Sensitivity to Hydrocarbons and Baselines of Exposure in Marine Birds on the Chukchi and Beaufort Seas

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ABSTRACT

With prospects of increasing development of oil and gas resources in the Chukchi and Beaufort Seas, the establishment of baselines and assessment of the sensitivity of arctic biota to hydrocarbon exposure provide key information needed for management and conservation of natural resources potentially impacted by development. Baseline exposure data can inform future monitoring of avian population health, and provide a metric for evaluating the success of remediation efforts, should they occur. Identification of sensitive species and populations can inform resource management, risk assessment of new development activities, and development of long-term monitoring strategies. We evaluated baselines of hydrocarbon exposure in select avian species of subsistence importance (king eider, common eider, and greater white-fronted goose) in the Chukchi and Beaufort Seas by measuring liver cytochrome P450 (CYP1A) activity. Results from this study provide the first assessment of baselines for hydrocarbon exposure in these species and form a basis for development of field programs for monitoring exposure levels in marine birds in the Chukchi and Beaufort Sea region. We also evaluated sensitivity to hydrocarbons in a broader suite of marine bird species using species-specific cell culture methods. These species offer a spectrum of candidate bioindicators of conservation and subsistence importance and included spectacled eider, Steller's eider, king eider, common eider, long-tailed duck, greater white-fronted goose, black brant, and three species of alcids. Liver cell culture sensitivity and CYP1A activity results from this project provide valuable tools and information for monitoring Arctic bird populations, identifying sensitive species, and planning future assessments in the event of an oil spill.

INTRODUCTION

A rich variety of marine bird species use the coastal habitats along the Chukchi and Beaufort Seas during their Arctic pre-breeding, breeding, and post-breeding seasons. These species represent multiple taxonomic groups, including marine-associated waterfowl (sea ducks, geese), alcids, loons, and shorebirds. The region supports a large number of migratory birds; the nearshore zone is a key migration corridor for many arctic-breeding species, and the marine waters provide key staging areas during spring and fall migration. Additionally, many of these species use the coastal tundra and nearshore barrier islands for nesting and brood/chick rearing, spending several months of their annual cycle in the Chukchi and Beaufort Seas and adjacent coastal habitats.

The coastal communities of the region rely on marine resources for subsistence, including several species of sea ducks and geese. King eiders (*Somateria spectabilis)* are the most abundant and most commonly hunted sea duck species, but common eiders (*Somateria mollissima)* and long-tailed ducks (*Clangula hyemali*) are used as well. Greater white-fronted goose (*Anser albifrons)*, black brant (*Branta bernicla)*, and Canada goose (*Branta canadensis)* are hunted in the region, with greater white-fronted goose the most frequently harvested by hunters (Robert Sarren, personal communication, June 10, 2014).

Documented population declines of several marine bird species have been identified as a conservation concern. The spectacled eider (*Somateria fischeri)* and the Steller's eider (*Polysticta stelleri)* are listed as threatened under the US Endangered Species Act due to significant population declines (Federal Register 1993, 1997). Population numbers of both king and common eiders are believed to be down by half compared to historical levels (Suydam et al. 2000), and long-tailed duck populations have declined (Hollmén et al. 2003 and references therein). The yellow-billed loon (*Gavia adamsii)*, a species of conservation concern, is also found in the region (Earnst et al. 2005). Reasons for these population declines are not well understood.

Arctic birds may be exposed to a variety of harmful contaminates in the Arctic and during life stages spent away from the Arctic (i.e., migration or on wintering grounds). For example, elevated, and potentially detrimental, levels of mercury exposure have been recorded in Arctic-breeding shorebirds (Perkins et al. 2016). Waterfowl are also at risk for lead poisoning by consuming lead shot left on the tundra or ponds on hunting grounds (Flint et al. 1997, Wilson et al. 2004). Male spectacled eiders wintering off of St. Lawrence Island, Alaska, had elevated levels copper, cadmium, and selenium, which may decrease fecundity or survival of young (Trust et al. 2000b). With prospects of resource development in the Chukchi and Beaufort Seas region, understanding current exposure levels and sensitivity to hydrocarbons is essential to inform monitoring and protection of these potentially vulnerable and culturally important marine bird populations.

Due to post-ingestion metabolism by the host, direct measurement of oil constituents in bird tissues cannot be used to assess exposure to polycyclic aromatic hydrocarbons (PAH) (Lee et al. 1985). Instead, cytochrome P450 (CYP1A) enzyme induction has been used as a biomarker of hydrocarbon exposure in wildlife, including marine waterfowl (Trust et al. 2000a, Miles et al. 2007, Esler 2008, Esler et al. 2010). CYP1A is induced by exposure to PAH constituents of crude oil, and analysis of liver 7-ethoxyresorufin-*O*-deethylase (EROD) activity has been used as an indicator of CYP1A induction. EROD activity measures the catalytic function of hydrocarbon-inducible CYP1A and is a standard and widely used measurement for assessing exposure to hydrocarbons from oil. Previous field and captive studies have linked increased EROD activity to oil exposure in harlequin ducks (*Histrionicus histrionicus*), Steller's eider, and Barrow's goldeneye (*Bucephala islandica*) (Trust et al. 2000a, Miles et al. 2007, Esler 2008, Esler et al. 2010). However, systematic baseline assessments of CYP1A induction in marine birds of the Chukchi and Beaufort Seas have not been reported to date.

Similarly, assessment of the sensitivity of marine birds of the Chukchi and Beaufort Seas to hydrocarbon exposure has not been systematically conducted. Understanding the comparative sensitivity of marine birds of the region would be valuable in identifying suitable candidate species for long-term monitoring and in assessing relative risks hydrocarbon exposure poses to the arctic biota. Recent advances in cell culture techniques provide analytical tools to characterize species-specific sensitivity and responses to hydrocarbon exposure. These techniques utilize species-specific liver cell lines and a suite of reference compounds, under controlled laboratory conditions, to test unique species-specific responses and enable comparisons among species. Measurement of EROD activity in liver cultures allows for species-specific assessment of magnitude and duration of CYP1A induction. In addition, EROD results can be combined with other measurements of cellular or genetic effects, allowing us to evaluate for potential cellular or genetic pathology associated with hydrocarbon exposure and CYP1A induction. Cell culture techniques, and specifically avian hepatocyte cell culture, have been used for a variety of purposes including investigations into possible injury or damage from toxic substances (Brendler-Schwaab et al. 1994, Kennedy et al. 1996, Hollmén et al. 2008–2012). For example, Kennedy et al. (1996) used CYP1A induction in hepatocyte cell cultures, measured by EROD activity, to predict the sensitivity of chickens, pheasants, turkeys, ducks, and herring gulls to several hydrocarbons.

In summary, this project established baselines for hydrocarbon exposure in marine waterfowl in the Chukchi and Beaufort Seas region. Species used in the study are important for local subsistence activities and represent a variety of potential exposure pathways due to differences in life history and habitat use. Measures of baseline exposure provide valuable background information for assessing future exposure levels. Additionally, using recently developed laboratory cell culture techniques, a broad suite of candidate species were screened for their sensitivity to hydrocarbon exposure. Results from these studies will help guide identification and selection of suitable priority species for monitoring marine birds for hydrocarbon exposure,

based on feasibility of field sampling and sensitivity of species. A better understanding of sensitivity among species will also provide information about relative risks of exposure to the arctic biota.

OBJECTIVES

1. Assess baseline levels of current CYP1A activity in selected marine bird indicator species of the Chukchi and Beaufort Seas.
2. Assess comparative sensitivity to hydrocarbon exposure in selected marine bird indicator species of the Chukchi and Beaufort Seas.

METHODS

Selection of Species

The criteria used to select candidate species for this study included population status, subsistence use, and role as a potential bioindicator in coastal marine areas as well as sampling logistics and feasibility for use in long-term of monitoring. We used common eider, king eider, and greater white-fronted goose for the baseline assessments. The Chukchi and Beaufort Seas and adjacent terrestrial and nearshore areas provide important habitat for these species (Suydam et al. 2000, Dickson and Gilchrist 2002, Oppel et al. 2008), and all three are important resources for subsistence and game hunters in the region. Population declines have been noted in king eider and common eider populations in the Beaufort Sea (Suydam et al. 2000). Due to differences in habitat use and foraging strategies, eiders and geese have the potential to serve as indicators of the at-sea benthic environment and more terrestrial/near shore environments, respectively.

We broadened the species selection included in laboratory sensitivity studies by adding Steller's eider (*Polysticta stelleri*), spectacled eider (*Somateria fischeri),* long-tailed duck (*Clangula hyemali*), black brant (*Branta bernicla),* common murre (*Uria aalge*), tufted puffin (*Fratercula cirrhata*), black guillemot (*Cepphus grylle*), and surrogate pigeon guillemot (*Cepphus columba*). We also planned to used mallard (*Anas platyrhunchos*) and chicken (*Gallus domesticus*) cell lines as a reference for the results from the marine birds of interest. These species were selected based on their conservation status, subsistence use, and potential to serve as bioindicators of different components of the aquatic environment and different exposure pathways. Due to significant population declines, the spectacled eider and the Alaska-breeding Steller's eiders are listed as threatened under the US Endangered Species Act (Federal Register 1993, 1997). Long-tailed duck populations are considered a species of concern due to population declines (Hollmén et al. 2003 and references therein). Both long-tailed ducks and black brant are used for subsistence by the arctic coastal communities, and black brant represents a goose species with a strong marine association. Due to their different foraging ecology (piscivorous vs. benthic foraging), alcids have the potential to serve as bioindicators for different components of the aquatic food web and exposure pathways.

Current CYP1A Activity

Field samples

Liver samples to measure hydrocarbon-inducible CYP1A activity were collected in collaboration with the North Slope Borough Department of Wildlife Management. Samples were collected opportunistically from hunter-killed birds and from lethal take during spring (pre-breeding) and fall (post-breeding) seasonal hunts near Barrow, AK, over three years (Figure 1). Our goal was to collect up to 20 liver samples per season/year/species from the three target species: king eider, common eider, and greater white-fronted goose. Eider samples were collected during fall hunts and greater white-fronted goose samples were collected during spring and fall hunts. Common eider and king eider represent species with a strong marine association during the pre-breeding migration to their arctic nesting grounds, and the greater white-fronted goose represents a species with more terrestrial and near shore association.

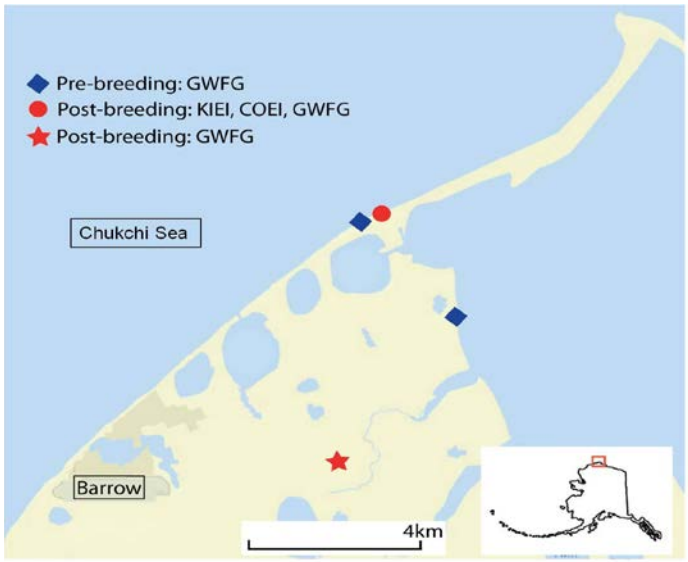

Figure 1: Pre- and post- breeding liver sample collection areas. Pre-breeding greater white-fronted goose (GWFG) locations were hunted from snow blinds (blue diamonds). Post-breeding GWFG locations were hunted inland on the tundra (red star), and at a pond near Pigniq (local hunting station; red circle). Post-breeding king eider (KIEI) and common eider (COEI) were taken also near Pigniq as birds flew toward the Chukchi Sea.

In previous studies, liver biopsies for CYP1A analysis have been frozen within 10 minutes of death/biopsy removal (Trust et al. 2000a), and information about potential post-mortem enzyme degradation beyond this timeframe has not been reported. To maintain a 10-minute post-mortem sampling window, we targeted birds with a verifiable time of death by direct-take or observing the death when collecting opportunistic samples. Post-mortem, livers were quickly removed through one or two incisions near the spine along the bird's back. Livers were cut into small pieces, samples placed in cryovials (~1 cm^3 tissue minimum), and cryovials immediately frozen in a cryogenic vapor phase liquid nitrogen dewar (-150ºC) in the field. In addition to a standard 10-minute vial, when possible, samples were also frozen at less than 10 minutes post-mortem. To

explore post-mortem degradation of CYP1A and determine an appropriate sampling window, additional liver vials were frozen at 20 and 30 minutes and 1, 2, 4, 6, and 24 hours post-mortem. Cryovials were kept at outdoor ambient temperatures until their assigned freeze time. Extra vials were also frozen at 10 minutes, and 1 and 2 hours to use for laboratory QA/QC and long-term freezer storage. While in the field, we collected whole blood and cloacal swabs for archival and recorded body mass, age/gender, wing length, culmen and tarsus measurements, and location data. Additionally, we collected organ and muscle tissue, bile, feathers, and fat for archival and health assessment. All liver samples were transported in the vapor phase liquid nitrogen dewar to the Alaska SeaLife Center (ASLC) in Seward, AK, for laboratory analysis. Once at the ASLC, liver samples were transferred to -80ºC for storage.

