Patterns in winter site fidelity and polycyclic aromatic hydrocarbon exposure risk in Barrow's goldeneye (*Bucephala islandica*) in the Pacific Northwest

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Abstract

Species inhabiting coastal areas can serve as indicators of marine pollution. Hydrocarbons occur naturally in marine ecosystems and wildlife have evolved detoxification systems to manage hydrocarbon exposure. Human activities may increase hydrocarbons in the environment, to the extent that they may be detrimental to biota. Elevated hydrocarbon exposure can be measured directly as increased concentrations in some species, or through biomarkers of active detoxification systems. I found that cytochrome P4501A induction in liver tissue of Barrow's goldeneyes (*Bucephala islandica*) and polycyclic aromatic hydrocarbon (PAH) concentration in their winter prey, blue mussels (*Mytilus* spp.) were correlated across coastal sites in British Columbia, despite generally low PAH concentrations. Using satellite telemetry, I determined that winter movements of Pacific goldeneyes were small, indicating that biomarkers reflected local hydrocarbon levels. These results indicate that the mussel – goldeneye system is useful for evaluating contemporary marine hydrocarbon contamination and recovery endpoints in the event of spills.

Keywords: Barrow's goldeneye; oil; Pacific; satellite telemetry; fidelity; cytochrome P450

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List of Acronyms

AHY	after hatch-year
AIC	Akaike's information criterion
ANOVA	analysis of variance
BC	British Columbia
BNF	β-napthoflavone
CYP1A	cytochrome P4501A
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
EPA	Environmental Protection Agency
EROD	7-ethoxyresorufin O-deethylase
ESRI	Environmental Systems Research Institute
g	gram
GC-MS	gas chromatography-mass spectrometry
GIS	Geographical Information System
GLM	general linear models
GOC	Government of Canada
HpCDD	1,2,3,4,6,7,8-heptachlorodibenzofuran
HpCDF	1,2,3,4,6,7,8-heptachlorodibenzofuran
HxCDF	1,2,3,7,8,9-Hexachlorodibenzofuran
HY	hatch-year
IBA	Important Bird Area
IQR	interquartile range
kg	kilogram
km	kilometer
LC	location class
MFO	mixed-function oxidase
µg	microgram
mg	milligram
min	minute
MRD	maximum redundant distance
M/V	marine vessel

NADPH	nicotinamide adenine dinucleotide phosphate
NOAA	National Oceanic and Atmospheric Administration
OCDD	octachlorodibenzodioxin
PAH	polycyclic aromatic hydrocarbon
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzo-p-dioxin
PCDF	polychlorinated dibenzofuran
pg	picogram
pmol	picomole
PTT	platform transmitter terminal
TCDF	2,3,7,8-tetrachlorodibenzofuran
US	United States
wwt	wet weight tissue

Chapter 1.

Introduction

1.1. Background

Marine industrial activities have been associated with catastrophic and chronic releases, or spills, of hydrocarbons into coastal environments (Nikolaou et al. 2009; Yunker et al. 2011). Catastrophic spills are accidental events typically of known origin (e.g., vessel groundings, pipeline ruptures) that result in large volumes of oil released into the environment over a relatively short period of time (i.e., hours to days) (Camphuysen 2007). Chronic spills are characterized as small to large discharges of oil that occur regularly or episodically over a longer period of time (e.g., weeks to years); chronic spills are usually associated with routine activities (e.g., discharges of oily waste water from vessels). However, because the sources and volumes chronic spills are often ambiguous, they can be difficult to detect or measure, complicating understanding the effects of recovery of oil from marine ecosystems (Camphuysen 2007).

Following catastrophic oil spills, response and monitoring programs are initiated to recover oil from the environment, and to investigate residual effects on impacted ecological receptors. Typically, monitoring programs are developed around focal taxa with known or expected sensitivity to hydrocarbon exposure, and for which evidence of exposure can be reliably measured over a prolonged period. For example, marine birds have presented indications of oil exposure for several years following the *Exxon Valdez*, *Selendang Ayu*, *Prestige*, and *Deepwater Horizon* oil spills (Trust et al. 2000; Esler et al. 2011; Flint et al. 2012). Hence, marine birds are widely used in monitoring programs as indicators of hydrocarbon exposure and provide a means for quantifying ecosystem recovery over time.

In some marine ecosystems, chronic spills can cause larger annual volumes of oil to enter coastal environments than catastrophic events; these spills have potential to have a greater contribution to avian mortality than occasional catastrophic spills (Szaro 1977; Nur et al. 1997; Henkel et al. 2014). Wiese and Ryan (2003) estimate that 300,000 marine birds are killed annually in Atlantic Canada from chronic hydrocarbon releases. Consequently, persistent exposure to chronic oiling can result in long-term adverse effects to marine bird populations (Wiese and Ryan 2003).

1.2. Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are comprised of two or more fused benzene rings (Neff 1979; CCME 1999; Douben 2003). Both the US and Canadian Environmental Protection Agencies (EPAs) have identified PAHs as priority pollutants due to their persistence in the environment, ability to act as carcinogens, mutagens, or teratogens and consequently, potential to cause adverse physiological effects in vertebrates (CEPA 1994; Bojes and Pope 2007; Pampanin and Sydnes 2013). The majority of PAHs are generally divided into two groups depending on their origin: pyrogenic and petrogenic. Pyrogenic PAHs are formed through the incomplete, high-temperature combustion of organic material and are commonly associated with facilities that burn petroleum, wood, and/or coal (Simpson et al. 1996). Petrogenic PAHs are found in petroleum derivatives (Burgess et al. 2003; Pampanin and Sydnes 2013). Low molecular weight PAHs (i.e., those with two or three benzene rings) cause acute toxicity in some organisms (e.g., fish and invertebrates) (Eisler 1987; Nagpal 2014). Higher molecular weight PAHs, are comprised of four to seven benzene rings and have lower likelihood of being acutely toxic but are known to cause carcinogenic, mutagenic, and teratogenic effects across many taxa (Eisler 1987; Nagpal 2014).

PAHs can enter the marine environment through natural processes (e.g., naturally occurring oil seeps) but complex mixtures of PAHs also are introduced from human activities (CEPA 1994; Kvenvolden and Cooper 2003; Latimer and Zheng 2003). Anthropogenic PAHs can be sourced via atmospheric transport and deposition of air-

borne particles emitted from smelters situated in coastal habitats. PAHs also enter the ocean through waste-water discharges or runoff; recreational marine activities; catastrophic spills; and deliberate or accidental chronic releases from industrial oil production, processing, and transportation (Eisler 1987; Latimer and Zheng 2003; Nikolaou et al. 2009; Yunker et al. 2011).

In the marine environment, the fate of PAHs depends on the solubility and hydrophobicity of individual constituents, and the availability of suspended particulate matter (Nikolaou et al. 2009). High molecular weight PAHs, which have low solubility in salt water, readily adsorb onto suspended organic and inorganic particles (Neff 1979; Eisler 1987; CEPA 1994). The deposition, accumulation, and persistence of particle-bound PAHs are further subject to ambient marine conditions. Seasonal or geographical effects of freshwater effluent, suspended particulate, wind and wave action, water temperature and salinity, and shoreline topography influence environmental availability of PAHs (Nikolaou et al. 2009). In general, because PAHs usually float on water, particles tend to settle in intertidal sediment of sheltered shoreline habitats, resulting in higher accumulation of PAHs in inlets, bays, and estuaries (Neff et al. 2006; Nikolaou et al. 2009). Following deposition in coastal habitats, PAHs can persist for years or decades due to slow-rate biodegradation or through bioaccumulation of PAH-laden particulate matter by intertidal biota (CEPA 1994; Meador 2003; Nikolaou et al. 2009; Li and Boufadel 2010).

Due to the volume, frequency, and general location of releases, PAHs tend to accumulate along industrialized coastlines, and wildlife that live or forage in these areas can be particularly vulnerable to sustained exposure to PAHs. The extent to which toxicity is expressed among marine birds, for example, is influenced by their annual or seasonal dependency on coastal habitats for foraging as well as the composition of their diet (Trust et al. 2000; Neff et al. 2006; Esler et al. 2010 2011; Velando et al. 2010).

1.3. Biological Indicators of Polycyclic Aromatic Hydrocarbon Exposure in Coastal British Columbia

Biological indicators are organisms with specific attributes that can be measured across time or space, and are frequently applied to monitor the status of environmental conditions (Noss 1990; Peakall et al. 1992; Altenbuger et al. 2003; Mallory et al. 2010; Ogden et al. 2014). To evaluate patterns in contemporary PAH exposure in marine birds, selection an appropriate biological indicator must consider several criteria:

- the sensitivity of the indicator to respond to changes in contaminant levels;
- the extent to which there is spatial and temporal overlap between the biological indicator and the known or predicted distribution of PAHs;
- the geographic scale across which the indicators are expected to be applicable; and,
- the feasibility of collecting sufficient samples from indicators to accurately reflect spatial and temporal patterns in contamination.

The following sections describe how Barrow's goldeneyes (*Bucephala islandica*), in conjunction with their primary prey, blue mussels (*Mytilus* spp.), fulfill these criteria. This justification provides rationale for applying Barrow's goldeneyes as indicators to characterize the vulnerability of this species to hydrocarbon exposure in the Pacific Northwest, and the extent of exposure to contemporary PAH contamination across coastal British Columbia.

1.3.1. Blue Mussels

Background Biology

Blue mussels are epibenthic organisms (i.e., they attach to substrata) inhabiting a range of intertidal and subtidal habitats that typically include steep rock faces or moderately sloping gravel or rocky beaches (Newell 1989). In British Columbia, a three-species complex of blue mussel can be found where ranges of individual species overlap in coastal habitats in British Columbia (*Mytilus edulis*, *M. trossulus*, and *M.*

galloprovincialis), and are hereafter described collectively as blue mussels (*Mytilus* spp.) (White et al. 2014). Adult mussels are predominantly sessile and anchor themselves to available substrate by secreting byssus threads from their foot (Newell 1989). In sheltered coastal environments, mussels can form densely packed aggregations, or "beds" of thousands of individuals, and can provide important foraging opportunities for marine wildlife. As suspension feeders, mussels obtain nutrients from the environment by filtering organic suspended particles from the water column and directing it across the surface of their gills (Newell 1989). Ingested particles travel to the stomach where they undergo mechanical and enzymatic digestion and can then be redirected for further digestive action or excretion. In general, the rate of filtration of suspended particles optimizes rates of ingestion and excretion; however, blue mussels can adjust their filtration rates based on a combination of endogenous factors (e.g., energy requirements, reproductive status) and exogenous conditions (e.g., concentration of suspended particles, temperature, and salinity) (Newell 1989; den Besten et al. 2003).

Metabolism of PAHs

Blue mussels and other filter-feeding bivalves have been used extensively to monitor contaminants in the environment due to their widespread distribution, sessile life history, ease of collection, and ability to demonstrate physiological or biochemical responses to pollutants (Newell 1989; Carls et al. 2001; Meador 2003; Galgani et al. 2011). One of the most recognized examples of this is the National Oceanic and Atmospheric Administration (NOAA) Mussel Watch Project. The Mussel Watch Project has used mussels and other bivalves as sentinel species for monitoring changes in biological contaminants at 300 coastal and freshwater sites across the US since 1986 (NCCOS 2014). Results from long-term mussel monitoring indicate that concentrations PAHs are highest in urbanized or industrialized coastal areas, and reveal long term trends of chronic oil availability (O'Connor 2002; Kimbrough et al. 2008; Lanksbury et al. 2014). For example, monitoring in Puget Sound, Washington has shown that mussel contamination levels have remained steady in recent decades, despite decreases in the number of reported oil spills (Puget Sound Action Team 2007). Consequently, monitoring sites in Puget Sound report some of the highest PAH burdens in blue mussels across the US (Kimbrough et al. 2008; Lanksbury et al. 2014).

Among mollusc species, blue mussels are poor metabolizers of PAHs and tend to bioaccumulate PAH compounds in their tissues (Boehm et al. 1996; Galgani et al. 2011). Partitioning of PAHs in mussel tissue is influenced by the bioavailability of individual PAHs but is also regulated by the foraging behaviour and physiology of the organism and exogenous environmental factors (Meador 2003). Dissolved and particulate PAHs are ingested from water and suspended particles that are filtered over the gill surface (Neff et al. 2006). In mussels, PAHs are readily absorbed through the gills due to a combination of their large surface area and lipid-rich membrane (Meador 2003). Mussels are capable of filtering large volumes of water and can concentrate PAHs by several orders of magnitude (Pruell et al. 1986; Newell 1989). The potential for accumulation increases with increasing hydrophobicity of PAHs; more hydrophobic compounds will show a stronger association with non-polar lipid-rich tissues (Meador 2003). PAH accumulation and biotransformation of parent compounds to metabolite forms can cause toxicity to benthic and epibenthic organisms subject to long-term exposure (Eisler 1987; Meador 2003). Further, parent compounds and metabolites can be bioavailable, and pose a toxicological risk to upper trophic-level consumers that prey on blue mussels (Newell 1989; Boehm et al. 1996).

1.3.2. Barrow's Goldeneye

Background Biology

Barrow's goldeneye is a species of sea duck that winters primarily in coastal areas of the Pacific Northwest from Puget Sound, Washington to Kodiak, Alaska (Campbell et al. 1990) (Figure 1-1). The Pacific Northwest is estimated to support >90% of the global population of Barrow's goldeneyes (Eadie et al. 2000). Approximately 60% of the global population is found in British Columbia; provincial population estimates range from 70,000 to 126,000 individuals. Barrow's goldeneyes winter throughout coastal British Columbia, and several locations in the province are known to host substantial concentrations of birds, including: Burrard Inlet, Indian Arm, the Strait of Georgia, Douglas Channel, Prince Rupert, and Haida Gwaii (Campbell et al. 1990; Horwood 1992; Important Bird Area [IBA] Canada 2016). The winter diet of Barrow's goldeneyes is comprised almost exclusively of blue

mussels, but can include minor components of aquatic vegetation and herring roe (Vermeer 1982; Eadie et al. 2000). Goldeneyes dive for mussels in intertidal waters along rocky shorelines in bays and estuaries (Eadie et al. 2000).

Because the Pacific Northwest constitutes a high proportion of the global distribution of Barrow's goldeneyes, habitat loss or degradation in the species' winter range has potential for large scale demographic consequences (Esler 2000; Calvert et al. 2009; Hostetler et al. 2015). Individual goldeneyes demonstrating a high-degree of fidelity to industrialized coastal areas are at higher risk of exposure to multiple or prolonged contamination events and other human activities. Conversely, birds that move between industrialized and undeveloped coastal sites, or use industrialized areas to a lesser degree, would be expected to have reduced exposure. Currently, little is known about the degree to which wintering Barrow's goldeneyes demonstrate interannual and intra-annual fidelity to coastal areas. The extent to which wintering aggregations occupy discrete coastal areas also is poorly understood. The degree of movement patterns within or among different coastal regions provides insights on the overall vulnerability of the Pacific population of goldeneyes.

The potential for contemporary PAH exposure among Barrow's goldeneyes is expected to be high in British Columbia. Here, goldeneyes show strong seasonal association with intertidal areas within sheltered coastal habitats that tend to accumulate PAHs, and their primary winter prey (blue mussels) are susceptible to, and capable of accumulating, high PAH burdens. The risk of lethal or sub-lethal effects from chronic ingestion of contaminated prey is greatest for species that forage on organisms with high PAH burdens (Newell 1989; Leighton 1993; Boehm et al. 1996). Consequently, British Columbia populations of Barrow's goldeneye are particularly prone to toxicological effects of PAHs and, hence, constitute a sensitive indicator of PAH exposure in coastal ecosystems in the province.

Metabolism of PAHs

All birds are equipped with a well-developed mixed-function oxidase (MFO) system that facilitates biotransformation and detoxification of exogenous chemicals, including

PAHs (Albers and Loughlin 2003). In contrast to mussels, Barrow's goldeneyes are readily capable of converting PAHs into more polar metabolites to facilitate rapid reduction and elimination of toxic body burdens (CEPA 1994; Albers and Loughlin 2003). This process is completed in two phases. In phase I, oxygen is added to the structure of the chemical. In phase II, the oxygenated product is conjugated with a water-soluble molecule within the cell; the resulting product is more water-soluble than the parent compound and thus easier to eliminate. PAH toxicity manifests in birds by interfering with normal function of cellular membranes and enzyme systems and processes. Metabolic forms of PAHs are able to bind to cellular proteins, however, that cannot be further metabolized or detoxified through additional conjugation. Consequently, metabolites readily bind covalently with cell proteins and deoxyribonucleic acid (DNA), promoting biochemical activation. Subsequent damage to cell or DNA structure can act as a precursor to abnormal tissue developmental, genetic mutation, and tumor or cancer formation (Albers and Loughlin 2003). The potential for mutagenic and carcinogenic effects is greatest among higher molecular weight PAHs (i.e., the four- to six-ring compounds and their alkylated forms).

Biomarkers

The bioavailability of PAHs to any ecological receptor is influenced by the site conditions for exposure (e.g., proximity to a discharge site), the chemical properties of constituents (e.g., hydrophobicity), and the biological capacity for the receptor to transform and excrete a particular contaminant (Altenburger et al. 2003). In combination, these factors influence the extent of contact that PAHs may have with a site of absorption, the amount absorbed, and the amount that is available to exert a toxic effect (Altenburger et al. 2003). Furthermore, because some species are able to rapidly metabolize PAH compounds, the portion of PAHs available in a biological sample (e.g., body tissue) is not necessarily an accurate measure of the quantity that is necessary to inflict a toxic effect on an organism (Altenburger et al. 2003). Evidence of in vivo responses to bioavailable compounds within an organism may be a more appropriate measure for understanding causal mechanisms of toxicity.

Biomarkers may be employed as an informative diagnostic tool to identify the extent to which PAHs can elicit defensive processes within an organism (Peakall 1992;

Altenburger et al. 2003). Biomarkers are defined as measurable molecular, cellular, physiological, or behavioural responses of an organism resulting from contaminant exposure or subsequent toxic effects (Altenburger et al. 2003). Whether within an organism or via excretory products, biomarkers provide quantifiable indices of response to a chemical. Accordingly, they can be important preliminary measures for characterizing physiological effects that ultimately influence the fitness of an organism.

An ecologically relevant biomarker should consider the specificity and sensitivity of a receptor to express exposure to target contaminants (Peakall 1992; Altenburger et al. 2003; Hanson et al. 2013). In birds, induction of the cytochrome P4501A (CYP1A) system is considered a precise biochemical means to assess exposure to PAHs, polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p¬-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) (Peakall 1992; Trust et al. 2000; Altenburger et al. 2003). The CYP1A protein is a member of the cytochrome P450 enzyme group, and is essential for the metabolic conversion, detoxification, and elimination of contaminants. Avian CYP1A induction is routinely measured in hepatic tissue because of higher metabolic capacity and enzyme concentrations found in bird livers (Altenburger et al. 2003). Here, induction of CYP1A by PAHs can be assessed through the catalytic activity of cytochrome enzymes, including 7-ethoxyresorufin O-deethylase (EROD). Assays that measure EROD activity can reliably indicate avian exposure to PAHs and provide early indications of the potential for toxicological effects (Trust et al. 2000; Altenburger et al. 2003).

1.4. Study Areas

To investigate patterns in coastal site fidelity, movement data of wintering Barrow's goldeneyes were collected from the Kenai Peninsula, Alaska south to Puget Sound, Washington and encompass most of the primary Pacific winter range for the species (Horwood 1992; d'Entremont 2010; IBA Canada 2016) (Figure 1-1). To investigate PAH contamination in blue mussels and subsequent exposure in Barrow's goldeneyes, this study focused on areas of the north and south coasts of British Columbia, where large congregations of goldeneyes occur (Horwood 1992; d'Entremont 2010; IBA Canada

2016). Within both areas of the coast, sampling was completed at sites located close to historical and contemporary marine industrial and commercial facilities that serve as known or putative sources of PAHs. Data from these locations were compared to samples obtained from reference locations for which PAH contamination is expected to be relatively low. Reference locations were selected as nearby areas without industrial or commercial activity or development and experience limited anthropogenic influence.

1.4.1. Northern British Columbia: Douglas Channel and Kitimat Arm

Douglas Channel is a deep-water fjord that extends approximately 150 km inland from the Pacific Ocean to the Kitimat Estuary (Figure 1-2). The Estuary is situated at the mouth of the Kitimat River and encompasses multiple tributaries and seasonally flooded wetlands along the river delta. Tidal mudflats line the Estuary; water depth increases rapidly as distance from the Estuary increases. These extensive areas of soft-sediment intertidal habitats support a variety of benthic and epibenthic species; blue mussels are among the mostly commonly occurring invertebrates (Levings 1976).

The Kitimat Estuary has a history of supporting several large industrial facilities dating back to the early 1950's, including the Rio Tinto Alcan aluminum smelter, the Eurocan pulp and paper mill, and the Methanex methanol production plant (Levings 1976; MacDonald and Shepherd 1983). Previous studies investigating PAH loads in marine sediments in the Kitimat Estuary have found that concentrations are highest in the vicinity of the smelter (Simpson et al. 1996; Yunker et al. 2011). The southern reach of Douglas Channel marks the confluence of several major shipping channels supporting bulk carrier, tanker, tug, fishing, commercial, and recreational traffic and may serve as an additional source of PAHs. In 2005, there were an estimated 552 annual vessel transits in Douglas Channel; deep sea ship transits (i.e., bulk carrier and tanker traffic) account for 34% of total vessel traffic in the region (ENGP 2010). Two notable shipwrecks have occurred in lower Douglas Channel. The M/V *USAT Brigadier General M.G. Zalinski* was a US Army transport ship that sank in southern Grenville Channel in 1946, containing 700 tonnes of bunker fuel. In 2012, emergency response was initiated to salvage residual oil reported to

be leaking from the vessel (DFO 2013). Approximately 35 tonnes of fuel were recovered by late 2013. In 2006, the M/V *Queen of the North* ferry had a catastrophic grounding along the northern end of Gil Island. At the time of sinking, the vessel was carrying approximately 220,000 litres of diesel fuel and lubricating oil (BC Ferries 2006). Efforts to contain subsequent oil releases have been implemented by BC Ferries, although the vessel remains sunk in Wright Sound with no immediate plans for salvage.

Douglas Channel also hosts several inlets that are likely to have relatively low PAH contamination. These inlets lie farther from industrial sites than other areas in the Channel and access is generally limited to local recreational fishing vessels due to their restricted depths and widths. Based on the relatively low anthropogenic impacts in these areas, inlets in Douglas Channel serve as reference sites to contrast against high vessel traffic areas and portions of Kitimat Arm that have undergone development. Kiskosh Inlet is a 15 km fjord extending northwest from Douglas Channel and serves as a representative reference location. A large lagoon is located at the northern extension of the inlet and borders Alty Conservancy; both the lagoon and the conservancy are recognized for providing valuable bird habitat (BC Parks 2015).

Douglas Channel and Kitimat Arm comprise an important area along the British Columbia coastline for Barrow's goldeneyes. This species forms annual congregations along shallow waters in Kitimat Arm and Douglas Channel between October and May feeding on dense mussel beds bordering the coastline (Horwood 1992). Goldeneye observations in upper Kitimat Arm have included localized abundances of approximately 1,500 birds (D. Horwood, personal communication, January 14 2014). Vessel-based surveys have similarly recorded between 900 and 1,600 individual Barrow's goldeneye in Douglas Channel during winter and spring (d'Entremont 2010). An estimated 2,700 individuals were recorded throughout Douglas Channel as part of this study, in April 2014.

1.4.2. Southern British Columbia: Burrard Inlet and Indian Arm

Burrard Inlet extends 25 km inland from the Georgia Strait to Indian Arm, encompassing all marine and intertidal habitats extending from Point Atkinson and Point

Grey east towards Port Moody Arm and Indian Arm, including English Bay and Vancouver Harbour (Figure 1-3) (Burrard Inlet Environmental Action Program 2011). The Indian Arm Basin is a steep-sloped fjord extending approximately 20 km north from Burrard Inlet. Burrard Inlet lies between several large municipalities, including Vancouver, Burnaby, Belcarra, Port Moody, West Vancouver, and North Vancouver resulting in large sections of marine riparian habitat that have been modified for human use. These uses include extensive industrial development (e.g., export terminals), marinas, residential areas, and regional and provincial parks (e.g., Belcarra Regional Park, Indian Arm Provincial Park, and Stanley Park). Residual natural habitat is generally restricted to rocky shorelines and tidal sand or mudflats are located at Spanish Banks, Maplewood Flats Conservation Area, and Port Moody Inlet (BC Parks 2015; IBA Canada 2016).

Several export terminals are located within Burrard Inlet, including six petroleum facilities (i.e., the Suncor Burrard Products, Ioco Refinery, Kinder Morgan Westridge, Shellburn, Stanovan, West Coast Reduction petroleum terminals) (Port Metro Vancouver 2014). In 2007, 100,000 litres of heavy synthetic crude oil was released into Burrard Inlet due to a rupture pipeline at the Westridge Terminal, depositing onto 15 km of shoreline (Stantec 2012). PAH profiles in mussels sampled during long-term monitoring following this spill show low levels of both pyrogenic and petrogenic PAHs, indicating mussels routinely bioaccumulate oil from a number of anthropogenic sources (Stantec 2012). Burrard Inlet also supports a high volume of vessel activity (including bulk carrier, tanker, tug, fishing, commercial, and recreational traffic), with 17,594 vessel transits reported in 2012 (Moffatt and Nichol 2013). Industrial activity is highest at the entrance of Burrard Inlet; human activity is generally limited to localized recreational traffic towards the northern end of Indian Arm.

