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Polycyclic Aromatic Hydrocarbons Detected in Common Loons (*Gavia immer*) Wintering off Coastal Louisiana

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Abstract.—On 20 April 2010, the *Deepwater Horizon* oil drilling rig located 66 km southeast of the Louisiana coast exploded and, by the time the pipeline was capped in July, estimates of 4.9 million barrels of oil were released in the northern Gulf of Mexico. Polycyclic aromatic hydrocarbons make up a small percentage of petroleum (< 5%), but are the most toxic with known negative impacts on wildlife and humans. Because of their lifestyle and trophic standing, seabirds are often impacted by marine spills. To test for the presence of polycyclic aromatic hydrocarbons, we captured and tested blood in Common Loons (*Gavia immer*), a winter migrant that spends 4-5 months in the Gulf of Mexico. Common Loons were captured at night, using spotlighting and a large dip net off the Louisiana coast, during January-March in 2011 and 2012. A total of 38 Common Loons were caught and sampled (17 in 2011 and 21 in 2012). Both the concentrations and frequency of polycyclic aromatic hydrocarbons present in Common Loons appear to be increasing between years; however, concentrations were low (< 10 ppb). In 2012, petrogenic alkyl polycyclic aromatic hydrocarbons, those derived from petroleum, were significantly higher than pyrogenic polycyclic aromatic hydrocarbons, derived from combustion and anthropogenic sources. It remains unknown if current levels have any adverse impacts on Common Loon health, reproduction and survival. *Received 30 January 2013, accepted 10 June 2013.*

Key words.—*Deepwater Horizon* oil spill, Common Loon, *Gavia immer*, Gulf of Mexico, Louisiana, polycyclic aromatic hydrocarbons.

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On 20 April 2010, the *Deepwater Horizon* oil drilling rig located 66 km southeast of the Louisiana coast exploded in the northern Gulf of Mexico (GOM). By the time the pipeline was capped in July, estimates of 4.9 million barrels of oil were released into the environment (Graham and Kelly 2011). The *Deepwater Horizon* oil spill (DHOS) was the worst marine oil spill on record and represents a large-scale disturbance (Graham and Kelly 2011). Winds and currents spread the oil over four Gulf Coast states (Louisiana, Mississippi, Alabama and Florida), with Louisiana receiving the majority. Oil reached a number of wetland habitats (Bik *et al.* 2012; Silliman *et al.* 2012) and entered some components of the planktonic base of the region's nearshore food web (Graham *et al.* 2010). Past oil spills have had both immediate and long-term impacts on seabird and other wildlife populations (Golet *et al.* 2002; Peterson *et al.* 2003; Alonso-Alvarez *et al.* 2007a; Esler *et al.* 2010). Immediate impacts with images of oiled individuals receive the greatest attention, but sublethal effects due to chronic exposure are only recently being explored (Esler *et al.* 2002; Alonso-Alvarez *et al.* 2007b).

The toxic effects attributed to petroleum exposure are due primarily to four classes of hydrocarbons (Albers 2003), but one class in particular, the polycyclic aromatic hydrocarbons or PAHs, is the most toxic to wildlife. PAHs are mutagenic, tumorigenic, and carcinogenic, causing a range of health effects that include liver damage, hemolytic anemia, weight loss, changes in salt gland weight, gut damage and immunosuppression (Rocke *et al.* 1984; Leighton 1993; Briggs *et al.* 1996; Troisi *et al.* 2006). The DHOS contained approximately 3.9% PAHs by weight for an estimated total 2.1 x 10^{10} g of total PAHs released in the environment (Reddy *et al.* 2011). PAHs can persist in the environment and diffuse across biological membranes and can enter organisms and the food web (Connell 1990; Carls and Thedinga 2010). Some studies have shown bioaccumulative effects in animals (e.g., mollusks and zooplankton; Meador *et al.* 1995), but generally

do not accumulate in vertebrates (Albers 2006). This is because vertebrates efficiently metabolize and excrete aromatic and non-aromatic hydrocarbons ingested during feeding, preening, and respiration via mixed function oxygenase systems (Hall and Coon 1988; Naf *et al.* 1992; Pérez *et al.* 2005). Consequently, only minor concentrations of parent PAHs are usually detectable in vertebrate tissues (Ariese *et al.* 1993), and accumulation of PAHs by birds is unlikely (Albers 2006).