Liver EROD assay

Embryonated mallard eggs were injected with a dose of 4mg beta-naphthoflavone (BNF; dissolved in peanut oil) at day 11 of incubation to act as a positive assay control. The shell was first cleaned with povidone iodine solution and two small holes were made at the top of the air cell (one hole for injection of a dose, one to alleviate air pressure during dosing) taking care not to break the internal air cell membrane. After dosing, holes were sealed with liquid Band-Aid© and eggs wereplaced upright for 10–30 minutes. Eggs were then returned to the incubator until liver extraction 24 hours later. Eggs were sterilized with povidone iodine, opened around the air cell using scissors, and the embryo quickly located and decapitated. The torso was placed in a petri dish and the liver, without the gallbladder, removed and placed in a cryovial. Embryo livers from 1–4 birds were pooled in cryovials and frozen in liquid nitrogen within 10 minutes of death. Samples were kept in liquid nitrogen until microsome extractions.

We used microsome EROD activity to measure CYP1A levels in liver samples following the methods of Trust et al. (2000a) and Miles et al. (2007) and standard laboratory QA/QC procedures. Microsomes were extracted within six months of collection from 50-100mg of liver tissue homogenized with 500 µL cold homogenizing buffer (0.05M Tris, 0.15M KCl, pH 7.4). Each homogenate was then centrifuged for 20 minutes at 10,000 xg at 2ºC. The resulting supernatant was transferred to a new cold vial and spun for 60 minutes at 20,800 xg at 2ºC. This supernatant was removed and the microsome pellet was resuspended in 100 µL cold resuspension solution [50 mM Tris, 1 mM ethylenediaminetetraacetic acid, 1 mM dithiothreitol, and 20% (v/v) glycerol, pH 7.4]. To determine EROD activity, microsomes were plated in triplicate in a 96-well plate and read using a Gemini EM Dual-Scanning Microplate Spectrofluorometer fluorescent plate reader (Molecular Devices, Sunnyvale, CA). Resorufin at 0, 5, 10, 20, 40 and 60pM acted as a positive control for each assay. Each well contained 200 µL consisting of 10 µL microsomes (or resorufin), 150 µL 2.5 µM 7-ethoxyresorufin (7-ER) in 50 mM Tris buffer (pH 8.0), and 40 µL 1.34 µM catalyst nicotinamide adenine dinucleotide phosphate (NADPH) in 50 mM Tris buffer (pH 8.0). Fluorescence was measured once every minute for six minutes at an excitation wavelength of 530 nm and an emission wavelength of 590 nm. EROD activity was expressed as pmol/min and results were averaged over the triplicates

with mean calculated. Protein levels (mg) were determined by diluting 1 µL microsomes with 4 µL dH2O and using the Bradford reagent (Sigma, St. Louis, MO). Final EROD activity was expressed as pmol/min/mg protein and calculated using the formula:

EROD activity = (EROD pmol/min) / EROD sample volume (0.01 mL) / mg protein

Sensitivity to Hydrocarbon Exposure

Source material

Eggs from several species were acquired from private or commercial breeders: mallard eggs from Murray McMurray Hatcheries (Webster City, IA), efowl.com (Denver, CO), and Metzer Farms (Gonzales, CA); chicken eggs from Murray McMurray Hatcheries, efowl.com, and Stromberg's Chick and Game Birds Unlimited (Pine River, MN); domestic goose from Murray McMurray Hatcheries; common eider and king eider eggs from Dry Creek Waterfowl (Port Angeles, WA); domestic goose and brant eggs from private breeders (Washington). Additionally, the ASLC (Seward, AK) provided eggs from captive research or collection birds including Steller's eider, spectacled eider, tufted puffin, horned puffin (*Fratercula corniculata*), long-tailed duck, and common murre. In 2015 and 2016, greater white-fronted goose and common eider eggs were collected on the North Slope (Barrow area and Kaktovik, respectively).

Egg development

After arrival at the laboratory, eggs were given an individual identification number, dipped in 1X chlorhexidine solution, and allowed to air dry. Eggs were either placed directly into an incubator or kept at room temperature at a 45° angle for 1–48 hours. Eggs kept longer than 12 hours were turned 180° every 12 hours. Artificial incubation occurred in a Grumbach incubator (Grumbach, Germany) at 37.5°C with 50–60% humidity. Eggs were placed on shelves in the incubator on top of rollers which slowly moved, gently rolling the egg over 24 hours. Incubator settings also included two 30-minute breaks with no heat per 24-hour period (at 10 and 14 hours). During development, eggs were monitored every 3–5 days by candling. Eggs that were infertile or stopped developing were removed from the incubator.

Hepatocyte cell extraction

Using established primary cell culture protocols (Hollmén et al. 2008–2012, Brendler-Schwaab et al. 1994) and following standard cell culture QA/QC (Freshney 2000), embryonic liver tissue was harvested at approximately 11–18 days of incubation for all species. Egg shells were sterilized with povidone iodine solution prior to opening. Using scissors, the top of the shell around the air cell was removed, the embryo located and quickly decapitated. The torso was removed and placed in a petri dish, and the liver, without the gallbladder, was removed and placed in cold 1X PBS. Livers were pooled in groups of 1–4 and rinsed with pre-perfusion buffer II [Hanks Balanced Salt Solution 1x (HBSS) without $CaCl_2$ and $MgCl_2$, 10 mM HEPES, and 0.5 mM EGTA (pH 7.4)]. The buffer was removed and livers were minced gently using sterile scalpels. Liver tissue and perfusion medium II [minimum essential medium (MEM) without L-

glutamine, collagenase IV (100U) and 0.125% trypsin] were added to a trypsinizing flask and digested for 30 minutes at 37°C with gentle stirring. Digestion was stopped with the addition of 20% heat inactivated FBS (HI FBS). The resulting mixture was filtered through nylon mesh (100 µM) into a sterile tube containing pre-perfusion buffer. The cell suspension was centrifuged at 63g and 4°C for 5 minutes. The supernatant was removed and the resulting cell pellet was gently resuspended in pre-perfusion buffer and spun at 110 xg and 4°C for 5 minutes. At this point, additional liver suspensions were combined, if necessary, to bring the total number of livers used per culture to 4–12. The supernatant was removed and the cell pellet was gently resuspended in low glucose media [high glucose Dulbecco's Modified Eagle Medium (DMEM), MEM, antibiotic mixture (nystatin, penicillin-streptomycin, and gentamycin) and L-glutamine] and 20% HI FBS. A cell count was then performed using a hemocytometer. Finally, hepatocyte cells were seeded in a black-walled 96-well plate at 30,000 cells/well and a total well volume of 200 µL. All cell lines were incubated at 37°C in 5% CO_2 atmosphere. Every 24 hours, cultures were given fresh media with 20% HI FBS and cells were checked by microscopy for confluency level, health, and morphology (Hollmén et al. 2002). Cell cultures were incubated at 37°C in 5% CO_2 atmosphere for 24–48 hours before dosing.

Cell culture QA/QC

Eggs, cell culture stocks, and reagents were all assigned individual lot and/or tracking numbers. Aseptic techniques were used for all cell culture protocols and work was performed in a biosafety cabinet. All buffer and media reagents were purchased from Invitrogen (Grand Island, NY), Sigma-Aldrich (St. Louis, MO), Fisher Scientific (Pittsburg, PA), or VWR (Radnor, PA).

Cell culture assays

Before dosing, media was removed from the wells and cells gently washed 1–2 times with 1X PBS to remove any cellular debris. Cultures were then given fresh media (100 µL with no FBS) and were dosed, in triplicate, with carrier solvent control dimethyl sulfoxide (DMSO) or ethanol, negative controls (media only and cells with no dose), and different concentrations of reference laboratory chemicals and compounds (BNF, chrysene, or phenanthrene) or compound mixture Alaska North Slope Crude Oil (ANS oil; Marathon Alaska Beaver Creek Crude Oil-Sweet). BNF is a standard laboratory chemical that is a known inducer of CYP1A activity and has been used in previous studies to measure avian EROD responses (Miles et al. 2007, Hollmén et al. 2008–2012). Phenanthrene and chrysene represent polycyclic aromatic hydrocarbons of different molecular weights (3- and 4-ring, respectively) found in crude oil and which induce CYP1A activity (Incardona et al. 2005, Short et al. 2008). Doses were diluted with low glucose media, when necessary, and stored in amber vials at 4°C. Following 12–36 hour incubation (typically 24-hour exposure) cells were first evaluated using microscopy for general cell morphology and cytopathic effects (CPE). Types of CPE observed were characterized and described for each cell line and test material used. Cells were scored on a semiquantitative response scale from 0–4 (0=none to 4=100 % effect) for a variety of CPE responses including vacuolarization, cytoplasmic swelling, and cellular debris (Hollmén et al. 2008–2012; Table 1).

Table 1: Cytopathic effects (CPE) measurements.

General culture evaluation Cell sheet confluency, general cell morphology	
Culture responses	**Scoring system**
granularization	Semiquantitative response grade 0-4 (0=none to 4=100% effect)
vacuolarization	
cytoplasmic swelling	
non-viability	
cellular debris	
other	

Adapting EROD methods from Hodson et al. (1996) and Hahn et al. (1996), a solution of 6 µM 7-ethoxyresorufin (7-ER) was freshly prepared from 100 µM 7-ER stock and $NaPO_4$ buffer and 25 µL added to each well. Next, catalyst NADPH was resuspended in 1.5 mL $NaPO_4$ buffer and 10 µL added to each well. Fluorescence was then immediately measured at excitation wavelength of 530 nm and an emission wavelength of 590 nm using a Gemini EM Dual-Scanning Microplate Spectrofluorometer fluorescent plate reader (Molecular Devices, Sunnyvale, CA). EROD activity was measured every 15 minutes for 3 hours to capture the threshold activity level. Results were averaged over the triplicates and standard error calculated. Cell count per well was uniform, and protein was not quantified (Hahn et al., 1996). Mallard results were used as an assay control and reference comparison.

Analytical Techniques

Curve fitting

Curve fitting has been used to characterize the dose-response relationships observed in cellular assays (Springman et al. 2008, Short et al. 2008) using regression analysis. Types of comparisons can include the relative potency of reference compounds within a species and the sensitivity of each species relative to a reference species. The method of comparison is based on the shape of the response among species and compounds, and then determining half maximum effective concentrations (EC_{50}), calculating concentrations that produce a response equivalent to an assigned % of the maximum response produced by the compound (for example, 10%), or comparing slope ratios (Finney 1978). We explore the use of five-parameter logistic curves to estimate dose-response values and variation between species and linear mixed models (Gottschalk and Dunn 2005). The five-parameter formulation allows for a range of response curves from the group of species and can be tested explicitly for goodness of fit before use in comparisons.

Sensitivity assessment

Species sensitivity is compared using data from controlled cell dosing experiments. The embryonic stage was monitored by candling and made comparable among species by adjusting the extraction time to incubation stage so embryos represented a similar stage of development at the time of cell line establishment. The responses were compared using reference reagents and standardized dosing concentrations and procedures. The fitted response curves for each of the species were compared. Mallard represented a reference responder (Hollmén et al. 2008–2012). Species were assigned a relative rank based on their responses. Additionally, assessment of CPE provided a metric to evaluate potential cellular-level toxicity relating to the experimental dose and associated enzyme response.

RESULTS

Current CYP1A Activity

Liver collections

Table 2 presents the sample sizes for birds sampled during pre- and post-breeding harvest in collaboration with the North Slope Borough Department of Wildlife Management. A total of 33 days in 2014, 30 days in 2015, and 12 days in 2016 were spent in Barrow for liver collections, with the first collections occurring in August 2014. All birds were collected using direct-take except two king eiders that were sampled opportunistically, one within 10 minutes of death. Complete time series samples were collected from all birds, excluding the king eider that was collected after 10 minutes post-mortem. In 2014, time series samples included 10, 20, 30 minutes and 1 and 2 hours. In 2015, the time series was adjusted to 10, 20, 30 minutes and 1, 2, 4, 6, and 24 hours. The longer time points were added in response to learning more about when hunters butcher their birds. Also, 81 of 93 birds had an additional liver sample collected and frozen at <10 minutes.

Table 2: Birds collected during pre- and post-breeding hunts over the course of this project.

Species	Birds sampled Post-breeding 2014	Birds sampled Pre-breeding 2015	Birds sampled Post-breeding 2015	Birds sampled Pre-breeding 2016
Greater white-fronted goose	9	20	13	18
King eider	7	---	15	---
Common eider	9	---	2	---

Microsome EROD

Table 3 shows median EROD results (pmol/min/mg protein) from microsome liver samples from pre-breeding 2015 (n=236), pre-breeding 2016 (n=210), post-breeding 2014 (n=141) and post-breeding 2015 (n=352) field seasons. Individual EROD results at 10 minutes post-mortem (the current collection protocol) ranged from 0–284 pmol/min/mg protein in king eiders, 27–367

pmol/min/mg protein in common eiders, and 0–915 pmol/min/mg protein in greater white-fronted geese. The highest EROD activity responses were found in post-breeding greater white-fronted geese in 2014.

Table 3: Median EROD results (pmol/min/mg protein) with sample size by species, season, and time vial was frozen post mortem for all collected liver samples. (KIEI=-king eider, COEI=common eider, GWFG=greater white-fronted goose).

