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Review

Effects of petroleum exposure on birds: A review

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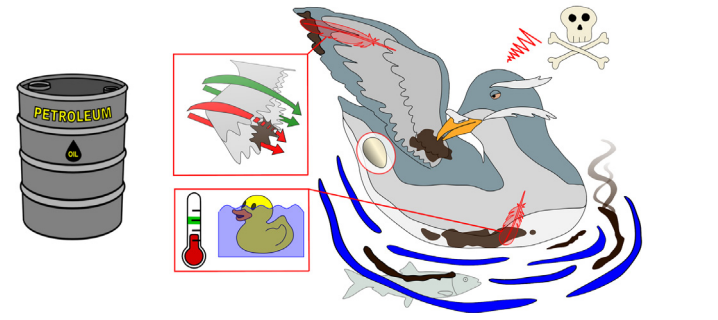
HIGHLIGHTS

- Oil disrupts feather structure, affecting function.
- Toxicity may result from ingestion, inhalation, or egg oiling.
- Environmentally relevant exposure rate estimates are needed, especially ingestion.
- Toxicity data for unconventional crude oil (e.g. oil sands bitumen) is needed.
- We propose best practices and methods to improve the quality of future studies.

GRAPHICAL ABSTRACT



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ABSTRACT

Birds are vulnerable to petroleum pollution, and exposure has a range of negative effects resulting from plumage fouling, systemic toxicity, and embryotoxicity. Recent research has not been synthesized since Leighton's 1993 review despite the continued discharge of conventional petroleum, including high-volume oil spills and chronic oil pollution, as well as the emergence of understudied unconventional crude oil types. To address this, we reviewed the individual-level effects of crude oil and refined fuel exposure in avifauna with peer-reviewed articles published 1993–2020 to provide a critical synthesis of the state of the science. We also sought to answer how unconventional crude petroleum effects compare with conventional crude oil. Relevant knowledge gaps and research challenges were identified. The resulting review examines avian exposure to petroleum and synthesizes advances regarding the physical effects of oil hydrocarbons on feather structure and function, as well the toxic effects of inhaled or ingested oil, embryotoxicity, and how exposure affects broader scale endpoints related to behavior, reproduction, and survival. Another outcome of the review was the knowledge gaps and challenges identified. The first finding was a paucity of oil ingestion rate estimates in birds. Characterizing environmentally realistic exposure and ingestion rates is a higher research priority than additional conventional oral dosing experiments. Second, there is an absence of toxicity data for unconventional crude petroleum. Although the effects of air and water contamination in the Canadian oil sands region have received attention, toxicity data for direct exposure to unrefined bitumen produced there in high volumes and other such unconventional oil types are needed. Third, we encountered barriers to the interpretation, replication, broad relevance, and comparability of studies. We therefore propose best practices and promising technological advancements for researchers. This review consolidates our understanding of petroleum's effects on birds and points a way forward for researchers and resource managers.

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Contents

1. Introduction

The harmful effects of petroleum exposure on birds have long been recognized and have been the subject of concerted research since the 1960s (Hartung, 1963). The resulting literature is voluminous and punctuated by waves of studies following large, high-profile spills such as the *Exxon Valdez*, *Prestige*, and *Deepwater Horizon* disasters (Table 1). Nonetheless, the continued economic importance of petroleum, the variable and complex chemical composition of hydrocarbon mixtures, and the economically-driven development of unconventional sources such as oil sands bitumen in Canada (Fig. 1) suggests that petroleum toxicity to marine birds will remain an important research subject (CAPP, 2017; Green et al., 2017; NEB, 2016a).

Petroleum has a broad range of harmful effects on birds which include the physical fouling of plumage, toxicity of ingested petroleum, and embryotoxicity (Leighton, 1993). Such effects can result from petroleum quantities as small as a few milliliters on the feathers or a few microliters on the eggshell (Leighton, 1993; Morandin and O'Hara, 2016). A number of older reviews in addition to Leighton's (1993) provide useful perspectives on avian petroleum toxicity and a wealth of references, as do a number of more recent reviews on closely related topics (Table 2). Despite that, a comprehensive summary of known toxicological effects of petroleum on birds has not been published since Leighton's (1993) seminal review 27 years ago, where he concluded:

> "It is difficult to draw general conclusions from the diverse, and often conflicting, results of the many studies of the effects of ingested petroleum oils. This difficulty makes the attempt all the more important,"

After the intervening decades of research, environmental scientists and resource managers would benefit from an updated overview of the effects of petroleum on birds.

This literature review provides a critical narrative synthesis of petroleum pollution in the environment and avian exposure (Section 2), the immediate harmful effects of crude and refined petroleum on avifauna (Section 3), longer-term and ecologically mediated effects that may emerge (Section 4), and finally an exploration of knowledge gaps, challenges, and how to address them (Section 5). On those respective themes we specifically synthesize exposure rate estimates (Section 2.3), indications of exposure (Section 2.4), and effects. Immediate effects include the physical effects of oil on feathers (Section 3.1), the toxic effects of oils (Section 3.2), the consequences of both, and

Table 1
Scientific highlights from research conducted since 1993 on the effects of petroleum exposure on birds.

Topic	Highlight	Reference(s)	Spill[a]
Reproduction	Reproduction study on the breeding grounds of an oil-impacted population	(Field et al., 1993)	*EV*
Immunotoxicity	Avian immunotoxicity demonstrated for a PAC[b]	(Trust et al., 1994)	Lab
Electrolytes & Reproduction	Reduced circulating calcium and eggshell thinning from ingested oil	(Stubblefield et al., 1995c)	*EV*
Reproductive hormones	Reproductive hormone effects in penguins with crude oil-contaminated plumage	(Fowler et al., 1995)	NI
Thermal and metabolic balance	Evidence of metabolic starvation in a wild seabird	(Oka and Okuyama, 2000)	NI
Behavior	Feeding and maintenance activity effects in birds rehabilitated after becoming oiled	(Anderson et al., 2000)	*UP*
Long-term survival	Decreased survival in birds overwintering in oil spill-impacted areas	(Esler et al., 2000b)	*EV*
Reproduction	Low quality chick diet in oil spill-impacted areas	(Golet et al., 2002)	*EV*
Reproduction	Reproductive success reduced by poor prey availability after oil spill	(Velando et al., 2005b)	*P*
Survival & Reproduction	Age- and sex-specific oil spill mortality projected to impair population recovery	(Martínez-Abraín et al., 2006)	*P*
Metabolism	Decreased blood glucose in oil-fed wild birds	(Alonso-Alvarez et al., 2007b)	*P*
Neurotoxicity	Depressed brain acetylcholinesterase activity in wild birds oiled at sea	(Oropesa et al., 2007)	*P*
Lungs & Immunotoxicity	Immunotoxicity from inhaled BTEX[c]	(Olsgard et al., 2008)	Lab
Behavior	Habitat use affected in oil spill-impacted area	(Banks et al., 2008)	*SE*
Reproduction & Survival	Evidence of increased cost of reproduction in oiled and rehabilitated birds	(Wolfaardt et al., 2008a)	*AS*
Feather structure	μm thin oil-on-water films disrupted seabird feather microstructure	(O'Hara and Morandin, 2010)	Lab
Oxidative balance & Reproduction	Ingested oil affected plasma antioxidant balance and carotenoid-based sexual ornamentation	(Pérez et al., 2010a)	*P*
Metabolism	Reduced hepatic fatty acid oxidation in embryos dosed with PACs[b] *in ovo*	(Westman et al., 2013)	Lab
Stress hormones	Dietary oil exposure decreased corticosterone secretion in response to an acute stressor	(Lattin et al., 2014)	*DH*
Exposure	EROD[d] activity indicated exposure to residual oil up to 22 years post-spill	(Esler et al., 2017)	*EV*
Flight performance	Migratory fight performance effects from feather oiling in a long-distance migrant	(Maggini et al., 2017c)	*DH*
Gastrointestinal damage	Experimental evidence of gastrointestinal damage from ingested oil	(Harr et al., 2017a, 2017b)	*DH*
Oxidative balance	Ingested oil affected hepatic oxidative balance	(Pritsos et al., 2017)	*DH*
Haemotoxicity	Hemolytic injury in spill-impacted wild birds	(Fallon et al., 2017)	*DH*
Cardiotoxicity	Cardiac function affected in oiled birds	(Harr et al., 2017c)	*DH*
Exposure	*Cyp1a5*[e] gene expression showed oil exposure in a passerine	(Perez-Umphrey et al., 2018)	*DH*
Lungs	Inhaled BTEX[c] induced pulmonary CYP1A[f] protein	(Dubansky et al., 2018)	*DH*
Reproduction	Ingested PACs affected pre-migratory mass gain dynamics in a long-distance migrant	(Bianchini and Morrissey, 2018a)	Lab
Metabolism	Targeted metabolomic analysis showed effects on fatty acid and amino acid metabolism from external oiling	(Dorr et al., 2019)	*DH*

[a] Oil spill events: *Exxon Valdez* (*EV*) crude oil spill, *Unocal pipeline* (*UP*) crude oil spill, *Prestige* (*P*) fuel oil spill, *Sea Empress* (*SE*) crude oil spill, *Apollo Sea* (*AS*) heavy fuel oil spill, *Deepwater Horizon* (*DH*) crude oil spill, Not identified (NI), or laboratory exposure (Lab).
[b] Polycyclic aromatic compound (PAC).
[c] Volatile monoaromatics that include benzene, toluene, ethylbenzene, and xylene (BTEX).
[d] Ethoxyresorufin-O-deethylase enzyme (EROD) activity, a biomarker of planar hydrocarbon exposure.
[e] The second of two main avian cytochrome P450 1A genes (*Cyp1a5*), inducible by planar hydrocarbon exposure.
[f] Cytochrome P450 1A protein CYP1A, a biomarker of planar hydrocarbon exposure.

embryotoxicity (Section 3.3). Emergent consequences at the individual level relate to behavior (Section 4.1), reproduction (Section 4.2), and survival (Section 4.3). The highest priority knowledge gaps for future research to address include, first, a paucity of environmentally relevant exposure data (Section 5.1), and secondly, a need for toxicity data for unconventional petroleum such as oil sands bitumen and other unconventional oil types (Section 5.2). Lastly, we identify specific research challenges and common shortcomings regarding study design and reporting for which we propose solutions to improve research (Section 5.3).

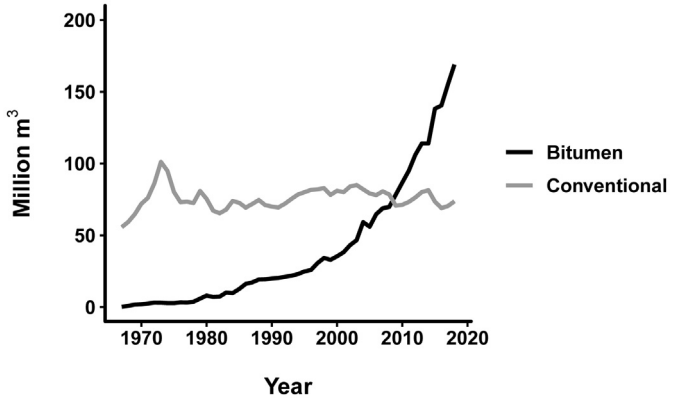

Fig. 1. Annual production (million m^3) of conventional crude petroleum (grey) and bituminous oil sands crude petroleum (black) in Canada from 1967 to 2018.
Data source: Canadian Association of Petroleum Producers (CAPP, 2020).

1.1. Methods

To review the effects of crude and refined petroleum exposure in avifauna, including all manner of study designs and individual level endpoints up to survival and reproduction, we conducted a systematized review (Grant and Booth, 2009). We searched for research articles in two databases, Web of Science and Academic Search Premiere. We iteratively optimized search terms until searches returned the maximum number of relevant hits that could reasonably be reviewed (hundreds to <3000 hits). The presence of highly relevant and often cited works among the search results were judged to be a good indicator of search terms that worked well. After optimization, we searched for peer-reviewed English language journal articles published 1993–2020 (inclusive) returned with the search terms by topic (petroleum OR fuel OR hydrocarbon* OR "oil spill" OR bitumen OR "crude oil") AND (avian OR *bird* OR *fowl*). A search on 1 April 2020 yielded 2290 hits in Web of Science and 1574 hits in Academic Search Premiere. We downloaded these search results, combined hits from the two databases, removed duplicates, and scored hits for relevance based on title and abstract as unrelated (zero), not relevant (one), somewhat relevant (two), and very relevant (three). We read and reviewed all articles with a score of two or three (83 and 101 articles, respectively). From the articles reviewed, we added relevant citations therein that met the above criteria and reviewed these as well (i.e. snowball method; 30 articles). While no peer-reviewed articles from this search were screened out a

Table 2
Peer-reviewed journal review articles since 1993 related to the topic of petroleum effects and toxicity in avifauna.

Content relevant to petroleum effects on avifauna	References
Petroleum pollution effects on birds of the northeast Pacific Ocean	(Burger and Fry, 1993)
Toxic and physical effects of petroleum on birds	(Leighton, 1993)
Thermoregulatory and metabolic effects of petroleum, as well as dispersants, and bird cleaning	(Jenssen, 1994)[a]
The fate and effects of the *Exxon Valdez* oil spill on birds	(Hartung, 1995; Stubblefield et al., 1995a; Wiens, 1995)[a]
Effects of contaminants on avian reproduction, including petroleum	(Fry, 1995)[a]
Possible mechanisms for petroleum immunotoxicity in birds	(Briggs et al., 1996, 1997)[a]
Beached bird surveys as a tool for monitoring chronic oil pollution	(Camphuysen and Heubeck, 2001)[b]
Avian endocrine disruption from contaminants, including petroleum	(Scanes and McNabb, 2003)[a]
Avian immunotoxicity from contaminants, including petroleum	(Fairbrother et al., 2004)
Effects of PACs[c] in avifauna	(Albers, 2006)[a]
Oil spill effects and human intervention in two species of marine birds	(Wolfaardt et al., 2009)[a]
Ecological and physiological effects of petroleum relevant to terrestrial vertebrates affected by shore-cast crude petroleum from the *Deepwater Horizon* oil spill	(Bergeon Burns et al., 2014)[b]
Avian toxicity of oil sands process-affected water	(Beck et al., 2015)[a]
Aquatic toxicity of diluted bitumen	(Dew et al., 2015)
Thyroid toxicity associated with petroleum in mammals and fish	(Fowles et al., 2016)[a]
Risks and effects of operational oil and gas discharge to marine birds	(Morandin and O'Hara, 2016)[a]
Avian toxicity studies conducted under the *Deepwater Horizon* Natural Resource Damage Assessment process	(Bursian et al., 2017a)
Wildlife population effects of the *Exxon Valdez* spill, their cause, and recovery status	(Esler et al., 2018)[a]

[a] Returned by database search (see methods).
[b] Cited by article in database search (see methods).
[c] polycyclic aromatic compounds' to make the meaning of the acronym clear.

priori based on quality criteria, any quality issues germane to the interpretation of results are dealt with in our narrative review and the findings given more or less weight according to the quality of the study in our synthesis. Where interpreting recent findings (since 1993) required consulting older articles and weighing their evidence, we primarily used sources in Leighton's (1993) review and cite them.

Petroleum doses are reported in units that differ from one study to another. To facilitate comparison among studies, we provide doses in milliliters per kilogram body weight (ml kg^{-1} bw), or for multiday and dietary studies in milliliters per kilogram body weight per day (ml kg^{-1} bw d^{-1}). Those rates are given on a body mass basis, so unfamiliar readers may more intuitively visualize the amount of petroleum in relation to a typical duck (e.g. *Anas platyrhynchos*) weighing approximately one kilogram where we discuss exposure rates. In many cases, conversions and estimates were necessary. In order to estimate the doses (as in Hartung, 1995), we based our conversions on several factors: petroleum dose per day, petroleum density, and bird body mass as given in the study. If not reported, we substituted the average body mass from the Birds of the World online database (Cornell Lab of Ornithology, 2020), petroleum density of 0.9 grams per milliliter, and a feeding rate of 10% body weight per day following Hartung (1995). Where external oiling was reported, we provide the percent surface area (SA) of the bird affected, and, where important to the context, the amount of petroleum in milliliters per kilogram body weight (ml kg^{-1} bw) if possible.

2. Petroleum in the environment and avian exposure

2.1. Petroleum is a broad class of complex hydrocarbon mixtures

The fact that petroleum encompasses a broad and heterogeneous class of hydrocarbon-based substances, typically mixtures, cannot be overemphasized. It is their shared chemical and physical properties that make types of petroleum comparable in a general sense, yet particular differences in properties and composition affect movement, fate, and toxicity in the environment (reviewed in Dupuis and Ucan-Marin, 2015; Lee et al., 2015). Crude oil is refined into various fractions and transformed into a wide array of products. This review primarily considers information about crude oil and refined fuels which are produced, transported, and used in the highest volumes, and therefore, most often discharged into the environment. For crude oil, physical and chemical properties vary by geological origin, production methods, and in some cases upgrading methods to create a more easily marketed or transported material. Fuels and especially crude oils typically contain thousands of compounds, but from a compositional perspective, the four main chemical classes of hydrocarbons in petroleum are saturates, aromatics, resins, and asphaltenes. From a toxicity perspective, the major classes of concern in vertebrates are naphthenic acids, metals, aliphatics, and aromatics, the latter being most associated with toxicity.

The aromatic fraction of petroleum is primarily implicated in avian toxicity (Albers, 2006; Gentes et al., 2007b; reviewed in Leighton, 1993; Walters et al., 1987). Aromatics include monoaromatic compounds like benzene, toluene, ethylbenzene, and xylene (BTEX), as well as polycyclic aromatic compounds (PACs) such as parent polycyclic aromatic hydrocarbons (PAHs), alkylated PAHs (alkyl-PAHs), and heterocycles of nitrogen, oxygen, and sulfur. Though petroleum studies have traditionally focused on parent PAHs, we subsume PAHs, alkyl-PAHs, and aromatic heterocycles within the more inclusive term PAC (reviewed in Achten and Andersson, 2015; Andersson and Achten, 2015). Not only must petroleum be considered as a complex mixture, but once released into the environment its composition is changed over time by interactions with air, water, temperature, light, and microbial activity, a process called weathering.

2.2. Oil sources, weathering, fate, and risk to birds

Human activity is a major source of petroleum to the environment. Roughly half the petroleum entering the marine environment comes from natural seeps on the seafloor, the other half from human sources related to oil and gas production, petroleum transportation, and consumption (Kvenvolden and Cooper, 2003; NRC, 2003). Accidents and conflict can result in large-scale, often well-publicized, spills from pipelines (Dew et al., 2015; McCrary et al., 2003), train tank cars (Dupuis and Ucan-Marin, 2015), wells (Bursian et al., 2017a; Evans et al., 1993), shipwrecks, especially tanker wrecks (Burger and Fry, 1993; Wolfaardt et al., 2009), and salvage (Camphuysen and Leopold, 2004) which impact birds. Though they seldom receive public attention, relatively small but regular petroleum discharges, known as chronic oil pollution, are documented in marine waters and can be an important source of avian mortality as well (Boersma, 1986; Camphuysen and Heubeck, 2001; García-Borboroglu et al., 2006; O'Hara and Morgan, 2006; Wiese and Robertson, 2004; Wilhelm et al., 2009). Sources of chronic petroleum discharge include oil and gas production (McCrary et al., 2003; Morandin and O'Hara, 2016; Rattner et al., 1995; Ronconi et al., 2015), vessel traffic and shipping, especially oil tanker shipping (tank cleaning particularly, see Hampton et al., 2003b; Bertazzon et al., 2014; Camphuysen, 2010; Dahlmann et al., 1994; García-Borboroglu et al., 2006; Lucas and MacGregor, 2006; O'Hara et al., 2009; O'Hara and Morgan, 2006; Wiese and Ryan, 2003), and submerged wrecks (Hampton et al., 2003a; Henkel et al., 2014b). Even where chronic pollution is low, natural seeps can constitute an import source of crude oil to birds (Henkel et al., 2014b).

At local and regional scales, the amount and dominant sources of chronic oil pollution likely vary. Tankers often carry loads of crude oil or refined petroleum products, whereas fuel oil (often called bunker fuel) normally powers larger ships; smaller vessels like fishing and pleasure craft use refined fuels such as diesel and gasoline (reviewed in Burger and Fry, 1993). A single shipwreck can release different petroleum types such as heavy fuel oil, diesel, and lubricating oil in various amounts (Crawford et al., 2000).