External exposure assessment of oiled seabirds is undertaken by assessing the percentage of oil covering the plumage, but measuring internal exposure is rarely undertaken (Troisi and Borjesson 2005). This is because traditional chromatographic methods for plasma PAH analysis require large sample volumes, more than what can be collected from smaller seabirds. Additionally, these methods are not cost-effective. Few studies have measured PAH concentrations in wild bird tissues after a marine oil spill, and those that have mostly examined birds either found dead or sacrificed (Broman *et al.* 1990; Kayall and Connell 1995; Custer *et al.* 2000; Troisi *et al.* 2006). Internal exposure to oil should be assessed using a nondestructive approach for assessing large numbers of birds in a short time period. Plasma samples are ideal for assessing petroleum exposure assessment because they represent that which is circulating to target organs and causing toxic effects. Blood sampling is nondestructive and can be repeated easily to monitor decontamination over time. Thus, monitoring blood PAH levels may be a less invasive yet instructive way to assess oil contamination.

Life history characteristics of marine birds make them particularly vulnerable to oil pollution. For example, they spend much of their lives on the ocean's surface and are situated in higher trophic positions (Pérez *et al.* 2005). Common Loons (*Gavia immer*) are long-lived (> 25 years), piscivorous waterbirds that breed in freshwater lakes of the northern United States and Canada and overwinter predominately along seacoasts (Evers *et al.* 2010), much like marine birds (Procellariformes). In addition, Common Loons (loons) behave like alcids in that they spend up to 4 to 5 consecutive months on the ocean, often a great distance from shore, and only come to land when sick or injured (Spitzer 1995). On their breeding grounds, loons have been used successfully as a bio-indicator species of heavy metals such as mercury (Evers 2006). On their wintering grounds, they may also be useful indicators of marine pollution. A substantial portion of the interior North America loon population winters in the GOM (Kenow *et al.* 2002; Evers 2007). Thus, researchers were concerned how this wintering population might be impacted by the DHOS. Our objective was to monitor PAH concentrations in loon blood as a potential tool for assessing the impacts of the DHOS, a large scale disturbance, on a wintering migrant marine-like bird.

METHODS

Study Area

The primary research area is located off the Louisiana coast west of Port Sulphur along the Mississippi Delta (Fig. 1). This area was chosen because it was closest to the DHOS and was one of the areas that saw the heaviest oiling (Graham *et al.* 2010). It consists of numerous bays (Adams Bay, Bay Batiste, Bastian Bay, and Bay Sanbois) and water courses (Grand Isle, Lake Washington) associated with the much larger Barataria Bay. Due to subsidence and proximity to the mouth of the Mississippi River, the water is typically shallow (1-3 m) and turbid (< 1 m using a secchi disc). In addition, the large influx of freshwater makes the entire coastline a large estuary with salinity levels between 5 and 20 ppt. Water temperatures during the winter ranges from 16 to 24 °C. The coastline in the study area (Fig. 1) consists of marshes and low-lying wetlands. The predominant underwater and emergent vegetation consists of eelgrass (*Zostera* sp.) and cordgrass (*Spartina* sp.).