Time	KIEI *post-breeding 2014*	KIEI *post-breeding 2015*	COEI *post-breeding 2014*	COEI *post-breeding 2015*	GWFG *pre-breeding 2015*	GWFG *pre-breeding 2016*	GWFG *post-breeding 2014*	GWFG *post-breeding 2015*
<10 min	112.07 (n=5)	25.70 (n=10)	257.95 (n=7)	73.36 (n=2)	69.93 (n=20)	87.52 (n=18)	417.72 (n=9)	20.03 (n=10)
10 min	92.65 (n=6)	19.97 (n=15)	254.51 (n=9)	32.97 (n=2)	89.80 (n=20)	82.78 (n=18)	518.57 (n=9)	25.04 (n=13)
20 min	135.71 (n=6)	20.27 (n=15)	225.42 (n=9)	36.93 (n=2)	104.82 (n=20)	68.79 (n=17)	406.69 (n=9)	23.62 (n=13)
30 min	152.34 (n=6)	16.92 (n=15)	236.02 (n=9)	81.01 (n=2)	108.43 (n=20)	85.19 (n=17)	379.59 (n=9)	22.50 (n=13)
1 hr	151.14 (n=6)	25.88 (n=15)	235.89 (n=9)	82.60 (n=2)	94.94 (n=20)	89.27 (n=18)	388.66 (n=9)	23.78 (n=13)
2 hr	157.54 (n=6)	22.47 (n=15)	260.24 (n=9)	87.31 (n=2)	122.71 (n=20)	71.46 (n=17)	535.01 (n=9)	28.64 (n=13)
4 hr	---	16.30 (n=15)	---	61.84 (n=2)	133.60 (n=20)	63.76 (n=17)	---	23.15 (n=13)
6 hr	---	14.32 (n=14)	---	53.16 (n=2)	156.08 (n=20)	87.35 (n=18)	---	23.23 (n=13)
24 hr	---	38.17 (n=2)	---	38.17 (n=2)	109.72 (n=16)	88.94 (n=17)	---	21.11 (n=13)

EROD results indicate enzyme activity throughout the post-mortem time series, including samples frozen 24 hours after death. Results for QA/QC duplicate microsome extractions (Table 4) show a similar response between the two extracts. Table 5 reports EROD results for the freezer storage experiment. The original samples from post-breeding collections in 2014 were extracted in February 2015 (within six months of collection). The same samples, from a vial that had not been previously thawed, were re-extracted in 2015 after 16 months in storage at -80°C. The EROD results suggest some decrease in enzyme activity during long-term freezer storage.

Table 4: Median EROD results (pmol/min/mg protein) with sample size by species, season, and time vial was frozen post-mortem for duplicate microsome extraction QA/QC. (KIEI=-king eider, COEI=common eider, GWFG=greater white-fronted goose).

	10 Minutes		60 Minutes		120 Minutes	
	First replicate	Second replicate	First replicate	Second replicate	First replicate	Second replicate
GWFG pre-breeding 2015	99.09 (n=16)	90.91 (n=16)	128.36 (n=15)	114.23 (n=15)	125.75 (n=15)	142.38 (n=15)
GWFG post-breeding 2015	19.99 (n=13)	28.01 (n=13)	22.48 (n=13)	20.32 (n=13)	21.50 (n=13)	31.62 (n=13)
GWFG pre-breeding 2016	88.83 (n=18)	73.36 (n=18)	87.17 (n=18)	90.96 (n=18)	66.65 (n=17)	67.27 (n=17)
COEI post-breeding 2015	34.66 (n=2)	31.27 (n=2)	65.05 (n=2)	100.14 (n=2)	110.67 (n=2)	63.94 (n=2)
KIEI post-breeding 2015	19.62 (n=15)	25.86 (n=15)	27.05 (n=15)	19.54 (n=15)	24.15 (n=15)	20.90 (n=15)

Table 5: Median EROD results (pmol/min/mg protein) for 10-minute post-mortem samples (2014 post-breeding) from freezer storage experiment.

Species	Activity (pmol/min/mg protein) Extracted Feb 2015 *EROD Feb 2015*	Activity (pmol/min/mg protein) Extracted Dec 2015 *EROD Feb 2016*
Greater white-fronted goose (n=9)	518.57	68.49
King eider (n=5)	94.64	36.62
Common eider (n=9)	254.51	30.23

Hydrocarbon Sensitivity

Source material

During the course of this study we received eggs from nine of our ten target species: Steller's eider, common eider, king eider, spectacled eider, long-tailed duck, common murre, tufted puffin, black brant, greater white-fronted goose, and surrogate domestic goose. We were unable to procure any eggs from black or pigeon guillemots. In 2015, we were able to acquire eggs from an additional species, the horned puffin. Chicken cultures were generally of low quality and we did not acquire adequate materials for high through-put assays. We purchased mallard eggs from several different vendors after noticing a variation in culture quality among sources. In 2015 and 2016, we received goose and eider eggs from the North Slope. Greater white-fronted goose eggs were collected near Barrow and common eider eggs were collected opportunistically around Kaktovik. In 2015, 19 of 20 twenty greater white-fronted goose eggs were damaged during

shipment or were not viable. The one remaining egg was fertile but the cells failed to grow after extraction.

Cell culture assays

We conducted a total of 51 EROD assays and assessed cytopathic effects (CPE) using liver cells cultured from ten marine bird species and mallard as a reference responder. Tables A1–A4 (see Appendix A) report the number of EROD repeats for each species and dose at 24-hour dose exposure.

We were able to obtain a large number of Steller's eider eggs during 2015 cell culture laboratory work. This allowed testing of many dose concentrations for our target compounds (chrysene, BNF, phenanthrene, and Alaska North Slope crude oil) and repeated testing of these doses. EROD Figures 2–8 and CPE tables B1–B7 (see Appendix B) summarize the results for Steller's eider.

Figure 2 shows enzyme responses of Steller's eider cells to a range of chrysene concentrations (0.5–5.0 µM) after a 24-hour dose exposure. Steller's eider had the highest response to 1.5 µM chrysene with responses decreasing with higher dose concentration. Cellular debris and amount of non-viable cells increased slightly with increasing dose concentration (appendix Table B1). Steller's eider cell culture results at 24- hour exposure to BNF concentrations (0.5–5.0 µM) are presented in Figure 3. The highest enzyme response was from 2.0 µM BNF and the lowest from 5.0 µM BNF. There was minimal change in cytopathic effects from the different BNF concentrations (appendix Table B2). Testing with the single hydrocarbon phenanthrene (0.5–50.0 µM) started in 2015, and results from Steller's eiders are shown in Figure 4 (24-hour dose exposure). Even with a higher concentration range then the other testing compounds, there was no enzyme response to this hydrocarbon in Steller's eider and only a slight response in mallard (data not shown). However, phenanthrene at higher concentrations produced a black material ("asphalting") that coated the well and obscured CPE readings of the cultures (appendix Table B3).

In 2015, we acquired Alaska North Slope Crude oil (NS oil) to use in dosing experiments. Figure 5 shows Steller's eider results from a 24-hour dose exposure to 0.5–20.0 µL neat NS oil. The highest enzyme response was at 5.0 µL oil with response decreasing with higher dose concentrations. These results show a low degree of variation in enzyme activity over the course of the EROD assay and indicate maximum cellular activity was potentially reached at these doses. Due to the high background from media with oil (no cells) controls, the fluorescent responses from these controls were removed from the cellular responses. Cytopathic responses were similar for all neat NS oil doses with a slight increase of non-viable cells observed at the higher doses (appendix Table B4). In experimenting with different concentrations of NS oil, we tested three doses diluted with media and carrier reagent DMSO (Figure 6) and used 100 µL per well: #1 20 µL 1:1 oil:DMSO with 2 mL media, #2 10 µL 1:1 oil:DMSO with 2 mL media, #4 5 µL 1:1 oil:DMSO with 2 mL media. These doses did not elicit a strong enzyme response in

Steller's eider but had a larger amount of non-viable cell and cellular debris (appendix Table B5) than neat NS oil doses. To further explore diluted NS oil doses, we also dosed Steller's eider cells with higher concentrations of oil with DMSO (1 part oil to 1 part DMSO up to 10 parts oil and 1 part DMSO, Figure 7). Dose 1:1 oil:DMSO had the lowest enzyme response but also the highest amount of cellular debris in the well (appendix Table B6). It is likely this dose overwhelmed the cell culture, limiting enzyme response. Lastly, using Steller's eider cells we were able to complete three replicates of a 12, 24, and 36-hour 1.0 µM chrysene dose exposure experiment (Figure 8). In general, the highest enzyme response was at 12 hours with the lowest responses at 36 hours with similar CPE over the course of the experiment (appendix Table B7).

Results for an alcid species (tufted puffin) response to a range of chrysene concentrations (0.5–2.0 µM) after a 24-hour dose exposure are shown in Figure 9. Tufted puffin responses increased as the chrysene concentration increased, and there was no noticeable change in CPE between the doses (appendix Table B8). Tufted puffin cell culture results at 24-hour exposure to BNF (0.5–2.0 µM) concentrations are presented in Figure 10. All three BNF doses produced a very similar enzyme response and no CPE change (appendix Table B9).

Results for all species tested (mallard, brant, spectacled eider, common eider, long-tailed duck, king eider, domestic goose, greater white-fronted goose, tufted puffin, horned puffin, common murre, and Steller's eider) at 1.0 µM chrysene at 24-hour dose exposure are shown in Figure 11. These results suggest differences in species response at this dose but showed no apparent difference in pathological response (CPE, appendix Table B10) between control wells and the 1.0 µM chrysene dose. The alcids showed a similar enzyme response and the ducks responded in a similar manner, with the possible exception of Steller's eider. EROD results for all species tested at 1.0 µM BNF (mallard, long-tailed duck, common murre, tufted puffin, common eider, domestic goose, greater white-fronted goose and Steller's eider) at 24-hour dose exposure are shown in Figure 12; mallard had the highest response and greater white-fronted goose the lowest. CPE results were similar for all tested species (appendix Table B11). Figure 13 represents EROD results for five target species (tufted puffin, common eider, common murre, greater white-fronted goose, and Steller's eider) and control species mallard dosed with 1.0 µL NS oil at 24-hour dose exposure. Tufted puffin had the highest enzyme response with the other tested species showing a similar, and lower, response. CPE results suggest that common murre had a higher amount of non-viable cells than other species (appendix Table B12). Finally, Figure 14 shows all tested species (tufted puffin, mallard, common murre, greater white-fronted goose, Steller's eider and common eider) responses to 5.0 µL oil after 24-hour dose exposure. All species showed a similar enzyme response and a response higher than at 1.0 µL oil. Again, common murre appeared to have a higher amount of non-viable cells, and common eiders showed a larger amount of cellular vacuolarization (appendix Table B13).

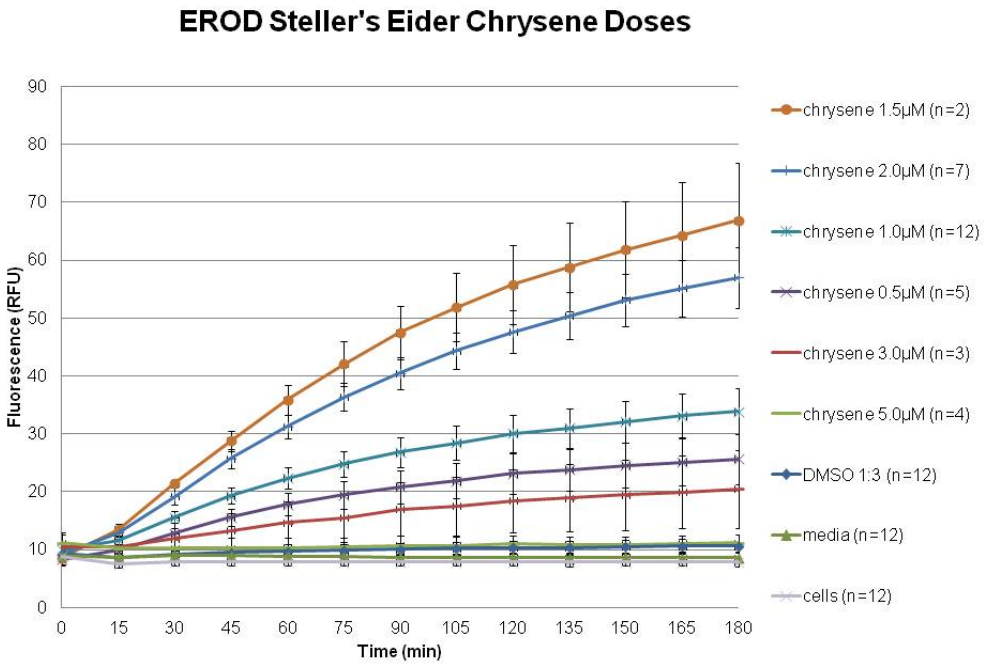

Figure 2: EROD responses (with standard error and sample size) in Steller's eider to different concentrations of chrysene doses with 24-hour dose exposure. Results from 2013-2015 for each dose were combined. Cell and media controls are also included.

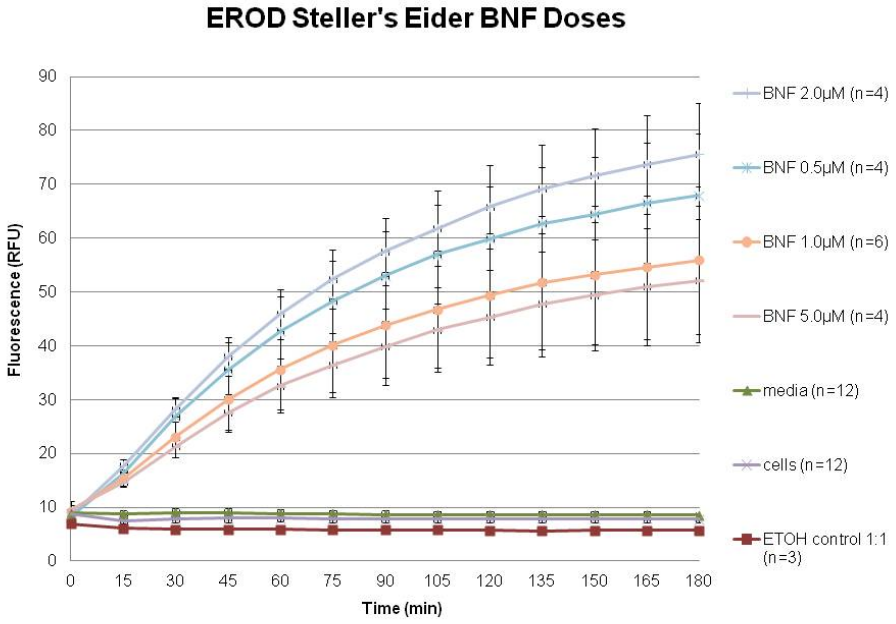

Figure 3: EROD responses (with standard error and sample size) in Steller's eider to different concentrations of BNF doses with 24-hour dose exposure. Results from 2013-2015 for each dose were combined. Cell and media controls are also included.