Burrard Inlet is designated as globally important habitat for Barrow's goldeneye within the English Bay and Burrard Inlet Important Bird Area (IBA Canada 2016). Between 800 and 7,100 individuals are estimated to overwinter in the Inlet (IBA Canada 2016). Blue mussels are located are located throughout Burrard Inlet and Indian Arm, with high densities found on terminal, wharf, and dock pilings and along rocky embankments. An estuarine complex is formed at the mouth of the Indian River at the northern most section

of Indian Arm. This area is recognized as an important spring staging area for Barrow's goldeneye as birds prepare to migrate to inland breeding sites (IBA Canada 2016).

1.5. Study Objectives

Despite the historical and contemporary prevalence of industrial activity in British Columbia, our understanding of the ecological risks of contemporary hydrocarbon exposure to marine bird populations remains nascent. In this context, this research proposes to investigate the relationship between fidelity to coastal habitats and variability in hydrocarbon exposure among individual Barrow's goldeneye. Similarly, few studies have attempted to characterize expressions of hydrocarbon exposure in relation to concentrations in marine bird prey (e.g., Miles et al. 2007). Here we examine the trophic relationship of PAH contamination in blue mussels and exposure in Barrow's goldeneyes in more detail.

1.5.1. Thesis Structure

In this first chapter, the thesis is introduced by providing rationale for this research followed by supporting information on the species and geographic areas of focus for this study, and provide context for the specific research objectives.

In Chapter 2, I use satellite telemetry data to explore patterns in movement and site fidelity of Barrow's goldeneyes overwintering in coastal regions of southcentral and south east Alaska, and northern, central, and southern British Columbia. To investigate how patterns in interannual and intra-annual fidelity vary, I examine differences in fidelity across sexes, age classes, and coastal regions.

In Chapter 3, I examine the relationship between enzymatic indications of hydrocarbon exposure in Barrow's goldeneyes and PAH concentrations measured in blue mussels, their primary winter food source. To test whether proximity to industrialized sites

influences rates of hydrocarbon exposure, tissue samples from Barrow's goldeneyes and blue mussels are compared from areas proximal to putative PAH sources relative to reference sites in northern and southern British Columbia. Subsequently, to investigate within-population patterns in PAH exposure, I examine how individual attributes (i.e., sex, age, and body mass) are related to EROD activity.

The concluding chapter summarizes the patterns of coastal site fidelity and contemporary hydrocarbon exposure in Barrow's goldeneyes and discusses management implications. These results are contextualized for their relevance to management of similar species and ecosystems that are currently, or have potential to, experience comparable effects from oil spills.

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1.7. Figures

Figure 1-1. Overview of Barrow's goldeneye study area on the Pacific coast of North America

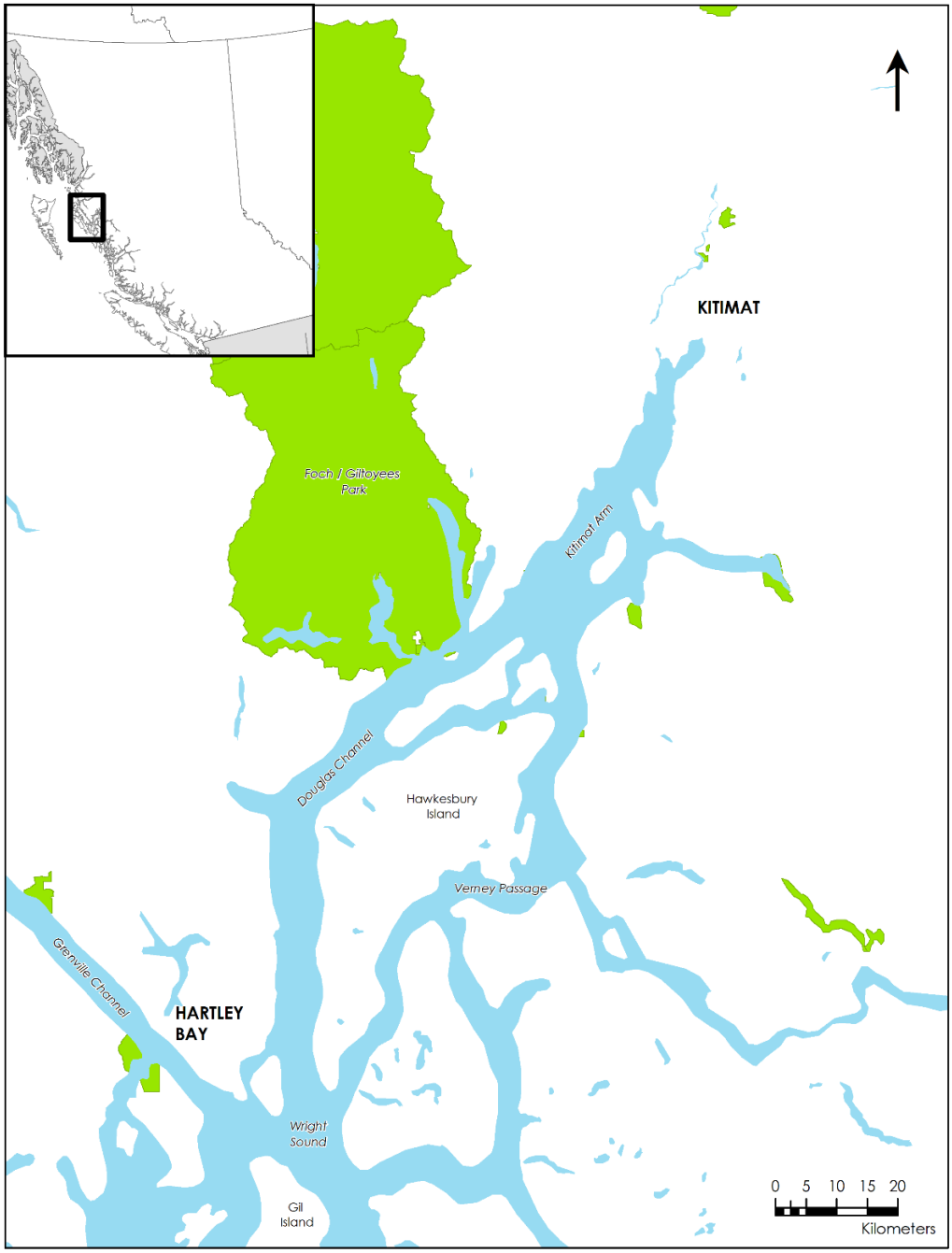

Figure 1-2. Northern British Columbia study area: Douglas Channel and Kitimat Arm

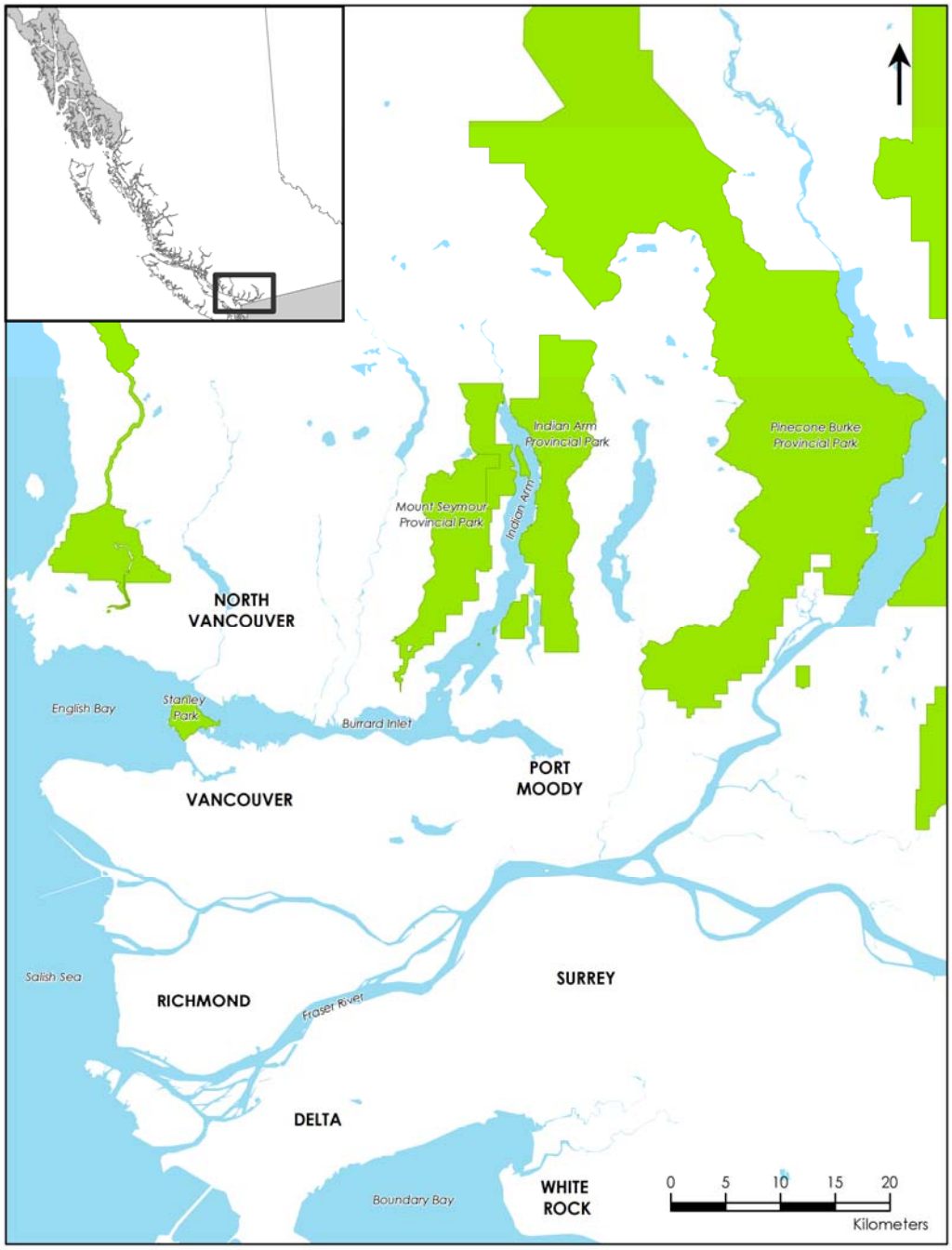

Figure 1-3. Southern British Columbia study area: Burrard Inlet and Indian Arm

Chapter 2.

Winter Movements and Interannual Site Fidelity of Barrow's Goldeneye (*Bucephala islandica*) along the Pacific Coast

2.1. Abstract

Coastal regions in the Pacific Northwest serve as important wintering, staging, and breeding habitats for marine birds and span areas that support a range of human activities. Our ability to understand interactions between marine birds and altered marine habitats, and the ecological consequences of such interactions, is limited by our lack of knowledge of their movement ecology. We used data obtained from satellite tagged Barrow's goldeneyes (*Bucephala islandica*), a sea duck, to investigate annual movements and interannual fidelity to coastal wintering areas. Between 2006 and 2015, goldeneyes were marked on breeding, molting, and wintering sites with platform transmitter terminals (satellite transmitters). The median arrival date of marked birds to wintering sites was November 7; goldeneyes remained on wintering sites for an average of 155 days. Half of tagged goldeneyes initiated spring migration by April 19. Goldeneyes demonstrated high interannual fidelity to coastal wintering sites; 75% of wintering areas were located within 30 km of sites used the previous winter. At wintering sites, distances between consecutive locations within winters were measured for each goldeneye. Within winters, female and after hatch-year goldeneyes moved shorter median distances (i.e., ≤10 km) compared to males and hatch-year birds (<11 km). Goldeneyes from southcentral British Columbia had the shortest distance measurements at wintering sites (<5 km) compared to goldeneyes from British Columbia or southeast Alaska (>9 km); winter home range sizes were similar. Results of this study revealed that Barrow's goldeneyes exhibited strong fidelity to coastal wintering sites and small seasonal winter movements relative to other waterfowl, which highlights the importance of spatial ecology as a useful metric for predicting and managing effects of human activities in coastal areas used by goldeneyes.

2.2. Introduction

Identifying patterns in fidelity to wintering, molting, staging, or breeding areas used by migratory birds is necessary for establishing effective conservation measures in key habitats throughout their range (Esler 2000; Calvert et al. 2009; Hostetler et al. 2015). Most migratory birds in North America spend a large portion of their annual cycle away from breeding grounds. While many studies focus on conservation activities on the breeding grounds, activities that degrade the availability or quality of wintering, molting, or staging habitat can have important ramifications on population dynamics (Esler 2000; Calvert et al. 2009; Hostetler et al. 2015). Migratory birds that express high interannual fidelity to wintering sites are susceptible to continuing effects from activities contributing to the degradation of important winter habitats (Hostetler et al. 2015). Demographically independent subpopulations are also more likely to exist in species that demonstrate high interannual site fidelity (Esler 2000; Calvert et al. 2009), with persistent effects of habitat degradation reflected in declining regional abundance. These effects may be geographically distinct, or can act cumulatively with impacts on staging, molting, or breeding sites (Hobson et al. 2005; Savard and Robert 2013). Similarly, birds that exhibit small levels of movements within winter can be chronically subject to prolonged habitat degradation or contamination at that locality.

Many bird species depend extensively on Pacific coastal habitats for wintering, staging, molting, or breeding activities (Horwood 1992; De La Cruz et al. 2014; Important Bird Area [IBA] Canada 2016). Due, in part, to the narrow distribution and patchy abundance of resources, small sections of coastline can support thousands of individuals. Marine bird species that consistently use industrialized coastal segments for part of their annual cycle may have higher exposure to industry-sourced contaminants (Burger and Gochfeld. 2004; Harris et al. 2007; Miles et al. 2007; Mallory et al. 2010). Contaminant exposure may result in sub-lethal physiological effects and large-scale demographic consequences for exposed populations (Trust et al. 2000; Esler et al. 2010). Individuals demonstrating a high-degree of intra-annual (i.e., within a single season) site fidelity to industrialized areas may be at risk of exposure to multiple or prolonged contamination events. In comparison, birds that move between industrialized and undeveloped coastal

sites, or exhibit greater fidelity to non-industrialized sites, are expected to interact with contaminated areas to a lesser degree. The extent to which movement patterns are consistent among different coastal regions can provide an insight into the overall susceptibility of a species.

Barrow's goldeneye (*Bucephala islandica*; hereafter goldeneyes) is a species of sea duck that winters primarily along the northern Pacific and Atlantic coasts of North America. Over 90% of the global population of goldeneyes winters in the Pacific Northwest, from Kodiak Island, Alaska south to northern California (Eadie et al. 2000) (Figure 2-1). In fall, goldeneyes migrate from molting and breeding sites on inland lakes to coastal areas, as freshwater lakes begin to freeze. The fall migration period can be prolonged, with most goldeneyes appearing on coastal wintering sites between early October and mid-November (Eadie et al. 2000). During winter, goldeneyes use bays, harbours, or inlets with rocky shorelines that support their primary prey, blue mussels (*Mytilus* spp.; Koehl et al. 1982; Vermeer 1982). Certain coastal regions support substantial congregations of goldeneyes, presumably due to the patchy distribution of prey resources. These can include areas with extensive human activity, such as Puget Sound, the Strait of Georgia, Prince Rupert, and Juneau (Horwood 1992; Eadie et al. 2000; De La Cruz et al. 2014; IBA Canada 2016). Deep-water ports located in Tacoma, Seattle, Vancouver, Kitimat, Prince Rupert, and Juneau serve as confluences of shipping routes for tankers, bulk carriers, tugs, barges, ferries, cruise ships, and recreational and commercial fishing vessels. The vulnerability of marine bird populations to human activity on the Pacific coast is of increasing concern given the extent of existing and proposed development (Eadie et al. 2000). Industrial expansion could see the addition of several marine terminals along the coast of British Columbia, with an estimated annual increase of up to 1,000 tankers and bulk carriers transiting coastal waters in this region (Nuka Research and Planning Group LLC 2013).

Because the Pacific Northwest hosts a high proportion of the global winter distribution of goldeneyes, habitat degradation (including contamination) in coastal areas has the potential for large scale demographic consequences that could be echoed in other marine bird species that share similar habitat requirements. Given their ubiquitous distribution along the northern Pacific coast, and interaction with marine habitats in

industrialized coastal settings, goldeneyes constitute a model species for investigating how patterns in site fidelity can inform sensitivity to human activities.

Currently, little is known about the degree to which goldeneyes demonstrate fidelity to coastal wintering areas. Savard (1985) described a single record of a breeding pair returning to the same wintering territory on the Pacific coast in consecutive years. To better predict ecological risk associated with human activity, additional information is needed to understand the temporal and spatial scale of coastal use by goldeneyes. The extent to which wintering aggregations occupy discrete coastal regions is similarly undocumented. These knowledge gaps limit our ability to identify distinct population units necessary to predict and mitigate the effects from existing and future habitat degradation or contamination (Eadie et al. 2000). In this context, the objective of this study was to determine the scale of intra-annual and interannual fidelity by goldeneyes to coastal sites throughout their primary Pacific winter range. Seasonal movement patterns and site fidelity in various waterfowl are known to differ by sex, age, or geographic area (Robertson and Cooke 1999; Savard and Robert 2013) and these differences have further potential to contribute to species' vulnerabilities in changes to wintering habitat from human activities. Accordingly, we evaluated site fidelity between sexes, age classes, and among coastal regions of wintering goldeneyes.

2.3. Study Areas

The winter range represented by marked birds in this study extended from the Kenai Peninsula, Alaska south to Puget Sound, Washington, and encompassed numerous coastal areas used by goldeneyes (Figure 2-1; Eadie et al. 2000). Primary wintering sites represented within the study area included Puget Sound, Burrard Inlet, Howe Sound, the Strait of Georgia, Douglas Channel, Juneau, Prince William Sound, and the Kenai Peninsula; we considered this extent of the Pacific coast as our study area.

2.4. Materials and Methods

2.4.1. Field Sampling Procedures

Barrow's Goldeneye Capture and Satellite Tag Implantation

Winter movements and winter site fidelity of goldeneyes that we captured and marked with platform transmitter terminal satellite tags (PTTs) on wintering sites in British Columbia in 2014 and 2015 were summarized with data previously collected at breeding, molting, and wintering sites in British Columbia, Alberta, and Alaska between 2006 and 2013. At Riske Creek, British Columbia, adult males were captured using a decoy and partially submerged mist net; adult females and their young were captured using drive traps. Molting adult males were captured at Cardinal Lake, Alberta, also using drive traps (Hogan et al. 2011). During winter, goldeneyes were captured using decoys and a floating mist net suspended between two poles (see Kaiser et al. 1995). For all sites and seasons, captured goldeneyes were immediately transported, using animal carriers, to a field research station or vessel for processing. We recorded the age, sex, and mass for each captured bird. Ages of goldeneyes were categorized as hatch-year (HY; defined as being within one year of hatching) or after hatch-year (AHY) individuals, as determined by depth of the bursa of Fabricius for females and bursal depth and plumage characteristics for males (Mather and Esler 1999). Sex of birds was determined by plumage and cloacal characteristics.

Surgical procedures for coelomic implantation of PTTs followed previously described methods (Mulcahy and Esler 1999). As part of the surgical procedure, birds were administered a subcutaneous dose of meloxicam (0.5 mg/kg) in advance of surgery to provide analgesia to recovering birds. Birds were mask-induced with an isoflurane/oxygen mixture at approximately 2 to 2.5% isoflurane to achieve effective anaesthesia. Anaesthetized birds were intubated with an endotracheal tube. Feathers were spread apart at the site of incision and tissue was sterilized using a topical 10% povidine-iodine solution. A 2 to 3 cm cutaneous midline incision was made on the ventral abdomen midway between the caudal sternum and the cranial extent of the pubic bones

exposing the linea alba. The linea alba was incised to provide access to the coelomic cavity. Transmitter terminals (26g [Microwave Telemetry] to 38g [Telonics] transmitters) were implanted in the abdominal cavity of each goldeneye. The body wall and skin were closed separately using 4-0 or 3-0 Polydiaxanone sutures. Tissue adhesive was applied over the sutured incision to enhance water resiliency of the incision. Birds recovering from surgery were placed in a shaded animal carrier and released at the field site (within one to two hours). Capture, banding, and surgical activities were performed under Environment Canada Scientific Permit 10673P and Simon Fraser University Animal Care Permit 1121B-06. The numbers of marked goldeneyes from each location used for this study are summarized in Table 2-1.

2.4.2. Satellite Telemetry

Date (calendar day), time, satellite location fix (i.e., latitude and longitude), location error index, body temperature (°Fahrenheit), and sensor voltage data were obtained from the Argos location and data collection system within 24 hours of a satellite receiving a transmission from a PTT (CLS America 2014). Each PTT was programmed to transmit a location for two to six hours every three to four days. The Argos system estimates locations by calculating the Doppler shift in transmission frequency received by the National Oceanic and Atmospheric Administration (NOAA) satellites as they move relative to a PTT. Argos (2015) rates the accuracy of each transmitted location using standard location classes (LCs) as an index value of 3, 2, 1, and 0, representing cases where at least four signals are received by the satellite, as <250 m, 250–500 m, 500–1,500 m, and >1,500 m, respectively. Accuracy is not provided for auxiliary locations indexed as LC A (3 messages), B (1 or 2 messages), and Z (where latitude and longitude are provided if >1 message received; Argos 2015).

Winter Locations

We applied a maximum redundant distance (MRD) algorithm to filter implausible locations (i.e., those that are spatial outliers and likely inaccurate locations) from the final dataset (Douglas et al. 2012). The MRD algorithm retained locations based on filtering

criteria that considered location accuracy (i.e., LC class) and distance travelled between consecutive locations. Standard locations were retained if their LC was 3, 2, or 1. Auxiliary locations are useful for reasonably informing short-distance gaps between consecutive highly accurate LCs (Douglas et al. 2012). Accordingly, the MRD filter was applied to locations indexed as 0, A, B, or Z; near-consecutive locations were retained if they occurred within 10 km of a preceding or subsequent standard location (i.e., MAXREDUN = 10). A value of 10 km was considered a reasonable distance for retaining near-consecutive locations of individuals on wintering grounds while removing implausible (i.e., outlier) locations (USGS 2012).

To further isolate probable winter movements, the remaining locations were filtered spatially and temporally. We retained PTT locations received between October 1 and June 1 in a given year (as a broad estimate of the wintering period; Eadie et al. 2000). However, goldeneyes occasionally demonstrated movements between one or several coastal areas before settling on, or departing from a wintering site. Accordingly, a bird was assumed to be migrating if consecutive locations in coastal habitats were >100 km apart. Locations also were plotted using ArcGIS 10.3 to remove improbable points that intersected land as delineated by the British Columbia Shorezone Mapping System and United States Boundaries (ESRI 2010; GeoBC 2015). Location data also usually included >1 useable location per bird on a given transmission day. To improve independence between consecutive locations, we selected a single location for each bird per transmission day following weight of evidence criteria described in Miller et al. (2005), where locations of LC 3 or 2 were favoured. Locations with similar accuracy were visually inspected for each individual in ArcGIS 10.3 and those that were closest to previous or subsequent high quality locations were retained. Finally, to ensure reliable estimates of fidelity in each region, individuals with <3 locations in a given winter (usually resulting from mortality or PTT failure) were removed from the final dataset. A total of 4,290 locations (hereafter referred to as Winter Locations) were retained for the final analyses on winter movements; 80% of final locations were of accuracy classes of LC 3 or 2. The numbers of original and final accepted locations for each PTT-marked cohort of goldeneyes are summarized in Table 2-2.

2.5. Data Analyses

2.5.1. Arrival and Departure Dates

We examined the dates goldeneyes arrived and departed coastal wintering sites, recognizing that the PTT duty cycle limits the temporal resolution of these dates to approximately three days. To ensure true arrival or departure periods were reflected, dates were excluded from our analyses if a bird's PTT stopped transmitting within 15 days of their last arrival or departure date (all other arrival and departure dates were retained for those individuals). We calculated the number of days individual goldeneyes spent on wintering sites where both arrival and departure dates were available for an individual within a winter season. To examine regional differences in arrival to and departure dates from wintering sites, we tested for a normal distribution of dates within each regional group using a Shapiro-Wilk test. The distribution of arrival or departure dates could not be transformed to meet assumptions of normality; hence we applied non-parametric methods to examine dates by region using a Kruskal-Wallis rank sum test. Migratory movements were considered to have started when consecutive PTT locations for a single individual were located >100 km from those retained in our Winter Locations (see Section 2.4.2). All statistical analyses were performed using R statistical computing language (Version 3.2.2; R Development Core Team 2015).