For crude oil that enters the environment, types and sources are increasingly associated with the production of petroleum reserves considered unconventional. Unconventional is a general term that refers to crude petroleum reservoirs of a less desirable quality or requiring a departure from conventional drilling methods to extract from geological formations (Zee Ma, 2016). Examples of unconventional oil resources are heavy crude oil, shale oil, and oil sands. The Canadian oil sands are the third largest known oil reserve in the world (NRC, 2017). It serves as a useful example of an unconventional bituminous crude oil resource that has been exploited for many years, has a massive footprint and production volume (Fig. 1), and has been studied for its impact to wildlife (reviewed in Beck et al., 2015; Dew et al., 2015). The properties of unconventional crude oil products differ from one another and conventional crude oil, and, therefore, their behavior in the environment and toxicity is potentially different.

When petroleum enters the environment, its composition and movement is dynamic over time as the oil weathers and is carried by water. Petroleum's hydrophobic nature and the adhesive properties of certain oils when weathered cause it to adsorb or adhere to solid surfaces. Albers (2003) and Lee et al. (2015) reviewed the transport and fate of petroleum in aqueous environments, which is highly relevant to birds which float on, swim in, and dive through water (Fig. 2). Initially, unweathered oil is less dense than water and under most conditions floats as a film on the water surface. The action of wind, waves, and surface currents spreads and thins the oil. During the first hours to days following release, low molecular weight compounds evaporate into the air and soluble components dissolve into the water (reviewed in Morandin and O'Hara, 2016). Sunlight photoxidizes petroleum and microbes actively biodegrade it. Together, these processes change the composition, texture, and density of oil over time, and importantly change the types of toxic compounds present through their loss, concentration (e.g. PACs) (Stubblefield et al., 1995a), or degradation. Energy from wind or waves, or the presence of suspended sediments, may entrain floating oil below the surface to form an oil-in-water emulsion. The fraction of petroleum that does not evaporate or dissolve typically sinks or is washed up on beaches. During these processes, from discharge to eventual fate, petroleum slicks (>3 μm) and sheens (≤3 μm) (Morandin and O'Hara, 2016) on the water's surface are considered most hazardous to avifauna in the short-term, while sediment-bound petroleum is thought to be the source of long-term exposure to spilled oil after the surface film dissipates (Day et al., 1995; reviewed in Esler et al., 2018; Paruk et al., 2016; Perez-Umphrey et al., 2018).

Petroleum discharge into the environment tends to occur where there is intensive maritime or industrial activity (e.g. oil sands), and areas of high petroleum spill risk that overlap with critical avian habitat is where birds are most at risk of exposure (Bertazzon et al., 2014; reviewed in Burger and Fry, 1993; Fox et al., 2016; Morandin and O'Hara, 2016). Oil spill volume correlates weakly with the number of oiled birds observed, and for spills of comparable volume, associated mortality estimates can vary enormously (Burger and Fry, 1993;

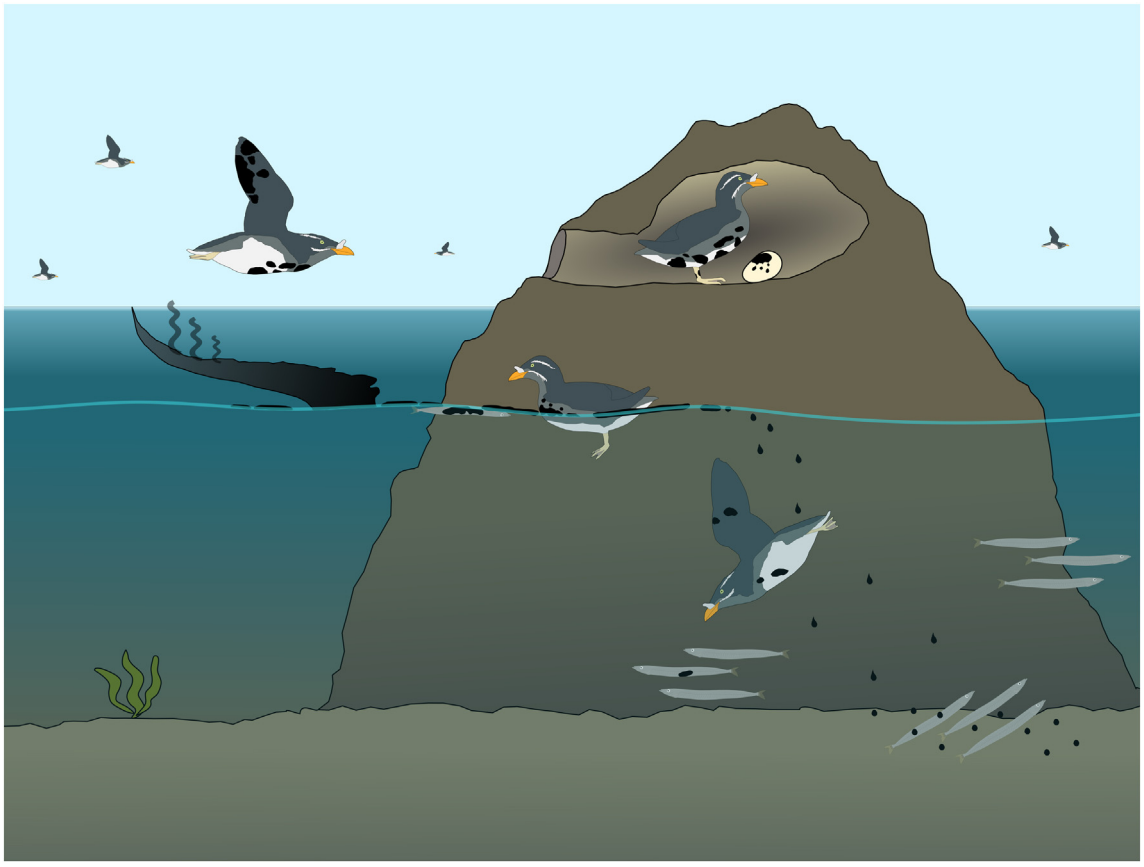

Fig. 2. Illustrated here in a marine bird, the most important effects of spilled petroleum on birds can include embryotoxicity in oiled eggs, compromised function of oiled feathers for flight performance, swimming performance, buoyancy, and thermoregulation, ingestion of petroleum via contaminated feathers and prey, and inhalation of volatile petroleum components. Indirectly, reduced prey availability can affect habitat use and chick provisioning.

Hampton et al., 2003b). This is because the proximity of a spill to feeding or breeding habitat, the type of oil, amount, time of year, duration, and the composition of the avian community can all affect the number and kinds of birds affected (Camphuysen, 2011; Day et al., 1995; Esler et al., 2018; Wiens, 1995). Oil spill management response and intervention to protect wildlife can also be a factor (e.g. Wolfaardt et al., 2009). Wildlife and spill response professionals have sought to score the vulnerability of individual bird species based on ecological factors and conservation concerns (Chilvers and Battley, 2019; King and Sanger, 1979; Romero et al., 2018; Williams et al., 1995), although ecological factors (e.g. feeding characteristics, migration) may not always predict oil spill impacts such as on habitat use for a given species (Day et al., 1995). Several studies using beached bird survey and aerial surveillance data point to regional decreases in mortality from chronic oil pollution at sea from improvements in environmental regulation, monitoring, and enforcement (reviewed in Camphuysen and Heubeck, 2001; Camphuysen, 1998, 2010, 2011; Larsen et al., 2007; O'Hara et al., 2009; Stienen et al., 2017; Wilhelm et al., 2009), though gaps and limitations may persist such as oil pollution from offshore shipping and smaller vessels (Bertazzon et al., 2014; Camphuysen, 2010; Hampton et al., 2003b; O'Hara et al., 2009).

Beyond bulk petroleum itself, industrial activity related to petroleum extraction and processing can also discharge petrogenic (i.e. oil source) contaminants into the water and air (Beck et al., 2015; Custer et al., 2000, 2001; King et al., 1987; Morandin and O'Hara, 2016). In particular, unconventional oil is often extracted by surface mining and industrial processes which produce massive volumes of tailings, tailings water, and air emissions. Oil sands production separates bitumen from water and other mineral particles (e.g. sand), generating tailings in the form of sediment and water, referred to as oil sands process-affected material or oil sands process affected-water, respectively (reviewed in Beck et al., 2015). Those terms refer to inherently variable classes of tailings that potentially contain residual bitumen itself or associated toxic compounds concentrated above background levels in the sediment or water phase (PACs, monoaromatics, metals, salts, and naphthenic acids) (reviewed in Beck et al., 2015; Dew et al., 2015). Thus, oil sands industrial activity is a source of airborne petrogenic PACs on a local scale (Cruz-Martinez et al., 2015a; Fernie et al., 2018b; Mundy et al., 2019) and may be a source of mercury and PAC contamination on a broader regional scale (Hebert, 2019; Hebert et al., 2011, 2013; Paruk et al., 2018). PAC concentrations in environments near oil sands industrial activity may be dominated by alkylated PAC and heterocycle PAC congeners rather than parent PAHs (Cruz-Martinez et al., 2015a; Fernie et al., 2018b; Mundy et al., 2019), so analyses of parent PAH compounds can underestimate exposure.

A common strategy to mitigate petroleum spilled on the water's surface is to apply chemical dispersants. These are chemical formulations (e.g. Corexit 9500, Corexit 9527) designed to act as a combination surfactant and solvent to emulsify oil films into droplets which more readily disperse, dissolve, and evaporate (Dave and Ghaly, 2011; Dupuis and Ucan-Marin, 2015; Fiocco and Lewis, 1999; Lee et al., 2015; McDougall and DeVink, 2020; Peakall et al., 1987). Dispersants thus alter the fate, and potentially the effects, of spilled oil. For instance, while successful dispersion of a surface slick could prevent oil from fouling a bird's plumage, subsurface organisms on which birds may depend could suffer greater impact from toxicity. Furthermore, dispersants may be applied to large petroleum spills in vast quantities and may modify the effects of oil on birds or have adverse effects of their own, including on feather function and toxicity (Finch et al., 2012; Jenssen, 1994; Peakall et al., 1987; Rocke et al., 1984; Stroski et al., 2019; Whitmer et al., 2018; Wooten et al., 2012). A discursive consideration of the effects of common dispersants and oil-dispersant-mixtures that may result from their use is beyond the scope of this review and given elsewhere (e.g. Jenssen, 1994; McDougall and DeVink, 2020; Peakall et al., 1987), so we focus on effects attributed primarily to petroleum.

2.3. Exposure routes, rates, and duration

Birds can be exposed (Fig. 2) directly to bulk petroleum via external oiling and feeding, and petroleum-derived contaminants may be inhaled or trace amounts ingested with soil, sediment, water, or prey. External oiling may cause harmful physical effects. It occurs when birds come into contact with petroleum (e.g. floating on the water surface) and it adsorbs to their feathers in amounts that may range from trace amounts to completely covering the body. Petroleum has seldom (Cunningham et al., 2017) been documented to irritate the skin of birds, and there is little evidence that dermal exposure alone is appreciably toxic, although eye leisions may result from contact with oils (reviewed in Hartung, 1995; Fiorello et al., 2016). Importantly, external petroleum contamination on the feathers may be aspirated (Camphuysen and Leopold, 2004; reviewed in Hartung, 1995) or ingested while preening oil-fouled feathers (Hartung, 1963). Even birds without visible oil on the plumage may ingest oil (Balseiro et al., 2005; Fallon et al., 2017), which may be toxic. Birds may consume petroleum-contaminated grit (King and Bendell-Young, 2000), water (reviewed in Beck et al., 2015), and prey such as invertebrates, fish, and oiled birds (Bonisoli-Alquati et al., 2016; reviewed in Burger and Fry, 1993; Cunningham et al., 2017; reviewed in Esler et al., 2018; Horak et al., 2017; Perez-Umphrey et al., 2018; Rattner et al., 1995; Seegar et al., 2015; Velando et al., 2010; Zuberogoitia et al., 2006). Although it has usually been assumed that the inhalation risk of volatile petroleum hydrocarbons (e.g. BTEX) is minor because of their rapid volatilization, mixing, and dilution in the air (reviewed in Hartung, 1995), recent studies suggest that airborne concentrations associated with oil sands industrial activity and crude oil spills at sea may cause toxic effects (Cruz-Martinez et al., 2015a, 2015b; Dubansky et al., 2018; Olsgard et al., 2008, 2009). Dietary exposure to atmospherically deposited petrogenic PACs may be an important exposure route, though the potential for such comparatively low concentrations to cause adverse effects remains unclear (Cruz-Martinez et al., 2015a; Fernie et al., 2018b; Mundy et al., 2019).

There is little information on the extent and rate that birds may actually ingest petroleum, though available estimates suggest rates on the order of several ml kg^{-1} bw d^{-1} are possible if birds are both oiled themselves and feeding on contaminated prey. Hartung's (1963) studies in waterfowl indicated that captive American black ducks (*Anas rubripes*) oiled with 0.8 to 16.6 ml kg^{-1} bw of a radiolabeled carbon and mineral oil mixture removed half of the application by preening within 8 days, most of which was ingested. Assuming complete ingestion, the maximum rate of ingestion when preening is most rapid over the first day after exposure is 3.32 ml kg^{-1} bw d^{-1} (Appendix A: Exposure Estimate 1). Hartung (1995) later used hydrocarbon residue in bivalve mussels to estimate that ducks feeding on the most contaminated mussels during the height of the *Exxon Valdez* spill ingested 0.14 ml kg^{-1} bw d^{-1} (Appendix A: Exposure Estimate 2). It thus seems reasonable that a duck with moderately oiled plumage and feeding on heavily contaminated invertebrates might ingest up to 4 ml kg^{-1} bw d^{-1} over several days. Alternatively, avoidance of food which is evidently oil-contaminated by means of olfaction, taste, or experience of prior intoxication could mitigate dietary exposure to oil. Results here are mixed. Mallards (*Anas platyrhynchos*) given crude oil in the diet did not avoid up to 10% (*w*/*w*) petroleum in feed (9.5 ml kg^{-1} bw d^{-1}) (reviewed in Stubblefield et al., 1995a), although laughing gulls (*Leucophaeus atricilla*) given fish injected with weathered crude oil in this range were observed to reject the food if they sensed the oil (Horak et al., 2017). Unfortunately, little has been done to quantify environmentally realistic petroleum ingestion rates beyond Hartung's (1963, 1995) work.

The most severe risk of exposure to petroleum spilled into the environment occurs in the days and weeks immediately after a large spill, but low-level exposure to lingering hydrocarbons may continue in some cases from many months up to several decades (reviewed in

Esler et al., 2018; Perez-Umphrey et al., 2018; Velando et al., 2010). Exposure to residual crude oil hydrocarbons in sediment is documented to occur even up to 2 to 20 years after the initial spill, for instance, likely through invertebrate prey (Esler et al., 2010, 2011, 2017, 2018; Golet et al., 2002; Trust et al., 2000; Velando et al., 2010). Storms may resuspend petroleum buried in sediment from past spills (Paruk et al., 2016; Perez-Umphrey et al., 2018). Petroleum hydrocarbon exposure, especially in the absence of visibly oiled birds, is inferred from the analysis of chemical residue in tissue or biomarkers.

2.4. Indicating exposure and effects: tissue residue concentrations and biomarkers

Petroleum exposed birds may not always appear to be oiled, and the chemical analysis of petroleum compounds in avian tissues is important for understanding rates of exposure and kinetics within the body, and for inferring thresholds of potential harm. Ingested petroleum passes rapidly through the gastrointestinal tract, is incompletely absorbed, and rapidly metabolized by biotransformation systems within the body (Dean et al., 2017b; reviewed in Hartung, 1995). Absorption and kinetics *in vivo* vary widely among the constituent compounds in petroleum (Lawler et al., 1978a, 1978b; Pérez et al., 2008). The amount of tissue residue data available has improved over recent years (reviewed in Leighton, 1993).

PACs are both implicated in petroleum toxicity and often detectable in the tissue of oil exposed birds. Wild birds may be regularly exposed to some background of pyrogenic- (i.e. combustion) (Custer et al., 2001; Vidal et al., 2011) and petrogenic- (i.e. oils) source PACs (Kayal and Connell, 1995; Pereira et al., 2009). Carbon isotope analysis (^{14}C) may help ascribe petrogenic origin to trace hydrocarbon contaminants found in tissue (Bonisoli-Alquati et al., 2016). Ingestion of oil can cause exposure to concentrated PACs on top of some shifting baseline of more chronic, low-level exposure in the diet (Custer et al., 2000; King et al., 1987; Michot et al., 1994; Miles et al., 2007; Willie et al., 2017). PAC analyses have historically focused on parent PAHs, though the recognition of the environmental and toxicological importance of alkyl-PAH and heterocycle congeners is increasingly recognized (reviewed in Achten and Andersson, 2015; Cruz-Martinez et al., 2015a; Fernie et al., 2018a; Mundy et al., 2019). Oiled seabirds can have quantifiable PAC concentrations in plasma and liver (Troisi et al., 2006, 2007, 2016; Troisi and Borjesson, 2005), and elevated petrogenic PAC residue has indicated exposure to trace petroleum contamination from oil spills at sea (Alonso-Alvarez et al., 2007b [erythrocytes]; Paruk et al., 2016 [plasma]; Pérez et al., 2008 [erythrocytes]; Seegar et al., 2015 [erythrocytes]; Zuberogoitia et al., 2006 [eggs]), produced water (Rattner et al., 1995 [stomach contents]), oil sands industrial activity (Fernie et al., 2018a [muscle and feces]; Gurney et al., 2005 [bile]; Paruk et al., 2018 [plasma]), and chronic oil pollution (Custer et al., 2000; King et al., 1987 [carcass]). Data from correlative, controlled dosing, and treatment-reference area studies provide evidence that elevated petrogenic PAC concentrations in tissue can cause biological effects on various endpoints including growth and metabolism, as well as circulating metabolites, erythrocytes, and thyroid hormones (Alonso-Alvarez et al., 2007a, 2007b; Fernie et al., 2018a, 2019; Gurney et al., 2005; Pérez et al., 2010a; Troisi et al., 2007, 2016). Beyond the brief summary above of petrogenic PAC residue and the effects of exposure, a more complete discussion of PAH residue in birds is found elsewhere (Albers, 2006).

Crude petroleum, some fuels, and waste oils may have high concentrations of potentially toxic metals such as arsenic, cadmium, copper, chromium, lead, manganese, mercury, selenium, vanadium and others, though compared to PAC tissue residue there is little data linking metal concentrations in tissue to petroleum exposure or any resulting adverse health effects. Most available studies since 1993 show little evidence of elevated metal residue associated with petroleum contamination in the environment or direct exposure (Burger et al., 2008; Godwin et al., 2016; Kammerer et al., 2004; Mochizuki et al., 2013; Pérez-López et al., 2006; Sanpera et al., 2008). However, the feathers of birds from areas affected by the *Exxon Valdez* crude oil spill 15 years earlier showed elevated cadmium and manganese in one of three species studied (Burger et al., 2007, 2008). An analogous study at bird breeding colonies impacted by the *Prestige* fuel oil spill examined the feathers of chicks from two species and showed a declining trend in copper and lead concentrations over subsequent years from putatively spill-caused increases (Moreno et al., 2011). A screening of metal concentrations in the blood of mallards (*Anas platyrhynchos*) experimentally exposed to oil sands process-affected water and exhibiting subclinical blood biochemistry and endocrine (thyroid and corticosterone) effects had higher vanadium than controls and sex-specific differences in molybdenum (Beck et al., 2014). As yet however, there is no evidence linking trace oil sands contaminant exposure *in situ*, or other bulk petroleum exposure, to elevated concentrations of vanadium or molybdenum in liver or kidney (Godwin et al., 2016; Kammerer et al., 2004; Mochizuki et al., 2013). As with hydrocarbons, the concentrations of metals will vary among different petroleum types, and inter-species differences among birds from the same areas are apparent (Burger et al., 2008; Moreno et al., 2011).