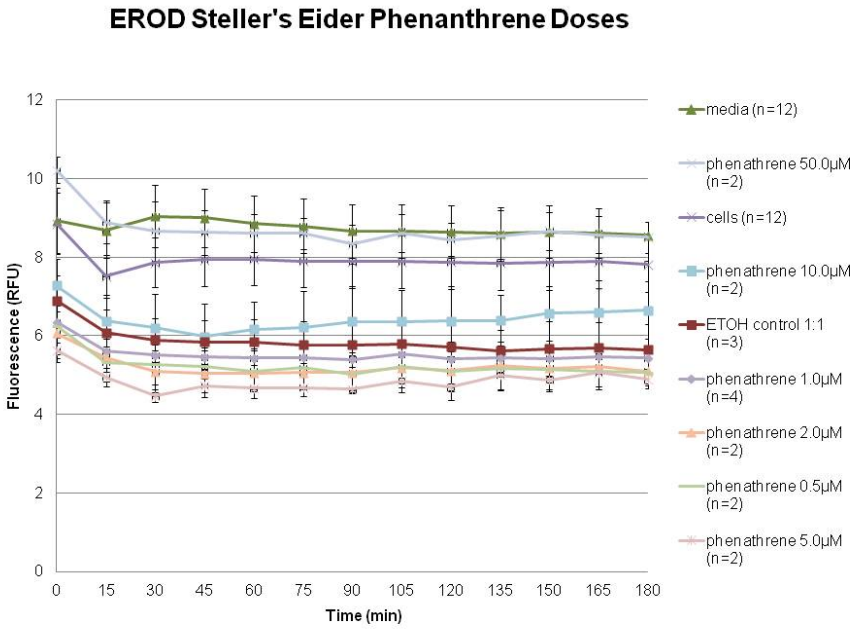

Figure 4: EROD responses (with standard error and sample size) in Steller's eider to different concentrations of phenanthrene doses with 24-hour dose exposure. Results from 2015 for each dose were combined. Cell and media controls are also included.

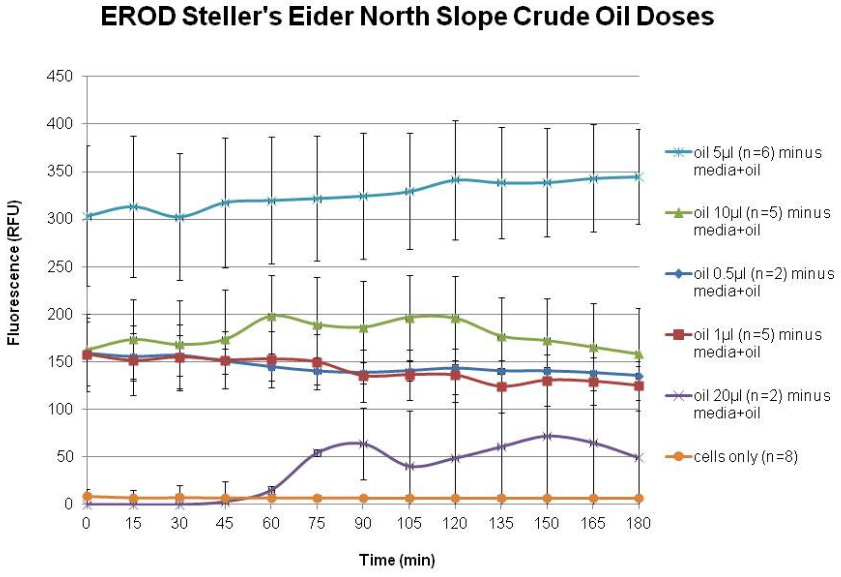

Figure 5: EROD responses (with standard error and sample size) to different amount of neat Alaska North Slope crude oil dose and cell control with 24-hour dose exposure in Steller's eider. Results from 2015 for each dose were combined. The fluorescence response of media + 0.5-20 µL oil (no cells) was removed from the cellular response for each corresponding dose.

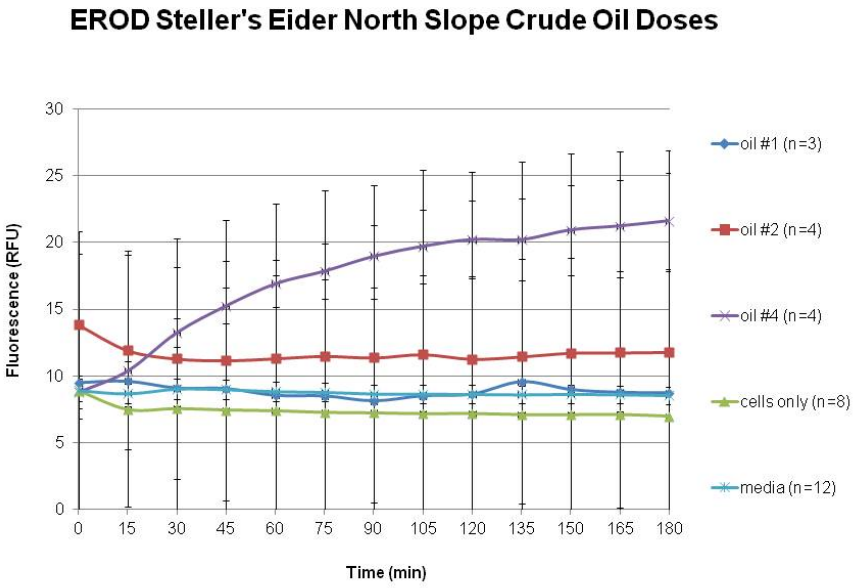

Figure 6: EROD responses (with standard error and sample size) to different amount of media diluted Alaska North Slope crude oil dose and cell and media control with 24-hour dose exposure in Steller's eider. 100 µL of each diluted dose was added to the well. Doses consisted of: #1 20 µL 1:1 oil:DMSO with 2 mL media, #2 10 µL 1:1 oil:DMSO with 2 mL media, #4 5 µL 1:1 oil:DMSO with 2 mL media.

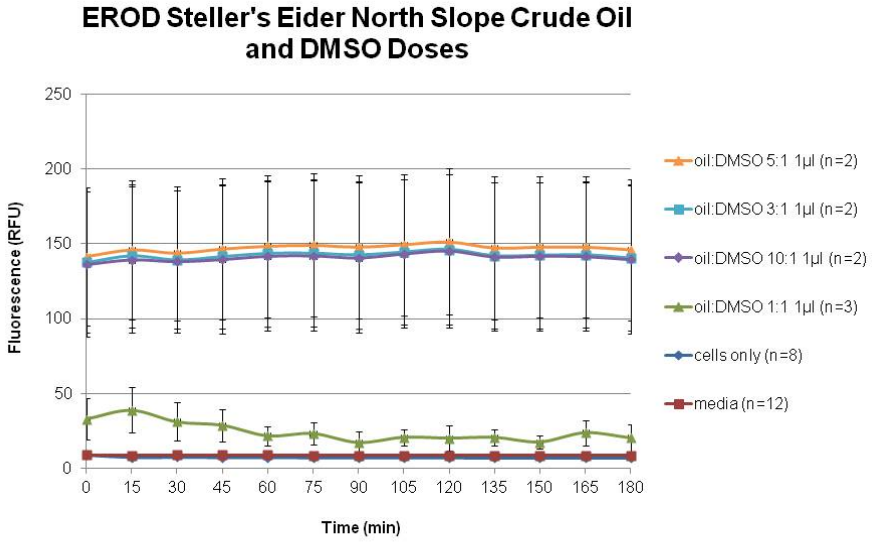

Figure 7: EROD responses (with standard error and sample size) to different amount of DMSO diluted Alaska North Slope crude oil doses and cell and media control with 24-hour dose exposure in Steller's eider.

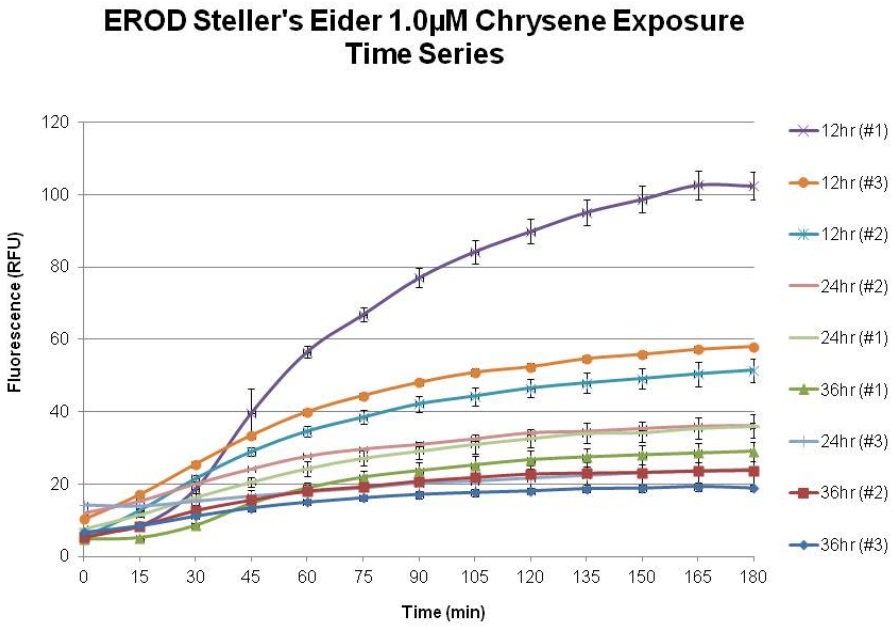

Figure 8: EROD responses (with standard error) in Steller's eider to 1.0 µM chrysene dose with 12, 24, and 36-hour dose exposure. Each of three replicates (#1, #2, #3) is labeled next to the exposure time.

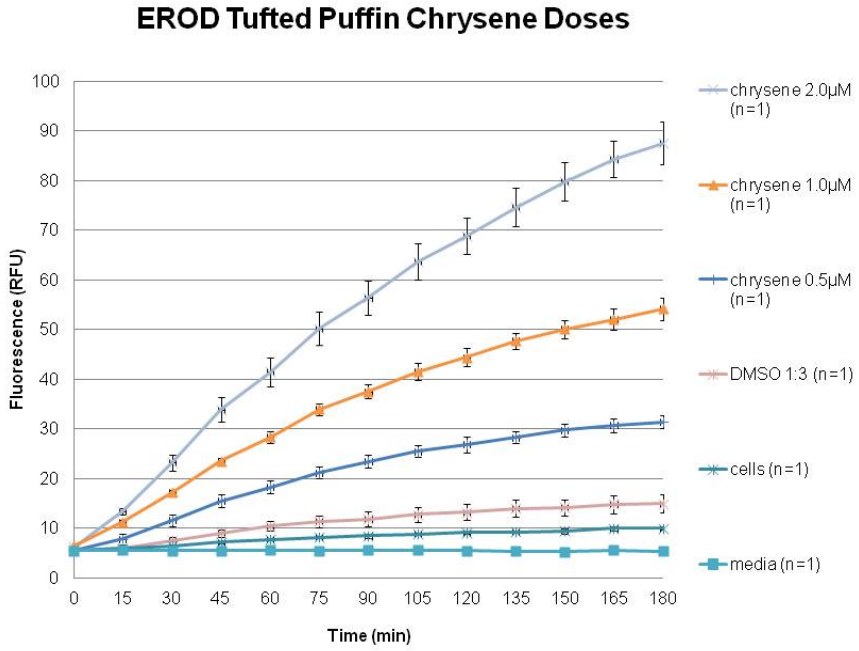

Figure 9: EROD responses (with standard error and sample size) in tufted puffin to different concentrations of chrysene doses with 24-hour dose exposure. Cell and media controls are also included.

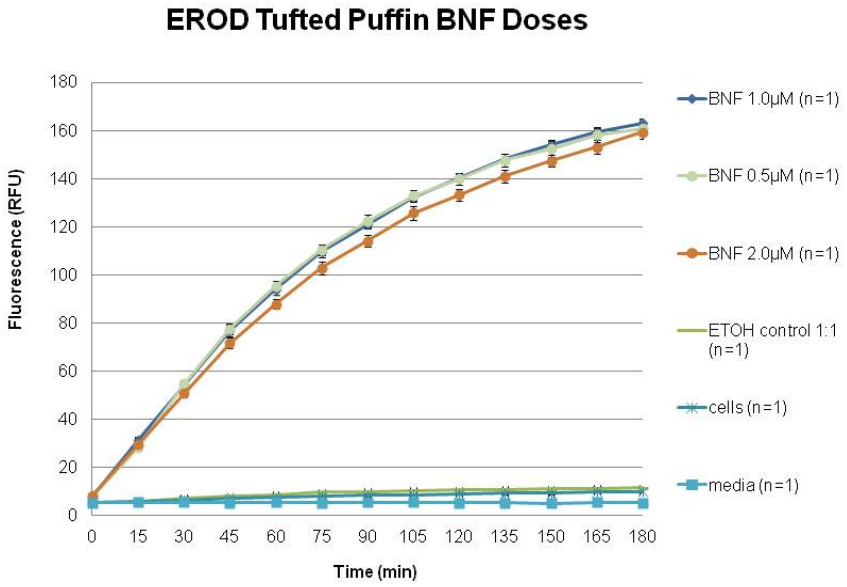

Figure 10: EROD responses (with standard error and sample size) in tufted puffin to different concentrations of BNF doses with 24-hour dose exposure. Cell and media controls are also included.