This region harbors some of the most productive fishing ports in the United States. For example, 72%, 66% and 16% of the nation's commercial shrimp, oyster, and fish catch, respectively, is obtained in the GOM, and five of the top 10 fishing ports in the GOM are located along the Louisiana coast (National Ocean Economics Program 2013). This area is diverse in fish species with > 200 species recorded (Hoese and Moore 1998). Some of the major fish species include the Gulf silverside (*Minidia peninsulae*), Gulf menhaden (*Brevoortia patronus*), striped bass (*Morone saxatilis*), winter flounder (*Paralichthys* spp.), striped mullet (*Mugil cephalus*), Atlantic croaker (*Micropogonias undulates*), red drum (*Sciaenops ocellatus*), black drum (*Pogonias cromis*), spot (*Leiostomus xanthrurus*), and bluefish (*Pomatomus salta-*

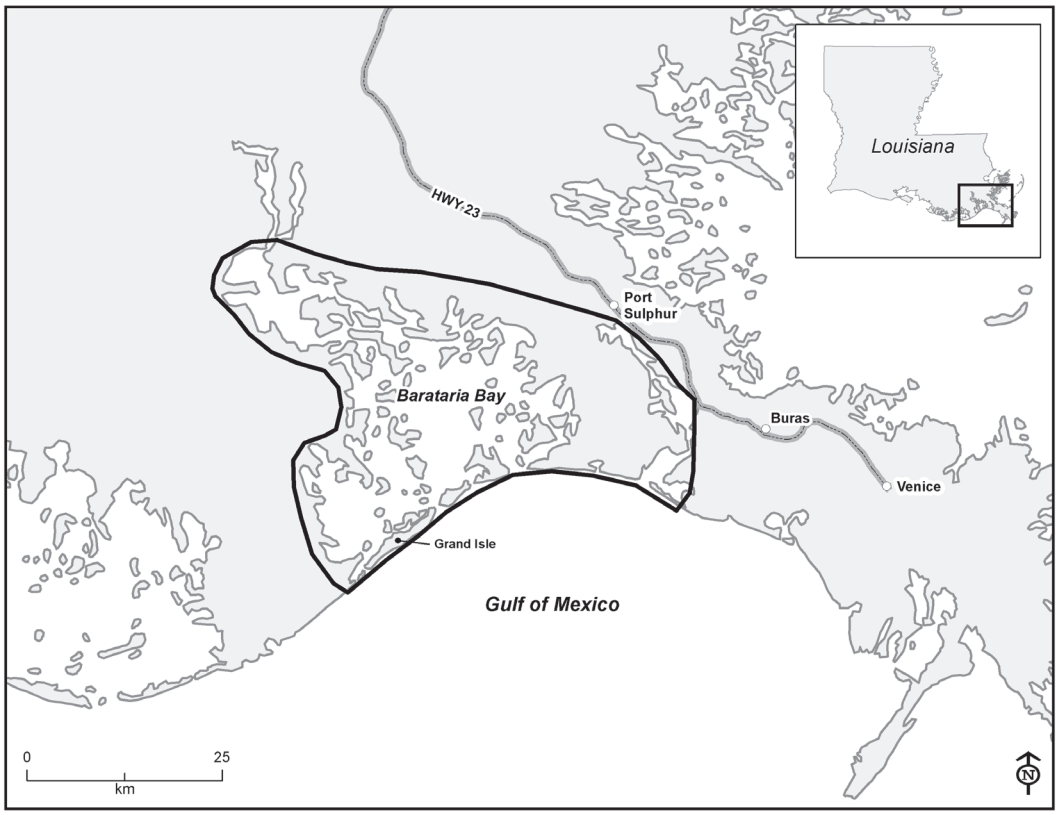

Figure 1. Wintering Common Loon study area off the coast of Louisiana west of Port Sulphur, Louisiana.

trix). Many of these species breed offshore, and their planktonic larvae drift inshore where they rely on the shallow coastal waters as hatcheries.

Loons were captured using well-established night-lighting techniques (Evers 2001) during the months of January-March of 2011 and 2012. Because of the opportunistic aspect of the capture technique, we did not discriminate among adult, subadult or immature birds. Blood was drawn from the metatarsal vein through a leur adapter directly into 5-10 cc vacutainers with sodium heparin and placed immediately on ice in a cooler. Birds were banded with U.S. Geological Survey aluminum bands and a unique combination of plastic colored bands. A suite of morphometric measurements was taken including bill width, length and depth; tarsal width and length; and weight. Each bird was also inspected for overall health and presence/absence of any external oil. Health assessments were made of keel condition, hydration, body weight, waterproof condition, molt stage, and alertness. Since hydrocarbons in oil can fluoresce, captured loons were placed under an ultraviolet light to determine if a loon was oiled and, if so, where. In addition, the amount of oiling was determined by visual inspection and placed in the following categories: 0 = not visibly oiled, 1 = Trace (< 5%), 2 = Light (6-20%), 3 = Moderate (21-40%) or 4 = Heavy (> 40%). Birds were released within 30 min of capture.