2.5.2. Interannual Fidelity to Wintering Sites

The duty cycle programmed for each PTT allowed us to monitor goldeneye movements through multiple annual cycles. We obtained multi-year data on winter areas of use for 106 goldeneyes. For most birds (82%), telemetry data were collected over two consecutive seasons; 18% of individuals had three or four consecutive seasons of wintering data. We determined the frequency at which individual goldeneyes returned to the same wintering location in subsequent years by analyzing their Winter Locations. The centroid of use for each individual's annual wintering site was calculated using the Mean Center tool for ArcGIS 10.3. For birds having data spanning more than one annual cycle,

we measured geodesic distances between their centroids of use between consecutive winter seasons (henceforth interannual pairwise distance). Interannual pairwise distances were used to characterize the degree and scale of between-year fidelity by grouping into distance categories. For all analyses, values were log transformed to meet assumptions of normality. Transformed values were investigated for patterns in interannual fidelity to wintering sites among sexes, age classes, and coastal regions of the Pacific coast (i.e., southcentral Alaska, southeast Alaska, northern British Columbia, southern and central British Columbia) using Welch's t-tests or analyses of variance (ANOVAs).

2.5.3. Intra-annual Movement Patterns During Winter

We also examined the scale of movements by goldeneyes within a wintering season. Using the Winter Locations of all PTT-marked birds, across all study years, we measured geodesic distances between consecutive PTT locations (fixes) for each individual (henceforth interfix distances). Average interfix distances were calculated for each bird and used to characterize the degree of within-year fidelity among sexes, age classes, and coastal regions. We tested for a normal distribution of interfix distances for each sex, age class, and regional group using a Shapiro-Wilk test. Based on results of the Shapiro-Wilk test, interfix distances were log transformed to meet assumptions of normality and evaluated using Welch's t-tests or ANOVAs.

To estimate the size of winter home ranges, annual wintering sites were delineated as the area of use for a single goldeneye within a winter season. We used the Concave Hull Estimator tool in ArcGIS 10.3 to create a polygon representing the area occupied by Winter Locations for each individual. We assumed goldeneyes used marine habitats exclusively during winter; accordingly, individual winter ranges excluded land using the Extract Clip tool for ArcGIS 10.3. Home ranges could not be transformed to meet assumptions of normality; hence regional differences in home range sizes between sexes and age classes were examined using a Wilcoxon rank sum test and differences among regions were evaluated using a Kruskal-Wallis rank sum test.

2.6. Results

2.6.1. Arrival and Departure Dates

A total of 225 fall arrival dates were estimated for 176 goldeneyes (inclusive of individuals with arrival data collected for multiple seasons). Across all birds, the median arrival date (and interquartile range [IQR]) to wintering sites was November 7 (October 29 – November 15), and with arrival dates ranging from October 3 (the earliest) to December 23 (the latest). Arrival dates varied significantly by region ($H_{(3)} = 55.62$, $p < 0.001$; Table 2-3). Arrival dates showed a latitudinal pattern where goldeneyes from southcentral Alaska arrived on wintering sites slightly earlier than did birds in other regions (Figure 2-2a). Half of all goldeneyes stayed on wintering sites for at least 155 days (135 – 172 days) before migrating to breeding grounds (n=154). The length of stay on wintering locations ranged from 83 to 223 days. We estimated 253 dates of departure from wintering sites for 183 goldeneyes. Across all birds, the median departure date was April 19 (April 5 – May 1). Departure dates ranged from February 11 (the earliest) to May 30 (the latest). Departure dates also varied significantly across regions ($H_{(3)} = 30.16$, $p < 0.001$; Table 2-3) and showed a similar, although less obvious latitudinal trend as arrival dates (Figure 2-2b). The median departure date for goldeneyes in southern and central British Columbia was earlier than birds in other regions. Departure dates for goldeneyes in northern British Columbia were similar to southeast and south central Alaska (Table 2-3).

2.6.2. Interannual Fidelity to Wintering Sites

Across all goldeneyes, 50% of interannual pairwise distances between winter centroids of use were ≤10 km; 75% of all pairwise distances were ≤ 30 km. The majority of goldeneyes expressed overlapping areas of use between coastal wintering sites. Interannual pairwise distances did not differ significantly between sexes (Welch's $t = -0.85$, $df = 89.09$, $p = 0.40$). Female goldeneyes expressed similar interannual fidelity (median = 8.64 km; IQR = 2.18 – 25.85 km) as males (median = 11.29 km; IQR = 3.50 – 32.99 km) (Figure 2-3). We also did not find a statistically significant difference between interannual

pairwise distances for HY (median = 17.85 km; IQR = 8.08 – 95.82 km) and AHY birds (median = 10.03 km; IQR = 2.76 – 28.43 km; Welch's t = -1.28, df = 6.55, p = 0.24) (Figure 2-4). Interannual pairwise distances between annual wintering sites differed among regions ($F_{(3, 121)}$ = 3.22, p = 0.03). The median interannual pairwise distance was smallest for birds wintering in southcentral Alaska (median = 4.64 km; IQR = 0.74 – 15.10 km). Interannual pairwise distances were similar for birds from southern and central British Columbia (median = 9.84 km; IQR = 4.38 – 26.33 km) and northern British Columbia (median = 10.01 km; IQR = 4.30 – 25.91 km). Median interannual pairwise distances were largest for birds wintering in southeast Alaska (median = 15.96 km; IQR = 7.72 – 41.93 km) (Figure 2-5).

Notably, we obtained data on wintering sites across four consecutive seasons for a single individual; this male goldeneye was observed along the west side of Culross Island, Alaska (approximately 15 km in length) for the winter seasons from 2008/2009 to 2011/2012. The average distance between annual centroids of use for this individual was 6.2 km. Nine goldeneyes selected wintering sites in subsequent seasons that were >100 km from their previous wintering sites. Of those individuals, we measured the largest distances between centroids of use for goldeneyes wintering in northern British Columbia. Two birds captured in Kitimat Arm during the 2013/2014 winter season relocated to inlets on east and west coasts of Haida Gwaii during the 2014/2015 winter (distances between wintering sites were 244 and 258 km, respectively). A third individual, tagged at Riske Creek, British Columbia in August 2011, wintered in Liberty Bay, Washington during the 2011/2012 winter and then relocated 178 km north to Gabriola Island, British Columbia for the 2012/2013 winter.

2.6.3. Intra-annual Movement Patterns During Winter

Intra-annual movement patterns of goldeneyes during winter differed between sexes, age classes, and coastal regions. Movement patterns, as indicted by interfix distances, differed between males (median = 5.77 km; IQR = 3.26 – 8.98 km) and females (median = 4.44 km; IQR = 2.59 – 7.02 km; Welch's t = -2.07, df = 169.14, p = 0.04) (Figure 2-6). Median interfix distances were similar between HY goldeneyes (median = 4.89 km;

IQR = 3.27 – 6.89 km) and AHY goldeneyes (median = 5.13 km; IQR = 3.06 – 8.76 km; Welch's t = 0.066, df = 46.121, p = 0.95) (Figure 2-7). We found statistically significant differences in interfix distances among goldeneyes from different coastal regions ($F_{(3, 211)}$ = 6.87, p <0.001) (Figure 2-8). The median interfix distance was smallest for birds wintering in southcentral Alaska (median = 3.06 km; IQR = 1.28 – 5.06 km). Interfix distances were similar for goldeneyes from northern British Columbia (median = 4.88 km; IQR = 3.59 – 9.34 km) and southern and central British Columbia (median = 4.89 km; IQR = 3.22 – 8.01 km). Goldeneyes from southeast Alaska had the greatest median interfix distances (median = 7.53 km; IQR = 5.51 – 12.44 km) (Figure 2-8).

There was a statistically significant difference between winter home ranges for male and female goldeneyes (W = 9,616, p < 0.001). The median winter home range was smaller for females (median = 9.66 km^2; IQR = 1.43 – 45.54 km^2) than for males (median = 25.99 km^2; IQR = 4.62 – 77.35 km^2; Table 2-4). Home range size did not differ between HY (median = 17.59 km; IQR = 3.86 – 46.37 km^2) and AHY goldeneyes (median = 15.38 km^2; IQR = 2.80 – 71.08 km^2; W = 5,017, p = 0.81) (Table 2-4). Differences in home ranges were statistically significant across regions (H(3) = 15.99, p = 0.001). We observed regional differences in winter home ranges that were similar to patterns observed among regional interfix distances. The median winter home range was smallest for goldeneyes from southcentral Alaska (median = 4.09 km^2; IQR = 1.37 – 41.07 km^2) and southern and central British Columbia (median = 13.77 km^2; IQR = 3.07 – 64.19 km^2; Table 2-4). Larger winter home ranges were observed among goldeneyes from northern British Columbia (median = 29.32 km^2; IQR = 7.19 – 74.15 km^2) and southeast Alaska (median = 41.28 km^2; IQR = 14.96 – 91.76 km^2).

2.7. Discussion

Barrow's goldeneyes wintering throughout the Pacific Northwest showed a high degree of interannual and intra-annual fidelity to their coastal wintering sites based on satellite telemetry data. Slight differences in site fidelity patterns were observed among sexes, age classes, and coastal regions, but overall the data indicate low propensity to

change locations, compared to many other birds. The range-wide sample of marked birds of all sex and age classes suggests our observations are representative of winter movements by goldeneyes, in general.

Goldeneyes from eastern North America were reported to arrive on wintering sites between late October and early November (Robert et al. 2000; Savard and Robert 2013). Eadie et al. (2000) reported that spring migration for goldeneyes from eastern North America peaked in late March or early April. We found that goldeneyes arrived on their coastal wintering sites between October and December, which is a more protracted period than has been documented for goldeneyes from eastern North America (Robert et al. 2000). This finding would be expected given the geographical range of goldeneyes represented in this study. Consistent with studies from the St. Lawrence Estuary, however, the majority of individuals in western North America arrived on wintering sites by mid-November (Robert et al. 2002; Savard and Robert 2013). We found the timing of spring migration reflected those reported by Robert et al. (2002) and Savard and Robert (2013), indicating that departure from wintering sites occurred between mid and late April. Only 28% of Pacific goldeneyes had initiated spring migration by the end of the first week of April, although dates of departure varied by latitude. On average, goldeneyes spent approximately 42% of their annual cycle in coastal areas during the non-breeding periods. Several birds appeared to stage at a secondary coastal site before migrating inland in the spring, likely as a means to reduce overland migration distances to breeding sites (Eadie et al. 2000). These stopover areas were located >100 km from primary wintering sites and were excluded from the final set of Winter Locations (see Section 2.4.2). However, they extend the period of time that some individuals rely on marine habitats during their annual cycle. The extensive amount of time that goldeneyes rely on coastal areas has implications for their interaction with marine contaminants or other human activities.

The timing of arrival and departure, and length of stay on coastal wintering sites may serve as an index of vulnerability. For example, these data could be used to predict the risk of oil exposure from a recent *M/V Marathassa* oil spill. The spill occurred in Vancouver on April 8, 2015, when the bulk grain carrier released >2,800 L of bunker C fuel in English Bay, an area used by large aggregations of goldeneyes during winter and spring (Butler 2015; IBA Canada 2016). Given the location of the spill relative to areas of

high goldeneye use, there was potential for extensive acute and chronic exposure. However, because the spill occurred immediately prior to the peak spring departure for goldeneyes, the number of individuals potentially exposed to residual oil declined rapidly in the days immediately following the spill. The high degree of interannual winter site fidelity expressed by goldeneyes in this study does, however, have consequences for potential chronic exposure to marine contaminants and other human activities. As demonstrated in previous studies (e.g., Trust et al. 2000; Miles et al. 2007; Esler et al. 2010; Esler et al. 2011), prolonged exposure to oiled habitats can result in adverse physiological and demographic effects.

Previous studies have indicated that goldeneyes express a high degree of fidelity to breeding and molting sites and have suggested similarly high fidelity rates to wintering locations (Eadie et al. 2000; Robert et al. 2002; Savard and Robert 2013). Our study provides the first empirical measures of the rates at which goldeneyes return to the same coastal sites in consecutive years. Here, 75% of goldeneyes were observed to select a wintering location within 30 km of the site used in the previous winter; in most cases, the area of use overlapped across years. Rates of winter site fidelity observed in goldeneyes were similar to those observed in some other sea ducks, geese, and swans (Limpert 1980; Robertson and Cooke 1999; Petersen and Flint 2002; Iverson et al. 2004). In contrast, some sea ducks appear to show larger distance movements between several wintering sites (Hestbeck 1993; Robertson and Cooke 1999; Phillips et al. 2006). Several studies contend that the flexibility in winter movement of sea ducks is greatest where foraging habitat is widely distributed, but access to prey resources is constrained by local sea ice conditions (Petersen and Flint 2002; Phillips et al. 2006; Savard and Robert 2013).

2.7.1. Management Implications

Effective management of marine bird populations requires knowledge of patterns in interannual and intra-annual fidelity. This is especially important for species are regularly subjected to human activities. For goldeneyes and other marine birds, understanding the extent of this interaction is necessary for prescribing appropriate conservation measures, including preventative and mitigative actions such as

contaminant monitoring and oil spill response planning. Prescribing appropriate management measures are further necessary to maintaining the viability of demographically independent subpopulations (Esler 2000; Calvert et al. 2009; Hostetler et al. 2015).

Savard and Robert (2013) suggested that some degree of spatial isolation occurs among goldeneyes wintering in eastern Canada, but with intermixing of birds on molting and breeding sites. Based on spatial and temporal patterns in fidelity, the authors concluded that while the presence of eastern subpopulations are unlikely, a disaster at a molting or wintering location could affect goldeneyes that breed in several different locations. Due to the high degree of wintering site fidelity expressed by goldeneyes in western North America, our results suggest a similar pattern in spatial segregation during part of their annual cycle. The extent to which individuals intermix on molting or breeding sites in the Pacific is to be determined.

Migratory birds that express high interannual or intra-annual fidelity to wintering sites are susceptible to enduring effects of activities that result in the loss or degradation of important coastal habitats (Hostetler et al. 2015). In risk assessments, for example, impacts of exposure to marine contaminants are traditionally assessed by considering the timing, location, and dispersal of contaminants in combination with the pathways that contribute to exposure (i.e., acute exposure direct contact, or chronic exposure through consumption of contaminated prey) (e.g., US EPA 1998; GOC 2012a). The capability of these methods to accurately predict both the immediate and long-term consequences of contaminant exposure can be improved by characterizing the abundance and distribution of animals within impacted areas and the degree of fidelity to those habitats. Understanding spatial and temporal patterns in fidelity across multiple species increases our ability to accurately forecast ecological risk of contamination events and other anthropogenic effects (US EPA 1998; GOC 2012b). The extent to which these effects are local, or can be carried forward and act cumulatively with impacts experienced during other parts of species' annual cycles are important for prescribing appropriate management measures (Hobson et al. 2005; Savard and Robert 2013).

Mitigating the risk of environmental contaminants (e.g., oil spills) also can be logistically challenging (Wiens 2013; Butler 2015). Knowledge gaps on abundance, distribution, and fidelity of marine wildlife limit the capability of government and industry to respond to, or mitigate effects from, current and future contamination events. To this end, judicious allocation of personnel and equipment can minimize response times in ecologically important areas. Our research demonstrates that goldeneyes could serve as a sensitive species for designing response plans that minimize ecological risk of certain marine activities. For example, understanding of high occupancy and fidelity by goldeneyes and other marine birds, relative to marine activities (e.g., shipping routes of tankers), can be used to identify areas of ecological importance that may be prioritized in oil spill response. Observed movement patterns further suggest that particular coastal regions in the Pacific Northwest may be more biologically important than others, at least from a management perspective and particularly where primary goldeneye wintering areas coincide with concentrated marine industry. This study provides an opportunity for resource managers to consider movement ecology, through the use of PTT location data, when identifying priority areas for future development. When considered in combination with hydrocarbon biomarkers (see Chapter 3), measures of site fidelity can be an informative tool for long-term monitoring of contaminant exposure. The differences in spatial movements observed among goldeneyes across regions provide context for adapting response strategies to ensure they are more broadly applicable.

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2.10. Tables

Table 2-1. The number of male and female PTT-marked Barrow's goldeneyes that were used in analyses of winter site fidelity by year of capture

Cohort			Number of Marked Birds[a]		Retained Birds[b]	
Capture Year	Capture Site	Annual Cycle Stage	Males	Females	Males	Females
2006	Riske Creek, BC	Breeding	22	-	6	-
2007	Burrard Inlet, BC	Wintering	10	10	9	10
	Riske Creek, BC	Breeding	15	-	5	-
2008	Riske Creek, BC	Breeding	22	18	14	9
2009	Prince William Sound, AK	Wintering	19	10	6	4
	Riske Creek, BC	Breeding	7	8	4	6
	Cardinal Lake, AB	Molting	20	-	9	-
2010	Cardinal Lake, AB	Molting	18	-	15	-
2011	Burrard Inlet, BC	Wintering	11	14	10	10
	Riske Creek, BC	Breeding	11	12	5	5
2012	Juneau, AK	Wintering	22	12	20	12
2013	Kachemak Bay, AK	Wintering	5	12	4	9
2014	Douglas Channel, BC	Wintering	19	12	12	9
2015	Burrard Inlet, BC	Wintering	8	16	7	11
Total	**-**	**-**	**209**	**124**	**126**	**85**

[a] The number of marked birds does not include goldeneyes with <3 accepted locations in any given wintering period (i.e., between October 1 through May 31) due to transmitter failure or bird mortality.

[b] The number of retained birds includes those with sufficient Winter Locations (described in Section 2.4.2) used for analyses.

Table 2-2. The number of Winter Locations retained for PTT-marked Barrow's goldeneyes, for each winter season

Wintering Season[a]	Retained Birds	Total Winter Locations	Mean Winter Locations Retained / Bird ± Standard Error	Location Class of Winter Locations						
				3	2	1	0	A	B	Z
2006 / 2007	25	373	14.92 ± 2.21	285	62	13	1	6	6	0
2007 / 2008	8	152	19.00 ± 4.98	112	33	6	0	1	0	0
2008 / 2009	35	402	11.49 ± 1.12	276	84	30	4	7	1	0
2009 / 2010	46	618	13.43 ± 0.84	382	142	47	6	27	14	0
2010 / 2011	38	550	14.47 ± 1.23	364	118	37	8	16	7	0
2011 / 2012	59	613	10.39 ± 0.90	326	153	31	10	59	32	2
2012 / 2013	48	626	13.04 ± 9.22	252	140	51	14	108	59	2
2013 / 2014	23	235	10.22 ± 1.93	164	28	11	3	16	13	0
2014 / 2015	38	555	14.61 ± 2.15	253	135	37	9	70	48	3
2015 / 2016	21	166	7.90 ± 0.83	75	32	10	15	18	13	3
Total	**-**	**4,290**	**-**	**2,489**	**927**	**273**	**70**	**328**	**193**	**10**

[a] Data for the 2015/2016 wintering season includes locations through December 31, 2015.

Table 2-3. Dates of arrival to and departure from coastal regions used by Barrow's goldeneyes on the Pacific coast

	Number of Birds	Median	1st Quartile	3rd Quartile	Range
Dates of Arrival					
Southcentral Alaska	25	Oct. 18	Oct. 16	Oct. 28	Oct. 8 – Oct. 30
Southeast Alaska	32	Oct. 27	Oct. 12	Nov. 3	Oct. 4 – Dec. 1
Northern BC	30	Nov. 9	Oct. 28	Nov. 15	Oct. 3 – Dec. 10
Southern and Central BC	138	Nov. 11	Nov. 4	Nov. 19	Oct. 14 – Dec. 23
Dates of Departure					
Southcentral Alaska	40	Apr. 30	Apr. 16	May 8	Mar. 16 – May 29
Southeast Alaska	50	Apr. 28	Apr. 7	May 14	Feb. 11 – May 30
Northern BC	31	Apr. 27	Apr. 12	May 5	Mar. 10 – May 24
Southern and Central BC	132	Apr. 15	Apr. 1	Apr. 25	Feb. 26 – May 30

Table 2-4. Size of winter home ranges (km^2) for sexes, age classes, and coastal regions used by Barrow's goldeneyes on the Pacific coast

	Number of Birds	Median (km^2)	1st Quartile (km^2)	3rd Quartile (km^2)
Sex				
Male	197	25.99	4.62	77.35
Female	125	9.66	1.44	45.54
Age				
After Hatch-year	288	15.38	2.80	71.08
Hatch-year	34	17.59	3.86	46.37
Coastal Region				
Southcentral Alaska	48	4.09	1.37	41.07
Southeast Alaska	57	41.28	14.96	91.76
Northern BC	38	29.32	7.19	74.15
Southern and Central BC	179	13.77	3.07	64.19

2.11. Figures

Figure 2-1. Overview of Barrow's goldeneye study area on the Pacific coast of North America

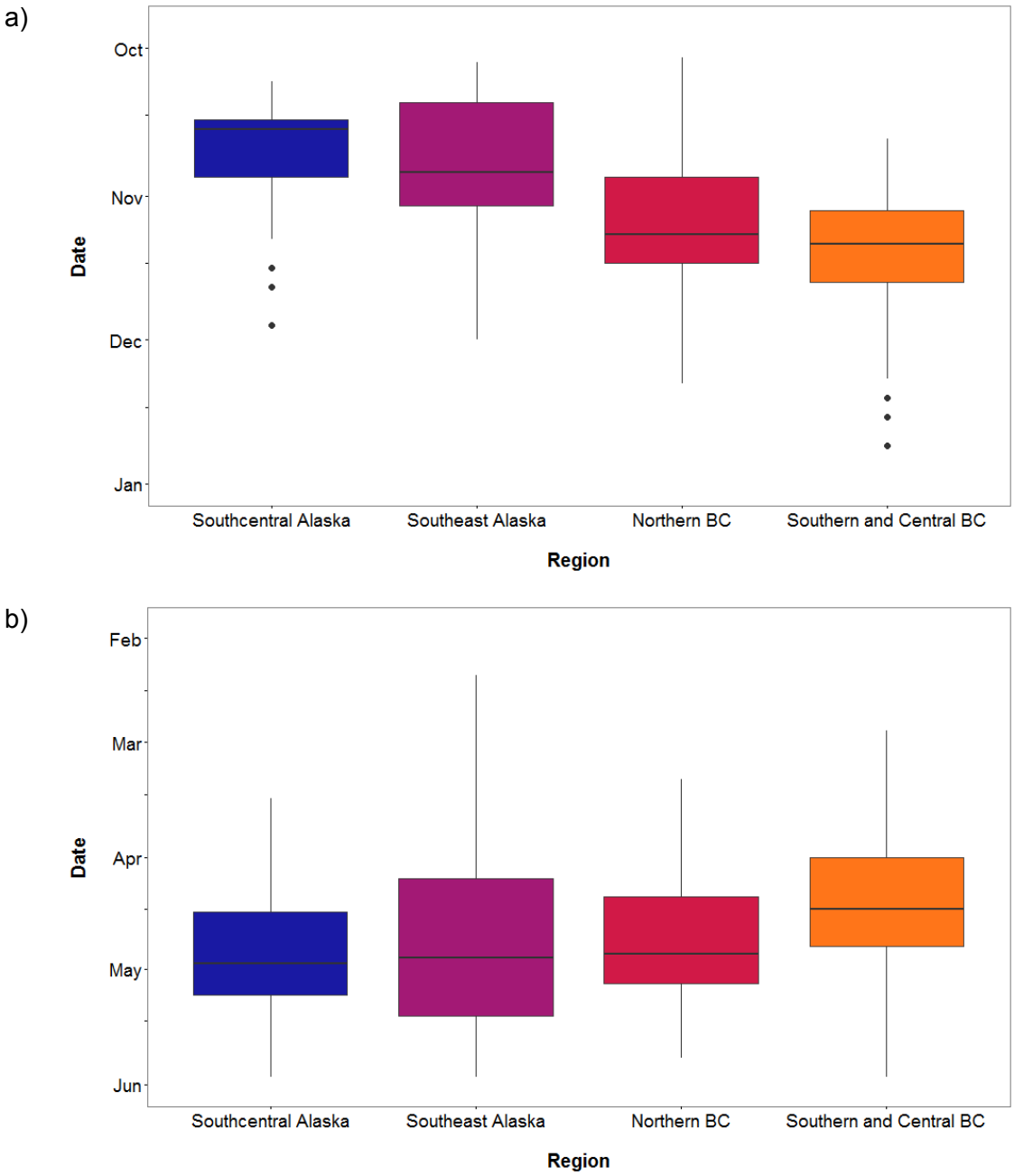

Figure 2-2. Dates of (a) arrival and (b) departure of Barrow's Goldeneye to and from coastal wintering sites. Box plots show median values (solid horizontal line), 50th percentile values (box outline); whiskers represent 1.5 * interquartile range; outliers are shown as circles (●)