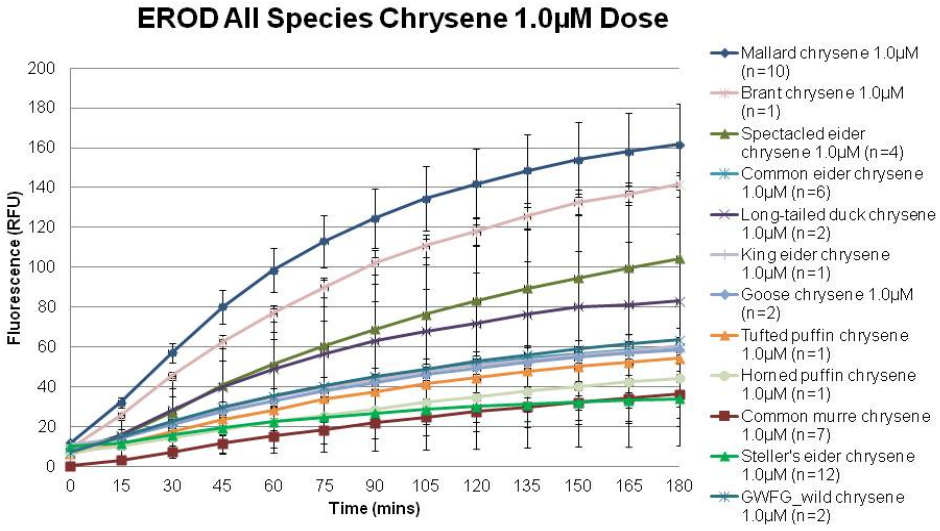

Figure 11: EROD responses (with standard error and sample size) in ten marine bird species and mallard control to 1.0 µM chrysene dose with 24-hour dose exposure. Results from 2013-2016 for each species were combined. Goose= domestic goose.

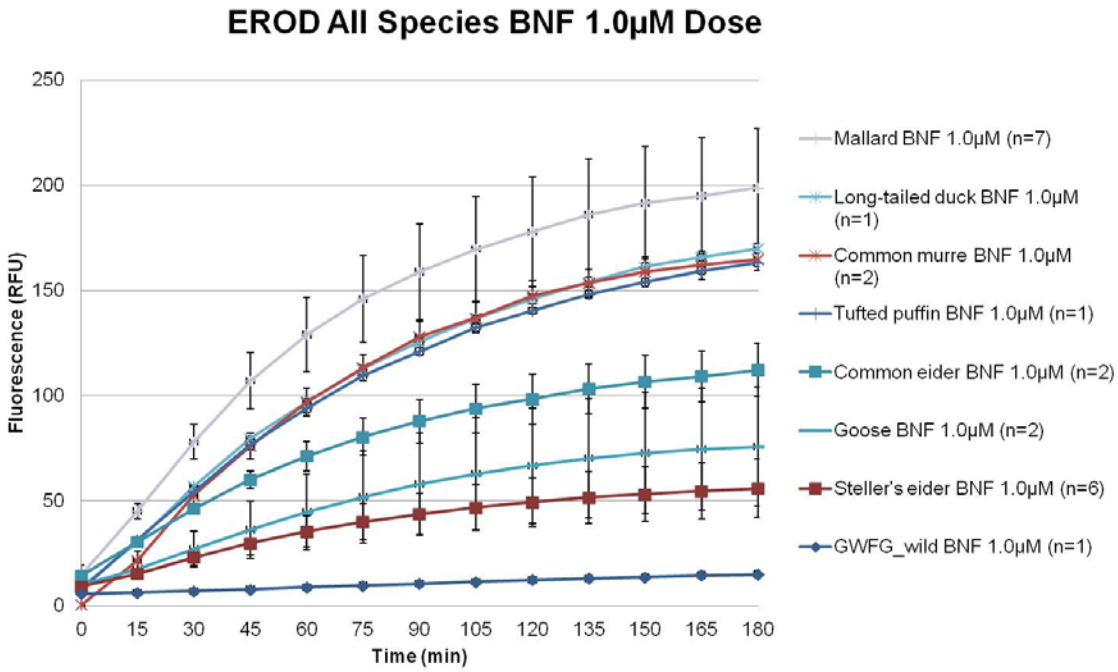

Figure 12: EROD responses (with standard error and sample size) in six marine bird species and mallard control to 1.0 µM BNF dose with 24-hour dose exposure. Results from 2013-2016 for each species were combined. Goose= domestic goose.

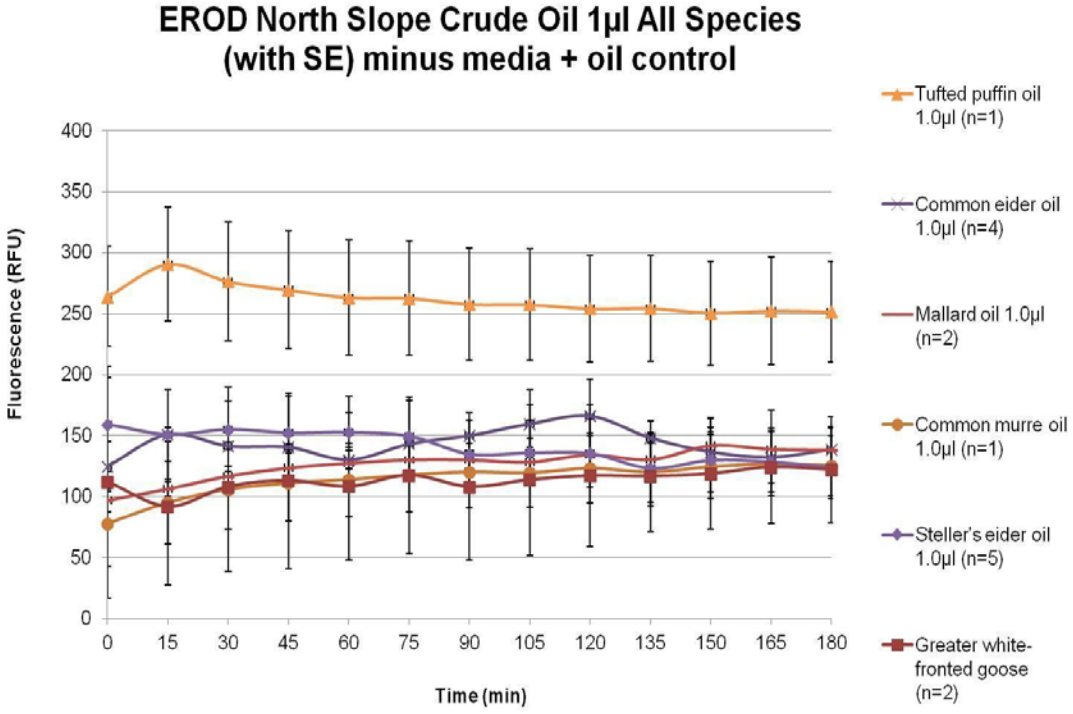

Figure 13: EROD responses (with standard error and sample size) to 1.0 µL neat Alaska North Slope crude oil dose with 24-hour dose exposure in five target species and one control species. The fluorescence response of media + 1.0 µL oil (no cells) was removed from the cellular response for each species.

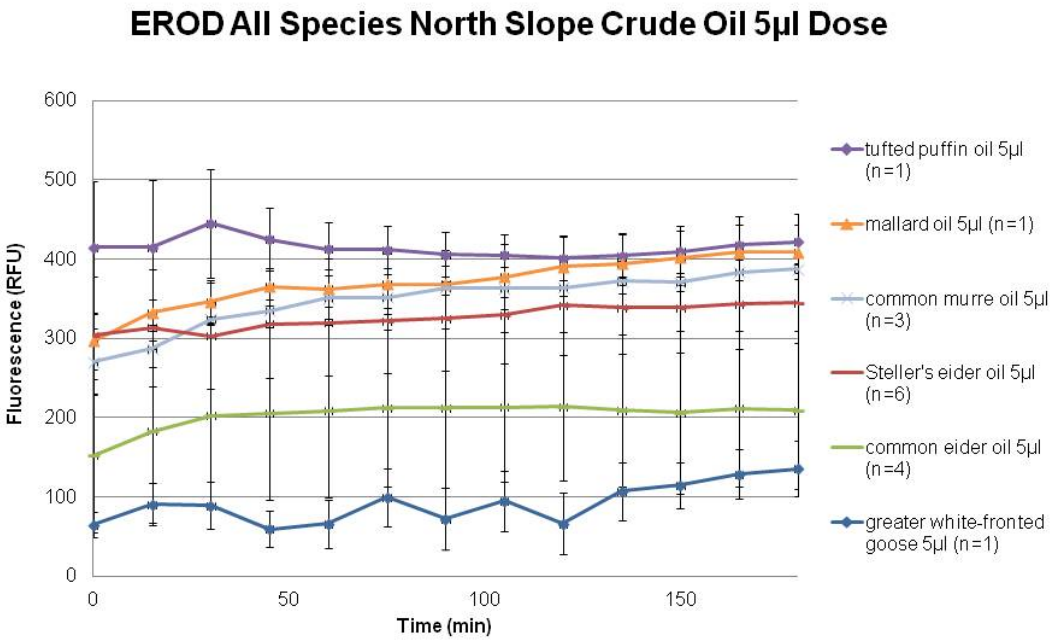

Figure 14: EROD responses (with standard error and sample size) to 5.0 µL neat Alaska North Slope crude oil dose with 24-hour dose exposure in four target species and one control species. The fluorescence response of media + 5.0 µL oil (no cells) was removed from the cellular response for each species.

Analysis

Curve fitting

After visualizing trends in the response for Steller's eider, we found it more appropriate to use a linear mixed modeling approach than a traditional logistic response. We tested models of response by grouping, time, and cell coverage with a random effect of date. The only significant trends in slope of the response were for seven oiled groupings; six had slight negative slopes (Figures 15 and 16). Curve fitting results are shown in Figures17-21.

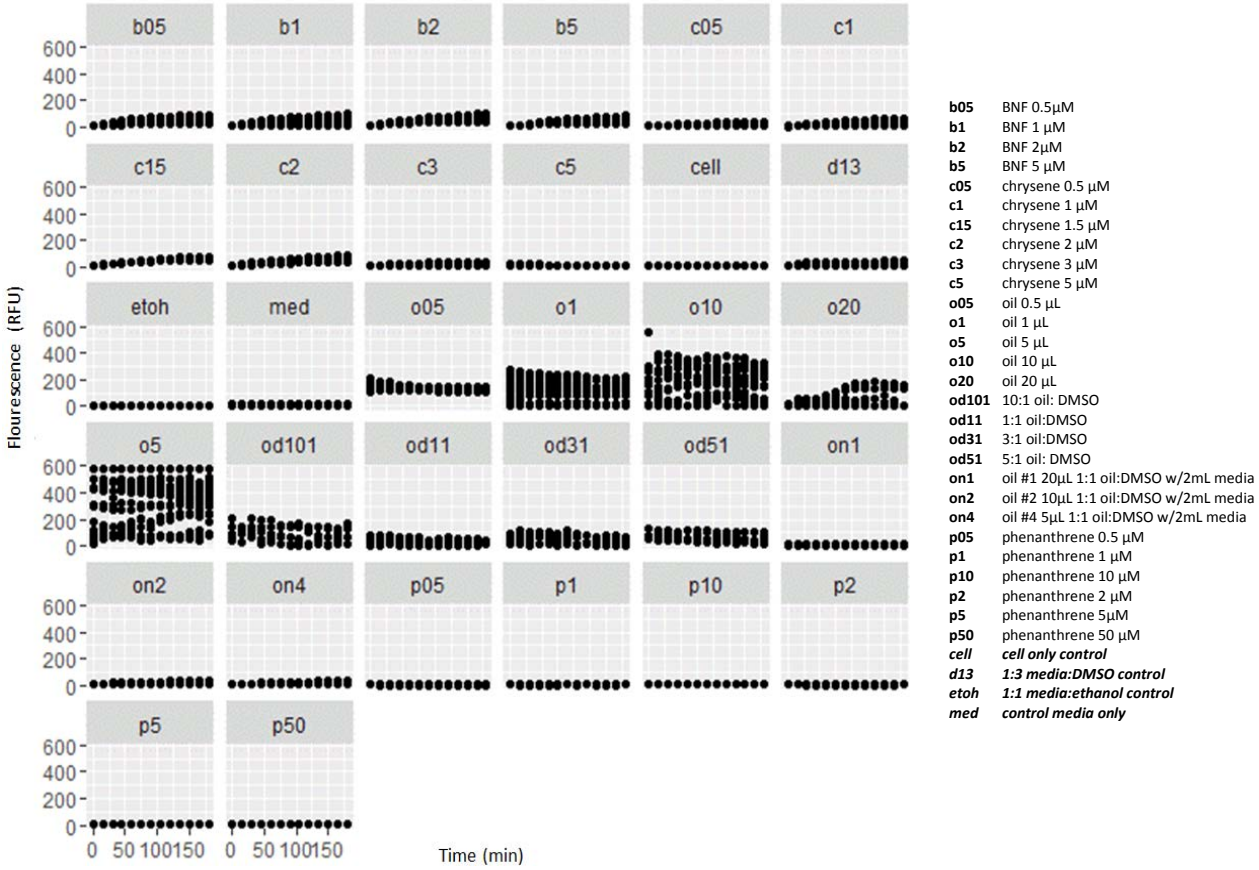

Figure 15: Statistical analysis of Steller's eider EROD results with assay time in minutes. Doses, except for the oil doses, show some minor effects within the group but are not statistically different from no effect.