After collection, one tube (4 cc) of blood was immediately centrifuged (spun at 3,000 rpm) for 10 min and the plasma was decanted and frozen separately from the cell elements. Plasma samples were used for PAH analysis conducted at the University of Connecticut. Two distinct types of PAHs were quantified: parent and alkyl. The most toxic parent PAHs according to the U.S. Environmental Protection Agency (Keith and Telliard 1979) were measured in 2011 and 2012 in loons. The toxic parent PAHs measured included naphthalene, acenaphthylene, acenaphthene, flourene, phenanthrene, anthracene, fluoranthene, pyrene, crysene, benzo(a) anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a) pyrene, dibenz(a,h)anthracene, benzo(g, h, i)perylene, and Indenol(1, 2, 3-c-d)pyrene. The alkylated PAHs were tested in 2012 individuals only and consisted of 2-methylnaphthalene, 2,6-dimethylnaphthalene, 3-methylphenanthrene, 9-methylphenanthrene, and 1,7-dimethylphenanthrene. Detectable PAH levels were pre-established at 1.0 ng/g (ppb).

Tissue (plasma) samples, approximately 0.25 g, for parent and alkylated PAH compound analysis, were extracted using liquid-liquid extraction in 2.5 ml hexane, vortex mixed for 5 min at 2,500 rpm, followed by centrifugation for 3 min at 4,000 rpm (Pleil *et al.* 2010; Yeudakimaua *et al.* 2013). The hexane extraction was repeated three times with the top organic

layer transferred to a clean vial after each step. Once completed, the combined extract was concentrated to 2.0 ml under a nitrogen stream, followed by sample clean-up using SEP Pak Alumina-N, 6-cc, solid-phase extraction columns (Waters Inc., Milford, MA) with methylene chloride as the elution solvent. The sample was then concentrated to a final volume of 0.5 ml and internal standard was added. Following extraction, the samples were analyzed using an Agilent 6890 gas chromatograph equipped with a Restek Rxi-5Sil MS column (30 m) using splitless injection coupled to a Waters Quattro Micro triple quadruple mass spectrometer. All peaks were quantified against an internal standard of chrysene-d12, and extraction efficiency was evaluated using a surrogate standard of naphthalene-d8. Standard quality assurance procedures were employed including analysis of duplicate samples, method blanks (Blank), post digestion spiked samples, laboratory control samples, and standard reference materials where available (Standard Reference Material (SRM)-2974a and SRM-1947; National Institute of Standards and Technology).

In all analyses, assumptions of normality (Shapiro-Wilk test) and homoscedasticity (Levene's test) were tested prior to analysis, and transformations of the data were performed if necessary. A Fisher Exact Test was used to compare the frequency of detectable PAHs in loons between 2011 and 2012. A Mann-Whitney Test was used to compare concentrations of PAHs between years. All results were considered significant at $P < 0.05$.

RESULTS

A total of 38 birds (21 adults, 17 subadults) were caught and tested for PAHs: 17 in 2011 and 21 in 2012. There was no difference ($P > 0.05$) in the proportion of adult and subadult loons that had detectable vs. non-detectable concentrations of parent PAHs between years nor was there any difference ($P > 0.05$) in PAH concentration levels between adults and subadults (Table 1). Therefore, adult and subadult data were treated as one group. The frequency of loons with detectable levels of parent PAHs significantly increased from 2011 to 2012 (Fischer Exact Test, $P < 0.008$). The frequency of loons with detectable levels of PAHs increased 3.5 fold from 17.6% (3/17) in 2011 to 61.9% (13/21) in 2012. Also, concentrations of parent PAHs were significantly higher in 2012 compared to 2011 (4.82 ± 0.36 ng/g vs. 2.03 ± 0.17 ng/g; Mann-Whitney, $P < 0.05$). The concentration of parent PAHs in loon blood was low, however. For exam-

Table 1. Sum of parent polycyclic aromatic hydrocarbon (PAH) plasma concentrations in Common Loons wintering off the Louisiana coast, January-March 2011 and 2012. ND = non-detectable.