The most sensitive and widely used biomarkers of petroleum hydrocarbon exposure are measures of the cytochrome P450 monooxygenase (CYP) system. This is an assemblage of enzyme isoforms concentrated and typically measured in the liver but also found in other tissues. The CYP enzyme system forms the primary phase I biotransformation system for a wide range of xenobiotics, including monoaromatic (e.g. BTEX) and PAC compounds found in petroleum (Alexander et al., 2017; Brunström et al., 1991; Cruz-Martinez et al., 2015a, 2015b; Dubansky et al., 2018; Gentes et al., 2007b; Head and Kennedy, 2007; Newman, 2010; Walters et al., 1987). CYP1A induction via the aryl hydrocarbon receptor is typically used as a biomarker of exposure in birds. The two avian CYP1A enzyme isoforms are CYP1A4 and CYP1A5 (Alexander et al., 2017). CYP1A induction is quantified by mRNA relative abundance (*Cyp1a4* and *Cyp1a5* gene expression), protein abundance (CYP1A immunoassay), and, most commonly, enzyme activity (alkoxyresorufin-O-deethylase activity). Those CYP1A biomarkers have provided evidence for exposure to petroleum hydrocarbons from large spills at sea (Esler et al., 2010, 2011, 2017; Golet et al., 2002; Perez-Umphrey et al., 2018; Trust et al., 2000; Velando et al., 2010), chronic petroleum pollution at sea (Miles et al., 2007; Willie et al., 2017), oil sands industrial activity (Cruz-Martinez et al., 2015a; Gentes et al., 2006; Smits et al., 2000), and laboratory inhalation studies with environmentally realistic volatile compound concentrations in air (Cruz-Martinez et al., 2015b; Dubansky et al., 2018). CYP1A system biomarkers have also been used extensively to characterize exposure in experiments (Alexander et al., 2017; Bianchini and Morrissey, 2018a; Gurney et al., 2005; Prichard et al., 1997; Trust et al., 1994). While elevated CYP1A enzyme activity itself is not necessarily deleterious (Leighton, 1993; Newman, 2010), studies have linked ethoxyresorufin-O-deethylase activity (EROD) evidence of petroleum exposure to decreased survival in free-living birds (Esler et al., 2000b; Gentes et al., 2006; Iverson and Esler, 2010; Velando et al., 2010). Other CYP enzyme systems like CYP3A may interact with PACs as well (Mundy et al., 2019).

Other biomarkers of oxidative stress and damage may be useful, though they are less specific to petroleum exposure, and possibly affected by infection, nutrition status, physical activity (e.g. migration, reproduction), age, and other factors (reviewed in Costantini, 2008; Skrip and McWilliams, 2016). The best studied and apparently sensitive example is the occurrence of oxidatively denatured hemoglobin (Heinz bodies) within erythrocytes (Fallon et al., 2013, 2017; Harr et al., 2017a; Horak et al., 2017; Leighton, 1986; see Section 3.2.6). This or other markers of oxidative stress and damage (Section 3.2.5) found in tissue or plasma may indicate petroleum exposure if corroborated by data to support ingested oil as the cause or strong circumstantial evidence, such as a known oil spill having impacted avian habitat.

3. Physical and toxic effects of petroleum exposure

The short-term negative impacts to birds from petroleum may be categorized as: 1) the physical effects of contaminated plumage, 2) the toxicological effects of petroleum that is ingested or inhaled, and 3) for contaminated eggs, potential embryotoxicity (reviewed in Leighton, 1993). Birds contacting bulk petroleum in the environment often face plumage fouling and ingestion simultaneously. The biological response *in vivo* (Fig. 3) includes immediate effects, a cascade of responses to compensate and overcome the challenges posed by oil exposure or, if these are unsuccessful, a terminal decline until death. Heavily oiled birds may succumb rapidly to the physical effects of oiling, while birds surviving the initial encounter will face sustained challenges from compromised plumage and potential toxicity.

3.1. Plumage fouling: the primary physical effect of petroleum

Birds are often the wildlife most visibly impacted by oil spill events, because as Leighton's (1993) terse synopsis makes clear, "oil floats on water and birds have feathers,". Feathers provide a lightweight and streamlined surface, water repellence, and trap air close to the skin, thus enabling flight, swimming, diving, buoyancy, and thermoregulation. Petroleum readily adsorbs to feathers, compromising these properties critical to their function in air and water (Fig. 4), and is thus likely the primary negative effect on birds rather than toxicity (see Section 4.3). This section deals with feather structure, how the structure of individual feathers is compromised by petroleum, and implications for thermoregulation, metabolism, locomotion, and recovery.

3.1.1. Disruption of feather structure

Feathers' light weight and water repellence results from their intricate, hydrophobic microstructure. From the central midline rachis, barbs branch off, and branching off from these, microscopic hooked barbules on the distal edge and smooth barbules on the proximal edge (Fig. 4). The barbules on the leading edge of a barb interlock with the barbules on the trailing edge of the neighboring barb to form a zippered matrix on a microscopic scale. This microstructural matrix opposes the surface tension of water and the hydrophobic, lipid-coated keratin they are comprised of resists water's polarity (O'Hara and Morandin, 2010; Stephenson, 1997). Barbules from adjacent feathers similarly interlock to form a contiguous, water repellent layer. However, because of its hydrophobic, oleophilic properties, this feather structure is extremely vulnerable to contact with other oils.

Petroleum compromises the function of the feather structure by lowering the surface tension of water while simultaneously adsorbing to the hydrophobic feather matrix, causing the interlocking barbules to become clogged and their supporting barbs to collapse (Fig. 4) (Jenssen, 1994; Morandin and O'Hara, 2016; O'Hara and Morandin, 2010; Stephenson, 1997). Decreased water surface tension and collapsed microstructure means that water can enter the gaps in the otherwise impenetrable feather matrix and displace the air that provides insulation and buoyancy (Jenssen, 1994; Morandin and O'Hara, 2016). The amount of oil adsorbed onto the feather sufficient to disrupt its structure is small. Collapse and clumping of neighboring barbules (quantified as amalgamation index) has been demonstrated for a majority of diverse marine species when feathers contact petroleum-on-seawater films as thin as 3 μm or less (Fritt-Rasmussen et al., 2016; Matcott et al., 2019; O'Hara and Morandin, 2010). Structural compromise can be apparent even when the amount of oil contaminating the feather is undetectable on a mass basis (several milligrams) (e.g. O'Hara and Morandin, 2010), though the threshold of effect varies somewhat among petroleum types and species. In addition to surface films, exposure to oil droplets suspended in water are also relevant to diving birds. A dive tank study with common murres (*Uria aalge*) showed effects on feather microstructure at crude oil-in-water concentrations of 0.2 ml l^{-1} and significant gross observations of plumage wetting at 2.0 ml l^{-1} (Whitmer et al., 2018). As opposed to barb amalgamation indices from manual counts, computer image analysis may prove useful for quantifying feather microstructure damage (Bigger et al., 2017). Studies to date have examined petroleum effects

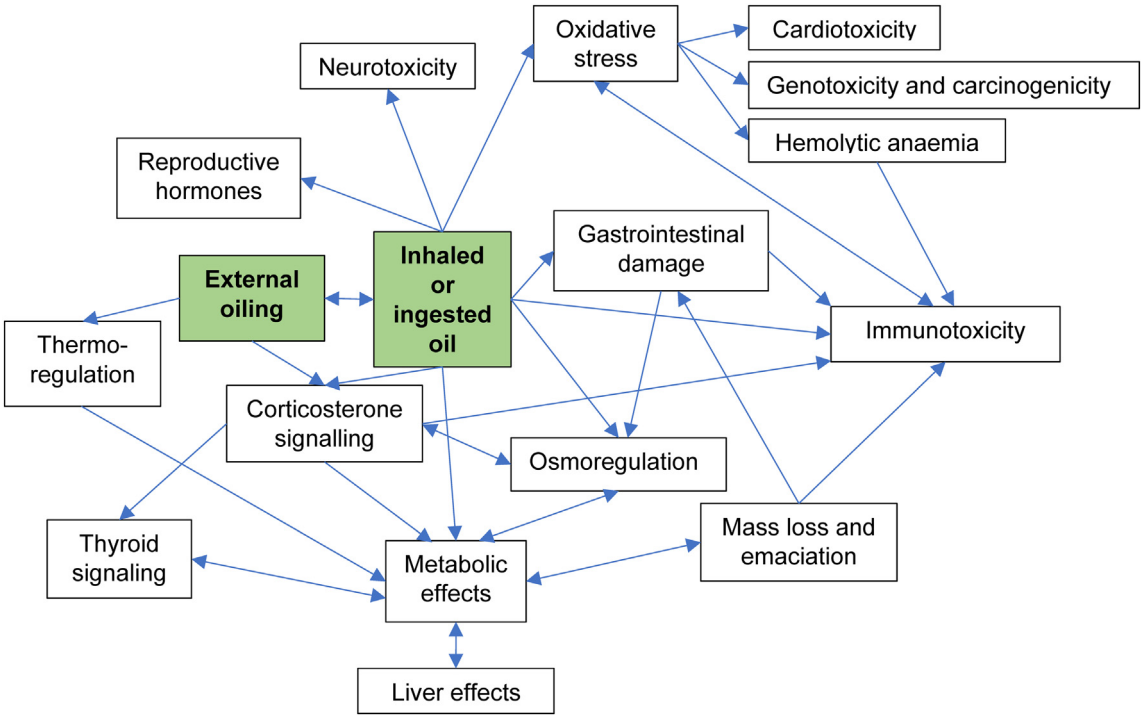

Fig. 3. Conceptual diagram of petroleum exposure's (green shaded boxes) effects (white boxes) on avian biology. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

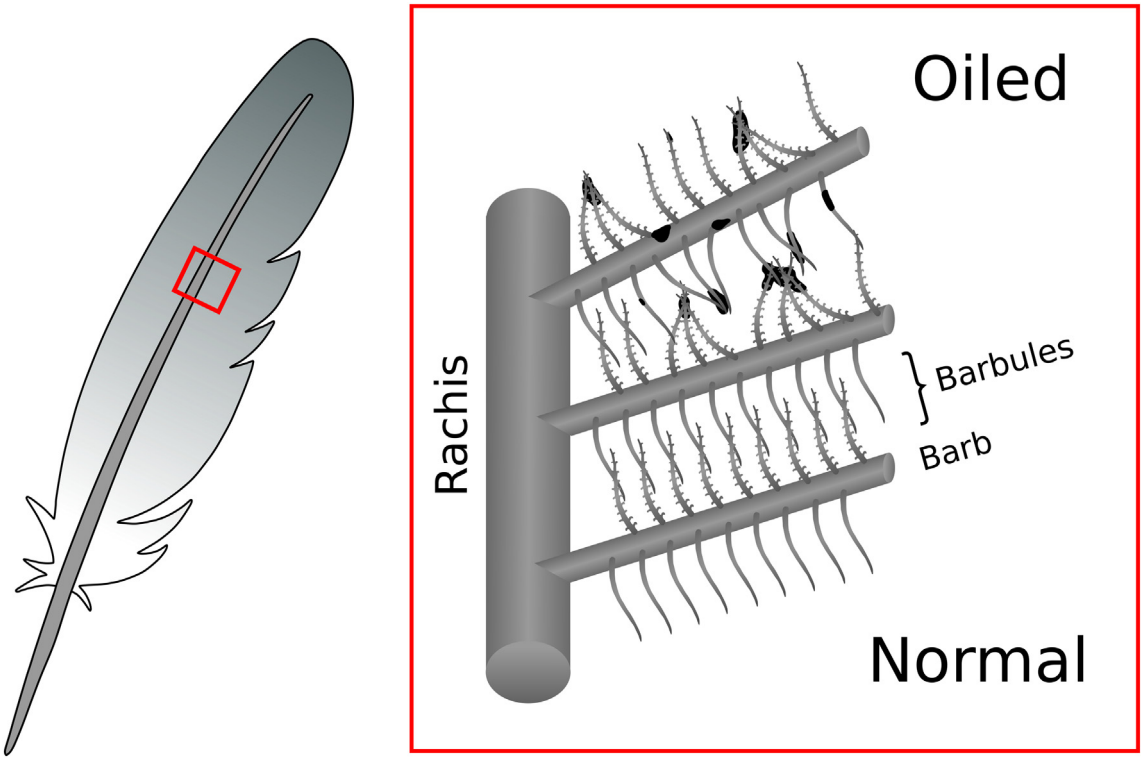

Fig. 4. Feather function is achieved by its lightweight, hydrophobic keratin structure which interacts with the surface tension of water. From the central rachis, barbs branch off. Branching off from these are microscopic hooked barbules on the distal edge and smooth barbules on the proximal edge. These interlock with barbules on the neighboring barbs. Petroleum is also hydrophobic and adsorbs to these structures, fouling them even at trace amounts. For instance, films of oil on the water surface <3 μm may clog barbule hooklets and cause neighboring barbules to adhere. Increasing amounts of oil progressively disrupt this hierarchical structural matrix to the point that barbs become disarranged with heavy fouling.

on individual feather barbs; it is not known if the barbule connections between feathers is affected to a comparable degree. In addition to petroleum itself, spill response approaches such as using chemical dispersants or burning oil slicks can affect feather structure and function through the same mechanisms by generating oil-dispersant-water mixtures and hydrophobic burn residue, respectively (Fritt-Rasmussen et al., 2016; reviewed in Jenssen, 1994; Whitmer et al., 2018).

The finding that even trace amounts of oil can disrupt feather structure is an important revelation, because visible amounts of oil on the plumage are known to affect thermal balance at a few milliliters or more, and heavy oiling can be fatal (Hartung, 1967; reviewed in Jenssen, 1994). Heavy oiling can collapse plumage to such an extent that a bird may drown or be effectively immobilized (Golightly et al., 2002; reviewed in Leighton, 1993), but contact with small amounts of oil may have more subtle effects. A bird swimming through a 1 to 3 μm thick oil film could pick up 1 ml of oil per 0.3 to 1.0 m^2 of surface swum, and surface sheens at offshore oil and gas platforms are associated with produced water discharges containing total hydrocarbon concentrations (≤0.15 ml l^{-1}) that approach thresholds for feather structure effects (0.2 ml l^{-1}) (Morandin and O'Hara, 2016; Whitmer et al., 2018). Thus, even petroleum that has thinned or become diluted to low concentrations at sea may still affect feathers. Larger amounts of petroleum ranging from several to tens of milliliters are associated with effects on energy balance in a dose-dependent fashion (reviewed in Jenssen, 1994; Oka and Okuyama, 2000; Whitmer et al., 2018). The highly adsorbent properties of feathers and spread of oil by preening suggests that the relationship between the volume of petroleum adsorbed and how much surface area (SA) of the bird appears oiled is indirect, but for the sake of example we calculate (see Section 1.1) from external dosing studies that 20% SA corresponds to contacting 5 to 10 ml kg^{-1} bw (Cunningham et al., 2017; Dannemiller et al., 2019; Maggini et al., 2017a, 2017c). This is consistent with the amount of petroleum contaminating dead, moderately oiled birds in winter (e.g. 6.4 ml kg^{-1} bw) (Hartung, 1963), so it seems reasonable that a bird encountering a thin film of oil could foul its plumage sufficiently to affect thermoregulation and metabolism under cold conditions (Hartung, 1967; also see Jenssen, 1994). Effect thresholds, or *how much oil*, remains an open question for most taxa. At present, quantitative links between the amount of oil contaminating feathers and organism energy balance do exist (reviewed in Jenssen, 1994), and the development of a general, unified model linking exposure, feather function, thermal balance, and metabolism would be invaluable. Feather type, age from molting, species, and petroleum type appear to be important factors (Fritt-Rasmussen et al., 2016; O'Hara and Morandin, 2010; Stephenson, 1997).

3.1.2. Thermal and metabolic balance effects

Because thermoregulation and metabolism are tightly coupled, reduced thermal insulation by oil-compromised plumage can increase heat loss and subsequently metabolic rate to compensate, which can be costly (Jauniaux et al., 1998; reviewed in Jenssen, 1994). Here we summarize Jenssen's (1994) review on the subject. Conductive heat loss is greater at lower temperatures, at higher surface area to volume ratios (i.e. smaller body size), and in water as opposed to air. To compensate for heat loss, metabolic rate increases up to a point, but if the rate of heat loss exceeds metabolic heat production, then hypothermia results. If hypothermia is avoided, elevated metabolic rate must be maintained by depleting lipid reserves or feeding more.

Should oiled birds at sea not succumb to drowning or hypothermia from heavy oiling, lesser amounts of oil may cause starvation from the increased metabolic cost of thermoregulation, or to use Hartung's (1967) term, accelerated starvation (reviewed in Jenssen, 1994; Leighton, 1993). For instance, Oka and Okuyama (2000) calculated that an oiled rhinoceros auklet (*Cerorhinca monocerata*), if unable to keep feeding,

would starve to death in 1.2 days at sea during winter at mid-latitudes. Recent studies of birds oiled at sea showed decreased body mass, emaciation, muscle atrophy, and depleted lipid reserves such as liver and subcutis, usually accompanied by empty stomachs (Balseiro et al., 2005; Fowler et al., 1995; Gandini et al., 1994; Jauniaux et al., 1997; Oka and Okuyama, 2000; Simpson and Fisher, 2017). Such *postmortem* findings are consistent with starvation in seabirds (Jauniaux et al., 1998). Data from oil spills and wildlife rescue operations support the link between low body mass or body condition and near-term morbidity and mortality (Anderson et al., 2000; Camphuysen and Leopold, 2004; Duerr et al., 2016; Gartrell et al., 2019; reviewed in Jenssen, 1994; Parsons et al., 2018; Sharp, 1996; Sievwright et al., 2019a). Paradoxically, seabirds with only light or moderate oil fouling appear to starve at sea, whereas more heavily oiled individuals that are forced ashore by completely compromised plumage are more likely to survive cleaning and rehabilitation (Camphuysen and Leopold, 2004; Duerr et al., 2016; Gartrell et al., 2019; Henkel et al., 2014b; Jauniaux et al., 1997).

Recent experiments where birds were externally exposed to moderate amounts of weathered crude oil show some results consistent with the large body of evidence for metabolic effects in externally oiled wild birds caused in part by altered thermal balance. Maggini et al. (2017a) found decreased body mass in western sandpipers (*Calidris mauri*) 3.5 d after a single 10 ml kg^{-1} bw (20% SA) external exposure, and during a cold temperature challenge those birds lost less fat mass and more lean mass than other treatments, perhaps because fat reserves were already depleted. However, no difference in metabolic rate was detected. While no body weight effects were seen in externally oiled (32 to 45 ml kg^{-1} bw, 14 to 40% SA) ring-billed gulls (*Larus delawarensis*) after several weeks (Dannemiller et al., 2019), double-crested cormorants (*Phalacrocorax auritus*) repeatedly oiled with 8 ml kg^{-1} bw (cumulatively 46 ml kg^{-1} bw, 20% SA) increased their food consumption compared to the control group and gained weight at a faster rate over the three week trial, likely due to increased energetic cost of thermoregulation in the oil-covered birds (Cunningham et al., 2017). The experimental effects on body mass, body composition, and food consumption at moderate, e.g. 20% SA, amounts of external oil exposure are less pronounced than the dramatic emaciation seen in oiled wild birds, presumably related to the easy access to dry land, food, and warmth (e.g. heat lamps) in captivity. Furthermore, besides the plausible effects on thermal balance from oiled plumage, the above metabolic endpoints can also be affected by the toxicity of ingested oil (see Section 3.2.4) and, as in those experiments mentioned, separating physical effects of oil on feathers from toxicity is often impossible.

3.1.3. Flight and swimming performance

Feathers enable bird species to move efficiently through water and, more uniquely, air by optimizing streamlining, buoyancy, and lift, but moderate amounts of oil impair flight (e.g. 20% SA) and swimming performance. Of course heavily oiled birds may be effectively immobilized by plumage collapse or the stickiness of viscous crude oils (Golightly et al., 2002), but comparatively small amounts of oil may cause birds to work harder to fly by reducing lift and thrust, increasing wing loading and drag, and making feathers susceptible to stress damage (Maggini et al., 2017c; Perez et al., 2017a). Experiments in Western sandpipers (*Calidris mauri*) and homing pigeons (*Columba livia*) with weathered crude oil (20 to 30% SA) on their feathers indicated impaired flight performance, increased energy expenditure, decreased body mass, and reduced take-off speeds (Maggini et al., 2017b, 2017c; Perez et al., 2017a, 2017b, 2017c). Analogous to oil-compromised flight performance, plumage oiling may hinder swimming and diving performance by increasing hydrodynamic drag or affecting buoyancy. Adélie penguins (*Pygoscelis adeliae*) lightly oiled with vegetable oil, similar in properties to mineral oils, may have had to work harder to swim in one experiment (Culik et al., 1991). Lower swim speeds, elevated energy expenditure evident from increased heart and metabolic rates, and an eagerness to exit the water were observed. While there are few recent studies on swimming and dive performance with oiled plumage, the common co-occurrence of emaciation and empty stomachs in dead, oiled seabirds may support a reduced ability to capture prey necessary to overcome increased metabolic costs (Gandini et al., 1994; Oka and Okuyama, 2000; Simpson and Fisher, 2017).

