	439 ± 368 AB ($n=14$)	408 ± 291 AB ($n=6$)	677 ± 344 A ($n=6$)	367 ± 407 B ($n=18$)
AST (IU/l)	323 ± 188 A ($n=14$)	456 ± 338 AB ($n=6$)	579 ± 321 B ($n=6$)	319 ± 236 A ($n=18$)
TP (g/dl)	5.4 ± 0.9 ($n=14$)	4.0 ± 0.8 B ($n=6$)	5.5 ± 1.1 AB ($n=6$)	4.3 ± 0.8 B ($n=18$)
Globulin (g/dl)	3.8 ± 0.7 A ($n=14$)	2.6 ± 0.5 B ($n=6$)	4.0 ± 1.1 AC ($n=6$)	2.9 ± 0.6 BC ($n=18$)
Glucose (mg/dl)	160 ± 42 A ($n=14$)	199 ± 28 AB ($n=6$)	164 ± 26 A ($n=6$)	209 ± 19 B ($n=18$)
Calcium (mg/dl)	10.2 ± 0.8 A ($n=14$)	8.9 ± 0.8 B ($n=6$)	9.4 ± 0.5 AB ($n=6$)	9.6 ± 0.6 AB ($n=18$)
Na (MEQ/l)	148 ± 4 A ($n=13$)	150 ± 4 AB ($n=6$)	147 ± 2 AB ($n=6$)	157 ± 25 B ($n=18$)
A:G ratio	0.42 ± 0.09 A ($n=14$)	0.55 ± 0.06 B ($n=6$)	0.4 ± 0.10 AB ($n=6$)	0.53 ± 0.07 B ($n=18$)

[a] WBC, white blood cell; PCV, packed cell volume; MCHC, mean cell hemoglobin concentration; Alk Phos, alkaline phosphatase; AST, aspartate aminotransferase; TP, total protein; A:G, albumin:globulin. Means across rows with letters in common do not differ statistically ($p \leqslant 0.05$, Kruskal–Wallis analysis of variance).

[b] Blood results from 13 RHB and seven REF coots which died were collected 56 days post oil exposure and one RHB coot blood sample was collected 81 days post oil exposure.

[c] Blood results from five RHB and 14 REF coots which survived were collected 140 days post oil exposure and two RHB and four REF coot blood samples were collected 108 days post oil exposure.

low glucose ($\leqslant$134 mg/dl). None of these parameters were linked to lower survival times of RHB birds alone, such that these blood changes were not likely caused by oil exposure or rehabilitation techniques, but rather were a result of captivity or monthly handling.

3.5. Necropsy findings from RHB coots

No pathopneumonic pathological lesions have been linked to mortality associated with oil toxicosis; however, previous studies of both free ranging and experimentally oiled marine birds (Szaro et al., 1978; Fry and Lowenstine, 1985; Leighton, 1986; Jessup and Leighton, 1996) have described similar pathological lesions to those found in this study (Table 4), including enteritis, coccidia parasitism, hepatitis, hepatocellular necrosis, hemosiderin deposition in splenic cells, hepatocytes, and renal epithelium, pneumoconiotic pigment present in the lungs and renal tubular necrosis. Six of 11 RHB birds examined were severely emaciated and had active bone marrow as well. Unfortunately, necropsy findings from REF coots were unavailable for comparison making it difficult to differentiate between lesions associated with oil exposure, captivity or a combination of factors.

There are many possible causes of hemosiderin deposition in the spleen, liver and kidney (Lowenstine and Munson, 1999). Rehabilitated coots may have had decreased RBC survival or ingested excessive dietary Fe prior to release from rehabilitation. Circumstances during the study which may have contributed to hemosiderin deposition included generalized catabolism and starvation which reduce erythropoiesis; and infection or inflammation, which decrease Fe utilization, reduce RBC survival and result in anemia of chronic disease (Lowenstine and Munson, 1999). Based on the findings in this study, anemia of chronic disease most likely contributed to hemosiderin deposition.

4. Conclusions

The results of this study suggested that blood analyses, in conjunction with post-release survival data, provided important information about long-term impacts of oil exposure and rehabilitation on marine birds. Differences detected between RHB birds which died and surviving REF birds indicated that WBC, PCV, MCH, MCHC, A:G ratio and concentrations of glucose, TP, Na and globulin may be valuable for examining causes of post-release mortality. Based on blood results and necropsy findings, inflammation, infection and Fe utilization or metabolism problems led to pathophysiological mechanisms which contributed to RHB coot mortality. Decreased duration of survival for RHB coots was predicted by high cholesterol and Cl concentrations meaning that mechanisms which result in these changes may also be related to higher mortality in oiled and rehabilitated wildlife.

As reference ranges are established for more species of marine birds, it will become easier to identify factors which place rehabilitated birds at risk, to gain a better understanding of the effects of oil and rehabilitation on wildlife and to determine reasons for post-release mortality.

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