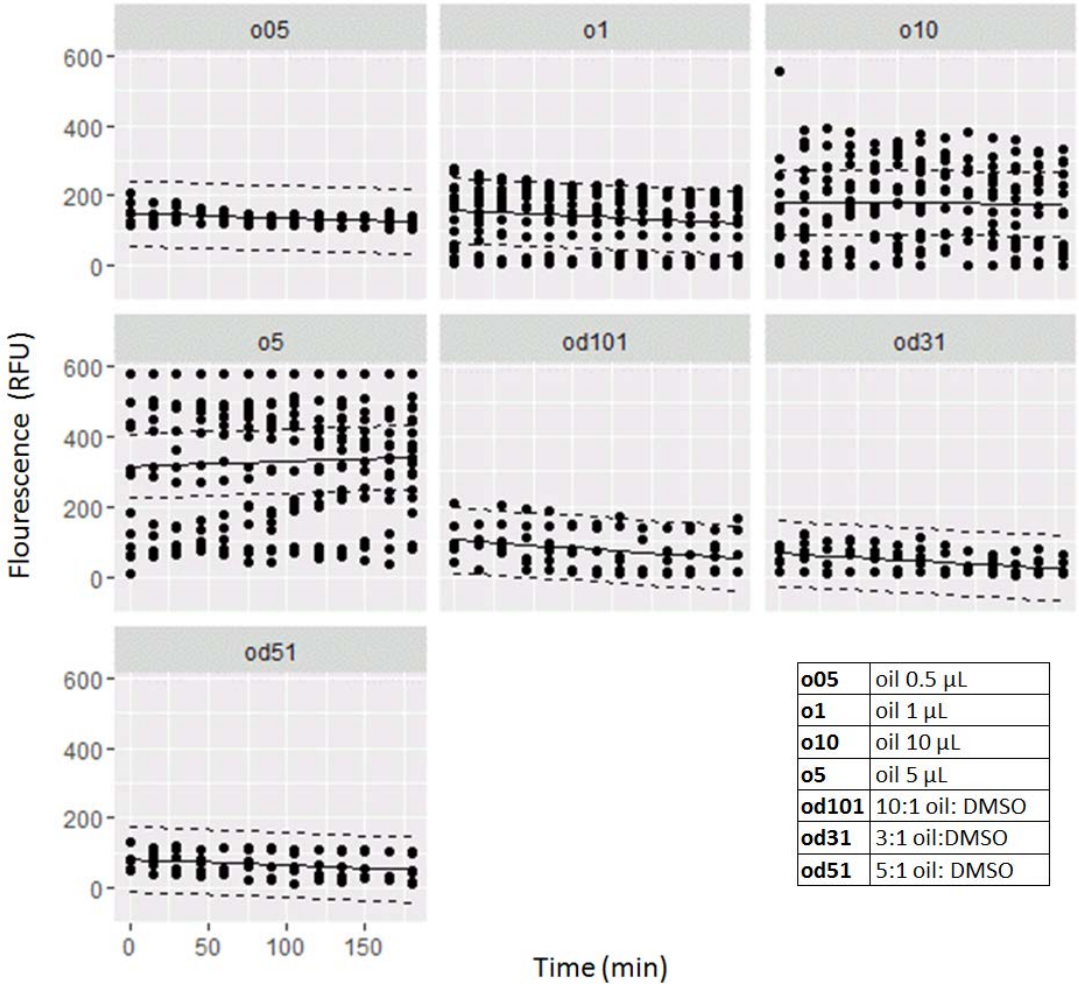

Figure 16: Statistical analysis of Steller's eider oil EROD results with assay time in minutes. Real data is represented in dots, the solid line is the estimated response trend over time, and the dashed line is the 95% confidence interval. These seven doses had slope and intercept parameters significantly different than 0 indicating some trend in fluorescence over time.

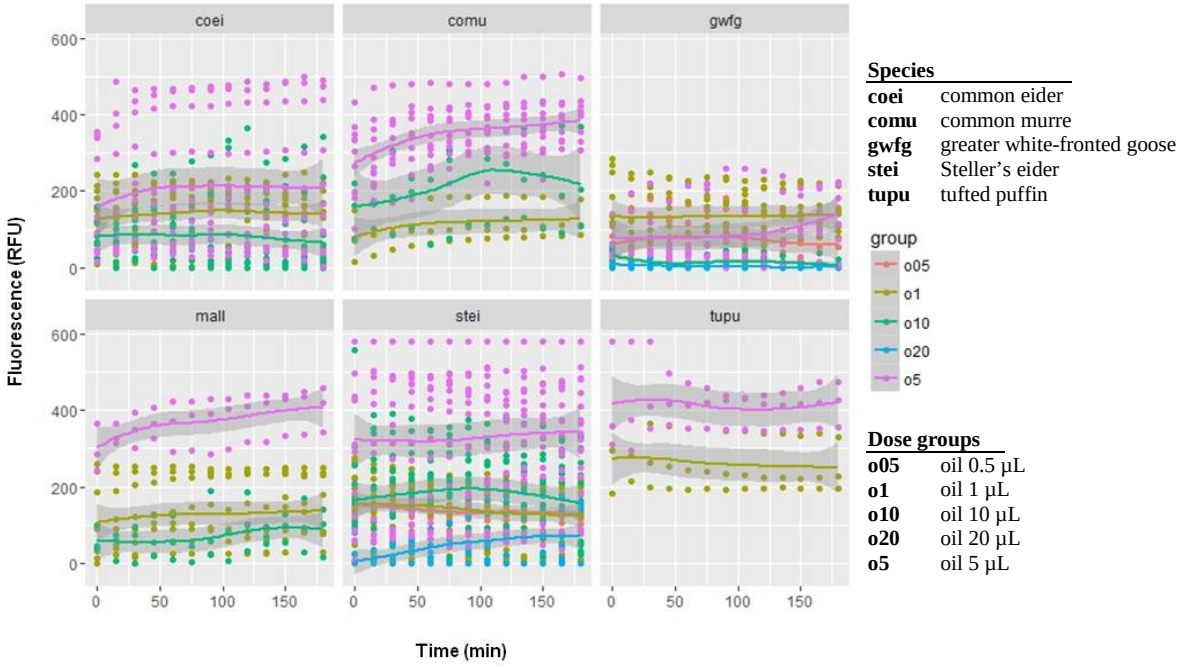

Figure 17: Statistical analysis of tested species EROD results to different amount of neat Alaska North Slope crude oil. Assay time in minutes is on the x-axis. Real data is represented in dots, the solid line is the estimated response trend over time, and the shaded area is the 95% confidence interval.

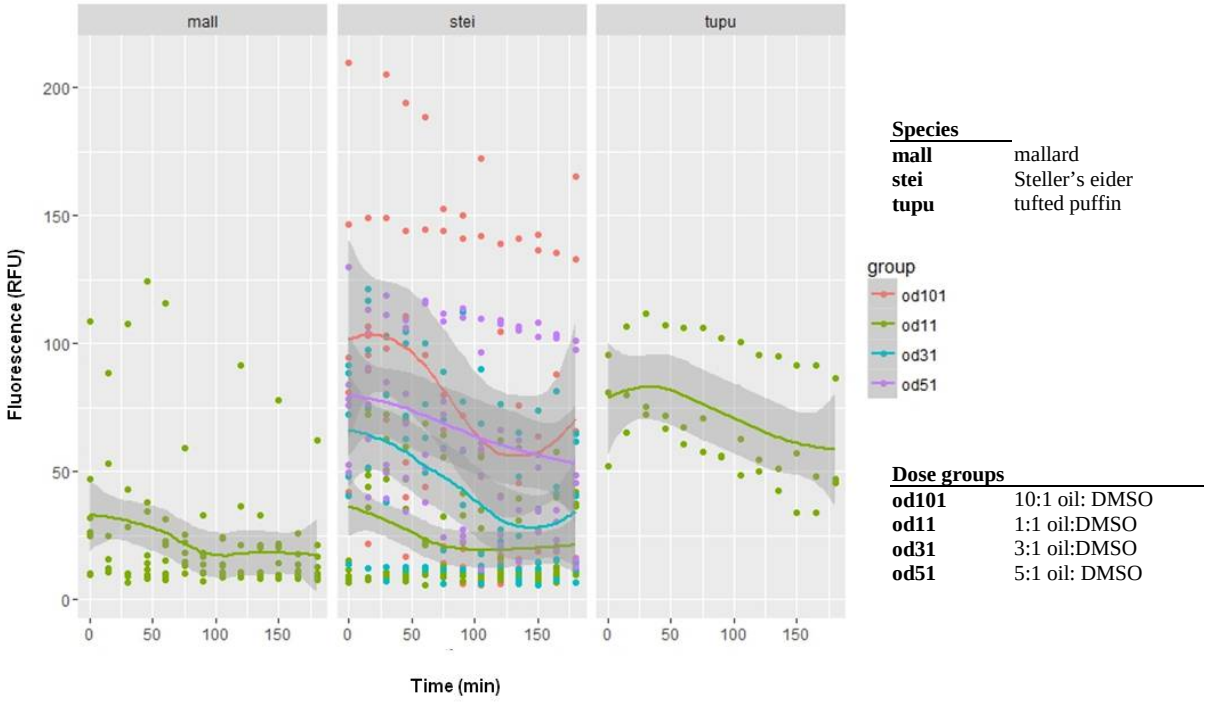

Figure 18: Statistical analysis of tested species EROD results to different amount of Alaska North Slope crude oil dissolved in carrier reagent dimethyl sulfoxide (DMSO). Assay time in minutes is on the x-axis. Real data is represented in dots, the solid line is the estimated response trend over time, and the shaded area is the 95% confidence interval.

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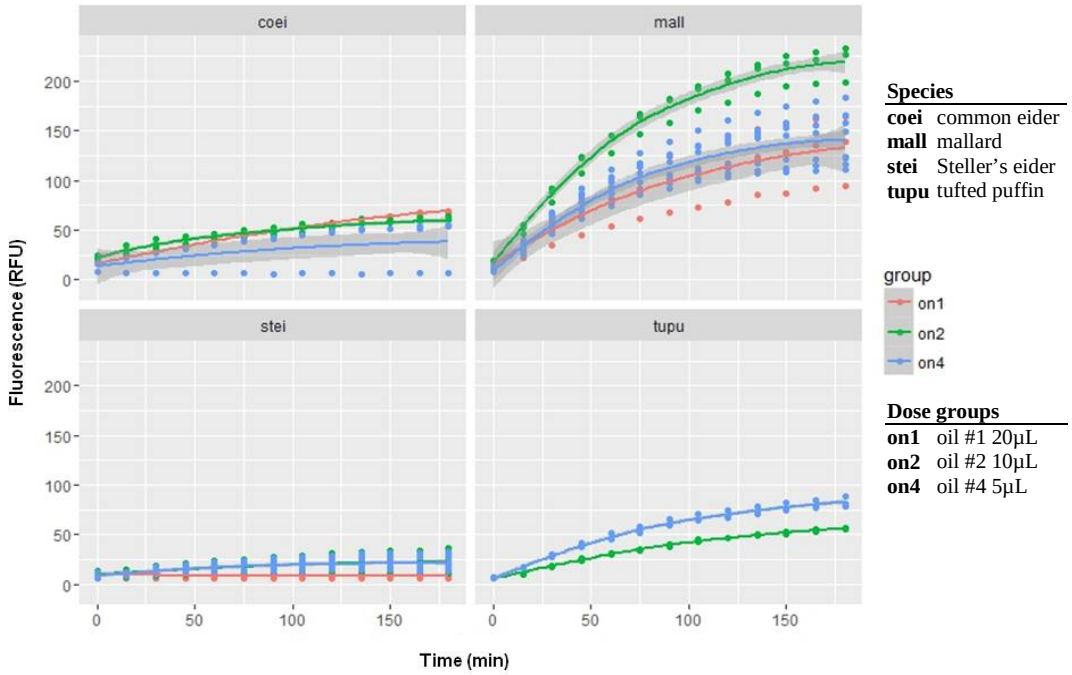

Figure 19: Statistical analysis of tested species EROD results to different amount of media diluted Alaska North Slope crude oil. Assay time in minutes is on the x-axis. Real data is represented in dots, the solid line is the estimated response trend over time, and the shaded area is the 95% confidence interval. Oil doses all in 1:1 oil:DMSO w/2 mL media.