3.1.4. Recovery by preening

Birds that survive with oil-fouled plumage appear to be able to remove visible traces of oil over several weeks by preening, however there is little information on the extent and speed with which function is restored. Observations from experimentally oiled shorebirds in captivity and observational studies in wild gulls and terns agree that birds were able to remove visible oil within about 2 to 10 weeks (Burger and Tsipoura, 1998; Camphuysen, 2011; Maggini et al., 2017c). Molting does not necessarily eliminate the oil, as it can transfer from the old feathers to the new (Kerley et al., 1985). It is widely recognized from work in rescued birds that when the plumage is cleaned of contaminating oil and allowed to dry, water repellence and insulation is restored (reviewed in Jenssen, 1994). One recent report shows that diving and foraging behavior in cleaned and rehabilitated little blue penguins (*Eudyptula minor*) was normal (Chilvers et al., 2015), suggesting a return to regular swimming and diving performance if contaminating oil is preened out or humans intervene. However, for birds that survive unaided by humans, the extent and time required for preening to fully restore feather structure and function has received relatively little attention, especially given the effects of trace amounts of oil on feather microstructure (Fritt-Rasmussen et al., 2016; Matcott et al., 2019; O'Hara and Morandin, 2010).

3.2. The primary effects of systemic petroleum toxicity

Petroleum toxicity is characterized as systemic (Leighton, 1993), affecting the structure and function of multiple organs, systems, and processes within the body (Fig. 3). While such physiological systems are not usually limited to a singular location or function, this section summarizes the proximate effects of petroleum toxicity, to the extent that they are known, on target tissues, organs, and body systems.

3.2.1. Inhalation and the lungs

Aspirated petroleum causes lipid pneumonia (Hartung and Hunt, 1966; reviewed in Leighton, 1993) and volatile components of petroleum which are inhaled plausibly affect the lungs (Dubansky et al., 2018; reviewed in Hartung, 1995). Pathological examination of avian lung and air sac tissue has not indicated obvious damage (Balseiro et al., 2005; Fry and Lowenstine, 1985; Simpson and Fisher, 2017; Szaro et al., 1978). Only recently have studies examined the toxicological effects of airborne petroleum contaminants in more detail. Inhaled volatile hydrocarbons, including monoaromatics (BTEX), from weathered crude oil increased CYP1A protein expression in lung epithelia (Dubansky et al., 2018). These phase I biotransformation enzymes may metabolically activate aromatic compounds as they pass through the epithelia, and studies on airborne petroleum contaminants point to effects on immune function, liver, thyroid, and stress response (Cruz-Martinez et al., 2015a, 2015b; Dubansky et al., 2018; Fernie et al., 2016; Olsgard et al., 2008, 2009).

3.2.2. Gastrointestinal damage

Ingested petroleum passes through the gastrointestinal tract, and evidence of damage there has been mixed, yet recent experimental work supports petroleum ingestion as a potential cause of gastrointestinal injury. Gastrointestinal lesions are common in dead marine birds suspected to have succumbed to oil exposure at sea (Balseiro et al., 2005; Gandini et al., 1994; Jauniaux et al., 1997, 1998; Simpson and Fisher, 2017), but the same lesions are also common where starvation is the cause of death and no oil exposure is suspected (Jauniaux et al., 1998). Past reviews have arrived at diverging conclusions as to whether

gastrointestinal damage and function is a primary effect of petroleum ingestion (Burger and Fry, 1993; Leighton, 1993). Common lesions include inflammation, petechiae, hemorrhage, and ulceration (Balseiro et al., 2005; Gandini et al., 1994; Jauniaux et al., 1997, 1998; Simpson and Fisher, 2017). The presence of oil-like substances in the affected intestinal lumen hints at a direct cause of such lesions in some cases (Balseiro et al., 2005). Experiments with double-crested cormorants (*Phalacrocorax auritus*) exposed orally (5 or 10 ml kg^{-1} bw d^{-1}) or externally (20% SA every 3 d) to weathered crude oil for several weeks passed bloody excreta and oral doses caused enteric lesions and intestinal inflammation (Cunningham et al., 2017; Harr et al., 2017a, 2017b). Some older dosing studies also indicate hemorrhage (Hartung and Hunt, 1966) and histopathological changes (edema) (Miller et al., 1978). The presence of gastrointestinal injury even with access to abundant food supports the case for some mechanism of gastrointestinal injury from ingested oil independent of acute starvation and tissue catabolism (Cunningham et al., 2017). Coagulopathy observed in some dosing studies could help explain gastrointestinal hemorrhaging (Cunningham et al., 2017; Dubansky et al., 2018; Harr et al., 2017b). The serious implications of intestinal epithelia damage to nutrient absorption and immune function was reviewed by Briggs et al. (1997), and the former is certainly consistent with observations of malnutrition and dehydration in oil-stricken birds (Balseiro et al., 2005; Duerr et al., 2016; Troisi et al., 2007).

3.2.3. Osmoregulation and excretion

The intestines, kidneys, and in some species, salt glands, are essential to osmotic balance in birds, and there is some evidence that petroleum is harmful to all of these organs (reviewed in Leighton, 1993). Osmotic stress could potentially be fatal in the presence of other stressors (reviewed in Burger and Fry, 1993). However, Leighton (1993) highlights the difficulty in separating primary and secondary toxic effects on osmoregulation, as well as the difficulty in drawing general conclusions from past studies, especially given the link between the corticosteroid stress axis and osmoregulation. Nevertheless, changes to circulating electrolyte or nitrogenous waste concentrations or changes in the kidney resulting from experimental petroleum exposure are consistent with dehydration observed among birds oiled at sea (Balseiro et al., 2005; Duerr et al., 2016; Troisi et al., 2007), supporting some nonspecific link between petroleum toxicity and osmoregulation as a whole.

There has not been consensus among reviews on whether the kidney is a major target of ingested oils (Briggs et al., 1996; Hartung, 1995), and opinions remain mixed. There is evidence from older studies of kidney lesions or damage in oil-exposed birds (e.g. Fry and Lowenstine, 1985; Hartung and Hunt, 1966; Szaro et al., 1978) and developing chicken embryos in oiled eggs (Couillard and Leighton, 1990a, 1990b). More recent experimental work demonstrated increased kidney size following weathered crude oil exposure for several weeks (5 to 10 ml kg^{-1} bw d^{-1} orally or 20% SA every 3 d externally) in double-crested cormorants (*Phalacrocorax auritus*) and laughing gulls (*Leucophaeus atricilla*), as well as histopathological lesions consistent with chronic irritation and inflammation in orally dosed groups (Harr et al., 2017b; Horak et al., 2017). In contrast, no histological changes were found in the kidneys of juvenile mallards (*Anas platyrhynchos*) in a similar experiment (9.5 ml kg^{-1} bw d^{-1}) (Stubblefield et al., 1995b). Increases in urea or uric acid consistent with decreased kidney function have been documented in the plasma or serum of double-crested cormorants experimentally exposed to weathered crude oil (Dean et al., 2017a, 2017b), although besides kidney function, metabolic changes (e.g. protein catabolism) or hydration status can also affect plasma urea and uric acid (Balseiro et al., 2005; Styles and Phalen, 1998).

Like changes to concentrations of circulating waste products, electrolytes such as calcium can be clinically useful in toxicological trials, but have done little to resolve the physiological mechanisms at work or their relation to toxic pathologies. Electrolyte effects associated with oil exposure vary among species, exposure route, duration, or dose, and likely some combination of these. As in the past, recent experimental results reporting circulating electrolytes (e.g. Ca^{2+}, Na^{+}, K^{+}, Cl^{-}, HCO_3^{-}) differ from one study to the next and are difficult to interpret collectively (Alonso-Alvarez et al., 2007a, 2007b; Beck et al., 2014; Bursian et al., 2017b; Dannemiller et al., 2019; Dean et al., 2017a, 2017b; Golet et al., 2002; Maggini et al., 2017a; Newman et al., 2000; Prichard et al., 1997; Seiser et al., 2000). Effects on Ca^{2+} are common, though divergent, possibly age- and sex-dependent, and linked to eggshell thinning (Alonso-Alvarez et al., 2007a, 2007b; Dean et al., 2017a; Golet et al., 2002; reviewed in Leighton, 1993; Seiser et al., 2000; Stubblefield et al., 1995c). Effects on circulating Ca^{2+} and eggshell thinning together with effects on the expression of genes related to intracellular Ca^{2+} regulation (*Rgn*) following *in vitro* hepatocyte exposures to PACs (Crump et al., 2017) suggests a direct link between petroleum toxicity and calcium homeostasis.

3.2.4. Metabolic effects and the liver

Metabolic effects from the toxicity of ingested petroleum are suggested by dysregulated body mass or food intake at the whole organism level, gross or histological changes at the organ level, and molecular level effects. Generalizations about the metabolic pathways affected and the mechanisms at work are complicated by myriad factors, including differences in species, dose, duration, oil composition, sampling, and the fact that metabolites participate not in discrete systems, but in multiple linked pathways to generate energy with available substrates and homeostatic mechanisms. However, the weight of evidence points to effects on glucose, protein, and fatty acid metabolism. The liver plays a central role in these effects. It is the first major organ in contact with substances brought into circulation via intestinal absorption, and is critical to metabolism, and especially biotransformation. Effects on the liver are common, yet evidence of actual damage is limited. Instead, petroleum appears to result in more subtle effects on homeostatic metabolic mechanisms.

3.2.4.1. Gross and histological effects. Recent studies reporting widespread effects on body mass, feeding, or growth, together with older studies (reviewed in Leighton, 1993) indicating changes to food consumption or decreased growth rates, suggest metabolic effects from ingested oil. Mass loss has resulted from oral dosing in studies with weathered crude oil and fuel oil (Bursian et al., 2017b; Dean et al., 2017b; Horak et al., 2017; Lee et al., 2012). Pre-migratory mass gain dynamics have been affected in an oral dosing study with PACs (Bianchini and Morrissey, 2018a), and field studies indicate negative associations between blood PAC burdens and body condition or body mass (Alonso-Alvarez et al., 2007a; Paruk et al., 2016), as well as sediment PAC concentrations and pre-migratory fattening (Bianchini and Morrissey, 2018b). Although significant mass loss was not associated with lethal toxicity (5 to 10 ml kg^{-1} bw d^{-1}) in double-crested cormorants (*Phalacrocorax auritus*) dosed orally with large amounts of weathered crude oil, that study did indicate an association between reduced feed intake and mortality (Cunningham et al., 2017). Paradoxically, avoiding oiled food items can restrict food intake sufficiently that survival is better among birds ingesting higher cumulative doses of oil (Horak et al., 2017). PAC exposure has resulted in growth effects (size or mass over time) in young birds (Gurney et al., 2005; Trust et al., 1994), though not in one dosing study with weathered crude oil (Prichard et al., 1997).

The liver and pancreas are organs important to digestion and metabolic regulation which gross and histological examinations reveal to be structurally altered or damaged by ingested petroleum. Dosing studies commonly report increases in liver mass in birds ingesting crude oil or fuel oil over several days or weeks (Dean et al., 2017b; Harr et al., 2017b; Horak et al., 2017; Lee et al., 2012; Stubblefield et al., 1995c). Metabolic starvation from oiled plumage can have the opposite effect on liver mass (Oka and Okuyama, 2000), and decreased liver mass is sometimes reported in wild birds in association with petrogenic PAC

exposure in the environment (Cruz-Martinez et al., 2015a; Rattner et al., 1995). It is, therefore, not surprising that reports of increased liver mass tend to come from captive dosing studies. Increased liver mass is not synonymous with toxic injury or damage *per se*. It can be caused simply by glucose or lipid accumulation, but also biotransformation activity (Cattley and Cullen, 2018). Recent dosing studies have helped to rule out pathological lesions or oxidative damage as the cause of increased liver weight in adult birds ingesting weathered crude oil (Bursian et al., 2017b; Dean et al., 2017b; Harr et al., 2017b; Pritsos et al., 2017; Stubblefield et al., 1995c). Liver failure reported in oiled and rescued birds (Burger and Fry, 1993) has not been supported by the large number of peer-reviewed petroleum dosing studies with adult birds. In fact, pathological signs of hepatic damage (e.g. necrosis, neoplasia, or inflammation) rarely result from petroleum exposure, including where increased liver mass is evident, and the few reports of hepatic damage are associated with fuels rather than crude oil (Cattley and Cullen, 2018; Fry and Lowenstine, 1985; Hartung and Hunt, 1966; Stubblefield et al., 1995b). However, young birds may be more sensitive, as liver necrosis has been identified as an important lesion in embryos (Couillard and Leighton, 1990a, 1991a; Westman et al., 2013, 2014) and nestlings (Leighton, 1986; Szaro et al., 1978). For pancreatic tissue, in contrast to liver, there has been evidence of cellular death in adult double-crested cormorants (*Phalacrocorax auritus*) ingesting 5 to 10 ml kg^{-1} bw d^{-1} weathered crude oil (Harr et al., 2017b).

3.2.4.2. Biochemical effects. Biochemical or molecular data can signal shifts in metabolic processes, and while findings vary appreciably among studies, the most salient and consistent results among avian petroleum studies suggest toxic effects related to glucose homeostasis, protein catabolism, fatty acid oxidation, and bile acid metabolism. In particular, a number of studies report metabolite concentrations in the plasma or serum fraction of the blood in birds, though their scattered findings are a challenge to interpret (Alonso-Alvarez et al., 2007a, 2007b; Bianchini and Morrissey, 2018a, 2018b; Bursian et al., 2017b; Dean et al., 2017a, 2017b; Duerr et al., 2016; Gartrell et al., 2019; Golet et al., 2002; Horak et al., 2017; Lattin et al., 2014; Newman et al., 2000; Parsons et al., 2018; Prichard et al., 1997; Seiser et al., 2000; Stubblefield et al., 1995b, 1995c). Decreased blood glucose concentrations were found in three species of birds in oral dosing experiments and wild individuals in oil spill-impacted areas, and glucose has been shown to be negatively correlated with $\sum$PACs in erythrocytes (Alonso-Alvarez et al., 2007a, 2007b; Dean et al., 2017a, 2017b; Golet et al., 2002). Protein metabolism effects, most likely skeletal muscle catabolism, may exist in birds orally exposed to petroleum, as suggested by titers of circulating total protein, creatinine, creatine kinase, urea, uric acid, and 3-methyl histidine in blood (Alonso-Alvarez et al., 2007a, 2007b; Bianchini and Morrissey, 2018a; Dean et al., 2017a, 2017b; Horak et al., 2017). Besides glucose and protein metabolism, increased fatty acid oxidation may occur, as has been shown in the hepatic tissue of avian embryos exposed to PACs (Westman et al., 2013, 2014). The toxic metabolic effects of any ingested petroleum may be further complicated in externally oiled birds because of their potentially increased energetic costs of thermoregulation and locomotion (Anderson et al., 2000; Cunningham et al., 2017; Dean et al., 2017a; Dorr et al., 2019; Maggini et al., 2017a; Perez et al., 2017b). Nonetheless, metabolic effects observed in externally oiled birds are consistent with the effects of ingested petroleum described so far: disturbance to glucose homeostasis, muscle catabolism, and increased fatty acid oxidation unexpected in non-fasting piscivorous birds (Dean et al., 2017a; Dorr et al., 2019; Maggini et al., 2017a). Indeed, both glucose and protein dysregulation are associated with mortality in oil-exposed birds undergoing rehabilitation (Anderson et al., 2000; Gartrell et al., 2019). Furthermore, the expression of genes linked to glucose and fatty acid homeostasis are affected by PAC exposure, as shown by *in vitro* avian embryo hepatocyte studies (Crump et al., 2017; Mundy et al., 2019). Altered glucocorticoid receptor density in liver and muscle resulting from chronic, dietary exposure to weathered crude oil has been shown, and might play a role in glucose and fatty acid metabolism, at least in stressed animals (Lattin et al., 2014; Lattin and Romero, 2014). There is some evidence that exposure to weathered crude oil or dissolved oil contaminants can affect bile acid metabolism (Beck et al., 2014; Bianchini and Morrissey, 2018a; Dorr et al., 2019; Harr et al., 2017b; Mundy et al., 2019), though data are few and their implications less clear.

Clinical enzyme biomarkers in the plasma or serum have often been used to screen for hepatic and bile system damage or metabolic shifts, but these results vary so much that their interpretation from past studies, utility as a robust and sensitive biomarker, and merit for continued use is questionable. Clinical enzyme assays are used for pathological diagnosis, and biomarkers of hepatocyte damage include alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and aspartate aminotransferase (AST), while alkaline phosphatase (ALP), and gamma glutamyl transferase (GGT) are considered markers of biliary damage (Cattley and Cullen, 2018). Among these however, the degree of specificity to hepatic or biliary damage, validation in avian species, and relevant clinical baselines vary and are lacking in most cases (Cattley and Cullen, 2018; Harr, 2002). Among all petroleum related studies, the presence and direction of significant biomarker effects vary to such an extent that their collective interpretation appears impossible, and data connecting effects to signs of hepatic or biliary damage is scant (Alonso-Alvarez et al., 2007a, 2007b; Bianchini and Morrissey, 2018a; Bursian et al., 2017b; Dean et al., 2017a, 2017b; Golet et al., 2002; Kertész and Hlubik, 2002; Newman et al., 2000; Prichard et al., 1997; Seiser et al., 2000; Stubblefield et al., 1995b, 1995c; Trust et al., 1994; Yamato et al., 1996). Even similar oral dosing studies with the same toxicant within the same species fail to produce consistent effects (Dean et al., 2017a, 2017b). Clinical enzyme biomarker effects appear to vary by time, age, sex, effects on extrahepatic tissue (e.g. skeletal muscle), and environmental factors, complicating their interpretation (Alonso-Alvarez et al., 2007a, 2007b; Dean et al., 2017a, 2017b; Dorr et al., 2019; Horak et al., 2017; Newman et al., 2000; Prichard et al., 1997; Seiser et al., 2000; Trust et al., 1994). Only a handful of petroleum related studies observe correlations between individual clinical enzyme biomarkers and other liver metabolism effects which seem individually robust (Alonso-Alvarez et al., 2007a, 2007b [AST and blood glucose]; Bianchini and Morrissey, 2018a [blood GGT, but not AST, and hepatic EROD]; Golet et al., 2002 [blood AST, LDH, and hepatic EROD]; Hartung and Hunt, 1966 [AST and hepatic dye clearance]). While one such study links diesel fuel ingestion to elevated AST and degenerative changes in liver tissue (Hartung and Hunt, 1966), the fact remains that substantial increases in markers like AST and ALT can occur without consistent histopathological indications of damage in the liver (Trust et al., 1994).

Because the liver plays a key physiological role in both metabolism and detoxification, there is a theoretical coupling between these two roles such that the biotransformation of petroleum hydrocarbons might also modulate baseline metabolic processes, although avian work establishing clear connections between the two remains scarce. As discussed previously, hepatic CYP1A enzyme system biotransformation activity is a reliable and sensitive indicator of exposure to aromatic petroleum compounds. It is not clear that hepatic CYP1A enzyme activity alone is deleterious (Leighton, 1993; Newman, 2010), although increased biotransformation activity logically comes at some cost to routine metabolism in the form of energy allocation, endogenous CYP1A enzyme functions (e.g. steroid hormone metabolism) (Lu et al., 2020), or the generation of reactive oxygenated species as by-products (Couillard and Leighton, 1993). PACs have been shown to affect hepatic clearance in dye assays (Hartung and Hunt, 1966; Patton and Dieter, 1980), though the extent and mechanisms by which petroleum hydrocarbon biotransformation activity is mechanistically coupled with broader metabolic effects in hepatic tissue or beyond remains unclear.

3.2.5. Oxidative balance and damage

Reactive oxygen species (ROS) that may damage cellular biomolecules arise as inherent by-products of metabolism (e.g. superoxide) or, in the case of petroleum exposure, foreign compounds and their metabolites. Like other vertebrates, birds have complex antioxidant systems consisting of a host of enzyme and non-enzyme antioxidants to quench ROS and thereby prevent oxidative damage to biomolecules such as lipids, proteins, and DNA (reviewed in Costantini, 2008; Skrip and McWilliams, 2016). PACs in petroleum are substrates for CYP biotransformation enzyme systems and may be transformed into potentially damaging ROS, which must be countered (i.e. balanced) by antioxidant systems if damage is to be prevented (Couillard and Leighton, 1993; Skrip and McWilliams, 2016; Xue and Warshawsky, 2005). Gene expression studies using *in vitro* hepatocyte exposures to PACs show modulated transcript abundance of genes that play a role in antioxidant defense system pathways (Crump et al., 2017; Mundy et al., 2019), which substantiates a mechanistic link between PACs and maintaining oxidative balance.