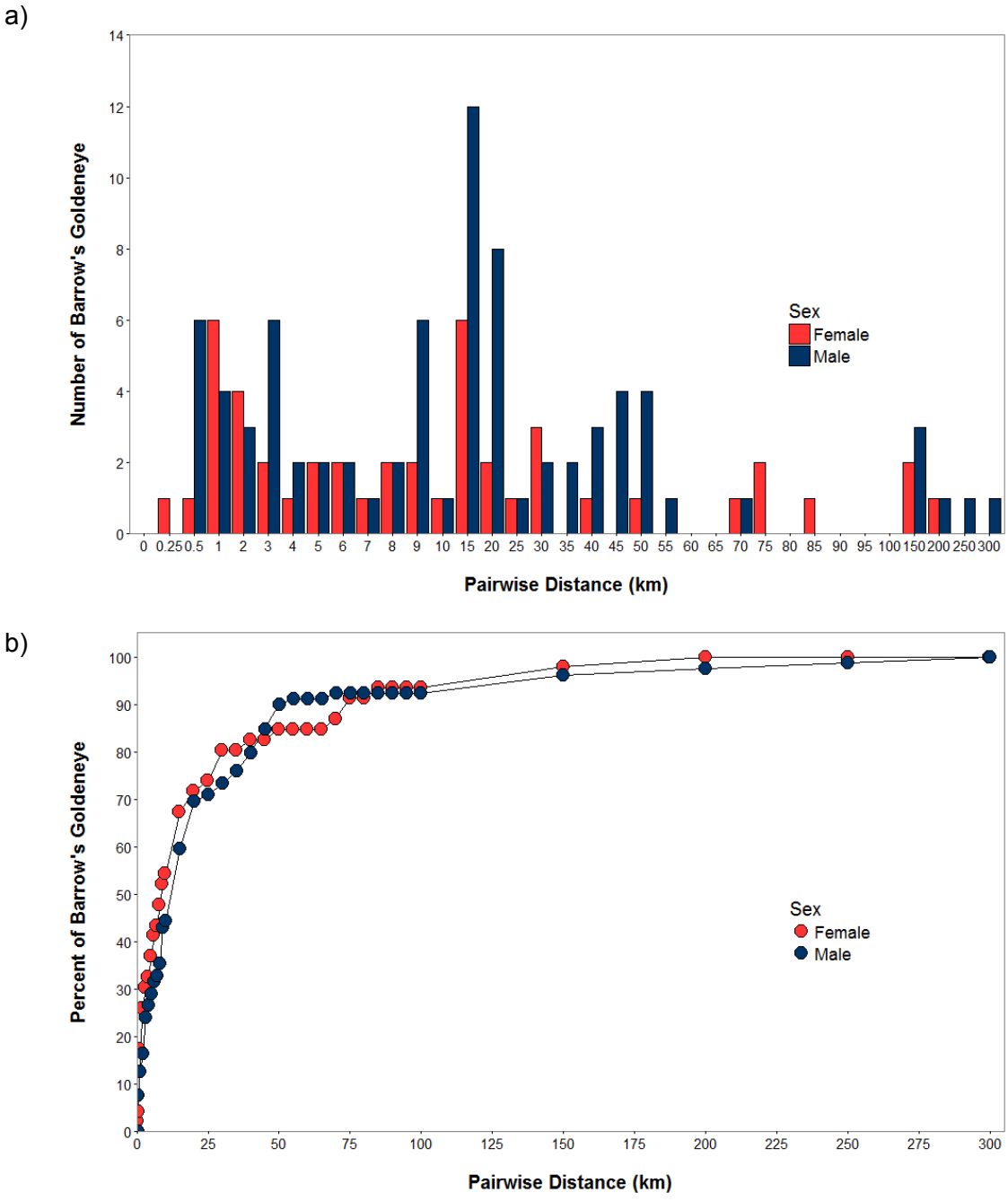

Figure 2-3. Frequency (a) and cumulative percent (b) of interannual pairwise distances between annual centroids of use for female and male Barrow's Goldeneyes on the Pacific coast

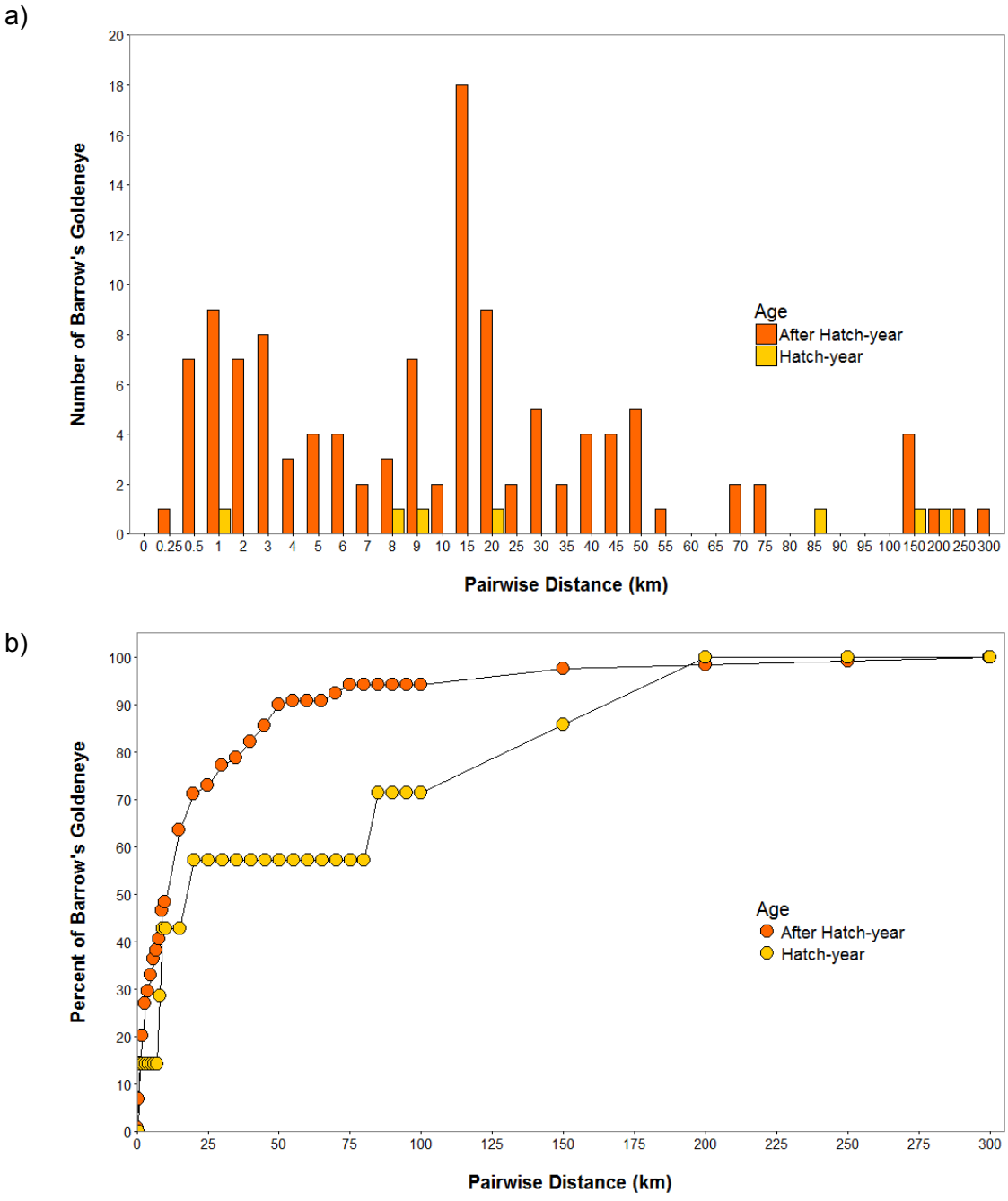

Figure 2-4. **Frequency (a) and cumulative percent (b) of interannual pairwise distances between annual centroids of use for after hatch-year and hatch-year Barrow's Goldeneyes on the Pacific coast**

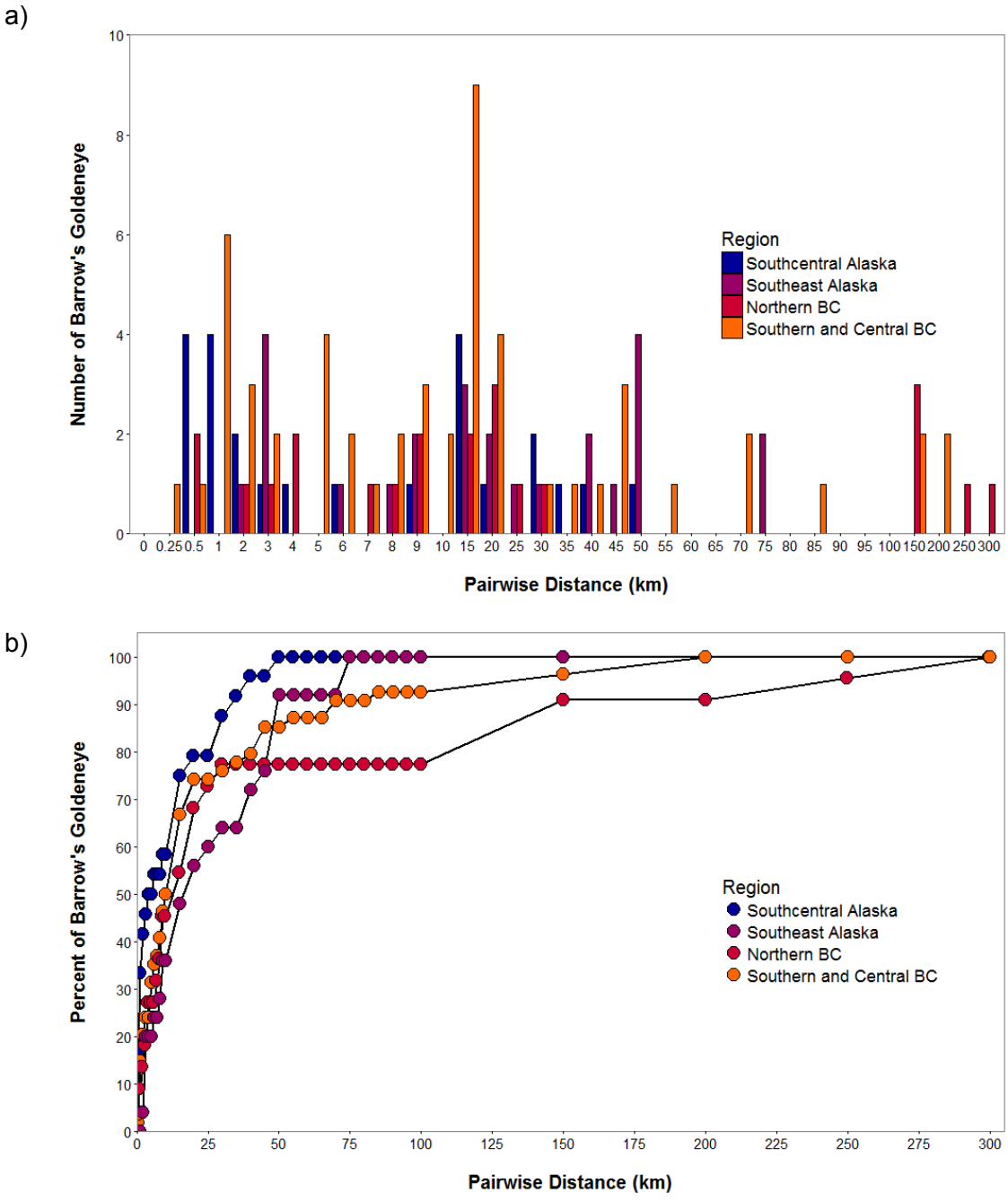

Figure 2-5. Frequency (a) and cumulative percent (b) of interannual pairwise distances between annual centroids for regional groups of Barrow's goldeneyes on the Pacific coast

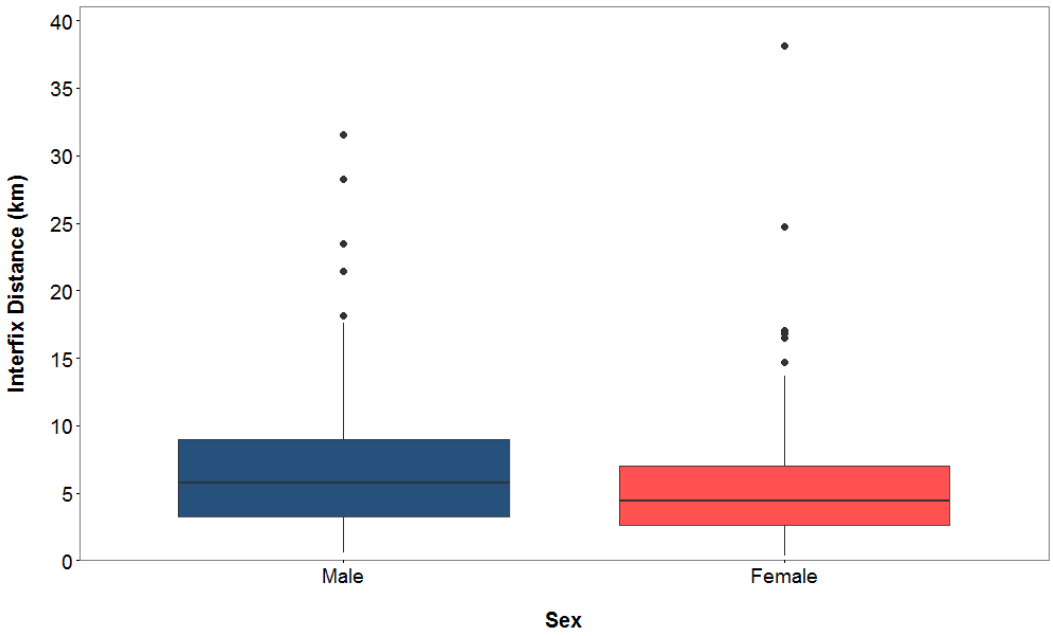

Figure 2-6. Average interfix distances for movements of male and female Barrow's goldeneyes during winter*.

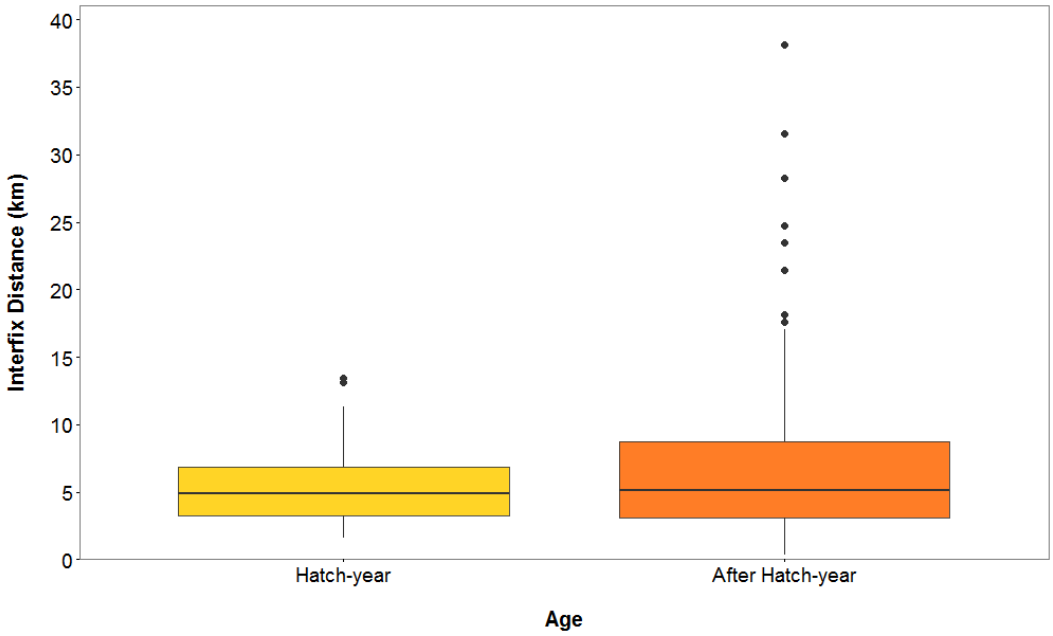

Figure 2-7. Average interfix distances for movements of hatch-year and after hatch-year Barrow's goldeneyes during winter*.

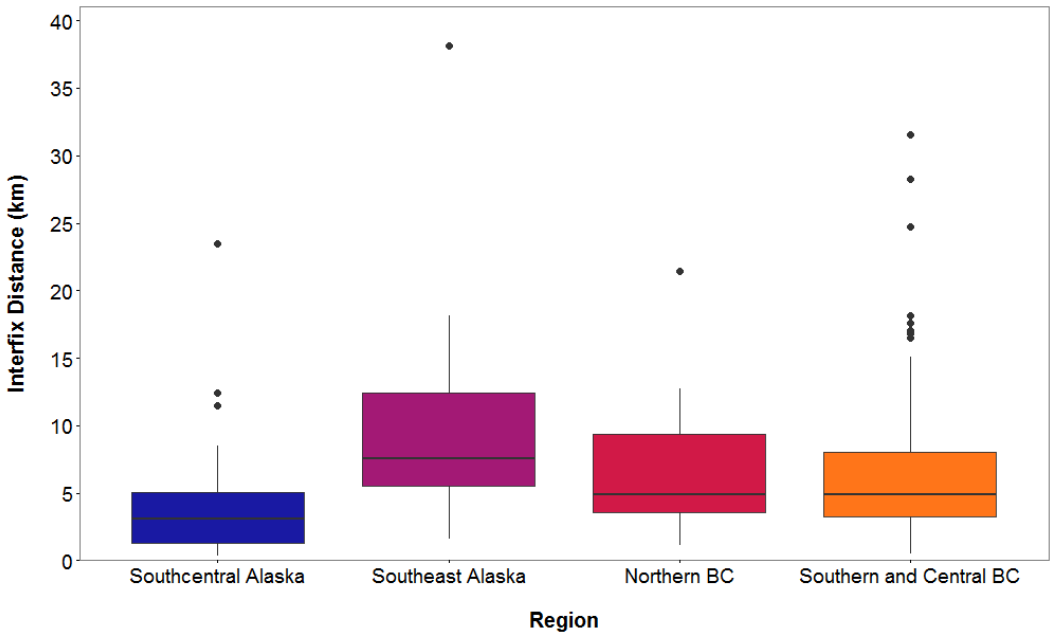

Figure 2-8. Average interfix distances for movements of Barrow's goldeneyes at regional coastal wintering sites during winter*.

* See Figure 2-2 caption for further information on boxplot characteristics.

Chapter 3.

Spatial Variation in Polycyclic Aromatic Hydrocarbon Exposure in Barrow's Goldeneye (*Bucephala islandica*) in Coastal British Columbia

3.1. Abstract

Oil pollution is an important cause of marine bird mortality in Canada and throughout the world. In particular, polycyclic aromatic hydrocarbons (PAHs) are known to exert a suite of deleterious physiological effects in exposed wildlife. Barrow's goldeneyes (*Bucephala islandica*) are sea ducks that winter in coastal British Columbia, across a range of degrees of industrial development. Because their winter diet is primarily comprised of filter-feeding blue mussels (*Mytilus* spp.), goldeneyes are susceptible to PAH exposure through contaminated prey. Hence, goldeneyes serve as a sensitive, upper trophic level indicator of PAH exposure in coastal ecosystems in the province. In 2014 and 2015, we compared the degree to which Barrow's goldeneyes wintering in undeveloped and industrialized coastal areas of British Columbia exhibited contemporary exposure to PAHs. As an indicator of PAH exposure, we measured 7-Ethoxyresorufin-*O*-deethylase (EROD) to determine cytochrome P4501A (CYP1A) induction in goldeneye liver tissue. To examine spatial patterns in dietary PAH availability, total PAH (∑PAH) concentrations were measured in blue mussels collected from the same wintering areas. Using information theoretic methods, we examined a combination of factors to evaluate variation in ∑PAH in blue mussels and EROD activity in goldeneyes. The ∑PAH in blue mussels was best explained by mussel lipid content and the level of industrialization (i.e., industrialized vs. intermediate and reference sites), although concentrations in this study were low relative to known polluted areas elsewhere. Region best explained goldeneye EROD patterns; birds from southern British Columbia demonstrated higher levels of EROD activity, overall. Little support was found for the hypotheses that variation in EROD activity was explained by the age, sex, and mass of goldeneyes. Our results suggest that

Barrow's goldeneyes wintering in coastal British Columbia were exposed to contemporary sources of PAHs through diet. Exposure was higher among individuals wintering in southern British Columbia, which generally has higher levels of human activity.

3.2. Introduction

Oil pollution in the marine environment is a leading cause of bird mortality in Canada (Wiese et al. 2004; O'Hara et al. 2009; Calvert et al. 2013). Among marine wildlife, birds are considered especially sensitive to lethal or sub-lethal effects resulting from direct contact, inhalation, absorption, or ingestion of oil (Leighton 1993). Birds that come into direct contact with oil will readily adsorb particles onto their feathers. Adsorbed oil reduces the waterproofing, insulating, and buoyancy properties that feathers provide; loss of these functions can result in death due to starvation or hypothermia (Leighton 1993; Wiese 2002). Similarly, oil that is inhaled, absorbed, or ingested can exert debilitating or lethal toxicity on internal tissues and organs (Eisler 1987; Leighton 1993; Piatt and Anderson 1996; Franci et al. 2014).

Catastrophic oil spills have resulted in sizeable marine bird mortality events. Mortality estimates following the *Amoco Cadiz*, *Exxon Valdez*, *Prestige*, *Selendang Ayu*, and *Deep Water Horizon* oil spills range from 100,000 to 300,000 birds (Piatt et al. 1990; Munilla et al. 2011; Haney et al. 2014). In some marine ecosystems, however, chronic oil discharges are reported to contribute larger inputs of hydrocarbons over time than catastrophic spills, with subsequent, enduring effects on birds (Leighton 1993; Wiese 2002; Camphuysen 2007). Chronic discharges are regularly occurring hydrocarbon releases typically associated with marine industrial activities (e.g., discharges of oily waste water from vessels) but also can be sourced from atmospheric emission and deposition; surface water runoff; and recreational, or commercial marine activities (Eisler 1987; Camphuysen 2007; Nikolaou et al. 2009; Yunker et al. 2011).

Among classes of hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), in particular, have potential to exert toxic effects on marine birds. Both the United States

(US) and Canadian Environmental Protection Agencies (EPAs) have classified PAHs as priority pollutants due to their persistence in the environment, ability to act as carcinogens or mutagens, and consequently, potential to cause adverse physiological effects in vertebrates (CEPA 1994; Bojes and Pope 2007; Pampanin and Sydnes 2013). Chronic PAH exposure has been attributed to a wide range of effects in marine birds, including: immune suppression, oxidative stress in the liver and kidneys, depressed reproductive performance, embryotoxicity, susceptibility to disease, and death (Eisler 1987; Leighton 1993; CEPA 1994; Piatt and Anderson 1996; Esler et al. 2000). Collectively, effects from chronic exposure can have wide-ranging demographic consequences on marine bird populations that exceed lethal effects of acute exposure experienced during catastrophic spills (Szaro 1976; Leighton 1993; Nur et al. 1997; Wiese and Robertson 2004; O'Hara et al. 2009; Esler and Iverson 2010; Henkel et al. 2014).

Birds that live and forage in industrialized coastal ecosystems are particularly vulnerable to sustained exposure to hydrocarbons (Miles et al. 2007). In western North America, British Columbia is heralded as the “Gateway to the Pacific”. Several regions of the British Columbia coast operate as major departure points for the transport of commercial and industrial commodities to primarily Asian markets. Deep-water ports located in Vancouver, Kitimat, and Prince Rupert serve as confluences of several major shipping routes. Shipping activity in British Columbia includes tankers, bulk carriers, tugs, barges, ferries, cruise ships, and recreational and commercial fishing boats. Through these activities, an estimated 110 million m^3 of petroleum are transported along the British Columbia coast each year (Nuka Research and Planning Group LLC 2013). Recent proposed industrial expansion could see the addition of more than 20 coastal facilities, including several oil and liquefied natural gas terminals. Collectively, these developments could result in an associated increase of approximately 1,000 tankers and bulk carriers transiting British Columbia waters each year (Nuka Research and Planning Group LLC 2013). Existing and future marine industries have potential to result in chronic or catastrophic PAH inputs into coastal ecosystems in the province. However, despite the history of industrial activity in the province, ecological risk of contemporary oil exposure to marine birds is poorly quantified. Little is known of the relationship between PAH concentrations in marine bird prey and rates of exposure in birds. The degree of contemporary PAH exposure among marine birds occupying industrialized coastlines is

similarly undocumented. These knowledge gaps limit efforts to predict and manage effects from existing and future hydrocarbon contamination.

In British Columbia, the potential for contemporary chronic PAH exposure in marine birds is well represented by Barrow's goldeneyes (*Bucephala islandica*). Barrow's goldeneyes are sea ducks that winter in the Pacific Northwest; an estimated 60% of the global population resides in coastal British Columbia during winter months (Campbell et al. 1990; Eadie et al. 2000). Several locations in British Columbia are recognized as supporting large concentrations of wintering birds, including: Burrard Inlet, Indian Arm, the Strait of Georgia, Douglas Channel, Prince Rupert, and Haida Gwaii (Campbell et al. 1990; Horwood 1992; Important Bird Area [IBA] Canada 2016), representing a wide range of degrees of industrialization and human activity. Barrow's goldeneyes show strong seasonal associations with intertidal habitats, where spilled oil tends to aggregate, and where they feed almost exclusively on blue mussels (*Mytilus* spp.) (Eadie et al. 2000). The risk of toxicological effects from sustained consumption of contaminated prey is greatest for species feeding on organisms with high PAH burdens (Newell 1989; Boehm et al. 1996). Among mollusc species, blue mussels are poor metabolizers of PAHs and as filter-feeders are highly susceptible to, and capable of accumulating, high PAH burdens in their lipid-rich tissues from their immediate surroundings (Boehm et al. 1996; Meador 2003; Galgani et al. 2011). Consequently, goldeneyes are expected to be particularly prone to toxicological effects of dietary sources of PAH and, hence, constitute a simple, sensitive vertebrate indicator of PAH exposure in coastal ecosystems in British Columbia.