2011				2012				
Bird No.	Sum PAH[1]	Anthracene	Other[2]	Bird No.	Sum PAH	Anthracene	Fluoranthene	Other
1	2.3	2.3	ND	1	6.7	5.1	1.6	ND
2	2.1	2.1	ND	2	3.6	3.6	ND	ND
3	1.7	1.7	ND	3	4.1	4.1	ND	ND
4	0	ND[3]	ND	4	3.4	3.4	ND	ND
5	0	ND	ND	5	3.9	3.9	ND	ND
6	0	ND	ND	6	4.4	4.4	ND	ND
7	0	ND	ND	7	5.3	5.3	ND	ND
8	0	ND	ND	8	5.1	5.1	ND	ND
9	0	ND	ND	9	5.0	5.0	ND	ND
10	0	ND	ND	10	4.7	4.7	ND	ND
11	0	ND	ND	11	4.2	4.2	ND	ND
12	0	ND	ND	12	8.0	8.0	ND	ND
13	0	ND	ND	13	4.2	4.2	ND	ND
14	0	ND	ND	14	0	ND	ND	ND
15	0	ND	ND	15	0	ND	ND	ND
16	0	ND	ND	16	0	ND	ND	ND
17	0	ND	ND	17	0	ND	ND	ND
				18	0	ND	ND	ND
				19	0	ND	ND	ND
				20	0	ND	ND	ND
				21	0	ND	ND	ND

[1]ng/g or ppb.
[2]A total of 16 different parent PAHs were measured, so Other represents 15 PAHs had ND concentrations.
[3]ND = 1.0 ng/g.

ple, in 2011 all individuals had < 5 ng/g, and in 2012 eight individuals had < 5 ng/g and five between 5-10 ng/g. Anthracene was the only parent PAH detected in 2011 and 2012 blood samples (Table 1).

Alkyl PAH concentrations (26.86 ± 8.86 ng/g) were significantly higher than parent PAH levels (4.82 ± 0.36 ng/g) ($t = 9.91$, $P < 0.01$). Three alkyl PAH compounds were detected in decreasing order of frequency: 1) 2-methyl naphthalene (88.9%); 2) 3-methyl phenanthrene (22.2%); and 3) 9-methyl phenanthrene (11.1%) (Table 2). Two individuals had slightly elevated levels (> 20 ng/g) and one loon measured fairly high levels (> 90 ng/g) (Table 2) relative to the others analyzed.

With the exception of one individual, all adult loons captured were generally within satisfactory health parameters. Conversely, most captured subadult loons showed one or more anomalies indicative of health issues (e.g., depleted pectoral muscle mass, lower than average weight, poor feather condition). A total of six loons (15.8%, $n = 38$) showed some evidence of external oiling, but the great majority of them did not (84.2%). Four of the six individuals (66.6%) had trace amounts of oil and two individuals had light levels (33.3%). The oil was located on the feet (6/6; 100%), tail (2/6; 33.3%) and belly (1/6; 16.6%). Presence of oil on captured loons was found on four individuals (23.5%, $n = 17$) in 2011 and two individuals (9.5%, $n = 21$) in 2012.