Although oxidative damage to erythrocytes has long been recognized as an important mechanism of petroleum toxicity (Leighton et al., 1983), only recently have studies begun to provide more data on oxidative balance and markers of damage. Dosing studies demonstrated effects of petroleum on oxidative balance and damage in avian species following the *Deepwater Horizon* crude oil spill and *Prestige* fuel oil spill. Across the five species studied, all exhibited effects on some antioxidant defense endpoint (total antioxidant capacity or some component thereof, e.g. glutathione) in at least one exposure scenario, and in many cases some signs of oxidative damage (Bursian et al., 2017b; Dean et al., 2017b; Horak et al., 2017; Maggini et al., 2017a; Pérez et al., 2010a; Pritsos et al., 2017). These effects included titers of antioxidant defenses in liver tissue as a result of acute oral exposure (i.e. one to several days) (Dean et al., 2017b), acute external exposure (Maggini et al., 2017a), and subacute (i.e. several weeks) oral exposure (Bursian et al., 2017b; Dean et al., 2017b; Horak et al., 2017) to *Deepwater Horizon* weathered crude oil. Acute exposure to *Prestige* fuel oil similarly produced plasma antioxidant defense titers (Pérez et al., 2010a). Among these studies, evidence of oxidative damage in the form lipid peroxidation markers in liver and plasma was weak (Bursian et al., 2017b; Maggini et al., 2017a; Pérez et al., 2010a; Pritsos et al., 2017) but red bill spot, a carotenoid-based and antioxidant-related sexual ornament, was smaller in gulls (Pérez et al., 2010a), and pathological signs of oxidative damage to erythrocytes and cardiac tissue were seen at high doses associated with mortality (5 to 10 ml kg^{-1} bw d^{-1}) (Harr et al., 2017a, 2017b; Horak et al., 2017).

3.2.6. Hemotoxicity

Oxidative damage to red blood cells and hemolysis is often a direct effect of petroleum toxicity, and the pathological condition that can result is hemolytic anemia (reviewed in Leighton, 1993). Leighton et al. (Leighton, 1985a, 1985b, 1986; Leighton et al., 1983) first described the model for petroleum-induced hemolytic anemia by exposing nestling birds to crude oil and showing large reductions in hematocrit after high doses (10 to 20 ml kg^{-1} bw d^{-1}) administered for several days. This response to ingested petroleum is characterized by oxidative damage by ROS metabolites of petroleum hydrocarbons (Couillard and Leighton, 1993) to erythrocyte hemoglobin, subsequent erythrocyte lysis, and an ensuing regenerative response. Evidence of these processes are oxidatively denatured hemoglobin (Heinz bodies), best observed with electron microscopy (Fallon et al., 2017; Harr et al., 2017a; Horak et al., 2017; Leighton, 1985a; although see Fallon et al., 2013), a transient decrease in hematocrit and hemoglobin, effects on blood antioxidant titers, effects on iron binding capacity (e.g. ferritin) (Fallon et al., 2017; Lee et al., 2012; Troisi et al., 2007), accumulation of erythrocyte material (hemosiderin) mainly in the liver, spleen, or kidney where damaged red cells are phagocytized (Balseiro et al., 2005; Fry and Lowenstine, 1985; Kammerer et al., 2004; Khan and Nag, 1993; Newman et al., 2000; Simpson, 1971; Yamato et al., 1996), and elevated reticulocyte counts from erythropoiesis. The latter regenerative response may alternatively result in elevated hematocrit (Alonso-Alvarez et al., 2007a; Fowler et al., 1995; Newman et al., 2000), decreased blood leukocyte counts, and histological effects in bone marrow where erythropoiesis occurs (reviewed in Briggs et al., 1996; Leighton, 1986). Such effects are consistent with, but not limited to petroleum exposure. While many studies report hematocrit modulations, definitive evidence of petroleum-induced hemolytic injury should be confirmed by 1) clinical anemia, meaningful reductions in hematocrit or hemoglobin (e.g. compared to reference intervals or controls), or a regenerative response with 2) evidence of hemolysis and/or oxidative damage to red blood cells. The potential consequence of hemolytic anemia clearly includes reduced aerobic capacity, and anemia is associated with near-term mortality and morbidity (Duerr et al., 2016; Gartrell et al., 2019; Newman et al., 2000; Parsons et al., 2018; Sharp, 1996; Sievwright et al., 2019a). The next paragraphs synthesize new information related to petroleum hemotoxicity with a combination of evidence from oiled birds brought into rehabilitation operations, measurements made in wild birds, and dosing experiments, which collectively allow for an improved understanding of hemolytic injury.

Since 1993, several studies of oiled birds brought into wildlife rehabilitation have provided useful evidence of hematological damage from oil exposure. Hematological injury has been suggested in fuel oil-exposed white-winged scoters (*Melanitta fusca*) and crude oil-exposed American coots (*Fulica americana*) (Newman et al., 2000; Yamato et al., 1996). Large clinical datasets from wildlife rehabilitation centers have found no direct association between oiling status and low hematocrit upon admission in African penguins (*Spheniscus demersus*) and common murres (*Uria aalge*), but data for the latter suggested a threshold effect such that at low body masses, oiled birds may have lower hematocrit than unoiled rescued birds of the same body mass (Duerr et al., 2016; Parsons et al., 2018). In common murres impacted by various unidentified sources of petroleum, Heinz bodies and plasma ferritin, a protein important for oxidant defense, iron storage, and inflammatory response, were both significantly and strongly correlated with $\sum$PACs in plasma (Troisi et al., 2007).

Effects consistent with the mechanism for hemolytic injury and anemia have been shown in wild birds inhabiting oil spill-impacted areas. This includes seven species in areas where fuel oil or crude oil was spilled in recent months, resulting in pronounced incidence or degree of anemia (Fallon et al., 2017; Nisbet et al., 2013, 2015; Walton et al., 1997). Remarkably, these effects were found in populations with little or no visible oil on the plumage (Fallon et al., 2017; Nisbet et al., 2013). In contrast to anemia, observations of increased hematocrit made in oiled Magellanic penguins (*Spheniscus magellanicus*) may have been an erythropoietic response to hemolysis from crude oil ingestion (Fowler et al., 1995). Available data suggest that hematological parameters return to normal as petroleum disappears from the environment (Alonso-Alvarez et al., 2007a; Nisbet et al., 2013). However, petroleum persisting in sediment may be an important source of prolonged hematological effects (Paruk et al., 2016; Walton et al., 1997). An association between PACs detected in plasma and lower hematocrit were described in common loons (*Gavia immer*) wintering in crude-oil impacted habitat years after the initial spill (Paruk et al., 2016). Altogether, there is good evidence that effects consistent with hemolysis and reticulosis occur in wild birds exposed to oil despite the complexities of real-world scenarios and inter-species variability.

Experimental evidence for oil-induced hemolytic injury in adult birds is mixed, however. Standard hematological endpoints show little to no response in a majority of recent oral dosing experiments with crude oil in five species (up to 2.4 to 20 ml kg^{-1} bw d^{-1}, with high doses of 5 ml kg^{-1} bw d^{-1} or more in most studies) (Bursian et al., 2017b; Dean et al., 2017b; Horak et al., 2017; Newman et al., 1999; Stubblefield et al., 1995b, 1995c). Signs of hemolytic anemia in adult birds exposed experimentally to petroleum are limited to fuel oil exposures in mallard ducks

(*Anas platyrhynchos*) (Hartung and Hunt, 1966; Lee et al., 2012) and weathered crude oil exposure in ring-billed gulls (*Larus delawarensis*) (Dannemiller et al., 2019) and double-crested cormorants (*Phalacrocorax auritus*) (Dean et al., 2017b; Harr et al., 2017a) at similar oral doses (2 to 20 ml kg^{-1} bw d^{-1}) or external applications (14 to 40% SA) over multiple days. In the oral exposure with cormorants, a pronounced hemolytic anemia was associated with mortality and morbidity at 5 to 10 ml kg^{-1} bw d^{-1} over several weeks (Harr et al., 2017a, 2017b). The wide-ranging results reported in these studies do more to highlight the multifactorial nature of hemolytic injury than actually resolve its complexities. It is clear that anemia frequently occurs in wild birds when brought into captivity (Dannemiller et al., 2019; Newman et al., 1999, 2000). Alternatively, increased food availability and reduced energetic demands in captivity may modulate antioxidant capacity and vulnerability to oxidative damage (reviewed in Skrip and McWilliams, 2016). Finally, inherent inter-species differences exist under similar exposure conditions (Dean et al., 2017b; Fallon et al., 2017).

Young birds may be more sensitive to hemolytic anemia than adults. The strong experimental evidence for hemolytic anemia in birds comes almost entirely from nestlings and juveniles dosed with conventional crude oil (reviewed in Leighton, 1993). In the wild, yellow-legged gull (*Larus michahellis*) chicks with lower hematocrit were at breeding colonies affected by the *Prestige* wreck which spilled predominantly fuel oil rich in naphthalene, a strong oxidant (Alonso-Alvarez et al., 2007a; Couillard and Leighton, 1993; Pérez et al., 2008). In addition, $\sum$PAC concentration in the erythrocytes was positively correlated with hematocrit although chicks were in similar in body condition, which suggested an erythropoietic cause (Alonso-Alvarez et al., 2007a). In contrast, no such hematocrit effect was found in adults from oiled areas, nor in a later dosing study with adults (Alonso-Alvarez et al., 2007b). The apparent resilience of breeding adult gulls to hematocrit effects in the face of direct naphthalene exposure suggests that adults may be less sensitive to oxidant injury by metabolically activated oil hydrocarbons than nestlings. Analogously, a difference in sensitivity between life-stages is apparent with single-PAC exposures of 7,12-dimethylbenz[*a*]anthracene in European starlings (*Sturnus vulgaris*), where reduced hemoglobin was found in nestlings given 100 mg kg^{-1} bw, but not adults (Trust et al., 1994). Furthermore, variation associated with PAC exposure in wild loons was age class dependent (Paruk et al., 2016). Finally, there is limited evidence that naphthenic acids in petroleum, despite their apparently low toxicity, may induce hepatic erythropoiesis in nestlings (Gentes et al., 2007b).

While the preceding paragraphs have synthesized the prevailing aspects of petroleum hemotoxicity, it is admittedly difficult to reconcile the often divergent findings related to hemolytic injury from petroleum exposure, a fact that has been noted in past reviews (Fry, 1995; Hartung, 1995). For instance, indicators of hemolytic anemia are conspicuously absent from the large datasets of hematological indices taken upon the admission of oiled birds to rehabilitation centers (Duerr et al., 2016; Newman et al., 1999; Parsons et al., 2018). That and the few documented hemolytic effects in species which forage by pursuing prey underwater, with the exception of double-crested cormorants (*Phalacrocorax auritus*), merits further examination (Newman et al., 1999; Parsons et al., 2018; Walton et al., 1997), especially in light of the inherent interspecies variability already identified (Dean et al., 2017b; Fallon et al., 2017). Pursuit divers might be more resilient to hemolytic injury, due for instance to high blood hemoglobin concentrations (Minias, 2020). Furthermore, hemorrhage (Balseiro et al., 2005), inflammatory response (Lee et al., 2012), body condition, starvation, and dehydration (Duerr et al., 2016) associated with petroleum exposure may affect hematocrit and hemoglobin in birds exposed to oil in the wild, as well as additional artefacts of captivity mentioned earlier. To improve our understanding of hemolytic injury, sensitive and robust methods are warranted for quantifying oxidative damage to erythrocytes and hemoglobin (see Fallon et al., 2013; Harr et al., 2017a; Skrip and McWilliams, 2016).

3.2.7. Cardiotoxicity

Recent studies have linked cardiac tissue damage to prolonged oil exposure. In double-crested cormorants (*Phalacrocorax auritus*) and laughing gulls (*Leucophaeus atricilla*) given weathered crude oil orally (5 or 10 ml kg^{-1} bw d^{-1}) for several weeks, the heart musculature appeared flaccid upon necropsy (Harr et al., 2017b; Horak et al., 2017). Gross pathology revealed heart lesions in those cormorants as well as externally oiled cormorants (20% SA area every 3 d) (Harr et al., 2017b). Despite such changes in appearance, heart mass is not greatly affected by crude oil or fuel oil exposure in birds (Harr et al., 2017b; Lee et al., 2012). However, echocardiography in the externally oiled cormorants revealed changes to cardiac structure and function that included arrhythmias and qualitative observations of labored breathing (Harr et al., 2017c). Cardiac findings were associated with an increase in plasma Ca^{2+} as well, which is important to muscle contraction (Harr et al., 2017c). Harr et al. (2017b) suggested that the high concentration of mitochondria in the cardiac muscle makes it vulnerable to oxidative damage caused by ingested petroleum. Damage to the membranes of myocardiocytes releases the calcium protein complex troponin I, which may be a useful biomarker for assaying cardiac damage from oil exposure (Daigneault et al., 2017). As with effects of hemolytic anemia on blood oxygen carrying capacity, cardiac output has clear implications for aerobic capacity.

3.2.8. Neurotoxicity

A limited number of studies suggest neurotoxic effects associated with refined fuel ingestion in birds, though this subject represents a knowledge gap. Hartung and Hunt (1966) observed signs of neurotoxicity in mallards (*Anas platyrhynchos*) given diesel fuel, including incoordination, ataxia, tremors, and pupil constriction. Effects on brain mass are unexpected even during substantial mass loss from toxicity (Cattley and Cullen, 2018), and the brain is less often included in reports of pathological examinations. However, one study did report decreased brain mass in mallards with substantial oral doses of fuel oil over 5 d (Lee et al., 2012), although another showed no histological findings in the brain of juvenile mallards given weathered crude oil in the diet over two weeks (Stubblefield et al., 1995b). Substantial decreases in acetylcholinesterase activity in the plasma and brain of birds exposed to diesel fuel and fuel oil, respectively, points to neurotoxic effects on acetylcholinesterase activity (Hartung and Hunt, 1966; Oropesa et al., 2007). The authors of those studies speculate that organic phosphates or PACs were responsible.

3.2.9. Immunotoxicity

Birds ingesting petroleum may exhibit signs of immunotoxicity, due at least in part to effects on cell-mediated immunity (reviewed in Briggs et al., 1996, 1997; Fairbrother et al., 2004; Leighton, 1993). Early studies concluded that changes to lymphoid organs and lymphocytes in those tissues, changes in circulating leukocytes, and reduced resistance to pathogens are evidence of immunotoxic effects. Recent experimental work and field studies support effects on the lymphatic organs and T-lymphocyte response, and low survival among birds that are cleaned of oil contamination, rehabilitated, and released suggests prolonged immunosuppression (Briggs et al., 1996; Newman et al., 2000; Sharp, 1996).

Changes in lymphoid organs and reduced T-lymphocyte inflammatory response suggest that some aspect of petroleum toxicity affects these, though not other common measures of immunity. Spleen mass was reduced in birds orally dosed with certain crude or fuel oil types over multiple days or weeks, which may relate to lymphoid blastogenesis (Holmes et al., 1979; Horak et al., 2017; Lee et al., 2012; Rocke et al., 1984; Stubblefield et al., 1995b, 1995c). Rather than decreased spleen mass, thymus and bursa of Fabricius (a lymphoepithelial organ in birds important for B-lymphocyte maturation) mass in nestlings may be decreased by PAC exposure (Cruz-Martinez et al., 2015a; Holmes et al., 1979; Leighton, 1986; Stubblefield et al., 1995b;

Trust et al., 1994). Counts of leukocytes circulating in blood are often reported, and significant effects on total circulating leukocytes or lymphocytes in particular have accompanied hemolytic injury (Dean et al., 2017a, 2017b; Harr et al., 2017a; Leighton, 1986; Newman et al., 2000; Nisbet et al., 2015), but are not necessarily altered by petroleum exposure (Beck et al., 2014; Bursian et al., 2017b; Dannemiller et al., 2019; Dean et al., 2017b; Horak et al., 2017; Maggini et al., 2017a; Newman et al., 1999; Seiser et al., 2000; Smits and Williams, 1999). However, abundance of circulating leukocytes may be hormetic, as is the case with inhaled monoaromatics (Olsgard et al., 2009). The inflammatory response of T-lymphocytes may be measured with mitogen injection assays. These have showed some mixed evidence of decreased T-lymphocyte inflammatory response associated with exposure to bulk petroleum or petrogenic oil sands contaminants, though antibody production was not affected (Cruz-Martinez et al., 2015a, 2015b; Harms et al., 2010; Olsgard et al., 2008; Smits et al., 2000; Smits and Williams, 1999; Troisi, 2013). Signs of inflammation in tissue among oil exposed birds are often attributed to toxicity (Harr et al., 2017b; Newman et al., 2000), and inflammatory response markers (e.g. acute phase proteins) in circulating serum and plasma can indicate immunological and inflammatory status (Fairbrother et al., 2004), but petroleum ingestion affects inflammatory response markers in a manner dissimilar to bacterial infection, and markers like haptoglobin appear to be ineffective indicators of exposure (Fallon et al., 2017; Lee et al., 2012; Prichard et al., 1997; Troisi et al., 2007).

Overall, there is ample evidence to support effects on cell-mediated immunity via lymphocytes and the lymphatic organs, and while the cause and functional relevance of this remains unknown, parallels between those findings and the immunotoxic effects of PAC exposure implicates that class of compounds in petroleum immunotoxicity. A study in European starlings (*Sturnus vulgaris*) with an immunosuppressive PAC congener, 7,12-dimethylbenz[*a*]anthracene, reported that oral exposure in nestlings caused effects on cell-mediated immunity similar to petroleum ingestion, including effects on viable spleen cell counts, bursal growth reduction, decreased lymphocyte proliferation (blastogenesis), and decreased macrophage phagocytosis (Trust et al., 1994). The known effects of PACs in fish and mammals on their lymphocytes, phagocytes, and non-specific cytotoxic cells, which are conserved across taxa, further suggests effects on cell-mediated immunity (Reynaud and Deschaux, 2006). Some likely mechanisms of reduced cell-mediated immunity include: reduced leukopoiesis to overcome anemia caused by petroleum ingestion, damage to gut mucosa and epithelia, altered metabolism and synthesis of acute phase inflammatory proteins, and effects secondary to impaired nutrient absorption and stress (Briggs et al., 1996, 1997; Dean et al., 2017a; Dorr et al., 2019; Harr et al., 2017a, 2017b; Leighton, 1986; Reynaud and Deschaux, 2006). Each of those factors could cause or contribute to immunosuppression, because the immune system is non-localized and complex. The potential for long-term immunosuppression encourages research into its mechanisms and consequences, as survival may be affected. Claims of decreased phagocytosis require further investigation, and tools more sensitive than organ weights, histopathology, and cell counts could provide valuable data (Briggs et al., 1996, 1997).

3.2.10. Genotoxicity and carcinogenicity

The potential for petroleum exposure to damage DNA or cause cancer in birds has received very little research attention even though many PACs that may be in petroleum are genotoxic. Metabolically activated PACs or related reactive oxygen species may damage DNA, potentially causing DNA mutations or cancer (reviewed in Cooke et al., 2003; Xue and Warshawsky, 2005). To date, avian studies have not reported cancer or investigated genetic damage but have showed changes to DNA structure that may relate to gene regulation. These include effects on histone acetylation markers, global DNA methylation, and methylation of specific genes following exposure to petroleum, PAC mixtures, and individual PACs, respectively (Brandenburg and Head, 2018; Dorr et al., 2019; Toochaei et al., 2019). Hartung (1995) reviewed the carcinogenicity of spilled crude oil to wildlife briefly. Crude oil has not been shown to be carcinogenic in mammals. Should cancer tumors occur in birds as a result of petroleum exposure, the probability of detecting them in individuals is low. This is because chemically caused tumors tend to occur during the latter part of an animal's lifespan after prolonged latency, yet wildlife populations tend to have proportionally few old individuals and experimental exposures are brief. As Hartung (1995) cautiously concluded about birds impacted by *Exxon Valdez* crude oil, there is an absence of evidence for carcinogenicity from crude oil, though cancer would be hard to detect. At present, genotoxicity even in comparatively long-lived humans affected by oil spills is not well understood (Laffon et al., 2016). Available assays for signs of DNA damage from ingested or inhaled petroleum PACs in birds could be informative.