The objective of this study was to evaluate the degree to which Barrow's goldeneyes wintering in coastal British Columbia exhibit exposure to PAHs and to identify whether ingestion of contaminated prey serves as a pathway for exposure. To investigate this, non-lethal liver biopsies were obtained from goldeneyes captured from sites representing varying degrees of industrialization (i.e., categorized as industrialized, intermediate, or reference sites). Because vertebrates rapidly metabolize hydrocarbon compounds, it is difficult measure PAH concentration in bird tissues directly. The cytochrome P4501A (CYP1A) system is reliably induced by metabolism of a small suite of contaminants, including PAHs, polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs). 7-

Ethoxyresorufin-O-deethylase (EROD) activity from liver samples was used to examine CYP1A expression. Previous studies have established EROD as an effective biomarker of exposure to PAHs and other contaminants (e.g., Trust et al. 2000; Miles et al. 2007; Esler et al. 2011; Franci et al. 2014). Measures of EROD activity were compared to sex, age, and body mass attributes from sampled goldeneyes to explore potential physiological relationships in birds exhibiting higher rates of exposure. Patterns of exposure also were hypothesized to correspond with PAH burdens in goldeneye prey. Accordingly, we measured the concentration of total PAHs in blue mussels obtained from goldeneye wintering sites in northern and southern British Columbia. Because PCDDs, PCDFs, and PCBs also serve as potential inducing agents, and previous studies indicate that these compounds are bioavailable to marine wildlife in British Columbia (e.g., Elliott and Martin 1998; Harris et al. 2003; JWA 2010), mussels also were evaluated for the potential occurrence of other CYP1A inducing agents. We predicted that birds wintering in industrialized coastal areas would demonstrate higher levels of EROD activity as a result of ingesting prey that are more likely to be chronically exposed to PAHs (Iverson and Esler 2006; Miles et al. 2007; Oros et al. 2007; Kimbrough et al. 2008). This pattern was expected to persist across industrialized coastal habitats in northern and southern British Columbia.

3.3. Study Areas

To investigate PAH contamination in blue mussels and exposure in Barrow's goldeneyes, sampling was completed in coastal areas of northern and southern British Columbia where large congregations of goldeneyes occur (d'Entremont 2010; Horwood 1992; IBA Canada 2016). In both regions, samples were collected at industrialized, intermediate, and reference sites. Industrialized sites were characterized by the historical and contemporary presence of industrial, commercial, and recreational shore-based facilities and associated vessel traffic. These sites serve as known or putative sources of PAHs. Intermediate sites were identified as areas without major shore-based infrastructure (with the exception of small residential communities) and support a lower intensity of marine-based activity. Samples collected from industrialized and intermediate

sites were compared to those obtained from reference locations. For this study, reference sites were defined as lacking shore-based infrastructure and encompassed areas where marine activity was entirely from recreational watercraft (i.e., small motorized and non-motorized vessels).

3.3.1. Northern British Columbia: Douglas Channel and Kitimat Arm

In northern British Columbia, goldeneye and mussel sampling was conducted in the Douglas Channel fjord system, extending 150 km inland from the Pacific Ocean to Kitimat Arm (Figure 3-1). Industrial activity in the Kitimat Estuary dates back to the early 1950's with the operation of the Rio Tinto Alcan aluminum smelter, the Eurocan pulp and paper mill, and the Methanex methanol production plant (Levings 1976; MacDonald and Shepherd 1983). PAH loads in marine sediments in Kitimat Arm are highest in northern parts of the estuary (Simpson et al. 1996; Yunker et al. 2011). The south end of Douglas Channel marks the confluence of several major shipping channels supporting tankers, bulk carriers, ferries, cruise ships, tugs, fishing boats, and recreational vessel traffic. Two notable shipwrecks have occurred in this area. The M/V *USAT Brigadier General M.G. Zalinski* was a US Army transport ship that sank in southern Grenville Channel in 1946, containing 700 tonnes of bunker fuel. In 2012, emergency response was initiated to partially salvage residual oil leaking from the vessel (DFO 2013). Thirty-five tonnes of fuel were recovered by late 2013. In 2006, the M/V *Queen of the North* ferry had a catastrophic grounding along the northern end of Gil Island. At the time of sinking, the vessel was carrying approximately 220,000 litres of diesel fuel and lubricating oil (BC Ferries 2006). Efforts to contain subsequent oil releases have been implemented by BC Ferries; however, the vessel remains sunk in Wright Sound with no immediate plans for salvage. Douglas Channel also hosts several narrow inlets which, due to their restricted depth and width, are generally only accessed by recreational fishing boats. For example, Kiskosh Inlet is a 15 km fjord extending northwest from Douglas Channel, and is geographically removed from industrialized areas of the Channel (Figure 3-1). Based on the negligible human activity in Kiskosh Inlet, it serves as a reference site to contrast against industrialized regions of Douglas Channel and Kitimat Arm.

3.3.2. Southern British Columbia: Burrard Inlet and Indian Arm

Burrard Inlet is a narrow inlet extending 30 km inland from the Strait of Georgia to Indian Arm, encompassing coastal areas from Point Atkinson and Point Grey east towards Port Moody (Figure 3-2). The Indian Arm Basin is a steep-sloped fjord extending 20 km north from Burrard Inlet. The Burrard Inlet and southern sections of Indian Arm lie between several large municipalities. Throughout the Inlet, large sections of coastal habitat have been modified to support industrial, commercial or residential use. Several export terminals are located in Burrard Inlet, including six petroleum facilities (i.e., the Suncor Burrard Products, Ioco Refinery, Kinder Morgan Westridge, Shellburn, Stanovan, West Coast Reduction petroleum terminals) (Port Metro Vancouver 2014). In 2007, 100,000 litres of heavy synthetic crude oil were released into Burrard Inlet due to a ruptured pipeline at the Westridge Terminal, which deposited onto 15 km of shoreline (Stantec 2012). Monitoring following the spill included PAH profiling in blue mussels. Results indicated that mussels routinely bioaccumulate oil from a number of anthropogenic sources beyond the Westridge spill (Stantec 2012). Burrard Inlet also supports a high volume of vessel activity, with 17,594 vessel transits reported in 2012 (Moffatt and Nichol 2013). An estuarine complex is formed towards the northern end of Indian Arm at the mouth of the Indian River. Shallow water in this area generally limits human activity to small recreational vessels, including non-motorized boats. Consequently, this area serves as an appropriate reference site to southern reaches of Indian Arm and Burrard Inlet.

3.4. Materials and Methods

3.4.1. Field Sampling Procedures

Blue Mussels

Samples of blue mussels were collected following the National Oceanic and Atmospheric Administration's (NOAA's) Sampling and Analytical Methods of the National Status and Trends Program for the National Benthic Surveillance and Mussel Watch

Projects (NOAA 1993). Twenty-four samples were collected at five locations in northern British Columbia on June 28 and 29, 2014 (Figure 3-1), and a further 22 samples at eight locations in southern British Columbia on May 16, 18, and 19, 2015 (Figure 3-2). In order to sample the full intertidal area sampling was scheduled to correspond with the lowest annual tides in each region. Mussels were collected during the low tide period on each sampling day from across the intertidal area.

Each sample comprised 60 mussels collected along a transect placed perpendicular to the shoreline and included 20 mussels hand-picked from surface substrates in the low, mid, and high intertidal regions along the transect line (NOAA 1993). Several samples (two to four) were collected at each location (Table 3-1), with transects spaced a minimum of 15 m apart. Inter-transect distance varied according to the topography and mussel availability at each site. Mussels were stored in sterilized sampling bags, kept on ice for transport, and once in the lab were frozen at -20°C until prepared for contaminant analysis (NOAA 1993). Mussel samples were collected under Fisheries and Oceans Scientific Permits SFU-XMCFR-9-2014 and SFU-XMCFR-10-2015.

Barrow's Goldeneye Capture and Liver Biopsy

Barrow's goldeneyes generally spend November through May along the British Columbia coast (Chapter 2). We captured goldeneyes in March and April, to maximize the period of time that sampled birds had spent foraging on mussels in coastal wintering habitats. In northern British Columbia, 40 goldeneyes were captured across six locations between April 12 and 25, 2014 (Figure 3-1; Table 3-2). Another 41 individuals were captured across five locations in southern British Columbia between March 10 and April 11, 2015 (Figure 3-2; Table 3-2).

Goldeneyes were captured using techniques described in Kaiser et al. (1995). Briefly, a floating mist net was installed at each capture location by suspending the net in shallow waters and extending it perpendicular to the shoreline. Each net was opened at dawn until late morning (approximately 5:30 am to 10:30 am). Captured goldeneyes were immediately extracted from mist nets and transported, using animal carriers, to the field research station for processing. We recorded the age, sex, and mass for each captured

bird. Ages of goldeneyes were categorized as hatch-year (HY) or after hatch-year (AHY) individuals, as determined by depth of the bursa of Fabricius for females and bursal depth and plumage characteristics for males (Mather and Esler 1999). Sex of birds was determined by plumage and cloacal characteristics.

Surgical procedures for liver biopsy followed methods described by Degernes et al. (2002) and have been successfully applied to Barrow's goldeneyes in previous research (e.g., Esler et al. 2011). As part of the surgical procedure, birds were administered a subcutaneous dose of meloxicam (0.5 mg/kg) in advance of surgery to provide analgesia to recovering birds. Birds were mask-induced with an isoflurane/oxygen mixture at approximately 2 to 2.5% isoflurane to achieve effective anaesthesia. Anaesthetized birds were intubated with an endotracheal tube. Feathers were isolated from the site of incision and tissue was sterilized using a topical 10% povidine-iodine solution. A 2 to 3 cm cutaneous midline incision was made on the ventral abdomen midway between the caudal sternum and the cranial extent of the pubic bones exposing the linea alba. The linea alba was incised to provide access to the coelomic cavity. A liver biopsy (<0.5 g and <5 mm diameter) was surgically removed from each anaesthetized bird; liver tissue was immediately transferred to a labeled cryovial and maintained at -80°C until ready to be prepared for analysis (Esler et al. 2011). The body wall and skin were closed separately using 4-0 or 3-0 Polydiaxanone sutures. Tissue adhesive was applied over the sutured incision to enhance water resiliency of the incision. Birds recovering from surgery were placed in a shaded animal carrier and released at the field site once sufficient time had passed for the isoflurane to be excreted from their systems (approximately one hour). Capture, banding, and surgical activities were performed under Environment Canada Scientific Permit 10673P and Simon Fraser University Animal Care Permit 1121B-06.

3.4.2. Statistical Analyses

Using R statistical computing language (Version 3.2.2; R Development Core Team 2015), least squares general linear models (GLMs), were used to estimate variation in contemporary hydrocarbon exposure explained by a set of models that included different combinations of variables of interest. An information-theoretic approach was used for

model selection and inference, where support for various model combinations was compared using Akaike's Information Criterion (AIC_c for small sample sizes; Burnham and Anderson 2002).

Higher lipid content in blue mussels can contribute to accumulation of lipophilic contaminants like PAHs (Meador 2003). Accordingly, we asked *a priori* if PAH concentration in blue mussels was best explained by degree of industrialization at individual coastal sites as well as percent lipid content in mussel tissue. Total PAH concentration in mussels was evaluated at five spatial scales to determine which was the most informative for understanding patterns in contamination. Spatial categories included:

- *Site*—Individual sites considered separately;
- *Condition*—Sites with similar hydrocarbon contamination histories were categorized as either industrialized, intermediate, or reference sites;
- *Industrialized*—Industrialized sites compared to intermediate and reference sites (i.e., where sites in Kitimat Arm and Burrard Inlet were compared to all remaining sites);
- *Reference*—Reference sites compared to intermediate and industrialized sites (i.e., where Kiskosh Inlet and Indian River were compared to all remaining sites); and,
- *Region*—Sites sampled in northern British Columbia were compared to all sites sampled in southern British Columbia.

To assess whether PAH exposure in Barrow's goldeneyes corresponded to geographic partitioning of PAHs in mussels, we developed a second model set to investigate if EROD activity was best explained by (a) spatial scale (using the same spatial category definitions as for blue mussels, above); (b) the sex, age, or body mass of sampled birds; or (c) a combination thereof. For each model set, sex, age, and body mass variables were included as a group, termed "individual attributes"; model sets either included all of these variables, or none of them (*sensu* Esler et al. 2011). A null model was included in each candidate model set to represent the mean and variance across all of the

data. Strong support for the null model would suggest that the added complexity contributed by explanatory variables considered in each model set was not justified in explaining variation in the response.

All candidate models used AIC_c to correct for small sample size (Burnham and Anderson 2002). Within each candidate model set, the model with the lowest AIC_c value was considered to have the strongest support from the data. Models with a ΔAIC_c less than two were considered to have strong support for explaining variation in the response variable (Burnham and Anderson 2002). AIC_c weights (w) also were calculated for models within each candidate set; AIC_c weights sum to 1.00 and indicate the relative support for each model. To determine support for each model relative to the best model of the set, evidence ratios of AIC_c weights were calculated (w_i/w_j, where w_i is the weight of the best-supported model and w_j is the weight of each of the next-best models in the model set; instances where w_i/w_j = 1.00 indicates equal support to the best-supported model). Parameter likelihoods were calculated by summing AIC_c weights for all models that include a given variable, across all models in the candidate set. A parameter likelihood approaching 1.00 indicates strong relative support for that particular variable. Model-averaged parameter estimates and associated unconditional standard errors also were calculated to estimate the size, direction, and variation of effects for each variable, and are calculated across the candidate model set. For linear models receiving support, diagnostic plots also were examined to ensure that model assumptions were not violated. Transforming ∑PAH or EROD values did not improve fit of best-supported top models. Accordingly, data were maintained untransformed throughout the analysis.

3.4.3. Contaminant Analyses

Composition and Concentration of Contaminants in Blue Mussels

Composite mussel samples were analyzed for lipid content and contaminant concentrations by gas chromatography-mass spectrometry (GC-MS) techniques performed by ALS Environmental in Burnaby, British Columbia. Analytical methods were adapted from the US EPA's Test Methods for Evaluating Solid Waste, Physical/Chemical

Methods (Methods 1613B, 3540, 3570, 3600, 8082, 8270, and 8290; US EPA 2015). To prepare each composite sample for analysis, mussels were thawed, shucked and tissue homogenized in a closed-cup blender. To measure lipid content, a portion of homogenized tissue was extracted with dichloromethane and evaporated to dryness; lipid content was measured gravimetrically and reported as a percent.

To determine spatial patterns in PAH exposure, concentrations were measured in mussel tissue. Samples were analyzed for 17 parent PAH compounds recognized to induce CYP1A activity in birds (Table A-1 and Table A-2). Individual PAH concentrations were reported as micrograms per kilogram of wet weight tissue (µg/kg wwt). Total PAH concentrations (i.e., ∑PAH) for each sample were calculated by summing the concentrations of each of the 17 individual PAH compounds. Method detection limits were 10 µg/kg wwt; concentrations of individual PAHs reported to be below method detection limits were treated as half of the detection limit value for all analyses (Galgani et al. 2011; Forsberg et al. 2014). There was no difference in our conclusions when a value of zero was used in place of half the detection limit. To characterize the extent to which other CYP1A-inducing compounds may potentially elicit a CYP1A response, a subset of mussel samples from different sites in each region were analyzed for the 17 2,3,7,8-substituted PCDDs and PCDFs as well as the coplanar group of PCBs (Table A-3). Individual PCDD/PCDF concentrations were reported as pg/g wwt; total PCDD/DF (∑PCDD/DF) concentrations were calculated by summing the concentrations of individual congeners measured above the effective detection limit. In the subset of mussels sampled for PCBs, all tested congeners were measured below the effective detection limit (50 µg/kg wwt). Consequently, we assumed that PCBs were not present in prey in quantities sufficient to influence EROD activity in Barrow's goldeneyes and were not considered further.

Cytochrome P4501A Induction in Barrow's Goldeneye Liver Tissue

We measured EROD activity from Barrow's goldeneye liver samples as an indicator of CYP1A expression. Analysis of EROD activity was completed at the University of Manitoba's Department of Biological Sciences following procedures adapted from Trust et al. (2000), Miles et al. (2007), and Rainio et al. (2012).

Between 40 and 250 mg (wet weight) of liver tissue per bird was analyzed for EROD activity. To prepare individual liver sections for analysis, samples were homogenized in 7 ml of homogenizing buffer (0.02 M HEPES, 0.07 M KCl, pH 7.5) at approximately 4°C to prevent the loss of enzymatic activity (Trust et al. 2000; Miles et al. 2007; Rainio et al. 2012). The cytosolic and microsome fraction was isolated from cellular debris by differential centrifugation (10,000 g for 10 minutes). The supernatant (S9) fraction was used in all EROD activity analyses. EROD activity is measured as an expression of CYP1A induction, where 7-ethoxyresorufin is converted to resorufin, a fluorescent product that can be measured using a fluorometer. EROD activity was measured by applying a kinetic modification to a standard plate-based assay (Trust et al. 2000). We ran the S9 fraction in triplicate in a 96-well plate at 25°C using a FLUOstar Omega microplate reader (BMG Labtech, Ortenberg, Germany). Individual wells contained 20 µl of S9 fraction and 20 µl of 7-ethoxyresorufin in 140 µl of reaction buffer (0.02 M HEPES, 0.07 M KCl, pH 7.5). Catalytic activity was initiated by adding 20 µl of 1.34 mM nicotinamide adenine dinucleotide phosphate (NADPH). Fluorescence was measured at an excitation wavelength of 530 nm and an emission wavelength of 590 nm, at one minute intervals for a total of 6 minutes. Final EROD activity is expressed as pmol/min/mg protein.

To ensure that goldeneyes were responsive to inducers, assays on positive and negative control samples were run in parallel with those on goldeneye liver tissue. Embryonated chicken (*Gallus gallus domesticus*) eggs were injected at 16 days of incubation with either 2 mg/egg of β-napthoflavone (BNF, a known CYP1A inducer) or 2 mg/egg of dimethyl sulfoxide (DMSO, for non-induced controls). Embryos were injected through a hole in the shell, at the air-cell. Livers were extracted from embryos after an additional 24 hours of incubation and maintained at -80°C. Seven samples per treatment (i.e., BNF or DMSO) were measured in parallel with assays performed on goldeneye livers. EROD activity in BNF-induced control tissue exceeded 1.00 pmol/min/mg protein in all cases, while all non-induced control samples measured below 0.50 pmol/min/mg protein.

3.5. Results

3.5.1. Blue Mussels

Composition and Concentration of PAHs in Blue Mussels

Total PAH concentration in blue mussels varied across industrialized, intermediate, and reference sites in northern British Columbia. Five of the 17 targeted PAHs were measured above detection limits in tissue samples (Table A-1). Benz[a]anthracene was measured in five of the eight samples obtained in Kitimat Arm (two samples from North Cove and three samples in Clio Bay), followed by single detections of acenaphthene, chrysene, dibenz[a,h]anthracene, and naphthalene from samples collected in North Cove and Stewart Narrows. Across all northern British Columbia sites, median ∑PAH was 90.0 µg/kg wwt (interquartile range [IQR] = 85.0, 100.5; n = 24). The highest ∑PAH concentrations were measured at sites closest to Kitimat: North Cove (median = 117.0 µg/kg wwt, IQR = 90.8, 144.8; n = 4) (Figure 3-3a) and Clio Bay (median = 115.5 µg/kg wwt, IQR = 97.8, 130.2; n = 4) (Figure 3-3a). PAH concentration was lowest at the reference site in Kiskosh Inlet (median = 85.0 µg/kg wwt, IQR = 85.0, 86.3; n = 8; Figure 3-3a). Among sites in northern British Columbia, ∑PAH and percent lipid content in blue mussels were modestly correlated (adjusted r^2 = 0.30, p = 0.003). Median percent lipid content was highest in Clio Bay (median = 2.3%, IQR = 2.1, 2.3), North Cove (median = 2.1%, IQR = 1.7, 2.0), and Malsey Bay (median = 2.1%, IQR = 2.1, 2.1); lipid content was lowest at Kiskosh Inlet (median = 1.5%, IQR = 1.3, 1.8) (Table A-1).

The total concentration of PAH in blue mussel samples from southern British Columbia were generally higher, and more similar between sites, compared to samples from northern British Columbia. Three of the 17 targeted PAHs were measured above detection limits in samples from southern British Columbia (Table A-2). Fluoranthene was detected in six samples collected from five separate sites, including four sites in Burrard Inlet. Acenaphthene was measured in a single sample from Stanley Park, while phenanthrene was measured in a single sample obtained near Mossom Creek. Across all

southern British Columbia sites, median ∑PAH was 95.0 µg/kg wwt, IQR = 91.0, 111.5; n = 22). Concentrations of ∑PAH were highest at Best Point (median = 133.5 µg/kg wwt, IQR = 129.2, 137.8; n = 2) and Mossom Creek (median = 113.0 µg/kg wwt, IQR = 112.0, 113.0; n = 2) (Figure 3-3b). Median ∑PAH was lowest for samples collected at the reference site near the Indian River (median = 95.0 µg/kg wwt, IQR = 92.5, 96.3; n = 4), Bedwell Bay (median = 95.0 µg/kg wwt; IQR = 93.8, 98.8; n = 4), and Stanley Park (median = 92.0 µg/kg wwt IQR = 88.8, 98.5; n = 4). Among sites in southern British Columbia, ∑PAH and percent lipid content in blue mussels were modestly correlated (adjusted r^2 = 0.26, p = 0.01). Median percent lipid content was highest at Best Point (median = 2.5%, IQR = 2.5, 2.6) and lowest at the Barnet Marine Park (median = 1.2%, IQR = 1.1, 1.2). PAHs were measured in low concentrations at both Kiskosh Inlet or Indian River, confirming that these sites appropriately represent reference conditions within each region.

Of the candidate models explored, variation of ∑PAH in blue mussels was best explained by the Industrialized + Lipid model (ΔAIC_c = 0.00, w = 0.37, evidence ratio = 1.00, adjusted r^2 = 0.41; Table 3-3). The second best model indicated support for effects of Region + Industrialized + Lipid (ΔAIC_c = 1.14, w = 0.21, evidence ratio = 1.76, adjusted r^2 = 0.41). The relationship between ∑PAH, Lipid, and Region for the two best-supported models is illustrated in Figure 3-4. Inclusion of a Region x Industrialized interaction term in a model with Region + Industrialized + Lipid as main effects also received some support (ΔAIC_c = 1.53, w = 0.17, evidence ratio = 2.18, adjusted r^2 = 0.43). The importance of these variables in explaining ∑PAH was supported by parameter likelihoods as well as model-averaged parameter estimates and their unconditional standard errors (Table 3-4). The parameter likelihoods for Lipid, Industrialized, and Region parameters were 1.00, 0.75, and 0.48, respectively. These results indicate that ∑PAH increased with mussel lipid content, was higher in mussels obtained from industrialized sites than intermediate or reference sites, and was higher in the southern region (Figure 3-4). Modest support was found for Condition + Lipid as parameters explaining variation in mussel ∑PAH (ΔAIC_c = 2.09, w = 0.13, evidence ratio = 2.85, adjusted r^2 = 0.40; Table 3-3). The remaining models had little support (ΔAIC_c > 3.58, w ≤ 0.06, and evidence ratios > 6.00; Table 3-3) and provide minimal contribution to describing variation in ∑PAH in blue mussels. No support was found for the hypothesis that mussel ∑PAH varied by Site, even when accounting for

lipid in mussels from each site (ΔAIC_c > 18.00, w < 0.00, evidence ratio > 37.00 for both the Site and Site + Lipid models, parameter likelihood = 0.00) (Table 3-3 and Table 3-4). Given the lack of support for the site parameter, the effect of mussel PAH on goldeneye EROD activity was not expected to vary at the site level and the Site parameter was removed from the subsequent candidate model set.

Composition and Concentration of PCDDs and PCDFs in Blue Mussels

Regional patterns in the total concentration of PCDD/DFs in blue mussel samples from northern and southern British Columbia were similar to total concentration of PAHs. Across all samples, 11 of the targeted PCDD/DFs were measured above detection limits in blue mussels (Table A-3). Nine congeners were detected in the single sample from North Cove, the most of any of the sites sampled in northern British Columbia. The congener 1,2,3,7,8,9-Hexachlorodibenzofuran (HxCDF) was measured above detection limits in four of the five samples from northern British Columbia. Across all northern British Columbia sites, median ∑PCDD/DF was 0.94 pg/g wwt, IQR = 0.25, 1.14; n = 5). The ∑PCDD/DFs was highest in Clio Bay (2.18 pg/g wwt) and North Cove (1.14 pg/g wwt), and was lowest in Kiskosh Inlet (0.10 pg/g wwt). Similar to northern British Columbia, 11 of the targeted PCDD/DFs were measured above detection limits (Table A-3). Overall, ∑PCDD/DF was higher in southern British Columbia (median = 4.17 pg/g wwt, IQR = 3.69, 8.31; n = 3). A single sample from Bedwell Bay contained the highest number of individual congeners above detection limits (n = 10) as well as the highest ∑PCDD/DF concentration (12.45 pg/g wwt). The ∑PCDD/DF concentration was 4.17 pg/g wwt at Stanley Park and 3.22 pg/g wwt at Camp Jubilee in Indian Arm. The congeners 1,2,3,4,6,7,8-heptachlorodibenzo-p-dioxin (HpCDD), octachlorodibenzodioxin (OCDD), 2,3,7,8-tetrachlorodibenzofuran (TCDF), and 1,2,3,4,6,7,8-heptachlorodibenzofuran (HpCDF) were reported in the three samples from southern British Columbia.