DISCUSSION

The GOM is a reservoir of underwater oil. It harbors > 30,000 oil rigs throughout and approximately 3,000 rigs are off coastal Louisiana (McClain 2010). The reservoir has natural seeps (Atlas and Hazen 2011), which results in a certain background level of petroleum (and PAH) contamination likely to have existed in the GOM marine environment prior to the DHOS. We did not sample loon blood prior to the DHOS so comparing pre-and post PAH concentrations in loons

Table 2. Concentrations of total alkyl polycyclic aromatic hydrocarbons (PAHs) in plasma from Common Loons wintering off the Louisiana coast, January-March 2012. ND = non-detectable.

Age	Total Alkyl PAH	2-Methyl Naphthalene	2,6-Dimethyl Naphthalene	3-Methyl Phenanthrene	9-Methyl Phenanthrene	1,7-Dimethyl Phenanthrene
Adult	18.4[1]	18.4	ND	ND	ND	ND
Adult	16.3	16.3	ND	ND	ND	ND
Adult	15.7	15.7	ND	ND	ND	ND
Adult	12.6	12.6	ND	ND	ND	ND
Adult	ND[2]	ND	ND	ND	ND	ND
Immature	91.8	21.0	ND	35.7	35.1	ND
Immature	31.9	ND	ND	31.9	ND	ND
Immature	24.3	24.3	ND	ND	ND	ND
Immature	17.3	17.3	ND	ND	ND	ND
Immature	13.4	13.4	ND	ND	ND	ND
Immature	ND	ND	ND	ND	ND	ND
Immature	ND	ND	ND	ND	ND	ND
Immature	ND	ND	ND	ND	ND	ND
Immature	ND	ND	ND	ND	ND	ND
Immature	ND	ND	ND	ND	ND	ND
Immature	ND	ND	ND	ND	ND	ND
Immature	ND	ND	ND	ND	ND	ND
Immature	ND	ND	ND	ND	ND	ND
Immature	ND	ND	ND	ND	ND	ND
Immature	ND	ND	ND	ND	ND	ND
Immature	ND	ND	ND	ND	ND	ND
Immature	ND	ND	ND	ND	ND	ND

[1]Concentrations listed are ng/g (ppb).
[2]ND = 1.0 ng/g (ppb).

was not possible. In addition, we were unable to establish background PAH levels in loons wintering outside the study area. Still, by comparing PAH levels in loons between years, we saw an increase in frequency and concentration level in 2012 compared to 2011. Polycyclic aromatic hydrocarbons can move through the food web, including presence in local finfish species (Xia *et al.* 2012), but they do not typically bioaccumulate in birds (Albers 2006). Loons could have acquired PAHs through consumption of contaminated prey (Allan *et al.* 2012) or ingestion through preening. Incorporation of oil products through trophic processes has been documented for seabirds after a large oil spill (Esler *et al.* 2002). It appears loons in our study area were exposed to more PAHs 21-23 months after the DHOS compared to 9-11 months afterwards. Allan *et al.* (2012) assessed free PAHs in GOM coastal waters in four states, pre- and post DHOS. In Louisiana, they found a significant increase in PAHs 3-4 months after the DHOS, but a return to pre-spill PAH levels 1 year later. Thus, it was surprising to observe an increase in frequency of loons with detectable PAH levels in 2012 compared to 2011. It is possible there was a year lag time for the movement of PAHs through the food chain. Another possibility is that Hurricane Isaac, which hit southeast Louisiana between the two sampling periods (29 August 2012; ~16 months after DHOS event), stirred up and remobilized oil that had settled in the sediments (tar balls had been observed at many locations, J. Paruk, pers. obs.).