3.2.11. Endocrine effects

Ingested contaminants can interfere with hormone signaling by affecting the secretion, synthesis, or catabolism of hormones, or affecting circulating hormones or target tissues (Scanes and McNabb, 2003). Petroleum can affect reproductive hormones, corticosterone signaling which may affect metabolism and the physiological stress response, and thyroid hormone signaling important to metabolism and growth. Because the role of hormone signaling includes controlling metabolism and stress responses necessary for daily survival, but also responses to life-threatening challenges like oil-fouled plumage, dysregulation of these systems from toxicity could be consequential to survival and reproduction.

3.2.11.1. Reproductive hormones. Several hormones regulate avian reproduction from copulation, to ovulation, to parental care, and early studies indicate numerous effects of petroleum on circulating hormones, fertility, and reproductive performance (reviewed in Leighton, 1993; Scanes and McNabb, 2003). Ducks fed 3 to 5 ml kg^{-1} bw d^{-1} crude oil in the diet showed decreased circulating progesterone, estradiol, estrone, prolactin, and luteinizing hormone levels and perturbations to their normal cycles that resulted in reduced reproductive performance, including laying, fertility, and behavioral changes (Cavanaugh et al., 1983; Cavanaugh and Holmes, 1982, 1987; Harvey et al., 1981). More recent research extends these findings to oiled birds in the wild. A study in wild Magellanic penguins (*Spheniscus magellanicus*) with spilled crude oil on their plumage (20% SA) had low circulating androgens in both sexes and decreased luteinizing hormone and estradiol in females, which together with increased corticosterone and greater mass loss in females, may have been related to low rates of laying (Fowler et al., 1995).

3.2.11.2. Stress hormones. Several reviews all point to adrenal gland and corticosterone effects from oil exposure (Burger and Fry, 1993; Hartung, 1995; Jenssen, 1994; Leighton, 1993; Scanes and McNabb, 2003), which more recent studies support. Corticosterone is the circulating glucocorticoid hormone in birds controlled by the hypothalamic-pituitary-adrenal (HPA) endocrine signaling axis which regulates energy in the body via glucose and fatty acid metabolism.

The adrenal gland that secretes corticosterone is particularly vulnerable to circulating toxins (reviewed in Hinson and Raven, 2006), and recent work substantiates evidence showing that this gland is a target of petroleum toxicity. As in older studies, petroleum exposure was associated with enlarged adrenal glands or interrenal cell hypertrophy (Bursian et al., 2017b; Simpson and Fisher, 2017). Though the incidence of adrenal lesions among exposed study organisms may be infrequent, perhaps apparent in only a few individuals, dose-dependent increases in the severity of lesions in western sandpipers (*Calidris mauri*) at doses where few other effects were observed (1 to 5 ml kg^{-1} bw d^{-1}) suggests that adrenal damage may be a primary toxic effect of ingested

hydrocarbons, and not simply a secondary response to physiological stress from systemic toxicity (Bursian et al., 2017b).

Prolonged petroleum exposure is associated with effects on circulating baseline corticosterone in plasma, and long-term exposure may affect the acute (i.e. elevated) corticosterone stress response itself. There is evidence of elevated baseline plasma corticosterone in female Magellanic penguins (*Spheniscus magellanicus*) externally oiled (20% SA) for three weeks (Fowler et al., 1995), as well as adult female mallards (*Anas platyrhynchos*) repeatedly ingesting oil-sands process affected water over several weeks (Beck et al., 2014). Airborne oil sands contaminants inhaled repeatedly for several weeks can also affect baseline corticosterone concentrations, although diverging effects between treatments and species within the same experiment were apparent (Cruz-Martinez et al., 2015b). Those studies highlight both the potential for age and sex effects on circulating baseline corticosterone, and the potential for a hormetic response with respect to dose, time, or species. A hormetic response is likely given the homeostatic mechanisms of the HPA system, as well as data from older avian petroleum toxicity studies (reviewed in Busch and Hayward, 2009; Hartung, 1995; Hinson and Raven, 2006; Leighton, 1993). Corticosterone increases above baseline cycles trigger physiological and behavioral responses (i.e. acute stress response) to help overcome energy-demanding, unexpected challenges that arise (reviewed in Busch and Hayward, 2009). The acute stress response (corticosterone measured immediately after an acute stimulus) was depressed in house sparrows (*Passer domesticus*) after four weeks of being fed a diet containing 1 ml kg^{-1} bw d^{-1} weathered crude oil (Lattin et al., 2014), a change associated with altered glucocorticoid receptor density in fat and liver (Lattin and Romero, 2014). No long-lasting effect on stress response was evident in little blue penguins (*Eudyptula minor*) three years after they were oiled, rehabilitated, and released (Chilvers et al., 2016).

The above studies, by examining circulating corticosterone titers in petroleum-exposure scenarios, and in particular the response to acute stress, add to older studies which showed 1) transient increases in circulating corticosterone with acute petroleum ingestion, 2) reduced adrenal corticosterone secretion, and 3) effects on baseline circulating corticosterone, often decreases, in birds ingesting petroleum over several days to months (Burger and Fry, 1993; Hartung, 1995; Jenssen, 1994; Leighton, 1993; Scanes and McNabb, 2003). Effects on corticosterone could affect baseline metabolism, immune function, or ability to overcome stressors successfully (reviewed in Briggs et al., 1997; Busch and Hayward, 2009), as well as behavior perhaps (reviewed in Hartung, 1995). Further targeted inquiry into the various components of the HPA axis and cross-talk with biotransformation systems could be useful for untangling the often diverging findings on baseline circulating corticosterone levels and how they may relate to metabolism, while additional study of the cause and consequence of decreased acute stress response would be insightful (e.g. Gorsline and Holmes, 1981, 1982; Lattin et al., 2014; Wang et al., 2009). Although feather corticosterone is increasingly used in avian studies of glucocorticoids and stress, it has not proven a useful indicator of oil exposure to date (Cruz-Martinez et al., 2015a; Lattin et al., 2014).

3.2.11.3. Thyroid hormones. Petroleum contaminant exposure may affect avian thyroid hormone signaling. Thyroid hormones participate in hypothalamic-pituitary-thyroid (HPT) signaling and are critical to growth, development, metabolism, and thermoregulation, including interaction with the HPA and hypothalamic-pituitary-growth hormone (HPGH) axes (reviewed in Decuypere et al., 2005; Scanes and McNabb, 2003). Study of toxic effects on the HPT has mainly focused on the thyroid gland and concentrations of important hormones. Those include thyroid stimulating hormone (TSH) which is secreted by the pituitary gland and regulates thyroperoxidase-mediated synthesis of triiodothyronine (T3) and tetraiodothyronine (T4) in thyroid follicular cells for secretion into plasma. Fowles et al. (2016) reviewed the potential for thyroid toxicity from exposure to raw and refined petroleum products with a combination of data from regulatory tests in mammals, human epidemiology, and wildlife studies. While most exposures showed low risk of thyroid toxicity, there were some exceptions, and certain PAC congeners present modulated *in vitro* mammalian thyroperoxidase activity. Until recently avian studies were few (reviewed in Leighton, 1993), and though the newly available information is difficult to interpret collectively, the weight of evidence indicates that thyroid toxicity from petroleum exposure cannot be ruled out.

A combination of effects on thyroid gland follicles, hormone concentrations, enzyme activity, and gene expression suggests a possible link between petroleum contaminant inhalation or ingestion and thyroid toxicity, though external oiling also modulates thyroid endpoints. Of the few studies that report on the thyroid gland in petroleum-exposed birds, no effect on thyroid mass was found following oral dosing with fuel oil in mallards (*Anas platyrhynchos*) over several days (Lee et al., 2012), nor histological lesions following oral dosing with weathered crude oil in double-crested cormorants (*Phalacrocorax auritus*) over several weeks (Harr et al., 2017b). In the latter experiment however, an external dosing trial (20% SA) yielded thyroid gland follicular hyperplasia. That finding, together with evidence of lower TSH and body mass associated with higher plasma $\sum$PAC concentrations in wild common murres (*Uria aalge*) found oiled (Troisi et al., 2016), suggests that thyroid signaling effects may relate more to metabolic changes or stress than oral toxicity (Dorr et al., 2019; Harr et al., 2017b). The remainder of thyroid endpoint data relates to oil sands contamination of air and water. These laboratory and field studies with three taxa show effects on thyroid gland follicles, hormone concentrations in the thyroid gland or plasma (T_3, T_4, and T_3 to T_4 ratio), and hepatic enzyme activity converting T_4 to T_3 (Beck et al., 2014; Fernie et al., 2016, 2019; Gentes et al., 2007a), although the endpoints affected and the direction of change vary considerably among studies. Age- and sex-specific effects on plasma hormone concentrations may exist (Beck et al., 2014). Finally, thyroid signaling effects are further supported by changes in the transcription of thyroid hormone related genes (e.g. *Thrsp*) in embryo hepatocytes exposed to environmentally relevant PAC mixtures (Crump et al., 2017; Mundy et al., 2019). Additional thyroid gland and hormone studies that are more robust to potentially confounding environmental (site, year) and biological factors (hormesis, age, sex) are needed.

3.3. Embryotoxicity

Incubating birds that become oiled may contaminate their eggs (Albers, 1980; Parnell et al., 1984), and oil that seeps through the eggshell can cause embryotoxicity. Leighton addressed the embryotoxicity of petroleum oils extensively (Leighton, 1993). In short, microliter quantities of petroleum applied to the eggshell exterior caused embryo mortality in several taxa, for instance 3 to 19 μl egg^{-1} of various petroleum types in one study with chicken (Couillard and Leighton, 1991a). Such estimates may be conservative because mortality at hatching can be high (Finch et al., 2011; Franci et al., 2018), but studies often evaluate pre-hatch mortality for logistical and ethical considerations. Embryos in later stages of development are less sensitive to oiling (Couillard and Leighton, 1991b; Lewis and Malecki, 1984), although egg neglect from the temporary abandonment of oiled, incubating parents is more consequential (Butler et al., 1988). *In situ*, embryos would be exposed to oil that has experienced some degree of weathering. Substantially weathered oil is often less toxic to embryos than unweathered oil (Finch et al., 2011; Macko and King, 1980; Stubblefield et al., 1995c; Szaro et al., 1980). For example, weathered oil was 5- to 20-fold less toxic in one experiment (reviewed in Stubblefield et al., 1995a), probably due to a combination of chemical and physical changes (e.g. evaporation of lower molecular weight compounds and resulting increase in viscosity) (reviewed in Albers, 2006). Oil dispersant formulations can modulate embryotoxicity as well (Finch et al., 2012; Wooten et al., 2012).

Aromatic hydrocarbons that permeate the eggshell and their metabolites are responsible for the most toxic effects of petroleum *in ovo* (reviewed in Albers, 2006; Ellenton, 1982; Hoffman, 1978, 1979; Lee et al., 1986; Walters et al., 1987). Recent studies further characterize embryonic exposure from petroleum in the environment. Oiled eggs can absorb detectable three to five ring PACs (Goodchild et al., 2020), and volatile aromatics (e.g. BTEX) can penetrate the eggshell to induce CYP1A protein expression (Dubansky et al., 2018). Because PACs are readily biotransformed *in vivo*, whether the maternal transfer of ingested PACs into the egg can reach concentrations that are embryotoxic, as is the case for external oil contamination of eggs, remains unclear. One laboratory study showed that only minor amounts (<0.1%) of ingested three to five ring parent PACs are deposited into eggs, though hydroxylated metabolites often responsible for PAC toxicity were present in higher amounts (Fournier et al., 2010). In wild birds breeding in oil spill-impacted areas, petrogenic PAC residue maternally transferred into the eggs is variable, though may reach considerable concentrations, as is shown in peregrine falcons (*Falco peregrinus*) (e.g. $\sum$PAC 21 to 461 ng g^{-1} ww) (Shore et al., 1999; Vidal et al., 2011; Zuberogoitia et al., 2006). Besides PACs, metals may also play a role in embryotoxicity, though fewer data related to petroleum exposure are available (Hoffman, 1979; Kertész and Hlubik, 2002). As the avian embryo is a common model for PAC toxicity, work is needed to put this laboratory data in a context of environmental relevance. Empirical measurements or estimates of the amounts of oil transferred to and absorbed by the eggs of incubating birds would be extremely valuable.

The harmful effects of petroleum exposure on embryos include growth, deformity, generalized edema, decreased fatty acid metabolism and necrosis in the liver, and changes to the spleen, kidney, and heart (Couillard and Leighton, 1989, 1990a, 1990b, 1991a, 1991b; reviewed in Leighton, 1993; Westman et al., 2013, 2014). Accumulating evidence points to aryl hydrocarbon receptor (AhR) agonism by PACs as an important mechanism of toxicity. Additionally, the physical blockage of gas exchange through the eggshell by oil covering sufficient surface area can cause hypoxia, which might explain findings of heart distention and decreased heart rate and metabolic rate, but cardiotoxicity could also be a factor (Couillard and Leighton, 1989, 1990a, 1990b; Goodchild et al., 2020; Harr et al., 2017b; Horak et al., 2017; Simpson and Fisher, 2017; Stubblefield et al., 1995c). If so, cardiotoxicity would be consistent with the adverse outcome pathway proposed for AhR-mediated mortality in early life stage organisms (Farhat and Kennedy, 2019). PACs are agonists of this receptor and its activation, measured as EROD activity, correlates with mortality (Franci et al., 2018). Related observations of generalized edema, increased heart mass, and potential effects on blood osmolarity support AhR-mediated mortality (Couillard and Leighton, 1989, 1990b; Dubansky et al., 2018). PACs injected into the egg are also shown to affect embryonic fatty acid metabolism and cause lesions similar to petroleum (Westman et al., 2013, 2014). Hemolytic injury, which can occur when petroleum is ingested at later life stages (Section 3.2.6) has not been documented in embryos (Dubansky et al., 2018; Kertész and Hlubik, 2002).

4. Emergent effects of petroleum exposure

Even where the molecular and physiological effects of petroleum exposure are not obvious or apparent, both external oiling and ingestion can result in effects on the individual that manifest in complex ways and on protracted timescales. Such emergent effects of oil contamination are evident in avian behavior, especially related to foraging and feeding, reproduction, and ultimately survival. In turn, emergent effects of petroleum contamination combined with acute mortality can contribute to population-level effects.

4.1. Behavior

External oiling or oil ingestion can affect the amount and type of certain routine behavioral activities, such as maintenance, feeding, and rest. Externally oiled birds attempt to remove petroleum from their feathers by preening or feather plucking (Camphuysen, 2011; Cunningham et al., 2017; Harr et al., 2017a; Hartung, 1963), and birds may spend more time preening and bathing, even after visible traces of petroleum are removed (Anderson et al., 2000; Burger and Tsipoura, 1998). Aggression, avoidance, and the overall amount of daily activity may be affected (Burger and Tsipoura, 1998; reviewed in Hartung, 1995). As a result, time spent resting or feeding may increase or decrease and lethargy is common, particularly in moribund birds (Anderson et al., 2000; Burger and Tsipoura, 1998; Cunningham et al., 2017; Holmes et al., 1979; reviewed in Leighton, 1993). Those observations come mainly from studies in captive animals. Such behavioral effects may occur in the absence of detectable adverse physiological effects. Accordingly, behavioral endpoints should be included in research studies. Tracking data (radio telemetry and GPS) from wild brown pelicans (*Pelecanus occidentalis*) that were oiled, rehabilitated, and released indicated that movement and habitat use were affected by some aspect of oil exposure or rehabilitation (Anderson et al., 1996; Lamb et al., 2018). One such study suggested that pelicans were perhaps choosing more profitable foraging areas over breeding grounds (Lamb et al., 2018). It is logical that feeding, resting, and maintenance behaviors, as well as aggression and risk-taking, would be affected by external oiling or oil ingestion and the ensuing stress or toxicity. Of course, in wild animals, ecological context mediates the broader behavioral consequences of petroleum exposure.

The ecological traits of a species can amplify or minimize the energetic costs of living with oil-fouled plumage; how those costs may vary among taxa that differ in traits such as feeding, roosting, and migrating has been observed and discussed extensively (Altwegg et al., 2008; Gartrell et al., 2019; reviewed in Hartung, 1995; Henkel et al., 2012; Henkel et al., 2014a; reviewed in Jenssen, 1994; Troisi, 2013; reviewed in Wiens, 1995; reviewed in Wolfaardt et al., 2009). Because of high conductive heat loss in water, reliance on marine or aquatic habitat greatly increases a species' vulnerability to the effects of oil-compromised plumage on thermal balance. Besides habitat, other ecological factors like ambient temperature and body size also govern thermal balance. To illustrate this with an example, the species most vulnerable to oiled plumage would be diving birds that occupy cold-water, pelagic habitats, roost on the water's surface, have a small body size, and have limited fat reserves (Altwegg et al., 2008; Gartrell et al., 2019; reviewed in Hartung, 1995; reviewed in Jenssen, 1994; Oka and Okuyama, 2000; Troisi, 2013; reviewed in Wiens, 1995; reviewed in Wolfaardt et al., 2009). By contrast, the least vulnerable birds would be those with larger body size resident in warm, terrestrial habitats. If oiled at sea, more terrestrial species may readily escape to land (Camphuysen, 2011; Munilla and Velando, 2010), whereas more pelagic species do so reluctantly (Camphuysen and Leopold, 2004; Duerr et al., 2016; Gartrell et al., 2019; Henkel et al., 2014b; Jauniaux et al., 1997). Obligate marine or aquatic birds are particularly affected by plumage oiling and may readily succumb to hypothermia or starvation (Jenssen, 1994). Beyond increased metabolic cost of thermoregulation, increased cost of transport for animals with oiled plumage would disproportionately affect birds where swimming or flight performance is critical to foraging, prey capture, predator escape, long-distance migration, or provisioning offspring (Culik et al., 1991; Maggini et al., 2017b, 2017c; Perez et al., 2017a, 2017b).

Petroleum contamination can affect prey availability and where birds forage. Long-term ecological effects can result from large petroleum spills (reviewed in Esler et al., 2018). Although the cause of trophic shifts could be multifactorial and hard to identify, they could include top-down effects or decreased prey populations. Changes in prey availability (Moreno et al., 2013; Sanpera et al., 2008; Velando et al., 2005b) and habitat use (Banks et al., 2008; Day et al., 1995; reviewed in Esler et al., 2000a, 2018) have been documented. Disturbance from clean-up activity may also impact habitat use (Burger, 1997; Henkel et al., 2014a). Decreased prey availability or quality have been linked to

reproductive effects (Golet et al., 2002; Moreno et al., 2013; Sanpera et al., 2008; Velando et al., 2005a, 2005b). Where birds themselves are exposed to oil, maintaining caloric intake with adequate prey seems important to overcoming the metabolic cost of oiled plumage and toxic effects (Esler et al., 2000b; Hartung, 1967; Horak et al., 2017; reviewed in Jenssen, 1994).

4.2. Reproduction

Mating and nesting success depend on attendance, demographics, and behavior at the breeding grounds, but petroleum exposure may adversely affect those processes either before or during breeding. Migration to breeding grounds or specific colonies is a life-history characteristic typical of birds. The physical and toxic effects of oil exposure at overwintering areas or *en route* (Esler et al., 2011, 2017; Gibson et al., 2017; Henkel et al., 2014a; Rattner et al., 1995; Seegar et al., 2015; Timoney and Ronconi, 2010) could affect the successful completion of migration or timely arrival in good condition for breeding, especially in long-distance migrants like shorebirds (Bianchini and Morrissey, 2018a, 2018b; Burger, 1997; Burger and Tsipoura, 1998; Bursian et al., 2017a; Henkel et al., 2012; Maggini et al., 2017a, 2017b, 2017c). The breeding grounds of petroleum-impacted populations may have fewer attending birds. Delay, absenteeism, emigration, and death affect pairing, nesting, and mate- and nest-site fidelity (Anderson et al., 1996; Camphuysen, 2011; Field et al., 1993; Walton et al., 1997; reviewed in Wiens, 1995; Wolfaardt et al., 2008c). Those may be influenced by other factors as well, including non-even sex ratio (Martínez-Abraín et al., 2006), or effects on physiologically-dependent sexual ornaments, such as the carotenoid-based red spot common to many species of gull, which can be affected by petroleum ingestion (Jaques et al., 2019; Pérez et al., 2010a, 2010b). Petroleum exposure may affect breeding over multiple seasons; even experienced breeders may not breed consistently or at all (Anderson et al., 1996; Lamb et al., 2018; Wolfaardt et al., 2008a, 2008b, 2008c). Furthermore, oiling appears to increase the cost of reproduction, as exposed African penguins (*Spheniscus demersus*) that bred had lower survival than oiled non-breeders and unoiled birds (Wolfaardt et al., 2008a, 2008b). This research suggests an effect of oiling on fitness, but available long-term breeding behavior data on spill-impacted individuals exists only in penguins (Giese et al., 2000; Sievwright et al., 2019b; Wolfaardt et al., 2008a, 2008b). More research in this area is needed, and modeling approaches, for instance, could be useful to predict effects on fitness. Also, more studies linking petroleum exposure at overwintering areas or on migration with effects at distant breeding grounds would be informative (e.g. Field et al., 1993).