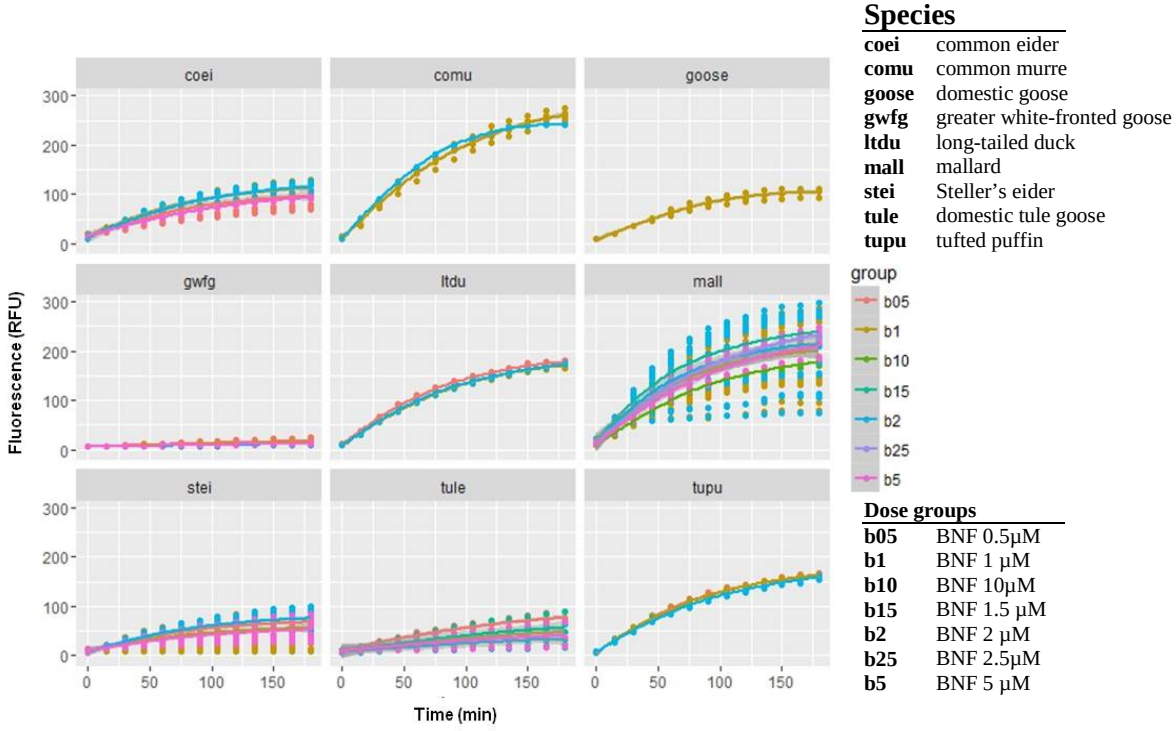

Figure 20: Statistical analysis of tested species EROD results to different amount of BNF. Assay time in minutes is on the x-axis and fluorescence on the y-axis. Real data is represented in dots, the solid line is the estimated response trend over time.

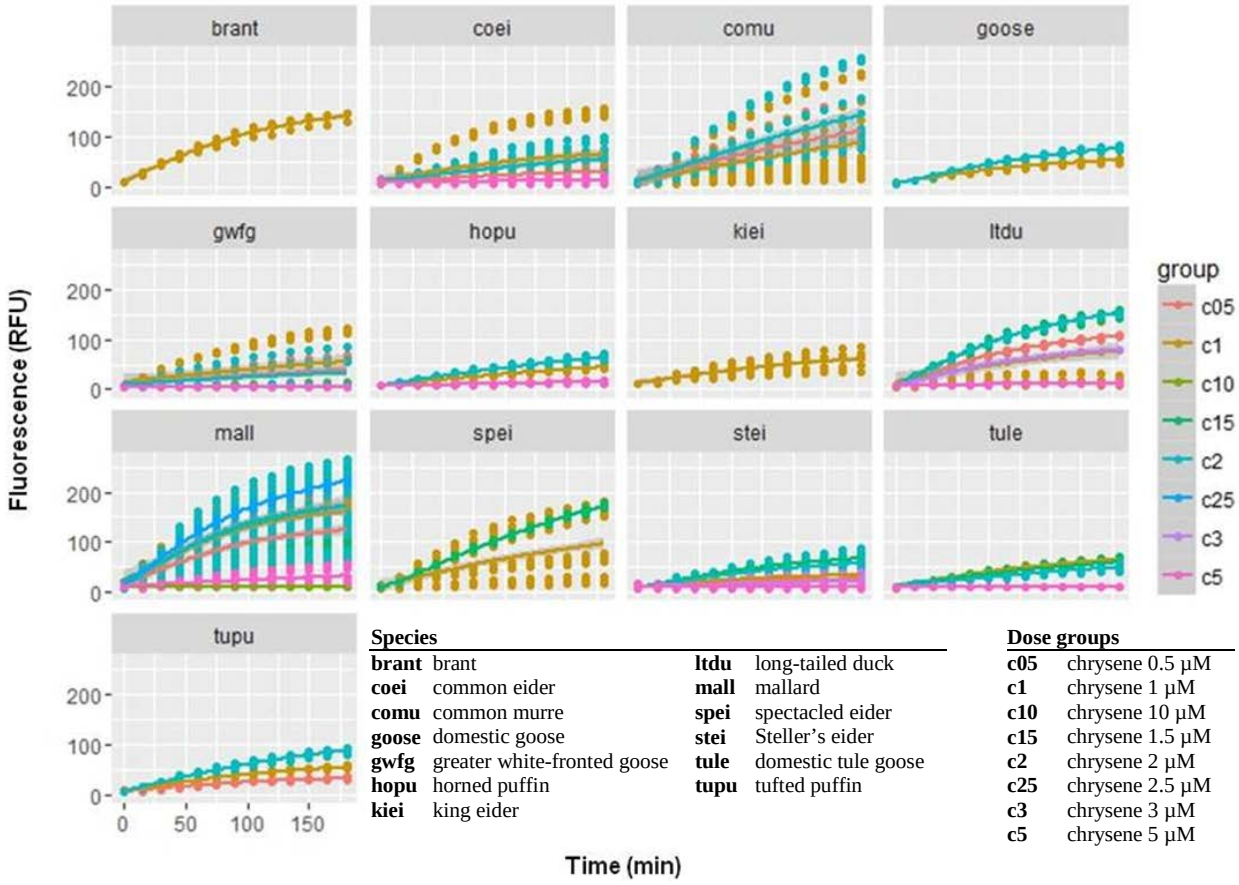

Figure 21: Statistical analysis of tested species EROD results to different amount of chrysene. Assay time in minutes is on the x-axis. Real data is represented in dots, the solid line is the estimated response trend over time.

DISCUSSION

Field Collections

Samples were primarily collected by direct-take during this project to ensure we were obtaining liver samples within the recommended 10-minute timeframe post-mortem. Using direct-take, collecting and sampling all three species within 10 minutes of death is feasible. We found that opportunistic sampling within 10 minutes of death may be challenging and requires a close relationship with a willing subsistence hunter. After spending many hours in the community hunting and talking with hunters, we have begun to form relationships that will likely lead to future collaborations. One such collaboration resulted in an opportunistic sample within the recommended 10 minute post-mortem window in fall 2015. Due to the nature of goose and eider hunting around Barrow, king and common eider samples will be most feasible to obtain in the fall due to the close proximity between hunters and more opportunities for social interactions (Figure 22). Based on discussions with hunters, we may have future opportunities for spring

eider sampling if we are able to team up with a whaling camp. During spring, the whaling camps are set up along ice edges, and eiders are hunted during slow periods. These birds are butchered on site. During spring goose hunts, hunters are spread out and hunt from solitary snow blinds or travel away from town for multi-day hunts. We also discovered there are very few hunters targeting geese in the fall due to their preference for eiders in that season.

Figure 22: Hunters outside their cars hunting flocking eiders near Barrow, AK, August 2015.

A key step to collecting samples less than and within 10 minutes of death was supply preparation and teamwork. This included pre-labeled vials, good organization of supplies that can be accessed easily and quickly and a minimum of two people. While the hunter retrieved the bird, typically by foot, occasionally by kayak, the second person prepared the workstation on the tailgate of the truck (in fall, Figure 23) or in the snow blind (in spring, Figure 24). The experience of the hunter was extremely valuable as he was skilled at targeting birds he could retrieve within the short timeframe and quick in extracting livers.

Figure 23: Slicing a greater white-fronted goose liver for sampling. Robert Sarren (North Slope Borough Department of Wildlife Management) cuts liver samples into smaller pieces in Barrow, AK, August 2015. We commonly used the back gate of the truck as our processing station.

Figure 24: Processing a greater white-fronted goose in a snow blind near Barrow, AK, May 2015. Pictured: Robert Sarren (North Slope Borough Department of Wildlife) and Ann Riddle-Berntsen (UAF CFOS graduate student).

For liver sample collections, we used a small (5 L), portable liquid nitrogen dewar and a large (20 L) dewar. The larger dewar proved more useful for our collections because it was able to hold more vials at temperature for over a month. Dewar size was not an issue during hunts as we often hunted very close to the truck or, during spring hunts, used snow machine sleds to haul gear to snow blinds. The smaller dewar was only able to hold tubes from 2–3 birds due to the volume of liver tubes collected for the time series sampling, limiting the birds we could collect during a day. Also, the temperature charge lasted less than two weeks. The length of temperature charge was very important as there are no liquid nitrogen facilities in Barrow to recharge dewars and we often were in Barrow for two or more weeks per hunt. The smaller dewar will be useful for future collections when fewer vials per bird need to be collected and vials can be poured into the larger dewar at base camp for storage and shipping. Additionally, the smaller dewar could be useful to send out with willing hunters for opportunistic collections due to its small footprint and weight and may be especially useful in more remote locations due to size and ease of handling.

We also found it was easier to store all liver vials in the larger dewar while in Barrow rather than transfer them to an onsite -80ºC freezer. Space was extremely limited in the -80ºC freezer and keeping samples in the dewar allowed for less sampling handling reducing the risk of samples partially thawing. We had no issues transporting samples from Barrow to the laboratory in Seward in the 20 L dewar. The dewars are not regulated as a hazardous material so the dewars could be safely shipped on passenger planes and ground transport.

Teaming with a North Slope Borough employee, who is also a local subsistence hunter, was extremely valuable to this project. His knowledge about the area, birds, and hunting was the key to the success of the liver collections. He also introduced us to local hunters who provided our two opportunistic samples. His support and enthusiasm for the project were passed onto other hunters and members of the community.

CYP1A Activity

We adapted previously published microsome extraction and EROD protocols (Trust et al. 2000a and Miles et al. 2007) for work in our laboratory. To validate the extraction process we tested CYP1A activity in the post-mitochondrial fraction before the 20,800 xg 60-minute spin and the supernatant resulting from that spin (a fraction that would normally be tossed). Each fraction showed some CYP1A activity but was on average 3–10 times lower than microsome fraction activity, confirming that we were correctly using the fraction with the highest CYP1A activity. Our assay validation and quality control included repeat assay of resorufin controls at different concentrations to verify consistency, and repeat assays at the same time points to identify variation among EROD assay runs.

Our EROD results indicate enzyme activity in liver samples through our time series samples, including those frozen 24 hours after death. Our goal of evaluating whether a recommendation could be made to allow an extension on sample collection window from 10 minutes needs further analysis. Due to a high degree of individual variability within and among species, we are exploring the use of a more complex model structure to assess the effects of time on enzyme response. Based on our current findings, we recommend maintaining the current protocol to freeze liver samples within 10 minutes of death if they will be used in enzyme analysis.

In a freezer storage experiment, samples kept at -80°C for over one year appeared to have lost enzyme activity when compared to the same samples extracted within six months of collection. Based on our findings, we recommend processing of samples within the currently used timeframes.

We consider most of our EROD results among species and seasons to represent activities that are similar to those reported for sea duck species sampled in areas considered unexposed to oil in Alaska, although absolute enzyme concentrations are not necessarily directly comparable among these different studies. Trust et al. (2000a) reported average activity of 49.5 and 70.7 pmol/min/mg protein in Barrow's goldeneye and harlequin ducks in areas untouched by the *Exxon-Valdez* oil spill in Prince William Sound, Alaska, and 94.3 and 204.6 pmol/min/mg protein in spill areas. Measuring EROD activity in harlequin ducks in Prince William Sound, Esler et al. (2010) reported activity of 15–25 pmol/min/mg protein in unoiled and 75–100 pmol/min/mg protein in oiled areas. EROD activity in Steller's eiders and harlequin ducks from clean reference site around Unalaska, Alaska, were 10–15 and 50 pmol/min/mg protein, respectively, and activity in birds from industrial areas were 20–50 and 100–275 pmol/min/mg protein, respectively.

In 2014, post-breeding samples showed the highest EROD activity. One king eider and one white-fronted goose were found in poor body condition, but all other birds in this group appeared healthy in post-mortem examination. Future research may involve supplementary tests, such as hormone analysis, to further determine the health of all sampled birds and physiological factors

potentially affecting seasonal variation in enzyme activity. In addition, archived liver and kidney samples could be used for direct measurement of potential contaminants.

Hydrocarbon Sensitivity

We were able to acquire greater white-fronted goose and common eider eggs from the field in 2015 and 2016. Transporting eggs from remote locations can be challenging, and in both years we experienced some breakage during transport. We recommend shipping eggs in multiple shipments, using large amounts of packing material, and using hard sided packing containers.

Liver cell culture extraction, EROD, and CPE assays were already validated for many species in our laboratory. During the course of this project, we expanded the work to new species, which required us to validate detailed protocols and reagents for each new species. Overall, species responded similarly to our protocols with the exception of common murre cells, which required extra washings with PBS 24 hours after seeding due to a large amount of red blood cells present on top of the culture (Figure 25). In addition, common murre cells occasionally had a high response to control doses of DMSO and ethanol (see Appendix C). To account for this background activity, EROD responses from DMSO and ethanol control wells were subtracted when these carrier reagents were used. To remove any dose specific background activity, any observed activity in oil+media controls was subtracted from test dose enzyme results in all tested species.