3.5.2. Barrow's Goldeneye

Cytochrome P4501A Induction in Barrow's Goldeneye Liver Tissue

Levels of EROD activity across all 81 sampled goldeneyes ranged from 0.08 to 77.24 pmol/min/mg protein. The majority (74 birds; 91%) of sampled birds had levels of EROD below 10.00 pmol/min/mg protein. EROD activity for goldeneyes from northern British Columbia ranged from 0.08 to 5.03 pmol/min/mg protein (n = 40). Median EROD activity in northern British Columbia was 0.68 pmol/min/mg protein, IQR = 0.36, 1.06; n=40). Across sites, median EROD activity levels were highest in individuals sampled from Hartley Bay (median = 0.96 pmol/min/mg protein, IQR = 0.33, 1.24; n = 5) and Clio Bay (median = 0.81 pmol/min/mg protein, IQR = 0.69, 0.91; n = 3; Figure 3-5a). Activity levels were lowest in North Cove (median = 0.50 pmol/min/mg protein, IQR = 0.22, 1.21; n = 15). In southern British Columbia, median EROD activity for goldeneyes was 2.12 pmol/min/mg protein, IQR = 1.21, 3.80; n = 41). Site-specific EROD activity was highest for a single goldeneye sampled from White Rock (median = 13.20 pmol/min/mg protein). For all remaining sites, median EROD activity was comparatively reduced, ranging from 1.85 pmol/min/mg protein, IQR = 0.98, 3.74; n = 25) at Best Point to 2.33 pmol/min/mg protein, IQR = 1.74, 5.14; n = 8) in Bedwell Bay; Figure 3-5b). Although median EROD activity was lowest for goldeneyes sampled from Best Point, birds from this site also demonstrated the greatest variability in individual activity levels (from 0.24 to 77.24 pmol/min/mg protein); across all 41 birds sampled in southern British Columbia, EROD activity was highest in four individuals sampled at Best Point (Figure 3-5b).

Overall, EROD activity was greater in birds sampled from southern British Columbia than from northern British Columbia. Variation in EROD activity in goldeneyes was best explained by the Region model (ΔAIC_c = 0.00, w = 0.54, evidence ratio = 1.00, adjusted r^2 = 0.08; Table 3-5). The parameter likelihood for Region was 0.90, indicating strong support for this parameter in explaining variation in EROD activity (Table 3-6). The second best model indicated modest support for Region + Industrialized in explaining variation in EROD (ΔAIC_c = 2.21, w = 0.18, evidence ratio = 3.00, adjusted r^2 = 0.06). The parameter likelihood for Industrialized was 0.28 (Table 3-6). Inclusion of the Reference category or Individual Attributes of goldeneyes as fixed effects in the remaining models

did not improve the fit of the best model ($\Delta AIC_c \geq 5.00$, $w \leq 0.04$, evidence ratios ≥ 18.00, and parameter likelihoods = 0.05). Three goldeneyes from Best Point, exhibiting the highest EROD levels, were identified as potential outliers in the regression of the top model. A secondary AIC analysis was performed, removing each of the highest EROD values, in sequence. In each case, EROD activity was still best explained by the Region model. Based on this result, EROD values for all goldeneyes were retained.

3.6. Discussion

Results of this study indicated regional differences in EROD activity among goldeneyes wintering in coastal British Columbia that were consistent with regional patterns in the concentration of ∑PAH in their prey. We observed contemporary levels of ∑PAH in blue mussels between 85 and 150 µg/kg wwt. PAH concentrations in this study were similar to values reported in mussels sampled from the Bering and Mediterranean Seas (e.g., Miles et al. 2007; Galgani et al. 2011), but considerably lower than in mussels or oysters sampled near densely populated coastal cities in North America (e.g., Seattle, Boston, Long Island, Galveston, and Honolulu) (Kimbrough et al. 2008; Lanksbury et al. 2014). Lipid content in blue mussels also appeared to be an important parameter in explaining ∑PAH concentration, particularly at industrialized sites where hydrophobic contaminants will readily associate with lipid-rich tissues in benthic organisms (Meador 2003). At the concentrations reported in this study, we found PAH burdens in blue mussels to be associated with CYP1A induction in regional goldeneye populations. Relative to sites in southern British Columbia, EROD activity was generally lower and less variable among goldeneyes sampled from industrialized, intermediate, and reference sites in the north. We found that the degree and incidence of elevated EROD activity in goldeneyes from southern British Columbia was similar to rates expressed by marine bird populations using oiled areas of Prince William Sound in years following the *Exxon Valdez* oil spill. Observed patterns in EROD activity likely reflect the magnitude and frequency of exposure to PAHs in prey. Integrating these results with telemetry data (see Chapter 2), we reason that PAH exposure is regulated by inter-site movements expressed by wintering goldeneye in each region. However, birds inhabiting highly developed coastal areas may have limited

potential to moderate PAH exposure through inter-site movements as a result of higher, and more ubiquitous, PAH burdens. This conclusion is supported by the elevated EROD levels observed in goldeneyes from southern British Columbia. Elevated EROD (>10 pmol/min/mg protein) activity was measured in goldeneyes from several locations, suggesting that a portion of goldeneyes in this region consume contaminated prey more regularly than others. Consequently, marine bird populations that strongly associate with contaminated coastal habitats are expected to be more susceptible to sub-lethal or lethal effects associated with sustained hydrocarbon exposure.

3.6.1. Blue Mussels

Composition and Concentration of PAHs in Blue Mussels

Our results support the hypothesis that industrialized sites are associated with higher levels of PAHs and that these contaminants penetrate marine food webs. Consistent with our prediction, we found that ∑PAH concentrations in northern British Columbia were higher among industrialized sites in Kitimat Arm compared to samples obtained from intermediate and reference sites in lower Douglas Channel. The highest ∑PAH concentrations were measured in four samples from North Cove and Clio Bay. In southern British Columbia, ∑PAH concentrations were relatively similar between industrialized sites in Burrard Inlet and intermediate or reference sites in Indian Arm, but overall higher than in northern reference sites. The even PAH concentration among sites in southern British Columbia suggests ubiquitous distribution of industrial, commercial, and recreational marine uses, and associated PAH inputs throughout this system. The highest mussel ∑PAH concentrations were recorded at Best Point, which is located approximately 15 km north of Burrard Inlet and hosts several small cabins. Based on the absence of major infrastructure and activity at this site, Best Point is unlikely to sustain chronically elevated levels of PAHs. The high concentration of fluoranthene observed at this location likely reflects a recent and localized source of this constituent that is anomalous with typical conditions in the area (Table A-2).

Consistent with findings in previous research (e.g., Eisler 1987, Meador 2003), mussel lipid content also was determined to be an important factor explaining PAH accumulation in blue mussels. Lipid content is known to influence the extent to which marine invertebrates accumulate lipophilic contaminants (Eisler 1987; Meador 2003). In this study, we found that percent lipid content and ∑PAH concentration were modestly correlated in mussels from northern and southern British Columbia. However, ∑PAH burdens were higher among mussels sampled from industrialized sites than from intermediate or reference sites, across the full range of lipid values reported in this study (Figure 3-4). Hence, goldeneyes foraging within industrialized sites in Kitimat Arm and Burrard Inlet have a greater likelihood of ingesting prey with higher PAH burdens.

One or two individual PAHs were detected in composite mussel samples from northern and southern British Columbia. Constituents measured above detection limits were primarily associated with the complete or partial combustion of coal tar and fossil fuels (e.g., benz[a]anthracene, chrysene, dibenz[a,h]anthracene, and fluoranthene). The composition and concentration of PAHs reported in this study are consistent with values previously described for industrialized coastal areas in northern and southern British Columbia. Earlier investigations of PAH contamination in blue mussels and other bivalves in the Douglas Channel system have generally focused on Kitimat Arm (e.g., JWA 2010; Yunker et al. 2011). As part of baseline surveys completed for the Northern Gateway Project in 2008, concentrations of 16 parent PAHs were measured in mussels collected in the Kitimat Estuary and Kitimat Arm (JWA 2010). In JWA (2010), ∑PAH in mussels ranged from 80 µg/kg wwt and 144 µg/kg wwt at sites in the Kitimat Estuary, and 120 µg/kg wwt at the marine terminal site in Kitimat Arm. These results are within the range of ∑PAH concentrations reported for North Cove and Clio Bay, as part of this study. However JWA (2010) reported elevated levels of naphthalene, phenanthrene, benzo[b]fluoranthene, chrysene, fluoranthene, and pyrene, suggesting that more complex mixtures of hydrocarbons were present historically. Yunker et al. (2011) measured complex mixtures of parent and alkylated PAHs in soft shell clams (*Mya arenaria*) collected at several locations in the Kitimat Estuary between 1995 and 2000. They reported similar trends to JWA (2010), where parent PAH concentrations were higher in clams sampled closest to industrial infrastructure in the Estuary. PAHs decreased in concentration along a southward gradient in Kitimat Arm. The authors identified primary sources of PAHs as

emissions from the Rio Tinto Alcan aluminum smelter, effluent discharge from the Eurocan pulp and paper mill, or chronic inputs of petroleum products associated with marine transportation. However, Yunker et al. (2011) concluded that soft-shell clams generally did not bioaccumulate smelter- or mill-derived sources of hydrocarbons; PAH concentrations measured in marine biota were most likely associated with petrogenic sources. Our results indicate that PAHs persist in Kitimat Arm and continue to be bioavailable to blue mussels in measurable concentrations, but are present in lower concentrations away from Kitimat Arm. Because PAHs typically occur in complex mixtures, and because few constituents were detected in individual samples in this study, it is difficult to attribute detections of PAHs to particular locations or activities. However, the presence of both low molecular weight PAHs (e.g., naphthalene and phenanthrene) and high molecular weight PAHs (e.g., dibenz[a,h]anthracene, fluoranthene) suggest contemporary sources of petrogenic and pyrogenic PAHs persist in this region.

The concentrations of ∑PAH in blue mussels measured from sites in southern British Columbia as part of this study also were consistent with previous sampling in Burrard Inlet. Blue mussels were collected from several locations in Burrard Inlet between 2007 and 2013 as part of monitoring following the Westridge Terminal oil spill (Stantec 2014). With the exception of the Westridge Terminal dock (in closest proximity to the spill site), concentrations of ∑PAH in mussels from elsewhere in Burrard Inlet typically measured below 100 µg/kg wwt throughout the sampling period (Stantec 2014). Stantec (2014) concluded that given the consistently low concentrations of PAHs over space and time in Burrard Inlet, hydrocarbons were being chronically introduced to the marine environment from multiple sources. Our results indicate that blue mussels throughout southern British Columbia continue to hold similar concentrations of PAHs. Here, comparable concentrations of ∑PAH between sites likely reflect the proximity of sampling sites in Burrard Inlet and Indian Arm, but also suggests that petrogenic and pyrogenic PAHs continue to be sourced from human activities throughout this region.

Concentrations of PAHs measured in mussel tissue from samples collected in both northern and southern BC serve as a contemporary baseline and can serve to evaluate changes in the magnitude and extent of contamination in each region from routine marine

activities (i.e., chronic spills) or applied as a recovery endpoint in the case of a large oil spill.

Composition and Concentration of PCDDs and PCDFs in Blue Mussels

PCDDs, PCDFs, and PCBs also serve as CYP1A inducing agents, and previous studies have observed measurable concentrations of these contaminants in sediments and biota in northern and southern British Columbia (e.g., Elliott and Martin 1998; Harris et al. 2003; JWA 2010). Accordingly, a subset of mussel samples in each region was also evaluated for the presence of these contaminants. While PCBs were not measured above detections limits, the regional patterns of ∑PCDD/DF in blue mussels were similar to those of ∑PAH. The highest concentrations of ∑PCDD/DF were measured in samples from industrialized sites in Kitimat Arm and were 12X to 23X higher than the reference site in Kiskosh Inlet. Relative to northern British Columbia, concentrations of PCDD/DFs were elevated at each sampled location in the south; with concentrations highest in mussels from Bedwell Bay. PCDD and PCDF concentrations in northern and southern British Columbia have showed steady declines in recent decades in relation to changing practices and restrictions adopted by the pulp and paper industry (Harris et al. 2003). We observed further declines in PCDD and PCDF concentrations in blue mussels from northern British Columbia compared to recent studies (i.e., JWA 2010), which are likely attributed to the closure of the Eurocan Pulp and Paper Mill in Kitimat in 2010. In light of these diminishing trends, PAHs appear to be the dominant contemporary CYP1A inducing agent that is bioavailable to goldeneyes in northern and southern British Columbia. However, given the persistence and similar regional patterning in ∑PCDD/DF, mixtures of PAHs, PCDDs, and PCDFs appear present in blue mussels in industrialized settings. Further investigation is necessary to evaluate the extent to which each class of contaminants contribute to CYP1A induction in goldeneyes.

3.6.2. Barrow's Goldeneye

Cytochrome P4501A Induction in Barrow's Goldeneye Liver Tissue

Our results suggested that variation in EROD activity among goldeneyes wintering in British Columbia was best explained at a regional level. In northern British Columbia, goldeneyes demonstrated consistently low levels of EROD activity (Figure 3-6). Levels of EROD activity were higher overall in southern British Columbia, but with more variability across sites. As with ∑PAH concentrations in blue mussels, EROD activity among goldeneyes in southern British Columbia likely reflects the relatively higher, and comparatively ubiquitous, distribution of hydrocarbon discharges throughout this system and subsequent exposure in sampled birds. We did not find strong evidence to suggest that age, sex, or mass contributed to the variation in EROD observed among goldeneyes sampled in this study. Our results are consistent with Miles et al. (2007) who found that gender did not contribute to explaining variation in EROD activity for harlequin ducks (*Histrionicus histrionicus*) or Steller's eiders (*Polysticta stelleri*). However, recent investigations suggest some support for sex- or mass-dependent sensitivities to oil exposure among yellow-legged gulls (*Larus michahellis*) and Barrow's goldeneyes (Velando et al. 2010; Esler et al. 2011). Trust et al. (2000) and Velando et al. (2010) suggest that female birds may experience greater sensitivity to oil exposure, as indicated by higher rates of mortality or increased EROD activity.

Relationship between Contaminants in Blue Mussels and EROD Activity in Barrow's Goldeneye

Blue mussels represent at least 80% of the winter diet of Barrow's goldeneyes (Eadie et al. 2000). Given the dietary preference of goldeneyes for blue mussels, we predicted that CYP1A induction, as measured by EROD activity, would reflect the spatial distribution of contaminant burdens in mussels in northern and southern British Columbia. It was not evident from our results that birds captured from industrialized sites in Kitimat Arm or Burrard Inlet had consistently higher EROD activity relative to birds from intermediate or references sites in Douglas Channel or Indian Arm.

Similar levels of EROD activity measured in goldeneyes captured from industrialized, intermediate, or reference sites in either region likely reflects the degree to which goldeneyes undertake moderate-scale movements among wintering sites (see Chapter 2 for more details). Miles et al. (2007) reported elevated EROD activity in harlequin ducks and Steller's eiders sampled from sites without known contemporary sources of hydrocarbon contamination. The authors suggested that local populations of both species were exposed to oil during short distance migrations to adjacent contaminated sites. Goldeneyes in this study were expected to exhibit strong fidelity to wintering sites in northern and southern British Columbia within years, and birds inhabiting industrialized areas would express higher rates of exposure. However, based on the detailed telemetry studies outlined in Chapter 2, site-level dissimilarities between ∑PAH in blue mussels and goldeneye EROD activity could be attributed to inter-site movements among goldeneyes. Although the majority of goldeneyes demonstrated high site fidelity, telemetry data indicate that a portion of birds regularly travel more than 30 km between wintering sites (see Chapter 2). Telemetry movements recorded during this study revealed that goldeneyes moved between Burrard Inlet and Indian Arm throughout a single wintering season. This idea is reinforced by field observations of goldeneyes in both northern and southern British Columbia adjusting site usage in response to localized herring spawn events.

Movement among several wintering sites is expected to limit the amount of contaminated prey ingested by a single bird over a short period, thereby moderating the overall toxicological burden placed on any individual. This concept is reinforced by observed patterns in northern British Columbia, where industrial activity is generally localized to areas of Kitimat Arm. However, birds occupying highly developed coastal areas in southern British Columbia showed indications of contemporary hydrocarbon exposure despite inter-site movements. Here, we collected telemetry data for two goldeneyes with EROD activity >10.00 pmol/min/mg protein. One individual was observed to consistently use eastern portions of Burrard Inlet, while the second bird appeared to use coastal areas between Boundary Bay and Semiahmoo Bay. The comparatively elevated levels of EROD activity measured among goldeneyes from southern British Columbia likely reflects the higher magnitude and frequency of PAH exposure from prey throughout this system (Figure 3-6). The few goldeneyes from Best Point exhibiting EROD

levels >50 pmol/min/mg protein suggests some individuals used contaminated sites more consistently, and either reflect increased exposure to PAHs, or were expressing exposure to multiple CYP1A-inducing agents. Interestingly, ∑PCDD/DF was highest in blue mussels from Bedwell Bay; however, goldeneyes sampled from this site did not exhibit similarly elevated levels of EROD activity. This finding provides further evidence for the concept that PAHs appear to be the primary CYP1A-inducing agent and that goldeneyes can moderate contaminant exposure by moving between several wintering sites.

The percentage of Barrow's goldeneyes demonstrating elevated levels of EROD activity in British Columbia was similar to rates measured in marine birds using areas of Prince William Sound, Alaska that were impacted by hydrocarbons for years following the *Exxon Valdez* oil spill. Applying a similar measurement of elevated EROD activity as Esler et al. (2011) (i.e., ≥2 times the average value of goldeneyes captured from reference sites in this study), 13% of goldeneyes from northern British Columbia and 71% of goldeneyes from southern British Columbia expressed elevated EROD levels. The degree and incidence of elevated EROD activity measured in birds from southern British Columbia was similar to those of harlequin ducks and Barrow's goldeneyes using oiled areas of Prince William Sound (Trust et al. 2000; Esler et al. 2010; Esler et al. 2011). Collectively, these results contribute support to the hypothesis that sustained exposure to contemporary chronically-sourced oil elicits a physiological response in exposed marine bird populations. Our present research further indicates that the exposure response observed in goldeneyes may be attributed to low levels of hydrocarbon contamination in goldeneye prey.

3.7. Conclusion

Understanding patterns in availability of, and risk of exposure to, anthropogenic sources of PAHs is vital given the persistence of these compounds in coastal ecosystems. To our knowledge, the present study constitutes the first investigation of contemporary hydrocarbon exposure among marine bird populations in British Columbia. Our results suggest that Barrow's goldeneyes are suitable for monitoring exposure to PAHs in

nearshore coastal habitats in the province. PAH concentrations measured in blue mussels sampled from sites in northern and southern British Columbia were influenced by the percent lipid content in mussel tissue, and were generally higher among industrialized sites. PAHs measured above detection limits suggest contemporary availability of hydrocarbons that includes petroleum- and combustion-based sources. We found that regional differences in EROD activity in wintering goldeneyes were consistent with regional patterns in the concentration of ∑PAH in their prey. Goldeneyes inhabiting coastal areas in southern British Columbia demonstrated higher levels of EROD activity, indicating that EROD may serve as a useful biomarker for exposure in areas with known or putative PAH contamination. We suggest that the comparatively elevated levels of EROD among goldeneyes from southern British Columbia are indicative of the magnitude and frequency of exposure of birds wintering in Burrard Inlet. Our present research further indicates that low levels of hydrocarbon contamination in blue mussel prey are associated with an exposure response in goldeneyes.

Together, these conclusions reinforce the use of Barrow's goldeneyes as a useful indicator for monitoring PAH contamination in nearshore coastal environments in British Columbia. Rates of elevated EROD activity among goldeneyes in this study, particularly among birds from southern British Columbia, were similar to those of marine bird populations exposed to residual oil in Prince William Sound following the *Exxon Valdez* oil spill. This finding contributes further support to the hypothesis that sustained exposure to chronically-sourced oil elicits a physiological response in marine bird populations, and provides preliminary insights into exposure rates among marine birds in chronic versus post-spill exposure scenarios. Notwithstanding, our findings are useful for future monitoring of both chronic and catastrophic oil spills in the marine environment that may be useful to apply in the assessment of ecological risk of various marine industrial activities. This research provides a contemporary baseline useful for informing recovery endpoints in the event of an accidental hydrocarbon release in British Columbia.

3.8. Acknowledgements

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3.10. Tables

Table 3-1. The number of composite blue mussel samples collected at (and the UTM coordinates of) industrialized, intermediate and reference sites in northern and southern British Columbia

Region	Site Condition	Site	Location (UTM)	Number of Composite Mussel Samples
Northern BC	Industrialized	North Cove	9U 518420 5976211	4
		Clio Bay	9U 521039 5972447	4
	Intermediate	Malsey Bay	9U 483180 5918777	4
		Stewart Narrows	9U 481676 5916300	4
	Reference	Kiskosh Inlet	9U 483570 5929883	8
Total for Northern BC			**-**	**24**
Southern BC	Industrialized	Mossom Creek	10U 509532 5460712	2
		Barnet Marine Park	10U 505344 5460010	2
		Burrard Inlet	10U 500535 5460098	2
		Stanley Park	10U 490862 5461205	4
	Intermediate	Best Point	10U 508667 5469744	2
		Camp Jubilee	10U 508098 5468837	2
		Bedwell Bay	10U 506917 5463475	4
	Reference	Indian River	10U 508921 5478879	4
Total for Southern BC			**-**	**22**

Table 3-2. The number of male and female Barrow's goldeneyes of different ages sampled at industrialized, intermediate and reference sites in northern and southern BC[a]

Region	Site Condition	Site	Male HY	Male AHY	Female HY	Female AHY	Total
Northern BC	Industrialized	North Cove	1	11	0	3	**15**
		Clio Bay	0	1	0	2	**3**
	Intermediate	Hartley Bay	1	1	1	2	**5**
		Malsey Bay	4	1	0	2	**7**
		Stewart Narrows	1	3	0	3	**7**
	Reference	Kiskosh Inlet	1	2	0	0	**3**
Total for Northern BC			**8**	**19**	**1**	**12**	**40**
Southern BC	Impact	Mossom Creek	1	0	0	0	**1**
		White Rock	0	0	0	1	**1**
	Intermediate	Best Point	4	9	5	6	**24**
		Camp Jubilee	1	4	1	2	**8**
		Bedwell Bay	2	0	3	2	**7**
Total for Southern BC			**8**	**13**	**9**	**11**	**41**

[a] Age cohorts are defined as: HY = hatch-year (i.e., within one year of hatching), AHY = after hatch-year.

Table 3-3. Summary of information-theoretic analyses using general linear models to evaluate variation in total PAH (∑PAH (µg/kg wwt]) in blue mussels in northern and southern BC

Model	k[a]	AIC_c[b]	ΔAIC_c[c]	w[d]	Evidence Ratio[e]
∑PAH = Industrialized + Lipid	4	374.78	0.00	0.37	1.00
∑PAH = Region + Industrialized + Lipid	5	375.92	1.14	0.21	1.76
∑PAH = Region + Industrialized + Lipid + Region * Industrialized	6	376.31	1.53	0.17	2.18
∑PAH = Condition + Lipid	5	376.87	2.09	0.13	2.85
∑PAH = Region + Condition + Lipid	6	378.36	3.58	0.06	6.17
∑PAH = Region + Condition + Lipid + Region * Condition	8	380.21	5.43	0.02	18.50
∑PAH = Reference + Lipid	4	380.76	5.97	0.02	18.50
∑PAH = Region + Reference + Lipid	5	382.21	7.43	0.01	37.00
∑PAH = Lipid	3	382.75	7.97	0.01	37.00
∑PAH = Region + Lipid	4	383.16	8.38	0.01	37.00
∑PAH = Region + Reference + Lipid + Region * Reference	6	384.70	9.92	0.00	> 37.00
∑PAH = Region + Industrialized + Region * Industrialized	5	388.80	14.02	0.00	> 37.00
∑PAH = Region + Condition + Region * Condition	7	391.31	16.52	0.00	> 37.00
∑PAH = Reference	3	391.37	16.59	0.00	> 37.00
∑PAH = Condition	4	391.76	16.98	0.00	> 37.00
∑PAH = Industrialized	3	392.64	17.86	0.00	> 37.00
∑PAH = Site + Lipid	15	393.27	18.49	0.00	> 37.00
∑PAH = Region + Reference	4	393.71	18.93	0.00	> 37.00
∑PAH = Region + Condition	5	394.25	19.47	0.00	> 37.00
∑PAH = Region + Industrialized	4	394.89	20.10	0.00	> 37.00
∑PAH = Region + Reference + Region * Reference	5	395.67	20.89	0.00	> 37.00
∑PAH = Null	2	396.38	21.60	0.00	> 37.00
∑PAH = Region	3	398.23	23.45	0.00	> 37.00
∑PAH = Site	14	401.12	26.34	0.00	> 37.00

[a] k = number of estimated parameters in the model.

[b] AIC_c = Akaike's Information Criterion, corrected for small sample size.

[c] ΔAIC_c = difference in AIC_c from the best-supported model.