For birds that do engage in breeding activity, the success of any breeding attempt will depend on successful laying and incubation, but adverse effects on those have been documented in association with spilled oil or exposed parents. Observational effects recorded among wild birds in petroleum-impacted areas include changes in clutch initiation timing, clutch size, egg abandonment, changes to eggshell volume and thickness, and reduced hatching success (Azkona et al., 2006; Velando et al., 2005b; Vidal and Domínguez, 2015; Zuberogoitia et al., 2006). Those recent findings are consistent with experimental evidence of effects on egg production, yolk deposition, eggshell thickness, fertility, egg rejection, abandonment, and hatching success (reviewed in Leighton, 1993). Eggshell thinning effects have been replicated in one more recent experiment at an environmentally relevant exposure rate with weathered crude oil (2.4 ml kg^{-1} bw d^{-1}), an effect concurrent with decreased blood Ca^{2+} in that experiment, as well a similar Ca^{2+} effect in laying wild female yellow-legged gulls (*Larus michahellis*) given small doses (0.04 ml kg^{-1} bw d^{-1}) of fuel oil (Alonso-Alvarez et al., 2007b; Stubblefield et al., 1995a, 1995c). Hatching success is mediated in part by parental care, though embryotoxicity (Section 3.3) can be a factor.

The most ecologically informative measure of reproductive success is the number of recruited offspring, and indirect proxies for this, the number and condition of fledged offspring, can be reduced by oil contamination in the environment (reviewed in Leighton, 1993). Widespread absenteeism of oil-affected parents in the immediate aftermath of a spill may cause mass chick mortality (Crawford et al., 2000). Chick rearing effects (i.e. condition, fledging) may result from reductions in the amount or quality of prey with which parents provision chicks and can persist many years after a spill (Barros et al., 2014; Golet et al., 2002; Moreno et al., 2013; Sanpera et al., 2008; Trivelpiece et al., 1984; Velando et al., 2005a, 2005b). Studies have indicated slower chick growth (Andres, 1999) and reduced chick condition and fledging success (Azkona et al., 2006; Bergeon Burns et al., 2014; Golet et al., 2002; Velando et al., 2005a, 2005b) in petroleum-impacted populations. Oiled and rehabilitated penguins have also shown decreased fledging success (Barham et al., 2007; Giese et al., 2000; Wolfaardt et al., 2008c), a longer fledging period (Barham et al., 2007), and lighter chicks at fledging (Giese et al., 2000). In those penguins, the sex of the oil-affected mate of the pair affected fledgling growth rate or overall success rate, and both parents being affected by oil was additive (Giese et al., 2000; Wolfaardt et al., 2008c). While lower food intake alone can explain growth and fledging effects, there is good evidence that oil ingestion by chicks reduced growth in some cases (Gurney et al., 2005; reviewed in Leighton, 1993), and industrial PAC contamination in the environment has been associated with non-predation nestling mortality (Bustnes, 2013; Gentes et al., 2006), suggesting that toxicity could be involved in effects on chicks.

Research indicates considerable variation in the severity and type of reproductive impacts on breeding success, even among apparently similar oil spill scenarios (reviewed in Wolfaardt et al., 2009). Reproductive effects are not always present or apparent following major oil spills (Altwegg et al., 2008; Andres, 1999; Burger, 2018; Camphuysen, 2011; Sievwright et al., 2019b) and in areas with considerable petroleum industry activity such as the oil sands (Cruz-Martinez et al., 2015a; Fernie et al., 2018b; Godwin et al., 2019). Breeding phenology and degree of contamination are important, and bird species which can forage over larger areas or on more diverse types of prey may be more resilient to toxic or ecological effects in petroleum-impacted habitats (Andres, 1999).

4.3. Survival

Information on how the degree of petroleum exposure relates to the ultimate fate of free-living individuals remains incomplete, but available examples highlight the importance of ecological factors and show potentially decreased survival from external oiling or ingestion of residual hydrocarbons after a spill. Of course, mass avian mortality, especially of marine taxa, during the initial days and weeks of large oil spills have been well-documented (e.g. reviewed in Burger and Fry, 1993; Bursian et al., 2017a; Crawford et al., 2000; Esler et al., 2018). Many studies report the total number of individuals killed, though these are estimates and subject to debate. For birds that escape oiling from floating petroleum during the initial phases of a spill, exposure to residual oil hydrocarbons at impacted areas may persist for many years (e.g. 2 to 20) (Esler et al., 2010, 2011, 2017, 2018; Golet et al., 2002; Trust et al., 2000; Velando et al., 2010) and can decrease survival in those areas (Esler et al., 2000b; Iverson and Esler, 2010). Few studies report on the survival of living birds shown to be exposed to oil in the wild, and still fewer report survival where exposure is quantified in some way.

As discussed earlier, external oiling can be catastrophic for heavily oiled birds, though smaller amounts of petroleum may still be immediately life threatening by primarily affecting thermal balance (Section 3.1.2). Lethal thresholds for thermal balance effects are complicated by the particularities of environmental conditions and species, and work on this is still needed. It is known that the amount of oil found on dead birds may vary widely, completely saturating the feathers of some individuals, while on other individuals insubstantial amounts of

oil (e.g. 6.3 to 21.5 ml kg^{-1} bw; 10% SA and above) are found on birds showing signs of death consistent with thermal dysregulation, though toxicity may contribute (Balseiro et al., 2005; Fry and Lowenstine, 1985; Hartung, 1963; Oka and Okuyama, 2000). Even if birds initially survive with only minor portions of their plumage affected by oil, long-term survival may still be decreased. In African penguins (*Spheniscus demersus*) with heavy fuel oil contaminating their feathers, those with 5% SA oiled or more were significantly less likely to be re-sighted in the next five years in a dose-dependent fashion, and none of the birds with oil penetrating to the skin were re-sighted (Wolfaardt et al., 2008b). Those observations may reasonably support the widespread assumption that survival is decreased from even light oiling and that heavy oiling is fatal in pelagic marine taxa. Yet, available data for more terrestrial species shows that survival in visibly oiled gulls and plovers is not lower than normal (Camphuysen, 2011; Gibson et al., 2017).

For birds ingesting petroleum by preening or through the diet, the weight of evidence from an abundance of captive oral dosing studies under normal husbandry conditions indicates that birds ingesting realistic amounts (e.g. <5 ml kg^{-1} bw d^{-1}) of petroleum over abbreviated time periods without oil-compromised plumage are likely to survive in the short term (Table 3), although ingestion rates remain inadequately characterized (Section 5.1). Most studies documented survival following exposure to petroleum amounts as high as 5 to 20 ml kg^{-1} bw d^{-1} for one to seven days (Dean et al., 2017b; Newman et al., 1999; Stubblefield et al., 1995b) or amounts up to 2.4 to 10 ml kg^{-1} bw d^{-1} for several weeks (Bursian et al., 2017b; Stubblefield et al., 1995b, 1995c), but two dosing studies in captive adult birds do point to death after oral exposure to 5 to 10 ml kg^{-1} bw d^{-1} of crude oil over 6 to 7 or more days (Cunningham et al., 2017; Horak et al., 2017). For birds in general, the rates of ingestion which are actually lethal probably ranges from a few to tens of ml kg^{-1} bw d^{-1} over at least several days for most bird species, a dose-response best characterized as a threshold effect (reviewed in Hartung, 1995). Differences in sensitivity among taxa are evident from multi-species studies using the same petroleum and comparable methodologies (Dean et al., 2017b). This may be due in part to moderate interspecies variation in sensitivity to PACs, as has been documented with embryonic exposures (Crump et al., 2017; Franci et al., 2018; Head et al., 2015; Mundy et al., 2019).

Importantly, the presence of factors such as age, stress, or food availability could affect lethal toxicity thresholds (Holmes et al., 1978; Horak et al., 2017). A number of older studies point to increased toxicity of ingested oils in young birds or in the presence of other stressors. While this information has been mentioned elsewhere (reviewed in Jenssen, 1994; Leighton, 1993), its importance merits emphasis. For instance, in ducks kept in crowded, cold conditions thought to be stressful, the LD_{50} of single oral doses of refined oils dropped dramatically (e.g. from 24 to 4 ml kg^{-1} bw for diesel fuel) (Hartung and Hunt, 1966). Analogous increases in mortality were apparent in experiments where

Table 3
For each of the various body systems affected by petroleum toxicty, we looked through the adult oral dosing studies published since 1993 for lowest observed effect levels for endpoints related to that body system and reports of mortality (data in bold font). This table reports nominal doses.

Outcome	Experimental endpoint	Species	Oil class	Dose range (ml kg^{-1} bw d^{-1})	Treatment duration (d)	Lowest observed effect level (ml kg^{-1} bw d^{-1})	Lowest lethal dose[a]		Reference
							Dose (ml kg^{-1} bw d^{-1})	Mortality occurrence (d)	
Gastrointestinal damage	Pathological lesions	*Phalacrocorax auritus*	Crude	5–10	*21*	**5**	**10**	***7–14***[b]	(Harr et al., 2017b)
	Increased kidney mass	*Phalacrocorax auritus*	Crude	5–10	*21*	**5**	**10**	***7–14***[b]	(Harr et al., 2017b)
Osmoregulation and excretion	Plasma Ca^{2+}	*Larus michahellis*	Fuel	0.04	*7*	**0.04**	–	–	(Alonso-Alvarez et al., 2007b)
	Serum Ca^{2+}	*Anas platyrhynchos*	Crude	0.02–2.4	*70*	**2.4**	–	[c]	(Stubblefield et al., 1995c)
	Body mass	*Leucophaeus atricilla*	Crude	5–10	*28*	**5**	**5**	***7–28***[b]	(Horak et al., 2017)
		Calidris mauri	Crude	1–5	*20*	**5**	–	[b]	(Bursian et al., 2017b)
Metabolic effects and the liver	Liver mass	*Calidris mauri*	Crude	1–5	*20*	**5**	–	[b]	(Bursian et al., 2017b)
	Plasma glucose	*Larus michahellis*	Fuel	0.04	*7*	**0.04**	–	–	(Alonso-Alvarez et al., 2007b)
	Glucocorticoid receptor density	*Passer domesticus*	Crude	1.1	*28*	**1.1**	–	–	(Lattin et al., 2014)
	Plasma vitamin E	*Larus michahellis*	Fuel	0.04	*7*	**0.04**	–	–	(Pérez et al., 2010a)
Oxidative balance and damage	Hepatic total antioxidant capacity	*Calidris mauri*	Crude	1–5	*20*	**1**	–	[b]	(Bursian et al., 2017b)
	Hepatic total, oxidized, and reduced glutathione	*Leucophaeus atricilla*	Crude	5–10	*28*	**10**	**5**	***7–28***[b]	(Horak et al., 2017)
Hemotoxicity	Decreased hematocrit and reticulosis	*Phalacrocorax auritus*	Crude	5–10	*21*	**5**	**10**	***7–14***[b]	(Harr et al., 2017b)
Cardiotoxicity	Gross pathology of cardiac structure	*Phalacrocorax auritus*	Crude	5–10	*21*	**5**	**10**	***7–14***[b]	(Harr et al., 2017b)
Immunotoxicity	Relative spleen size	*Anas platyrhynchos*	Crude	0.02–2.4	*70*	**2.4**	–	[c]	(Stubblefield et al., 1995c)
Endocrine effects	Adrenal interrenal hypertrophy	*Calidris mauri*	Crude	1–5	*20*	**1**	–	[b]	(Bursian et al., 2017b)
	Acute corticosterone stress response	*Passer domesticus*	Crude	1.1	*28*	**1.1**	–	–	(Lattin et al., 2014)

[a] We considered the lowest lethal dose to be 10 ml kg^{-1} bw d^{-1} for Harr et al. (2017b) because only one out of nine birds died in the 5 ml kg^{-1} bw d^{-1} treatment, inconsistent with the pattern of mortality from toxicity observed with the other birds.
[b] Another similar experiment showed no mortality with doses up to 20 ml kg^{-1} bw d^{-1} for 5 days in this species (Dean et al., 2017b).
[c] Another similar experiment showed no mortality with doses up to 9.5 ml kg^{-1} bw d^{-1} for 14 days in this species (Stubblefield et al., 1995b).

ducks fed otherwise sublethal amounts of petroleum were stressed with saline water and cold temperatures, or a bacterial challenge (Holmes et al., 1978, 1979; Rocke et al., 1984). Furthermore, workers at rehabilitation centers have made the anecdotal observation that common murres (*Uria aalge*) with oiled plumage are unable to survive clinically low hematocrit and plasma protein as unoiled murres commonly do, suggesting a decreased resilience to physiological crisis (Duerr et al., 2016). Exposure to low levels of petroleum contamination in the environment may thus lead to decreased survival when birds are faced with physiologically precarious situations (Esler et al., 2000b; Gentes et al., 2006), and we have described previously the ways that petroleum exposure can affect food intake, body mass, thermoregulation, and the corticosterone-mediated acute stress response via the HPA hormone axis. While dosing studies show that birds are likely to survive estimated rates of *in situ* petroleum exposure under normal conditions in captivity, that resilience to environmentally realistic amounts of ingested petroleum may quickly vanish in the presence of environmental stressors, and ingested petroleum may cause or contribute to death in birds that are unable physiologically to handle a stress-inducing, life-threatening challenge.

Data on the fate of oiled birds that had been rescued in large numbers, cleaned in rehabilitation centers, and released shows lower long-term survival in some cases that may relate to some aspect of petroleum exposure. During care, oiled birds undergoing rehabilitation do not necessarily fair worse than birds rescued for non-oil related reasons (Gartrell et al., 2019; Montesdeoca et al., 2017; Parsons et al., 2018), though mortality rates may be high (Duerr et al., 2016). Post-release survival in controlled studies (i.e. compared to unoiled wild birds kept temporarily in rehabilitation settings as a control group) was substantially lower over the tracking period in some cases, but no different in other cases (reviewed in Henkel and Ziccardi, 2018; Sharp, 1996; reviewed in Sievwright et al., 2019a; Underhill et al., 1999; reviewed in Wolfaardt et al., 2009). Such considerable variation in post-release survival appears related to species and scenario. Individuals of more resilient species may live out their expected lifespan (reviewed in Wolfaardt et al., 2009), while low post-release survival in other species may relate to petroleum exposure.

Survival often differs by age class or sex in petroleum-impacted bird populations. Sex- and age-specific effects on mortality or survival rates are evident in many studies (Anderson et al., 1996; Azkona et al., 2006; Balseiro et al., 2005; Camphuysen and Leopold, 2004; Duerr et al., 2016; Jauniaux et al., 1997; Martínez-Abraín et al., 2006; Nevins and Carter, 2003; Votier et al., 2008). Those observations often were attributed to different use of habitat. For instance, the carcasses of European shags (*Phalacrocorax aristotelis*) thought to be killed by oil from the *Prestige* wreck pointed to disproportionately higher mortality among juveniles and females in spill-affected waters compared to adult males that attend the breeding colony earlier to stake and defend nesting territory (Martínez-Abraín et al., 2006). Although sex- or age-specific mortality is usually attributed to differences in habitat use, it is also possible that individuals with smaller body sizes, such as subadults or females, may suffer greater effects from exposure to a given amount of petroleum (Camphuysen and Leopold, 2004; Fowler et al., 1995). How such differences in sensitivity among demographic classes relate to survival remain incompletely known despite numerous dosing studies. Mortality-driven demographic shifts may subsequently affect population dynamics.

4.4. Populations

Mortality from petroleum spills can reduce bird populations and long-term effects, direct or indirect, may prolong population recovery. Although an examination of mortality estimates and population dynamics over time is beyond the scope of this review, it bears mentioning that the slow intrinsic population growth common to seabirds can put their populations at risk from large mortality events (reviewed in Morandin and O'Hara, 2016). Indeed, the *Exxon Valdez* crude oil spill heavily impacted marine bird populations, which recovered to pre-spill numbers only after several decades, if at all (Esler et al., 2018). Beyond acute mortality among the general population, age- or sex-specific mortality (above paragraph) and sub-lethal effects (Alonso-Alvarez et al., 2007b; Giese et al., 2000; Wolfaardt et al., 2008c), as well as long-term reproductive effects (Barros et al., 2014; Velando et al., 2005a) could each affect population size and structure, and thereby constrain population size or growth. Continued exposure to residual oil or changes in prey availability can be important factors (Barros et al., 2014; Esler et al., 2018; Moreno et al., 2013; Sanpera et al., 2008; Velando et al., 2005a, 2005b).

5. Research priorities: bridging knowledge gaps and forging ahead

5.1. Where are the exposure estimates?

Despite an abundance of oral toxicity studies, there is a glaring scarcity of estimates for how much petroleum birds that are free to feed and preen actually ingest over time, and more research is profoundly needed here. In this literature review, we found only one paper since 1993 that provided a new estimate of oral ingestion rate. In that work, hydrocarbon residue in bivalve mussels was used to estimate that sea ducks feeding on the most contaminated mussels during the height of the *Exxon Valdez* spill ingested 0.14 ml kg^{-1} bw d^{-1} of crude oil (Hartung, 1995) (Appendix A: Exposure Estimate 2). A further examination of pre-1993 works yielded only one other primary study estimating ingestion rate. That pioneering and often-cited, though extremely limited, study with three ducks showed that a bird with 16.6 ml kg^{-1} bw of oil on its plumage would consume up to 3.32 ml kg^{-1} bw d^{-1} from preening (Hartung, 1963) (Appendix A: Exposure Estimate 1). So, even when birds may be substantially oiled themselves and feeding on contaminated prey, it is hard to imagine ingestion rates far beyond 4 ml kg^{-1} bw d^{-1} or persisting at this level over more than a few days without surreptitious external exposure. Again, data on the duration of exposure expected are also needed. Many conventional oral dosing studies reviewed herein have employed doses, including 'low' doses, well above 4 ml kg^{-1} bw d^{-1}, often over multiple weeks. Until petroleum ingestion rates are more thoroughly characterized, the relevance of toxic effects described at such substantial doses remains uncertain and those studies may be best considered as worst-case scenario examples. Related to the need for ingestion rate data, quantitative data linking surface area of plumage appearing oiled, what observers actually see in the field, to the amount of oil affecting plumage (ml kg^{-1} bw) would be extremely useful for modeling plumage effects, ingestion from preening, and ultimately survival.

An important caveat is that sublethal effects may occur well within the range of environmentally relevant ingestion rates (e.g. 4 ml kg^{-1} bw d^{-1}), as shown by the lowest observed effect levels for endpoints relevant to the body systems affected by petroleum toxicity among the studies we reviewed (Table 3). For instance, exposures within this range can cause effects on circulating calcium and eggshell thinning (2.4 ml kg^{-1} bw d^{-1}), plasma glucose (0.04 ml kg^{-1} bw d^{-1}), hepatic antioxidants (1 ml kg^{-1} bw d^{-1}), corticosterone stress response (1.1 ml kg^{-1} bw d^{-1}), and others (Alonso-Alvarez et al., 2007b; Bursian et al., 2017b; Lattin et al., 2014; Stubblefield et al., 1995c).

After nearly 60 years of petroleum dosing studies, the need for better estimates of what constitutes realistic petroleum exposure seems to far outweigh the need for further conventional laboratory oral dosing studies. When one considers that high rates of oral ingestion are only expected in heavily oiled birds, and that the physical and metabolic effects of heavy external oiling alone are disastrous (Hartung, 1967), *the use of conventional lethal oral dosing studies and high doses* (i.e. *10 to 20 ml*) *are nearly unjustifiable*, at least until their relevance is better demonstrated. Thus, refined and broadly relevant estimates of environmentally realistic ingestion rates, and how concurrent stressors may

modify toxicity, are a higher research priority. Related to this, emerging data showing the potential toxicity of airborne petroleum compounds associated with spills at sea or industrial activity (e.g. oil sands) highlights a need for putting air contaminant measurements in an avian exposure context (Cruz-Martinez et al., 2015a, 2015b; Dubansky et al., 2018; Fernie et al., 2016; Olsgard et al., 2008).