There are few data on blood/plasma PAH levels in wild birds following a major oil spill. In Europe, blood PAH concentrations were determined for Yellow-legged Gulls (*Larus michahellis*) shortly after the largest oil spill in Europe. In 2002, the *Prestige* oil tanker sank off the Galencia Coast, releasing over 60,000 tonnes of oil equivalent. Pérez *et al.* (2005) investigated blood PAH concentrations in Yellow-legged Gulls at three unoiled and four oiled sites along Spain and Portugal coastlines. Parent PAH concentrations were significantly higher in gulls at the oiled (136-228 ng/g) vs. unoiled (72-101 ng/g) sites, but concentrations at the unoiled site were higher than what we observed in loons at our contaminated site. The lower-than-expected plasma PAH concentrations in loons after DHOS may be explained in several ways. First, the well exuded Macondo oil, a light crude with a higher proportion of simple lower molecular weight hydrocarbons and, therefore, inherently more biodegradable than the heavy crude oil released from the *Prestige* and the Exxon *Valdez* (Atlas and Hazen 2011). Second, further biodegradation of PAHs may have been assisted by the enormous amounts (6,500 tonnes) of dispersant (Corexit) that served to increase the surface area of the oil and potentially increase its biodegradation (Atlas and Hazen 2011). Third, the GOM is known for harboring pre-existing microbial communities that biodegrade oil, and a large cloud of microbes was detected moving in response to the oil (Atlas and Hazen 2011). Fourth, it has been suggested that food webs are often comprised of multiple compartments or pathways, some of which may not be linked (Pimm and Lawton 1980). This is thought to be true in species-rich regions such as the northern GOM and has been previously suggested as the reason for a lack of clear trophic cascades in tropical ecosystems (Pace *et al.* 1999). Lastly, Yellow-legged Gulls are residents in southern Europe and would be exposed to oil year-round, instead of a few months, like the winter migrant Common Loon.

Interspecies comparisons of blood PAH concentrations, such as between loons and gulls, are difficult to make and challenging to interpret. More data are needed from other species and other situations (e.g., different spill environments and oil type, uncontaminated sites) before comparisons can be better interpreted. What remains unknown is the PAH threshold level or levels where significant impacts can be observed, or expected, in wild birds. Alonso-Alvarez *et al.* (2007b) showed sublethal toxic effects, such as differences in blood parameters (glucose and inorganic phosphorus levels), that suggested liver and kidney damage on gulls from the oiled site 17 months after the oil spill.

Although loon blood PAH concentrations in Louisiana were lower than those found in Yellow-legged Gulls, it is unknown if they are high enough to cause sublethal effects. Even low levels of PAHs can have sublethal effects, such as impairment of both immune and osmoregulatory functions, on birds (Albers 2003). The biological consequences of the DHOS are unknown for most avian residents and migrants, but may be more severe for migrants (Henkel *et al.* 2012). Whitehead *et al.* (2011) found that oil-contaminated waters in coastal Louisiana marshes affected gene expression and gill immunochemistry in resident Gulf killifish (*Fundulis grandis*). Loons exhibit strong winter site fidelity (D. Long and J. D. Paruk, unpubl. data) and inhabit these areas for a minimum of 4 months (mid-November to mid/late March). As a result, these individuals are exposed annually to PAHs and other local contaminants in the northern GOM for extended periods of time.

More loons die during the winter in marine environments than the summer in freshwater environments (Evers 2007). Organisms in marine environments face both physiological and environmental stressors that they do not face on the breeding grounds (Murphy and King 1992). For example, the leading cause of loon winter mortality is likely emaciation syndrome, which may be directly linked to these stressors (Alexander 1991; Spitzer 1995; Forrester *et al.* 1997; Evers 2007). In addition, loons undergo a synchronous wing molt mid-winter, which can be energetically expensive, requiring additional nutritional needs and higher than normal metabolic rates (Howell 2010). Energy reserves are needed to meet this increased demand and, if in short supply, may compromise health and lead to emaciation syndrome. Loons wintering in coastal Louisiana waters are also long-distant migrants, coming as far as Saskatchewan, Canada (Paruk *et al.* 2014), so health and energy reserves are likely at a premium, and additional stressors such as PAH exposure may compromise loon health and survivorship. Indirect effects of oil spills on birds are difficult to quantify, but necessary and important to better understand the potential long-term effects on survivorship and reproduction, especially in migrant species. To effectively evaluate the potential long-term effects of the DHOS on loon survivorship and reproduction, we recommend the following: 1) link wintering and breeding locations; 2) evaluate and measure potential reproductive impacts on the breeding grounds; and 3) assess winter health of loons in the northern GOM with a control area.

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