[d] w = AIC_c weight.

[e] Evidence ratio = w_i / w_j, where w_i is the best supported model.

Table 3-4. Parameter likelihoods, model-averaged parameter estimates, and unconditional standard errors (SE) derived from information-theoretic analysis using general linear models to evaluate variation in total PAH (∑PAH (µg/kg wwt]) in blue mussels in northern and southern BC

Parameter[a]	Parameter Likelihood	Model-averaged Estimate	± Unconditional SE
Intercept	1.00	70.77	9.73
Lipid	1.00	20.25	4.54
Site – Barnet Marine Park	0.00	73.25	11.65
Site – Bedwell Bay	0.00	63.69	11.71
Site – Best Point	0.00	88.96	14.91
Site – Burrard Inlet	0.00	76.97	12.84
Site – Camp Jubilee	0.00	65.34	13.15
Site – Clio Bay	0.00	71.67	12.78
Site – Indian River	0.00	60.35	11.66
Site – Kiskosh Inlet	0.00	58.22	10.23
Site – Malsey Bay	0.00	55.40	12.35
Site – Mossom Creek	0.00	83.32	167.52
Site – North Cove	0.00	83.45	11.91
Site – Stanley Park	0.00	64.74	11.36
Site – Stewart Narrows	0.00	58.19	11.71
Region – north	0.48	66.96	9.06
Region – south	0.48	71.44	4.04
$\text{Industrialized}_{[\text{Condition}]}$	0.21	70.82	5.53
$\text{Intermediate}_{[\text{Condition}]}$	0.21	55.94	5.13
$\text{Reference}_{[\text{Condition}]}$	0.21	57.70	4.05
$\text{Intermediate + Industrialized}_{[\text{Reference}]}$	0.03	69.48	5.83
$\text{Reference}_{[\text{Reference}]}$	0.03	59.54	5.02
$\text{Industrialized}_{[\text{Industrialized}]}$	0.75	69.14	5.44
$\text{Intermediate + Reference}_{[\text{Industrialized}]}$	0.75	56.02	4.05
$\text{North * Industrialized}_{[\text{Condition}]}$	0.02	76.08	11.55
$\text{North * Intermediate}_{[\text{Condition}]}$	0.02	58.26	7.28
$\text{North * Reference}_{[\text{Condition}]}$	0.02	55.79	6.63
$\text{South * Industrialized}_{[\text{Condition}]}$	0.02	72.62	6.95
$\text{South * Intermediate}_{[\text{Condition}]}$	0.02	93.21	9.61
$\text{South * Reference}_{[\text{Condition}]}$	0.02	81.04	11.28
$\text{North * Industrialized + Intermediate}_{[\text{Reference}]}$	0.00	65.08	11.01

Parameter[a]	Parameter Likelihood	Model-averaged Estimate	± Unconditional SE
North * Reference$_{[Reference]}$	0.00	57.64	6.83
South * Industrialized + Intermediate$_{[Reference]}$	0.00	70.48	5.22
South * Reference$_{[Reference]}$	0.00	61.03	10.66
North * Industrialized$_{[Industrialized]}$	0.17	76.24	10.86
North * Intermediate + Reference$_{[Industrialized]}$	0.17	57.16	5.94
South * Industrialized$_{[Industrialized]}$	0.17	72.74	6.83
South * Intermediate + Reference$_{[Industrialized]}$	0.17	88.94	8.83

[a] Spatial categories are described in square brackets "[ ]"; please refer to Section 3.5.3 for description of each spatial category considered as a fixed effect in the candidate model set (e.g., [reference] refers to the reference spatial factor).

Table 3-5. Summary of information-theoretic analyses using general linear models to evaluate variation in hepatic EROD activity of Barrow's goldeneyes sampled in northern and southern BC

Model	k[a]	AIC_c[b]	ΔAIC_c[c]	*w*[d]	Evidence Ratio[e]
EROD = Region	3	622.07	0.00	0.54	1.00
EROD = Region + Industrialized	4	624.28	2.21	0.18	3.00
EROD = Region + Condition	5	626.55	4.48	0.06	9.00
EROD = Region + Industrialized + Region * Industrialized	5	626.55	4.48	0.06	9.00
EROD = Null	2	627.38	5.32	0.04	13.50
EROD = Region + Individual Attributes[f]	6	627.66	5.59	0.03	18.00
EROD = Industrialized	3	627.85	5.78	0.03	18.00
EROD = Region + Condition + Region * Condition	6	628.88	6.82	0.02	27.00
EROD = Reference	3	629.23	7.16	0.01	54.00
EROD = Condition	4	629.55	7.48	0.01	54.00
EROD = Region + Industrialized + Individual Attributes	7	630.04	7.98	0.01	54.00
EROD = Individual Attributes	5	631.14	9.07	0.01	54.00
EROD = Region + Industrialized + Individual Attributes + Region * Industrialized	8	632.48	10.41	0.00	<54.00
EROD = Region + Condition + Individual Attributes	8	632.50	10.43	0.00	<54.00
EROD = Industrialized + Individual Attributes	6	632.73	10.67	0.00	<54.00
EROD = Reference + Individual Attributes	6	633.34	11.27	0.00	<54.00
EROD = Condition + Individual Attributes	7	634.88	12.82	0.00	<54.00
EROD = Region + Condition + Individual Attributes + Region * Condition	9	635.00	12.94	0.00	<54.00

[a] k = number of estimated parameters in the model.

[b] AIC_c = Akaike's Information Criterion, corrected for small sample size.

[c] ΔAIC_c = difference in AIC_c from the best-supported model.

[d] w = AIC_c weight.

[e] Evidence ratio = w_i / w_j, where w_i is the best supported model.

[f] Individual attributes = grouping of variables describing attributes of individual Barrow's goldeneye (i.e., age, sex, mass).

Table 3-6. Parameter likelihoods, model-averaged parameter estimates, and unconditional standard errors (SE) derived from information-theoretic analysis using general linear models to evaluate variation in hepatic 7-ethoxyresorufin-*O*-deethylase (EROD) activity (pmol/min/mg protein) of Barrow's goldeneye sampled in northern and southern BC

Parameter	Parameter Likelihood	Model-averaged Estimate	± Unconditional SE
Intercept	1.00	1.46	4.96
Age – AHY	0.05	6.82	17.29
Age – HY	0.05	8.20	3.66
Sex – female	0.05	7.40	7.37
Sex – male	0.05	6.24	7.37
Mass	0.05	-0.01	0.02
Region – north	0.90	2.93	5.38
Region – south	0.90	9.62	2.57
$\text{Industrialized}_{[\text{Condition}]}$	0.09	1.25	4.82
$\text{Intermediate}_{[\text{Condition}]}$	0.09	2.14	3.58
$\text{Reference}_{[\text{Condition}]}$	0.09	1.05	6.93
$\text{Intermediate + Industrialized}_{[\text{Reference}]}$	0.01	4.00	5.97
$\text{Reference}_{[\text{Reference}]}$	0.01	0.41	6.77
$\text{Industrialized}_{[\text{Industrialized}]}$	0.28	1.29	5.13
$\text{Intermediate + Reference}_{[\text{Industrialized}]}$	0.28	1.97	3.42
$\text{North * Industrialized}_{[\text{Condition}]}$	0.02	1.15	4.73
$\text{North * Intermediate}_{[\text{Condition}]}$	0.02	1.39	3.69
$\text{North * Reference}_{[\text{Condition}]}$	0.02	1.09	6.98
$\text{South * Industrialized}_{[\text{Condition}]}$	0.02	7.96	8.35
$\text{South * Intermediate}_{[\text{Condition}]}$	0.02	0.94	8.92
$\text{North * Industrialized}_{[\text{Industrialized}]}$	0.06	1.23	5.08
$\text{North * Intermediate + Reference}_{[\text{Industrialized}]}$	0.06	1.42	3.54
$\text{South * Industrialized}_{[\text{Industrialized}]}$	0.06	8.02	8.30
$\text{South * Intermediate + Reference}_{[\text{Industrialized}]}$	0.06	1.07	8.82

3.11. Figures

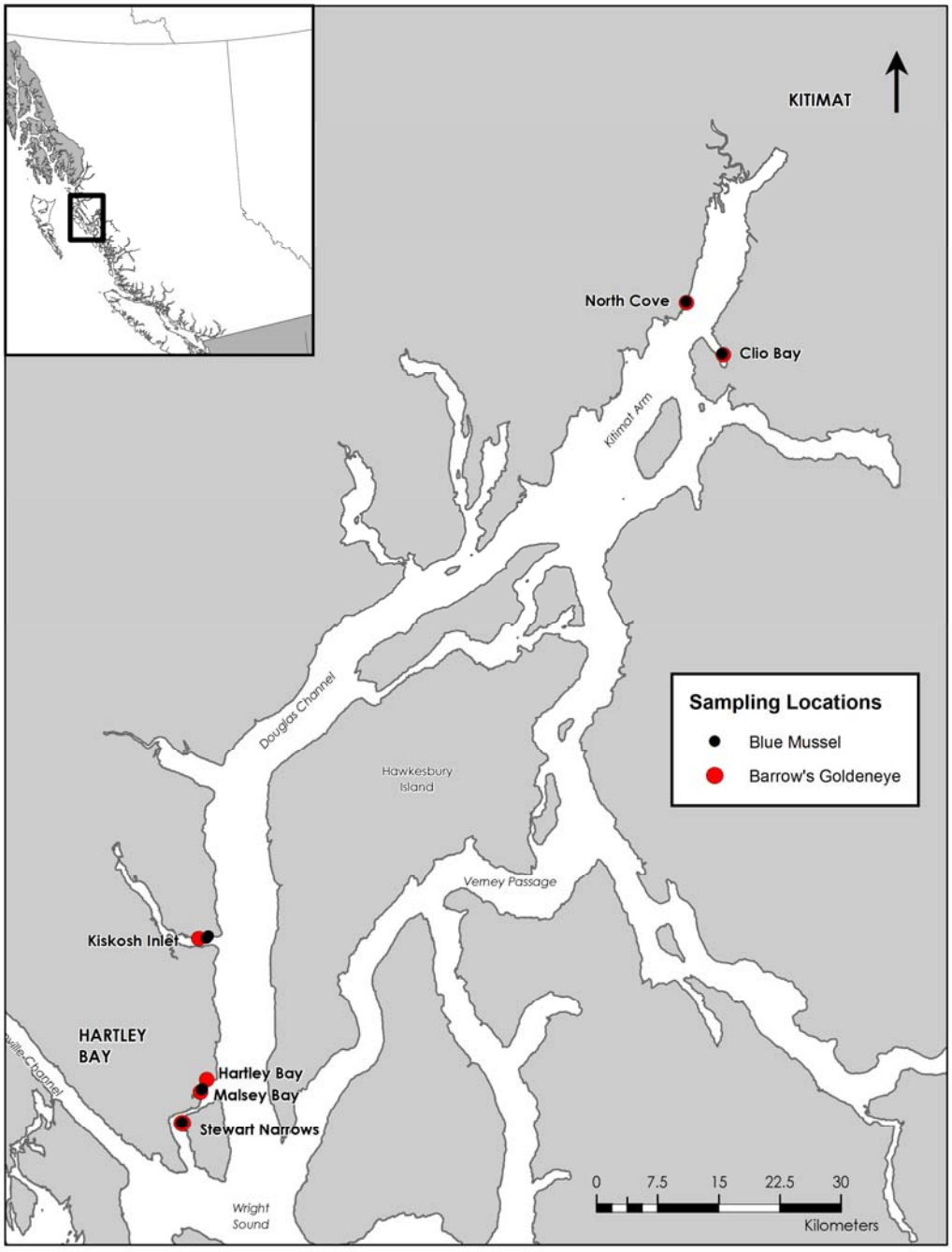


Figure 3-1. Blue mussel and Barrow's goldeneye sampling locations in northern British Columbia: Douglas Channel and Kitimat Arm

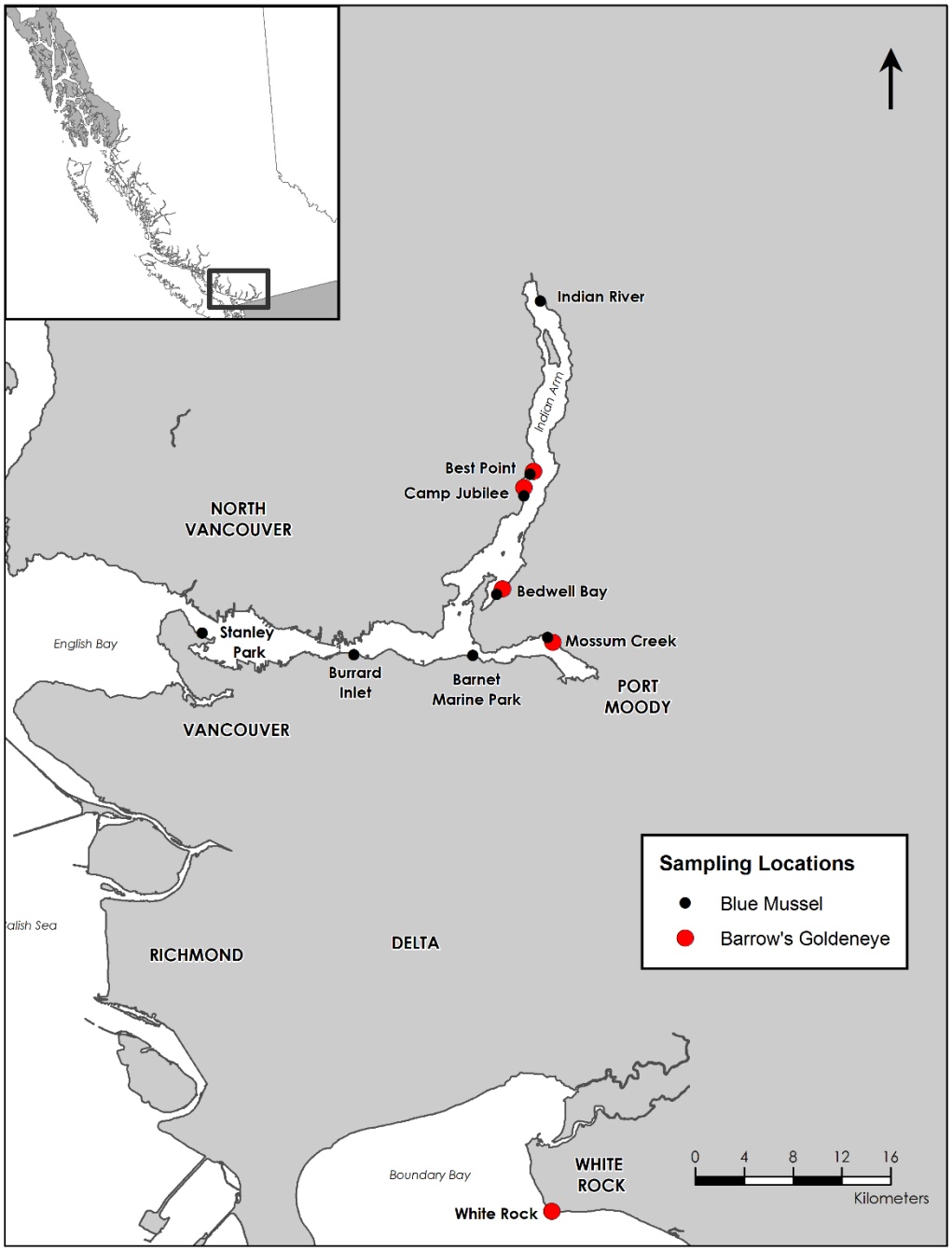


Figure 3-2. Blue mussel and Barrow's goldeneye sampling locations in southern British Columbia: Burrard Inlet and Indian Arm

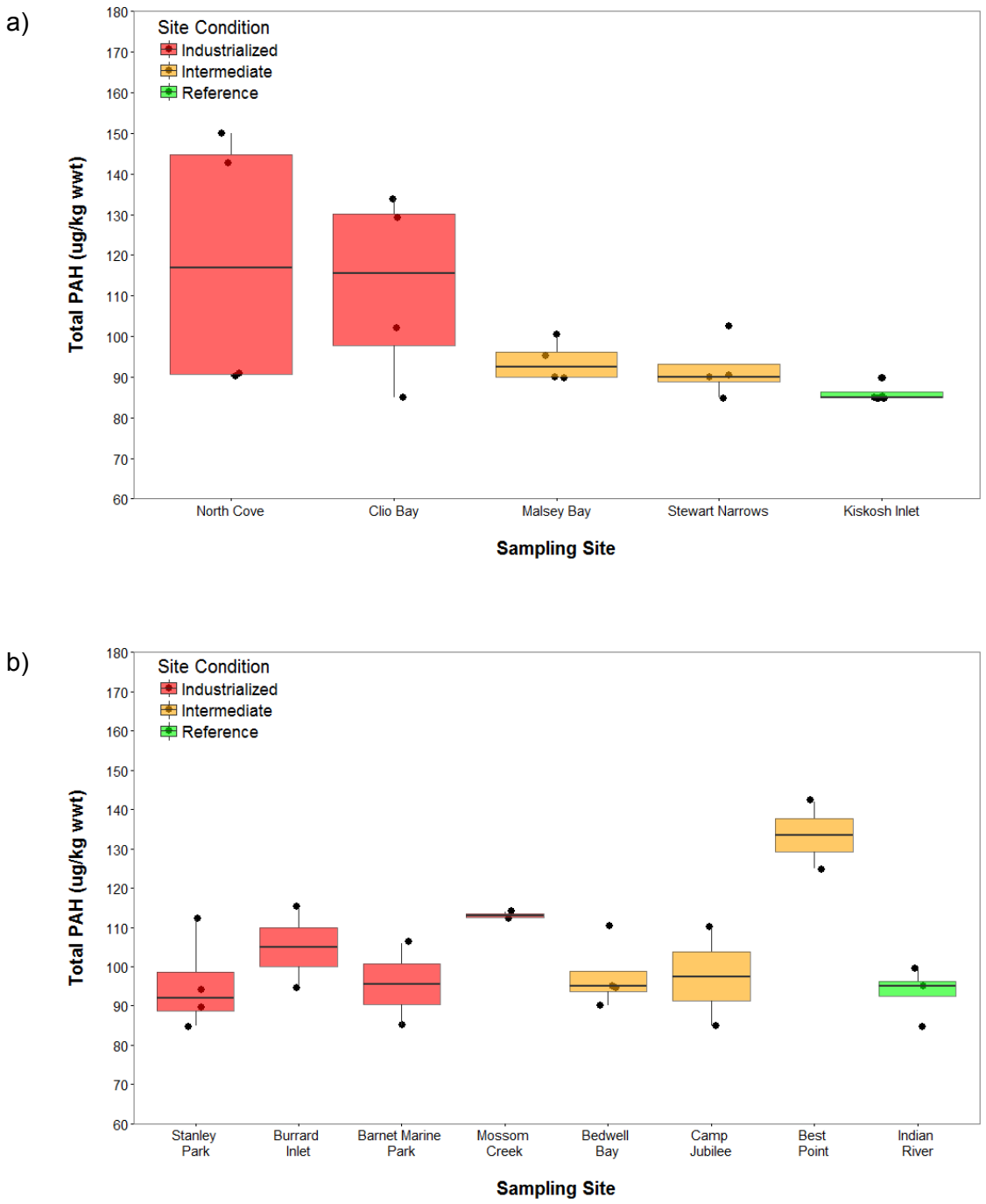


Figure 3-3. **Total PAH (∑PAH; µg/kg wet weight tissue (wwt]) in blue mussels from sampling sites in (a) northern BC and (b) southern BC. Box plots show median values (solid horizontal line), 50th percentile values (box outline); whiskers represent 1.5 * interquartile range; data points are shown as solid black circles (●). Shading represents the Condition of each site (i.e., Industrialized, Intermediate, or Reference)**

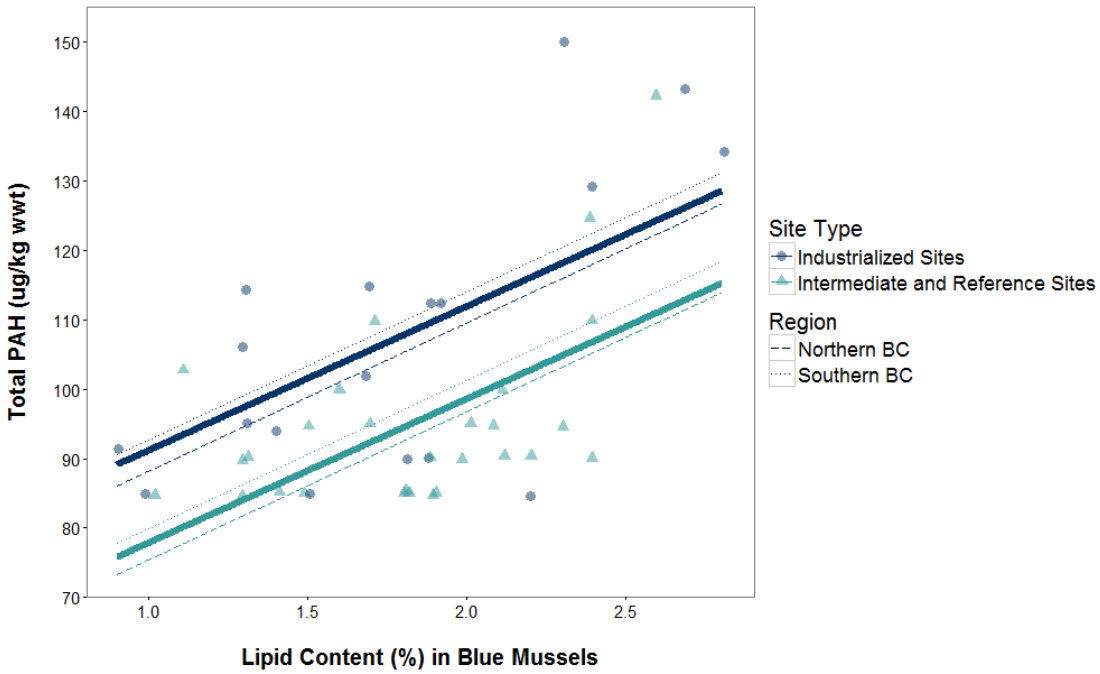
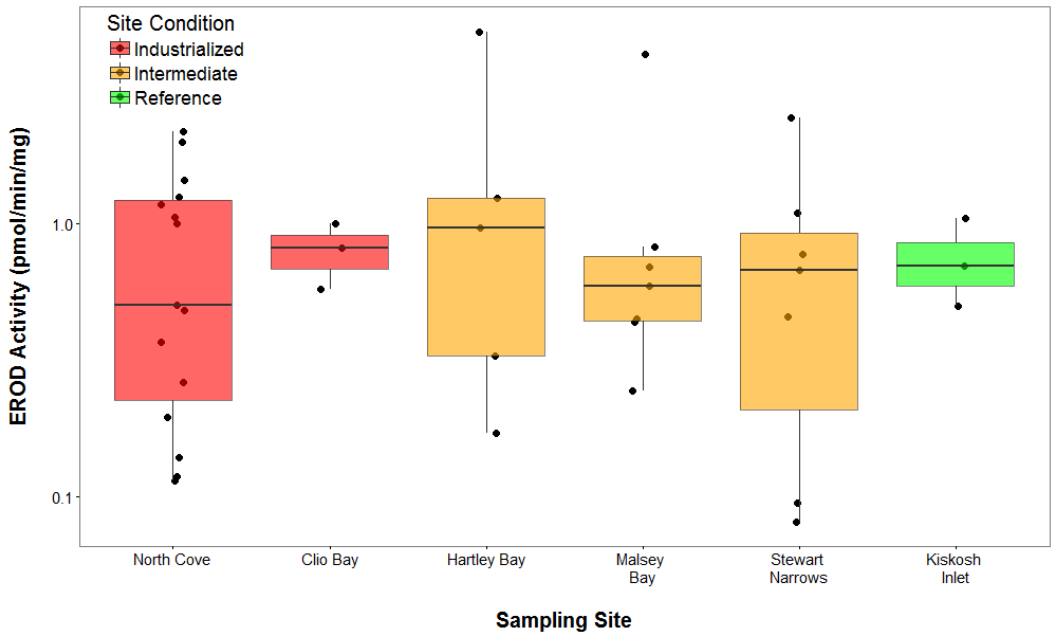


Figure 3-4. **The relationship between lipid content (%) and total PAH (∑PAH; µg/kg wet weight tissue [wwt]) in blue mussels in northern and southern BC based on the top two models in the candidate set (see Table 3-3). Solid lines represent the top model (∑PAH ~ *Industrialized* + Lipid), dashed lines represent variation of top model by region (∑PAH ~ *Region* + *Industrialized* + Lipid)**

a)



b)

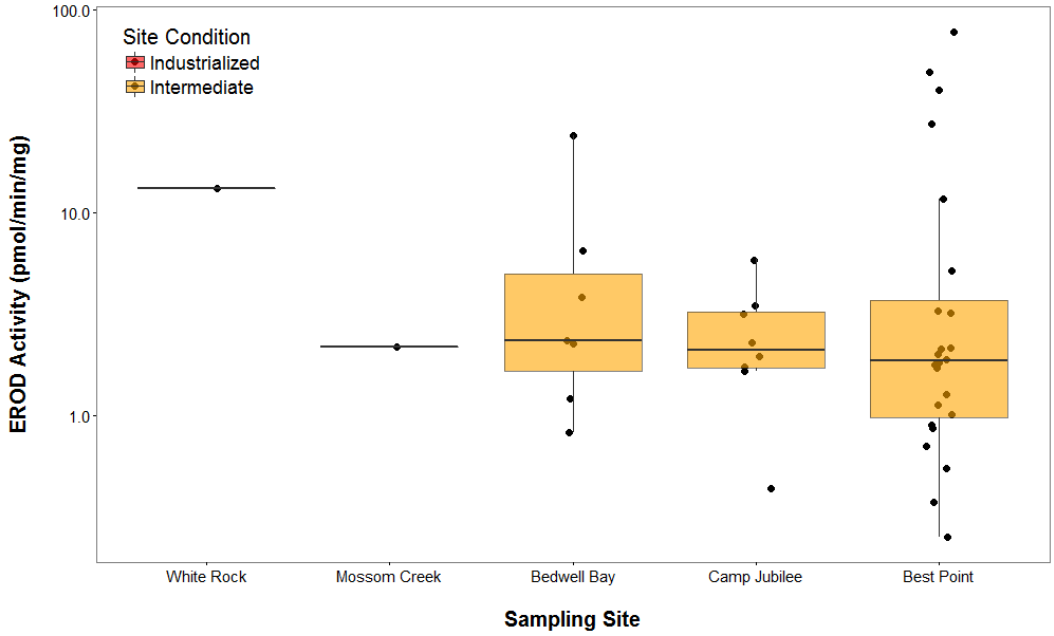


Figure 3-5. EROD activity (pmol/min/mg protein) in Barrow's goldeneyes from sampling sites in (a) northern BC and (b) southern BC*.