5.2. Unconventional crude oil

Unconventional crude petroleum types, though increasingly common, have not been studied for their direct toxicological effects in avifauna, as existing research has focused exclusively on the effects of oil sands process-affected materials and airborne contaminants in the environment near Canadian oil sands mining and processing industrial activity. Oil sands process-affected water has received substantial research attention in particular, work that Beck et al. (2015) has reviewed, concluding that adverse effects, including growth and reproduction, thyroid and stress hormone function, and immune function were weak at best or equivocal and inconsistent between years. Since then, unconventional crude oil research has focused on airborne oil sands industry contaminants that may be inhaled or deposited into aquatic ecosystems. These do show some evidence of toxicity in laboratory and field studies, though field studies with replicate reference sites and years are needed in many cases. *In vitro* hepatocyte exposures with PACs atmospherically deposited to lakes in the oil sands region or found in petroleum coke, a thermal hydrocracking by-product of bitumen upgrading subject to wind erosion, both affect the expression of genes associated with toxicity (Crump et al., 2017; Mundy et al., 2019). Laboratory and field studies in nestling and adult birds report some indications of immune (Cruz-Martinez et al., 2015a, 2015b; Olsgard et al., 2008, 2009), thyroid (Fernie et al., 2016, 2019), and stress hormone (Cruz-Martinez et al., 2015b) effects in association with exposure to airborne or atmospherically deposited oil sands industry contaminants. However, available data shows no consistent reproductive effects in field studies (Cruz-Martinez et al., 2015a; Fernie et al., 2018b; Godwin et al., 2019), and no evidence of decreased survival exists.

Despite the above research suggesting avian toxicity from petrogenic oil sands contaminants in environmental matrices, the toxicity of direct avian exposure to bituminous oil sands crude petroleum and how it may compare to conventional crude petroleum remains unknown. Direct exposure to bulk petroleum occurs when birds such as migratory waterfowl contact residual bitumen in the sediment or floating on the surface water of tailings ponds at industrial sites (reviewed in Beck et al., 2015; Timoney and Ronconi, 2010). Exposure may occur from accidental spillage along transportation routes as well. At present, the transportation of bituminous petroleum to distant areas and overseas by tanker vessel are projected to increase (NEB, 2016b). As this oil sands example demonstrates, how unconventional petroleum types compare in toxicity to the current understanding of avian petroleum toxicity remains an open question. Yet, it is important to answer in order to adequately assess and manage risk to wildlife resources.

5.3. Towards an improved avian model of petroleum effects

Studies on the effects of petroleum exposure on birds are valuable, though technically challenging, and barriers to the replication and broader scientific application of research results abound. There are methodological obstacles related to quantifying exposure, delivering doses, and potential experimental artefacts. Design and reporting deficiencies often limit the interpretability and applicability of a given study. Given these pitfalls, what is the prudent empiricist to do? Fortunately, the available literature contains many good examples and instructive lessons. Careful study design, improved reporting standards, and novel tools and approaches will benefit this area of research, each of which we discuss below. To enhance comparability among studies, we propose a list of minimum information and design criteria for publishing avian petroleum toxicity studies (Table 4).

Among petroleum dosing studies, major barriers to interpreting, replicating, and applying results to other exposure scenarios are caused by insufficient information about or replication of the toxicant. A type of petroleum such as fuel oil or crude oil is more a category than an invariable substance. The composition of, for example, a given crude oil can

Table 4
In order to make experiments replicable and their results more broadly relevant, the following should constitute the minimum information and design criteria for publishing avian petroleum toxicity studies. Some items are essential (E), while others are at least desirable (D).

Design aspect	Criteria	Importance
Toxicant	Type of petroleum and source	E
	Age and storage conditions	D
	Degree of weathering, weathering conditions, percent mass or volume loss	E
	The experimental relevance of weathering	E
	Petroleum density	E
	Petroleum viscosity	D
	Replication of petroleum sample(s) used (e.g. replicate samples of a spilled crude oil, or samples of from multiple production fields and seasons, composited samples, statistical design)	D
Test organism	Species name	E
	Average body mass	E
	Life stage	E
	Controls for sex-specific effects	D
	Pre-experiment baseline data for endpoints of interest	D
	Domestic birds:	
	Husbandry conditions	E
	Strain	D
	Generations in captivity	D
	Any prior exposure to the toxicant	E
	Wild birds:	
	Source population	E
	If brought into captivity, duration and husbandry conditions	E
Oral doses	Administration method (e.g. oil-in-food, gelatin capsule, slurry gavage, neat)	E
	Appropriate controls (e.g. food-grade mineral oil)	E
	Range of at least two or more doses	D
	Dose amounts as g or ml per kg body weight, per day if over multiple days (e.g. g kg^{-1} bw d^{-1} or ml kg^{-1} bw d^{-1})	E
	Report any regurgitation and defecation that could affect exposure	E
	Pre-dose fasting duration	D
	Doses per day	D
	Explanation of environmental relevance of dose	E
External application	Percent surface area oiled and whether this is with folded or stretched wings	D
	Application amounts as g or ml per kg body weight (e.g. g kg^{-1} bw or ml kg^{-1} bw)	E
	Any reapplication or cleaning	E
	Temperature	E
Test conditions	Duration of exposure	E
	Time elapsed from treatment to sampling	E
Embryotoxicity	Average initial fresh egg mass	E
	Dosage on a μl or mg per g egg basis (e.g. μl g^{-1} egg)	E
	Percent egg surface area oiled	D
	Appropriate nontoxic oil control	E
	Whether the embryos could be determined to be alive when the dose was applied	E
	Age at sampling	E
	Hatching success endpoint	D
Endpoints	All endpoints evaluated and their result, even if not significant	E
	Effect sizes	D
	Variability	D
	Power	D
	Context of clinical or literature reference values	D

vary among production fields (e.g. Prudhoe Bay versus South Louisiana), and weathering is important to toxicity (Couillard and Leighton, 1991a; Finch et al., 2011; Macko and King, 1980; Stubblefield et al., 1995a; Szaro et al., 1980). Though common, it is inherently problematic to use a single can of petroleum from a particular source in a battery of laboratory studies to predict the effects of a category of petroleum on an entire species or birds in general. Detailed information about the petroleum, such as what year and season it was produced, and storage conditions are essential (DFO, 2017; Lee et al., 2015). Beyond those details, results are more broadly applicable if the experimental design accounts for variation in the petroleum's exact composition. That could include using composite petroleum samples, using the petroleum sample as the unit of replication or treatment group, or using statistical models that account for petroleum sample.

Quantifying the amount of petroleum on plumage or that birds ingest has always been a challenge, but can be addressed. Quantifying visible oil on plumage is difficult, subjective, and has no universally recognized standard methodology. Oil scoring methods reported in research papers are seldom comparable, although good examples of scoring methods exist (Camphuysen, 2011; DHNRDA Trustees, 2016), and study skins can be useful (e.g. Cunningham et al., 2017; Maggini et al., 2017b). Oil washed from plumage can be quantified, and methods like Hartung's (1963) would be valuable to further quantify oil ingestion rates. Even in oral exposures, the net amount ingested may be somewhat uncertain because petroleum can be excreted or regurgitated within minutes, making the amount available for absorption less certain (Cunningham et al., 2017; Dean et al., 2017b). Delivery methods can be optimized (e.g. incorporating into food items or slurry) but these, in turn, can affect toxicity. For instance, oil that is administered neat via gavage or in gelatin capsules is more toxic than when incorporated into the diet (reviewed in Hartung, 1995). Employing low doses and routes (e.g. food items) that are environmentally realistic is one obvious solution.

Consistent reporting of critical exposure information like animal weight, oil density, and analytical chemistry methodology enables ready comparison among studies and can facilitate replication of results, meta-analyses, literature reviews, and risk assessments. Petroleum doses are usually given on the basis of grams or milliliters per unit body weight per day (e.g. ml oil kg^{-1} bw d^{-1}). Doses on a body weight basis, or some indication of average animal body mass, are necessary to compare among studies accurately. Like body mass, egg size varies among taxa. Egg oiling studies should measure the initial fresh weight of eggs used and include petroleum application data on a μl g^{-1} egg or mg g^{-1} egg basis, as well as the proportion of egg surface area affected (e.g. Finch et al., 2011). To convert between oil weight and volume, petroleum density is necessary. Dose and density data must be reported in future studies. Lastly, chemical analysis of PAC residue, especially in avian tissue, is extremely valuable. Unfortunately, there is often insufficient analytical information to compare the data between studies. PAC concentration data would be more beneficial to other researchers if only slightly more information were reported in supplementary files, including target PAC analytes, their detection limits, whether concentrations are given on a wet or dry weight basis, and relevant quality control and quality assurance data. Fortunately, the aforementioned problems found commonly in the literature are easily corrected.

Animal studies are useful, where ethically justifiable, and researchers can benefit from appreciable consistency in the literature regarding experimental conditions and the choice of species used. Some standard toxicity test methods have been successfully adapted to petroleum (Stubblefield et al., 1995a). The current versions of those may be a useful framework (ASTM, 2019; OECD, 1984a, 1984b, 2016; USEPA, 1996, 2012a, 2012b, 2015) and we recommend other good experimental designs in the peer-reviewed literature that can serve as a template (e.g. Couillard and Leighton, 1989; Cunningham et al., 2017; Dean et al., 2017b; Kononen et al., 1986; Maggini et al., 2017c; Perez et al., 2017a). Guidelines exist for aquatic petroleum toxicity studies, many of which are applicable to avian research (DFO, 2017). Because petroleum can exert effects via the physical fouling of plumage or blockage of eggshell gas exchange, toxicity studies should use non-toxic control substances with matching physical properties (e.g. vegetable oil) to attempt to separate physical effects of oil from actual toxicity. In terms of test organisms, studies have historically relied on the mallard (*Anas platyrhynchos*), wild caught or domestic, as the laboratory model of choice for petroleum toxicity studies. Wild caught double-crested cormorant (*Phalacrocorax auritus*), and captive-bred zebra finch (*Taeniopygia guttata*) and homing pigeon (*Columba livia*) have also proven to be tractable models (Cunningham et al., 2017; Goodchild et al., 2020; Perez et al., 2017c). While each of these animal models has its respective advantages, studies should make use of species most relevant to the ecological context of the research question at hand.

Experimental artefacts from the handling or captivity of birds may affect results and their interpretability, which should be taken into account. Handling can affect plasma biochemistry related to stress and markers of muscle injury (Chilvers et al., 2016; Dean et al., 2017b; Lattin et al., 2014), and captivity can affect the biology of wild birds, for instance causing anemia (Burco et al., 2014; Cunningham et al., 2017; Dannemiller et al., 2019; Dean et al., 2017a; Newman et al., 1999, 2000). For wild birds studied in captivity, appropriate acclimatization period, sampling methods, pre-treatment baselines, and statistical procedures are warranted.

Researchers need not confine avian petroleum ecotoxicology studies to captive birds. The realism of field experiments done *in situ* is invaluable. Homing flight studies in domestic pigeons (*Columba livia*) have proven informative and there are many examples in truly wild birds (Alonso-Alvarez et al., 2007b; Butler et al., 1988; Perez et al., 2017a, 2017b, 2017c). Future studies could take advantage of advances in tracking device technology (e.g. Perez et al., 2017c). Wild birds almost certainly work harder to move and forage on their own and are without the safety net of ad libitum access to food in captivity. Furthermore, *in situ* studies integrate important behavioral effects and ecological factors, and if desired, the effects of plumage fouling on flight performance and metabolism that simplified studies on oral toxicity necessarily exclude. Thus, field experiments provide an essential bridge between the results of conventional laboratory dosing studies and effects on free-living birds which may be exposed to petroleum at lower levels but face the challenges of survival in the wild.

While there are many studies on oiled birds that were rescued, rehabilitated, and released, many do not include the necessary controls to distinguish the effects of oiling from handling and captivity. Where this is the case, any inference of oil-related effect should be avoided or made cautiously. Data on pre-spill baselines, effects in oil exposed animals left unaided, and unoiled control groups receiving the same rehabilitation regimen would make studies in rescued birds more valuable, and good examples do exist (e.g. Golightly et al., 2002; Wolfaardt et al., 2008b).

Petroleum toxicity is systemic, affecting multiple body systems in subtle ways rather than an obvious or localized common mode of action, yet there is a chance that recent technological advances may further improve our understanding of the mechanisms at work. Advances in the study of gene expression that measures RNA within tissue and the emerging field of omics are particularly promising (reviewed in Gonzalez and Pierron, 2015). For example, gene expression analysis methods employ quantitative PCR (qPCR) to look at transcript abundance of genes of interest within a tissue, for instance the *Cyp1a4* and *Cyp1a5* genes that code for their respective CYP1A phase I biotransformation proteins. Gene expression with qPCR is useful for examining one or a few genes of interest, examining a suite of genes involved in a single pathway, or high-throughput gene arrays which employ a panel of genes, such as genes commonly implicated in adverse outcome pathways (Crump et al., 2016, 2017). RNA sequencing (RNA-Seq) is a transcriptomic tool that determines the sequence and quantity of thousands of RNA strands or whole transcriptomes present in a

Table 5
Strategic recommendations for researchers and decision makers to prepare for and respond to a petroleum spill affecting avian wildlife resources. We make these suggestions to aid in documenting impact, assessing recovery, and ensuring that data from the incident will further the scientific understanding of the effects of petroleum on birds.

Spill phase	Goal	Strategy recommendations
1. Oil spill preparedness *Before spill* (Years prior)	Maintain evidence to demonstrate potential environmental contamination	Monitor PAC and metal contaminant concentrations in avian tissues, prey, and relevant environmental matrices
	Predict exposure risk	Document avian taxa and habitat use
	Maintain evidence to demonstrate potential effects on reproduction, populations, and demographics	Monitor avian populations, demographics, productivity, and prey at high spill risk and reference areas
2. Acute phase of spill *Visible spilled oil and oiled birds* (Days to months)	Monitor oil contamination to estimate exposure	Oil fate studies should consider avian habitat, oil deposition to benthos, and its persistence there
		Chemical residue analysis: • Oil grab samples • Air monitoring • Avian prey monitoring, especially during height of oil spill contamination to generate dietary exposure estimates • Sample any oiled eggs available to determine amount on shell and PAC concentration inside
	Evaluate sublethal toxic and physical injury to bird populations	In oiled individuals and oil impacted populations: 1. Take blood samples to assess: • Hematological injury • Oxidative damage • Neurotoxicity • Cardiac damage • Corticosterone stress response • Metabolomic effects • PACs in erythrocyte fraction 2. Take liver biopsy samples to assess: • Transcriptomics and proteomics, including CYP1A and other relevant genes 3. Evaluate body condition and feather (micro)structural integrity Include reference populations and control groups
	Evaluate sublethal behavioral effects	Observe oiled wild birds to assess: • Behavioral effects • Persistence of visible plumage oiling to estimate external exposure and ingestion from preening • Breeding activity and productivity
	Maximize information collected from oil-killed birds	For *postmortem* analyses: • Include unoiled reference birds • Quantify amount of oil on plumage and percent area affected for comparison with live oiled birds • Analyze oil residue in liver tissue to confirm and quantify exposure
	Maximize information collected from rescued and rehabilitated oiled birds	Leave some oiled birds as positive control (track survival) and take some unoiled birds into captivity as control. Quantify oil on plumage and take clinical data at intake.
3. Chronic phase of spill *After visible oil disappears* (Months to decades)	Determine the long-term fate of oil impacted individuals	Tracking studies of survival, movement, habitat use, and reproduction in wild and rehabilitated birds: • Locally breeding birds • Migratory birds tracked to breeding grounds Monitor the incidence of cancerous tumors in birds and evaluate long-term genotoxicity
	Determine cessation of avian exposure to residual contamination	Monitor hydrocarbon exposure via hepatic CYP1A and other relevant biomarkers Compare PAC and metal contaminant concentrations in avian tissues, prey, and relevant environmental matrices to pre-spill baselines
	Assess recovery of oil impacted populations	Compare avian populations, demographics, productivity, prey, and habitat use to pre-spill baselines and multiple reference areas.

sample, a method called transcriptional profiling. Transcriptional profiling (i.e. gene expression profiling) and differential gene expression can be used without a sequenced genome or known target sequences (Grabherr et al., 2011). By examining the abundance (often relative abundance) or distribution of RNA which is transcribed into proteins within the cell, qPCR and RNA-Seq offer the molecular tools to look at the effects of petroleum exposure which are the most upstream in a pathways sense. Other analogous omics tools are proteomics, which examines the abundance of proteins within tissue, and metabolomics which typically focuses on metabolites within a tissue or in circulation (Dorr et al., 2019). Collectively, qPCR, RNA-Seq, and their related omics approaches can offer snapshots of biological processes that span several levels of organization from the transcriptome to the metabolome. A caveat is that the resulting datasets can be huge and require bioinformatic methods to analyze. Applied astutely, these molecular methods may advance avian petroleum toxicology research by elucidating effects on biochemical pathways underlying the known physiological effects of toxicity.

High volume oil spills can be catastrophic to birds, yet one challenge to studying the effects of large spills is the urgent and often *ad hoc* nature of the response to wildlife resource damage. Based on what has been learned from past spills, including the large body of research we have synthesized in this review and older studies (Leighton, 1993), we recommend strategies for researchers and decision makers to aid the assessment of damage and recovery, while ensuring that data from the incident will further the scientific understanding of the effects of petroleum on birds (Table 5). Further developing the model for the effects of petroleum on birds is an unfortunate necessity, as exposure is likely to continue to occur as long as production, transportation, and

consumption persist, especially near water bodies that facilitate the spread of discharged oil.

6. Conclusions

Birds are practical and publicly visible indicators of pollution (Elliott and Elliott, 2013; Furness and Camphuysen, 1997), and our understanding of how petroleum affects them has improved by degrees over the 27 years since last reviewed by Leighton (1993). In conclusion, we attempt to give an overview of key advancements. Feather fouling alone is harmful to thermoregulation and locomotion, and even small amounts of oil may affect feather integrity and locomotive efficiency. Adverse toxic effects usually result from oral doses on the order of milliliters to tens of milliliters kg^{-1} bw d^{-1} over several days (Table 3), although the presence of concurrent stressors can increase sensitivity. The previously held notion that inhalation is an unimportant route of petroleum hydrocarbon exposure has been overturned by evidence of effects. The aromatic (PAC and BTEX) fraction of ingested petroleum causes systemic toxicity, yielding complex effects among multiple body systems. Metabolic effects of toxicity are suggested by mass loss and multiple biochemical endpoints, an important finding which merits further study by itself and how it may interact with the metabolic effects of oil-compromised plumage. As metabolism and endocrine signaling are linked, feedback effects between the two are likely. Not only would metabolic changes be expected to affect endocrine signaling, but there is also evidence that petroleum ingestion alters endocrine signaling directly, at least corticosterone signaling and stress response, but also potentially reproductive hormone and thyroid hormone signaling. In addition to the metabolic and endocrine effects of toxicity, recent work shows effects on antioxidant capacity, and there is abundant evidence that petroleum ingestion frequently results in oxidative damage to erythrocytes. As a result of the latter, hemolysis and erythrocyte regeneration may occur, at least in wild birds and nestlings. There is also emerging evidence of cardiotoxic effects, which together with hemolysis could reduce overall aerobic capacity. Cardiac effects may also be an important driver of embryotoxicity, which can occur from the exposure of eggs to small amounts of oil. Finally, the weight of evidence suggests immunotoxic effects, at least on the cells and organs of the lymphatic system. The toxic and physical effects of petroleum exposure can translate to emergent, ecological-level effects on behavior, reproduction, and survival. One important cause of those, though indirect, is reduced prey availability in contaminated areas.

Thoughtfully designed investigations will accelerate research progress on the myriad and often subtle ways in which petroleum and petroleum-source contaminants affect avifauna. To investigate toxicological questions in a context of environmental realism, quantitative data on petroleum exposure, estimated or empirical, are sorely needed. This includes effect thresholds for the adverse physical effects of petroleum adsorbed to the plumage, how much is ingested through preening and the diet over time, inhalation, and how much may be transferred by incubating parents to eggs. The emergence of unconventional crude petroleum warrants an evaluation of its toxicity. Robust study design and consistent reporting will improve comparability across experiments, species, regions, and petroleum types, and the continued development of powerful methods like gene expression, omics, and tracking devices may help further resolve the effects of petroleum on free-living birds. Ultimately, the better the consequences of petroleum exposure on birds are understood, the better such effects are mitigated.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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