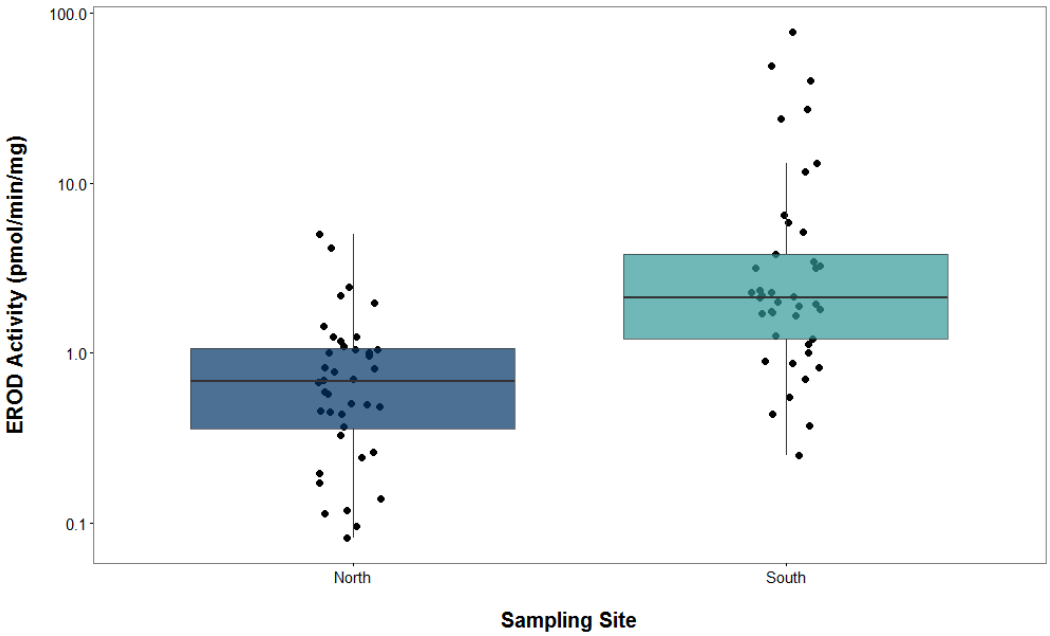


Figure 3-6. EROD activity (pmol/min/mg protein) in Barrow's goldeneye from northern and southern BC*.

* See Figure 3-3 caption for further information on boxplot characteristics.

3.12. Supplemental Data

Table A-1. Polycyclic aromatic hydrocarbon (PAH) concentrations (µg/kg wet weight tissue [wwt]) in blue mussels from each sampling location in northern British Columbia

Site	Sample	% Lipid	PAH (µg/kg wwt)*																
			Acenaphthene	Acenaphthylene	Anthracene	Benz[a]anthracene	Benzo[a]pyrene	Benzo[b]fluoranthene	Benzo[g,h,i]perylene	Benzo[k]fluoranthene	Chrysene	Dibenz[a,h]anthracene	Fluoranthene	Fluorene	Indeno[1,2,3-c,d]pyrene	2-methylnaphthalene	Naphthalene	Phenanthrene	Pyrene	Total PAH
North Cove	**NC1**	1.9	10.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	**90.0**
	NC2	0.9	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	**11.0**	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	**91.0**
	NC3	2.3	5.0	5.0	5.0	**35.0**	5.0	10.0	5.0	5.0	20.0	5.0	10.0	5.0	5.0	5.0	5.0	10.0	10.0	**150.0**
	NC4	2.7	5.0	5.0	5.0	**33.0**	5.0	10.0	5.0	5.0	20.0	5.0	10.0	5.0	5.0	5.0	5.0	5.0	10.0	**143.0**
Clio Bay	**CB1**	2.8	5.0	5.0	10.0	**19.0**	5.0	10.0	5.0	5.0	20.0	5.0	10.0	5.0	5.0	5.0	5.0	10.0	5.0	**134.0**
	CB2	2.4	**10.0**	5.0	5.0	**14.0**	5.0	10.0	5.0	5.0	15.0	5.0	10.0	5.0	5.0	5.0	5.0	10.0	10.0	**129.0**
	CB3	1.7	5.0	5.0	5.0	**12.0**	5.0	5.0	5.0	5.0	10.0	5.0	10.0	5.0	5.0	5.0	5.0	5.0	5.0	**102.0**
	CB4	2.2	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	**85.0**
Kiskosh Inlet	**KI1**	1.4	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	**85.0**
	KI2	1.8	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	**85.0**
	KI3	1.9	10.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	**90.0**
	KI4	1.8	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	**85.0**
	KI5	1.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	**85.0**
	KI6	1.5	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	**85.0**
	KI7	1.3	10.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	**90.0**
	KI8	1.3	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	**85.0**
Malsey Bay	**MB1**	2.1	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	10.0	5.0	5.0	5.0	5.0	5.0	5.0	10.0	10.0	**100.0**
	MB2	2.1	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	10.0	10.0	**95.0**
	MB3	2.1	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	10.0	**90.0**
	MB4	2.2	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	10.0	**90.0**
Stewart Narrows	**SN1**	1.1	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	10.0	**12.0**	5.0	5.0	5.0	5.0	**11.0**	5.0	5.0	**103.0**
	SN2	2.4	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	10.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	**90.0**
	SN3	1.9	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	**85.0**
	SN4	2.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	10.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	**90.0**

* Method detection limits (DL) set at 10 µg/kg wet weight tissue; individual PAH concentrations reported below detection limit were set at 0.5*DL for all analyses; values in bold denote PAH measures above DL.

Table A-2. Polycyclic aromatic hydrocarbon (PAH) concentrations (µg/kg wet weight tissue [wwt]) in blue mussels from each sampling location in southern British Columbia

Site	Sample	% Lipid	PAH (µg/kg wwt)*																	
			Acenaphthene	Acenaphthylene	Anthracene	Benz[a]anthracene	Benzo[a]pyrene	Benzo[b]fluoranthene	Benzo[g,h,i]perylene	Benzo[k]fluoranthene	Chrysene	Dibenz[a,h]anthracene	Fluoranthene	Fluorene	Indeno[1,2,3-c,d]pyrene	2-methylnaphthalene	Naphthalene	Phenanthrene	Pyrene	Total PAH
Indian River	IR1	2.3	15.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	**95.0**
	IR2	1.9	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	**85.0**
	IR3	1.6	20.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	**100.0**
	IR4	1.5	15.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	**95.0**
Best Point	BP1	2.6	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	**37.0**	5.0	5.0	5.0	5.0	25.0	10.0	**142.0**
	BP2	2.4	40.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	10.0	5.0	**125.0**
Camp Jubilee	CJ1	1.8	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	**85.0**
	CJ2	1.7	30.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	**110.0**
Bedwell Bay	BB1	2.0	15.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	**95.0**
	BB2	2.4	20.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	10.0	5.0	5.0	5.0	5.0	10.0	5.0	**110.0**
	BB3	1.7	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	10.0	5.0	5.0	5.0	5.0	10.0	5.0	**95.0**
	BB4	1.3	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	10.0	5.0	**90.0**
Mossom Creek	MC1	1.9	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	**22.0**	5.0	5.0	5.0	5.0	10.0	10.0	**112.0**
	MC2	1.3	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	10.0	5.0	**21.0**	5.0	5.0	5.0	5.0	**13.0**	5.0	**114.0**
Barnet Marine Park	BMP1	1.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	**85.0**
	BMP2	1.3	5.0	5.0	5.0	10.0	5.0	5.0	5.0	5.0	5.0	5.0	**11.0**	10.0	5.0	5.0	5.0	10.0	5.0	**106.0**
Burrard Inlet	BII1	1.3	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	10.0	5.0	5.0	5.0	10.0	5.0	**95.0**
	BII2	1.7	10.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	**15.0**	10.0	5.0	5.0	5.0	10.0	10.0	**115.0**
Stanley Park	SP1	1.8	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	10.0	5.0	**90.0**
	SP2	1.5	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	**85.0**
	SP3	1.4	**14.0**	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	**94.0**
	SP4	1.9	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	**17.0**	5.0	5.0	5.0	5.0	10.0	15.0	**112.0**

* Method detection limits (DL) set at 10 µg/kg wet weight tissue; individual PAH concentrations reported below detection limit were set at 0.5*DL for all analyses; values in bold denote PAH measures above the DL.

Table A-3. Polychlorinated dibenzo-p-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF) concentrations (pg/g wet weight tissue[wwt]) in blue mussels from sampling locations in northern and southern British Columbia

Region	Site	Sample	PCDD or PCDF (pg/g wwt)*																	
			2,3,7,8-TCDD	1,2,3,7,8-PeCDD	1,2,3,4,7,8-HxCDD	1,2,3,6,7,8-HxCDD	1,2,3,7,8,9-HxCDD	1,2,3,4,6,7,8-HpCDD	OCDD	2,3,7,8-TCDF	1,2,3,7,8-PeCDF	2,3,4,7,8-PeCDF	1,2,3,4,7,8-HxCDF	1,2,3,6,7,8-HxCDF	1,2,3,7,8,9-HxCDF	2,3,4,6,7,8-HxCDF	1,2,3,4,6,7,8-HpCDF	1,2,3,4,7,8,9-HpCDF	OCDF	Total PCDD / PCDF
North	North Cove	NC2	<0.071	<0.081	<0.070	<0.064	<0.066	**0.160**	**0.210**	**0.247**	**0.110**	**0.092**	**0.034**	**0.061**	**0.130**	**0.098**	<0.025	<0.051	<0.130	1.142
	Clio Bay	CB2	<0.072	<0.065	<0.058	<0.050	<0.054	**0.220**	**0.590**	**0.153**	<0.088	<0.076	<0.050	<0.045	**0.186**	**0.830**	**0.200**	<0.099	<0.230	2.179
	Kiskosh Inlet	KI5	<0.015	<0.014	<0.010	<0.010	**0.020**	<0.026	<0.052	**0.051**	**0.025**	<0.012	<0.022	<0.024	<0.027	<0.021	<0.027	<0.042	<0.059	0.096
	Malsey Bay	MB2	<0.091	<0.120	<0.084	<0.076	<0.079	**0.170**	**0.380**	<0.073	<0.093	<0.078	<0.043	<0.037	**0.180**	**0.131**	**0.080**	<0.120	<0.210	0.941
	Stewart Narrows	SN2	<0.073	<0.066	<0.058	<0.049	<0.053	<0.120	<0.250	<0.068	<0.065	<0.056	<0.028	<0.025	**0.208**	<0.030	**0.043**	<0.065	<0.18	0.251
South	Camp Jubilee	CJ1	<0.035	<0.042	<0.030	<0.023	<0.025	**0.501**	**2.270**	**0.155**	<0.034	**0.037**	<0.038	<0.034	**0.069**	<0.038	**0.035**	<0.037	**0.150**	3.217
	Bedwell Bay	BB3	<0.042	**0.054**	**0.077**	**0.070**	<0.046	**2.220**	**8.990**	**0.200**	<0.032	**0.047**	<0.078	<0.069	<0.100	**0.105**	**0.180**	<0.077	**0.510**	12.453
	Stanley Park	SP2	<0.042	**0.043**	**0.045**	**0.033**	<0.027	**0.645**	**2.810**	**0.063**	<0.063	<0.049	<0.028	<0.025	<0.040	<0.027	**0.228**	<0.049	**0.300**	4.167

* Effective detection limits (DL) vary by sample due to sample interferences (EPA 2015); individual PCDD/DF concentrations reported below detection limit are considered negligible; values in bold denote PCDD/DF measures above the DL.

Chapter 4.

Final Summary, Synthesis, and General Conclusions

4.1. Overview

Marine birds can serve as early indicators of environmental pollution and are often used as a sentinel species for understanding effects of different contaminants in marine ecosystems (Burger and Gochfeld 2004; Wiese et al. 2004; O'Hara et al. 2009; Calvert et al. 2013). Based on their natural history, marine birds are considered especially sensitive to lethal or sub-lethal effects resulting from direct or indirect contact with oil (Leighton 1993). Direct oiling reduces the waterproofing, insulating, and buoyancy properties that feathers provide and can result in death due to starvation or hypothermia (Leighton 1993; Wiese 2002). Similarly, oil that is inhaled, absorbed, or ingested can exert debilitating or lethal toxicity on internal tissues and organs (Eisler 1987; Leighton 1993; CEPA 1994; Piatt and Anderson 1996; Franci et al. 2014).

Birds that live or forage in industrialized coastal ecosystems are particularly vulnerable to sustained exposure to hydrocarbons through catastrophic and chronic oil spill events (Nur et al. 1997; Miles et al. 2007; Henkel et al. 2014). In the Pacific Northwest, the potential for polycyclic aromatic hydrocarbon (PAH) exposure in marine birds is well represented by Barrow's goldeneyes (*Bucephala islandica*). Here, goldeneyes exhibit strong seasonal associations with coastal habitats (Eadie et al. 2000). These areas tend to accumulate PAHs, and primary goldeneye winter prey (blue mussels; *Mytilus* spp.) are highly susceptible to, and capable of accumulating, high PAH burdens (Boehm et al. 1996; Meador 2003; Galgani et al. 2011). Consequently, goldeneyes are expected to be

particularly susceptible to toxicological effects from dietary sources of PAH and, hence, constitute a simple, sensitive indicator of PAH exposure on the Pacific coast.

In this context, I used Barrow's goldeneyes as a model species to investigate the relationship between fidelity to coastal habitats and expressions of hydrocarbon exposure. Specifically, I investigated susceptibility to oiling by measuring fidelity of Barrow's goldeneyes to wintering sites in the Pacific Northwest (Chapter 2). I also measured the extent to which goldeneyes wintering in coastal British Columbia demonstrate exposure to PAHs, evaluating the degree to which ingestion of contaminated prey serves as a pathway for exposure (Chapter 3). My findings are summarized in the following sections.

4.1.1. Winter Site Fidelity

In Chapter 2, I examined intra-annual and interannual fidelity by Barrow's goldeneyes to coastal sites in the Pacific Northwest to explore how these behaviours influence hydrocarbon exposure risk. Site fidelity was measured using satellite telemetry data from Barrow's goldeneyes captured between 2006 and 2015. A total of 211 goldeneyes were marked with platform transmitter terminals (PTTs) to document intra-annual and interannual movement patterns. Based on winter locations from marked birds, the majority of goldeneyes appeared to have arrived on wintering sites by mid-November, staying for a period of approximately 22 weeks before initiating spring migration by mid- to late April. I observed a latitudinal trend in arrival and departure dates, with birds from northernmost latitudes arriving earlier on coastal sites and departing later. Goldeneyes demonstrated high interannual fidelity to coastal wintering sites; 75% of goldeneyes selected a wintering location within 30 km of the site used in the previous winter. I observed differences in interfix distances and home ranges among sexes, age classes, and coastal regions. Shorter interfix distances and smaller home ranges were measured for female goldeneyes than for males, but were similar between hatch-year and after hatch-year birds. Goldeneyes from southcentral Alaska appeared to use sites most discretely, while birds from southeast Alaska had the largest interfix distances and winter home range measures compared to goldeneyes from southcentral Alaska or British Columbia. Because Barrow's goldeneyes exhibit strong and consistent associations with

coastal wintering sites, these data highlight the importance of site fidelity as a useful method for predicting and managing effects of current and potential future effects from environmental contaminants and other human activities.

4.1.2. Spatial Variation in PAH Exposure

Chapter 3 focused on the degree to which Barrow's goldeneyes wintering in coastal British Columbia demonstrated exposure to PAHs and whether ingestion of contaminated prey served as an exposure pathway. From non-lethal liver biopsies, I investigated PAH exposure by measuring 7-Ethoxyresorufin-O-deethylase (EROD) activity in goldeneyes wintering in industrialized, intermediate, or reference (i.e., undeveloped) coastal areas of northern and southern British Columbia. To examine spatial patterns in dietary sources of PAH, ∑PAH (or the sum of 17 individual constituents) was measured in blue mussels collected from the same goldeneye wintering areas. I determined ∑PAH in blue mussels was best explained by mussel lipid content and the level of coastal industrialization (i.e., industrialized vs. intermediate and reference sites). Region best explained EROD patterns in goldeneye, with birds from southern British Columbia demonstrating higher levels of EROD activity, overall. Little support was found for the hypotheses that variation in EROD activity was explained by intra-site differences in ∑PAH in blue mussels, or by the age, sex, and mass of sampled goldeneyes. These results suggested that goldeneyes wintering in coastal British Columbia were exposed to contemporary sources of PAHs through their diet. Exposure was higher among individuals wintering in southern British Columbia, and likely reflects the magnitude and frequency of exposure to PAHs through consumption of contaminated prey in this region. Placing my PAH exposure results into context with observed patterns in site fidelity, it is reasonable to conclude that hydrocarbon exposure is regulated to some degree by inter-site movements expressed by wintering goldeneyes in each region. However, because industrialized areas typically support greater and more ubiquitous PAH burdens, goldeneyes inhabiting highly developed coastlines have reduced capability of moderating hydrocarbon exposure through inter-site movements.

4.2. Recommendations for Future Studies

Collectively, my study results demonstrate that Barrow's goldeneyes serve as a sensitive indicator for evaluating risk of PAH exposure in nearshore coastal environments in the Pacific Northwest. While this study reasonably characterizes oil spill vulnerability, my findings highlight several areas of research that warrant further investigation.

Using measures of fidelity to inform response planning—Traditional environmental risk assessment techniques assess impacts from oil spill events by developing spill fate models (e.g., US EPA 1998; GOC 2012a). These models characterize oil dispersion based on the physical properties of oil as well as the conditions of the ambient environment during a spill event. However, most models overlook other biological measures that can be applied to increase the accuracy of predicting the ecological consequences of a spill (Burger and Gochfeld 2004). Understanding the spatial and temporal patterns in marine bird movement is essential for forecasting and mitigating effects of catastrophic or chronic oil spill events (GOC 2012b). In this study, satellite telemetry data were used to identify coastal areas that consistently support higher use by goldeneyes during winter months relative to marine industrial activities. Other marine bird species (e.g., surf scoter [*Melanitta perspicillata*] and harlequin duck [*Histrionicus histrionicus*]) also demonstrate a reasonable degree of fidelity to coastal habitats and may have similar patterns of vulnerability to oil spill events (Robertson and Goudie 1999; Anderson et al. 2015). Species that demonstrate lower degrees of site fidelity (e.g., geese, swans, and other sea ducks) may have a reduced likelihood of interacting with contaminated areas, depending on the extent of their coastal habitat use. Accordingly, measures of site fidelity can provide information on the seasons and sites where marine birds, more generally, are most vulnerable to oil spills. Complimentary long-term monitoring on the abundance, distribution, and patterns in mortality of marine birds on the British Columbia coast (e.g., Bird Studies Canada's British Columbia Coastal Waterbird and Beached Bird Surveys) can be used to further prioritize areas of high ecological importance (Bird Studies Canada 2015). Together, these data should be integrated into regional spill response plans developed by government and industry to address areas of increased risk to goldeneyes and other sensitive species.

Expanding the spatial and temporal relevance of exposure studies—Results of this study indicate that contemporary hydrocarbon discharges are associated with an exposure response by goldeneyes in coastal British Columbia. By comparison, the Mussel Watch Project indicates that PAH burdens in densely populated coastal cities elsewhere in North America are higher and, consequently, may pose greater toxicological risk to bird species that strongly associate with marine habitats in these areas (Kimbrough et al. 2008; Lanksbury et al. 2014). Additional research should consider assessing hydrocarbon vulnerabilities at other industrialized coastal sites, using regionally-appropriate indicator species. Conducting multi-year surveys in these areas would further facilitate monitoring of temporal changes in exposure resulting from changing industrial activities and practices each region.

Integrating contaminant and biomarker studies—Cytochrome P4501A (CYP1A) has been successfully applied as a biomarker of hydrocarbon exposure in several studies on marine birds (e.g., Trust et al. 2000; Miles et al. 2007; Velando et al. 2010; Esler et al. 2011). However, to establish a causal relationship between environmental contaminants and CYP1A activity, studies should incorporate measurements of CYP1A-inducing agents in water, sediments, or prey species that are spatially and temporally linked to measurements of exposure (Miles et al. 2007; Wiens 2013). Similarly, CYP1A enzymes serve to detoxify and eliminate chemical burdens and do not necessarily indicate toxicity (Wiens 2013). Studies that combine biomarkers of exposure (e.g., CYP1A) with those of effect (e.g., DNA adduct formation, reproductive effects, reduced survivorship) will have greater capacity to identify adverse toxicological effects in organisms that have been exposed to PAHs (Wiens 2013).

Investigating effects of contaminant mixtures—Contaminants typically occur in complex mixtures in industrialized coastal environments and are simultaneously bioavailable to marine biota (Elliott and Martin 1998; Harris et al. 2003; JWA 2010). In Chapter 3, we identified higher levels of EROD activity among goldeneyes from southern British Columbia and found that they corresponded with regionally elevated levels of PAHs, polychlorinated dibenzo-p-dioxins, and polychlorinated dibenzofurans in blue mussels. Further research is necessary to understand the spatial and temporal availability of multiple CYP1A-inducing agents; and whether co-occurrence of inducing agents elicits

additive, synergistic, or antagonistic toxicological effects in exposed organisms (Burger and Gochfeld 2004). Accurate characterization of potential mixture effects further underscores the need for integrated contaminant and biomarker studies.

4.3. Conservation and Management Implications

The *International Convention for the Prevention of Pollution from Ships* (MARPOL) is the primary regulatory instrument through which ship-sourced pollution is regulated. In Canada, oil discharges in the marine environment are further regulated through the *Canada Shipping Act*, the *Environmental Protection Ac*t, the *Oceans Act*, and the *Fisheries Act*. The *Migratory Birds Convention Act* specifically prohibits releases of deleterious substances, including oil, into waters that are used by migratory birds. The Government of Canada enforces compliance to these pieces of legislation through several means, including: aerial surveillance, increasing the financial liability of polluters, development of regionally-directed area response plans, and using trained local personnel to navigate tankers in inner waterways (GOC 2015). However, despite these legislative protective measures, catastrophic and chronic oil spills persist in coastal waters in Canada and continue to pose a toxicological risk to birds and other marine wildlife (Wiese 2002; GOC 2015). Further actions are necessary to understand the extent to which marine oil spills persist in Canada as a means to improve the approaches taken by federal, provincial, and municipal governments to respond to oil spill events.

This research can be used to help guide the management of coastal communities and ecosystems in the Pacific Northwest that are, or could become, impacted by hydrocarbon contamination. Given the increases in hydrocarbon exportation proposed for British Columbia, oil spill preparedness and response has been identified by federal and provincial governments as a priority objective to preserve the biological integrity of marine ecosystems in the province (GOC 2015; Province of British Columbia 2015). Results of this study support government spill response and recovery objectives. Inclusion of reference sites in my study design provides further information on relative measures of change in contaminant burdens over time and space.

Results of this study are most applicable to nearshore environments in the Pacific Northwest, where industrial, commercial, and recreational marine activities are concentrated. Shifts in the distribution of marine activities from near-shore to far-shore environments (e.g., increased offshore tanker traffic) may influence the distribution of PAH inputs, and the species most likely to be exposed to catastrophic or chronic spill events. Accordingly, Barrow's goldeneyes may not suffice as a surrogate for exposure by other species that may have different degrees of interaction with marine-based human activities. Notwithstanding, results of this research would be of interest to regulators, industry, and public stakeholders for understanding contemporary PAH exposure risk. Results of this study are useful in providing a baseline for evaluating changes in PAH exposure as proposed coastal development occurs. Conclusions of this study could further be applied to guide regulatory commitments, long-term monitoring programs, and inform recovery endpoints in the event of an accidental hydrocarbon release.

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