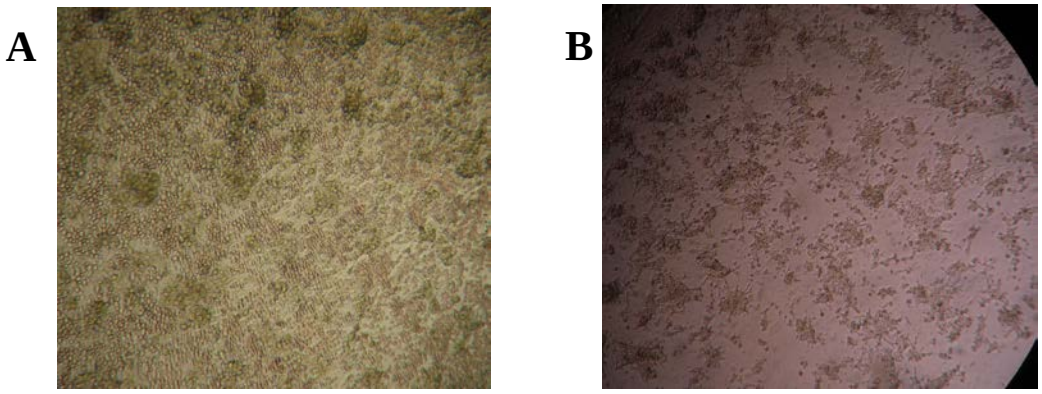

Figure 25: Common murre liver cell culture with red blood cell contamination before (A) and after (B) washing with phosphate-buffered saline (PBS).

CONCLUSIONS

We developed and tested field protocols for collection of liver samples from hunter-killed specimens of Arctic marine birds for EROD analysis. Our study offers guidelines and field sampling protocols and refined techniques for sample collection from hunter-killed specimens. Due to a high degree of individual variability within and among species, we are exploring the use of a more complex model structure to assess the effects of post-mortem sampling time on enzyme response. Based on our findings, we recommend maintaining the current protocol to

freeze liver samples for enzyme analysis within 10 minutes of death. We present a reference data set for three marine bird species from the Arctic: common eider, king eider, and greater white-fronted goose. Common eider and king eider represent species with a strong marine association during the pre-breeding migration to their Arctic nesting grounds, and greater white-fronted goose represents a species with more terrestrial and near shore association. All three species are important subsistence resources for communities in the Arctic.

Our study contributes to the development of future monitoring plans by providing information about the sensitivity of a suite of candidate species to CYP1A induction by crude oil and its reference compounds. Species that rank sensitive to induction are potentially sensitive indicators and ecosystem sentinels (i.e., first species to show evidence of exposure or physiological effects). Our recommendations and conclusions about species sensitivity to hydrocarbons are based on a large amount of liver cell culture EROD data from our target species. Overall, common murre exhibited consistently high responses at tested doses. Long-tailed duck, while not dosed with oil, had a high enzyme response to chrysene and BNF. Tufted puffin had high responses to BNF and oil dosing and a moderate response to chrysene. Common eiders responded high to 1 μM chrysene but had moderate enzyme response to BNF and oil doses. Greater white-fronted geese and surrogate domestic geese were low responders at all test doses. Steller's eider response to chrysene and BNF doses were low but showed a variable (low to high) response to oil dosing. Brant and spectacled eider had high responses to chrysene while horned puffin and king eider responses were low to chrysene. Unfortunately, we were unable to acquire enough cell material to conduct the full dosing regimen.

In summary, common murre and common eider represented species that showed moderate to high responses to a suite of testing doses. Therefore, our findings suggest they are potentially sensitive candidate species to target in monitoring programs. However, our response results suggest that multiple species are likely suitable candidate species so, as a group, marine birds offer a diversity of options for ecological indicator species.

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STUDY PRODUCTS

Riddle A.E. Assessing Hydrocarbon Sensitivity and Establishing Current CYP1A Activity in Select Marine Birds and Waterfowl of the Beaufort and Chukchi Seas. M.S. Thesis. University of Alaska. 2017. *In progress*.

Riddle A.E., Hollmén T.E., Suydam R., Sarren R., and Stimmelmayr R. 2016. Sensitivity to Hydrocarbons and Baselines of Exposure in Marine Birds on the Chukchi and Beaufort Seas. Coastal Marine Institute Annual Review, Anchorage, AK, January 29, 2016 (presentation).

Riddle A.E., Hollmén T.E., Suydam R., Sarren R., and Stimmelmayr R. 2016. Assessing Hydrocarbon Sensitivity and Establishing Current CYP1A Baselines in Arctic Marine Birds and Waterfowl. Alaska Marine Science Symposium, Anchorage, AK, January 25–28, 2016 (poster).

Riddle A.E., Hollmén T.E., Suydam R., Sarren R., and R. Stimmelmayr. 2015. Assessing Hydrocarbon Sensitivity and Establishing Current P450 Baselines in Select Marine Birds and Waterfowl of the Beaufort and Chukchi Seas. Effects of Oil on Wildlife Conference, Anchorage, AK, May 18–22, 2015 (poster).

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APPENDICES

Appendix A: Number of EROD assays performed for species/dose combinations.

Table A1: Number of EROD assays for each species and BNF doses at 24-hour dose exposure. Some replicates may have been removed from analysis due poor culture quality. COEI=common eider, COMU=common murre, goose=domestic goose, GWFG=greater white-fronted goose, LTDU=long-tailed duck, MALL=mallard, STEI=Steller's eider, TUPU=tufted puffin.

Dose	Species	Number of dose repeats
BNF 0.5 µM	COEI	2
	GWFG	2
	LTDU	1
	MALL	3
	STEI	4
	TUPU	1
BNF 1.0 µM	COEI	2
	COMU	2
	Goose	1
	GWFG	2
	LTDU	1
	MALL	7
	STEI	6
	TUPU	1
BNF 1.5 µM	GWFG-tule	1
	MALL	1
BNF 2.0 µM	COEI	2
	COMU	1
	GWFG	2
	LTDU	1
	MALL	6
	STEI	4
	TUPU	1
BNF 2.5 µM	MALL	1
BNF 5.0 µM	COEI	1
	GWFG	2
	MALL	2
	STEI	4
BNF 10.0 µM	MALL	1

Table A2: Number of EROD assays for each species and chrysene doses at 24-hour dose exposure. Some replicates may have been removed from analysis due poor culture quality. COEI=common eider, COMU=common murre, goose=domestic goose, GWFG=greater white-fronted goose, HOPU=horned puffin, LTDU= long-tailed duck, MALL=mallard, STEI=Steller's eider, TUPU=tufted puffin.

Dose	Species	Number of dose repeats	Dose	Species	Number of dose repeats
chrysene 0.5 µM	COEI	4	chrysene 2.0 µM	COEI	3
	COMU	2		COMU	4
	GWFG	3		Goose	1
	LTDU	1		GWFG	3
	MALL	6		HOPU	1
	STEI	5		LTDU	1
	TUPU	1		MALL	10
chrysene 1.0 µM	Brant	1		STEI	7
	COEI	5		TUPU	1
	COMU	7	chrysene 2.5 µM	MALL	1
	Goose	1	chrysene 3.0 µM	COEI	1
	GWFG	3		LTDU	1
	HOPU	1		STEI	3
	KIEI	1	chrysene 5.0 µM	COEI	1
	LTDU	2		GWFG	3
	MALL	10		HOPU	1
	SPEI	4		LTDU	1
	STEI	12		MALL	3
	TUPU	1		STEI	4
chrysene 1.5 µM	GWFG	1	chrysene 10.0 µM	MALL	1
	LTDU	1			
	MALL	4			
	SPEI	1			
	STEI	2			

Table A3: Number of EROD assays for each species and phenathrene doses at 24-hour dose exposure. Some replicates may have been removed from analysis due poor culture quality. MALL= mallard, and STEI= Steller's eider.

Dose	Species	Number of dose repeats	Dose	Species	Number of dose repeats
phenathrene 0.5 µM	MALL	1	phenathrene 5.0 µM	GWFG	1
	STEI	2		STEI	1
phenathrene 1.0 µM	GWFG	1	phenathrene 10.0 µM	GWFG	1
	MALL	1		STEI	2
	STEI	4	phenathrene 50.0 µM	GWFG	1
phenathrene 2.0 µM	MALL	1		STEI	2
	STEI	2			

Table A4: Number of EROD assays for each species and North Slope crude oil doses at 24-hour dose exposure. Some replicates may have been removed from analysis due poor culture quality. COEI=common eider, COMU=common murre, MALL=mallard, STEI=Steller's eider, TUPU=tufted puffin

Dose	Species	Number of dose repeats	Dose	Species	Number of dose repeats
oil 0.5 µL	GWFG	1	oil #1 20 µL 1:1 oil:DMSO w/2 mL media	COEI	1
	STEI	2		MALL	1
oil 1 µL	COEI	4		STEI	3
	COMU	1	oil #2 10 µL 1:1 oil:DMSO w/2 mL media	COEI	1
	GWFG	2		MALL	1
	MALL	2		STEI	4
	STEI	5		TUPU	1
	TUPU	1	oil #4 5 µL 1:1 oil:DMSO wi/2 mL media	COEI	1
oil 5 µL	COEI	4		MALL	2
	COMU	3		STEI	4
	GWFG	1		TUPU	1
	MALL	1	oil:DMSO 1:1 1 µL	MALL	2
	STEI	6		STEI	3
	TUPU	1		TUPU	1
oil 10 µL	COEI	4	oil:DMSO 10:1 1 µL	STEI	2
	COMU	1	oil:DMSO 3:1 1 µL	STEI	2
	GWFG	1	oil:DMSO 5:1 1 µL	STEI	2
	MALL	1			
	STEI	5			
oil 20 µL	GWFG	1			
	STEI	2			

Appendix B: CPE results by species/dose combinations.

Table B1: CPE results in Steller's eider to different concentrations of chrysene doses with 24-hour dose exposure. Results from 2013-2015 for each dose were combined. Semiquantitative response grade 0-4 (0=none to 4=100 % effect). Cell and carrier material (DMSO) controls are also included.

Species	Dose	Dose Concen-tration	Sheet Confluency	Granular--ization	Vacuolar--ization	Rounded up (non-viable)	Cyto-plasmic Swelling	Debris	Asphalt-ing
Steller's eider	chrysene	0.5 µM	20-90%	0	0	0-2	0	1	0
Steller's eider	chrysene	1.0 µM	40-80%	0	0-1	0-2	0	0-1	0
Steller's eider	chrysene	1.5 µM	10-40%	0	0	1-2	0	1-2	0
Steller's eider	chrysene	2.0 µM	10-70%	0	0	1-2	0	1-2	0
Steller's eider	chrysene	3.0 µM	20-60%	0	0	1-2	1-2	1	0
Steller's eider	chrysene	5.0 µM	20-80%	0	1	1-2	0	1-3	0
Steller's eider	DMSO	1:3	40-90%	0	0-2	0-2	0	0-1	0
Steller's eider	cells	none	30-90%	0	0-1	0-2	0	0-1	0

Table B2: CPE results in Steller's eider to different concentrations of BNF doses with 24-hour dose exposure. Results from 2013-2015 for each dose were combined. Semiquantitative response grade 0-4 (0=none to 4=100 % effect). Cell and carrier material (ethanol) controls are also included.

Species	Dose	Dose Concen-tration	Sheet Confluency	Granular--ization	Vacuolar--ization	Rounded up (non-viable)	Cyto-plasmic Swelling	Debris	Asphalt-ing
Steller's eider	BNF	0.5 µM	30-70%	0	0-1	1-2	0	0-2	0
Steller's eider	BNF	1.0 µM	20-80%	0	0-1	0-3	0	0-2	0
Steller's eider	BNF	2.0 µM	20-70%	0	0-1	1-3	0	0-2	0
Steller's eider	BNF	5.0 µM	50-60%	0	0-1	1-3	0	0-2	0
Steller's eider	ethanol	1:1	60-80%	0	0-1	0-2	0	0-1	0
Steller's eider	cells	none	30-90%	0	0-1	0-2	0	0-1	0

Table B3: CPE results in Steller's eider to different concentrations of phenanthrene doses with 24-hour dose exposure. Results from 2015 for each dose were combined. Semiquantitative response grade 0-4 (0=none to 4=100 % effect). Cell and carrier material (ethanol) controls are also included.

Species	Dose	Dose Concen-tration	Sheet Confluency	Granular--ization	Vacuolar-ization	Rounded up (non-viable)	Cyto-plasmic Swelling	Debris	Asphalt-ing
Steller's eider	phen-anthrene	0.5 µM	60-80%	0	0	1-3	0	1	0
Steller's eider	phen-anthrene	1.0 µM	50-80%	0	0-1	1-3	0	0-2	0
Steller's eider	phen-anthrene	2.0 µM	50-80%	0	0	3	1-2	0	0
Steller's eider	phen-anthrene	5.0 µM	50-60%	0	0	3-4	0	2	0
Steller's eider	phen-anthrene	10.0 µM	obscured by debris	0	0	0	0	0	3-4
Steller's eider	phen-anthrene	50.0 µM	obscured by debris	0	0	0	0	0	3-4
Steller's eider	ethanol	1:1	60-80%	0	0-1	0-2	0	0-1	0
Steller's eider	cells	none	30-90%	0	0-1	0-2	0	0-1	0

Table B4: CPE results different amount of neat Alaska North Slope crude oil dose and cell control with 24-hour dose exposure in Steller's eider. Results from 2015 for each dose were combined. Semiquantitative response grade 0-4 (0=none to 4=100 % effect).

Species	Dose	Dose Concen-tration	Sheet Confluency	Granular--ization	Vacuolar--ization	Rounded up (non-viable)	Cyto-plasmic Swelling	Debris	Asphalt-ing
Steller's eider	NS crude oil	0.5 µL	50-80%	0	0-1	0-2	0	0-1	0
Steller's eider	NS crude oil	1.0 µL	70-80%	0	0-1	0-2	0	0-1	0
Steller's eider	NS crude oil	5.0 µL	50-80%	0	0-1	0-3	0	0-1	0
Steller's eider	NS crude oil	10.0 µL	50-80%	0	0-1	1-3	0	0-1	0
Steller's eider	NS crude oil	20.0 µL	50-70%	0	0-1	2	0	0	0
Steller's eider	cells	none	30-90%	0	0-1	0-2	0	0-1	0

Table B5: CPE results to different amount of media diluted Alaska North Slope crude oil dose and cell and media control with 24-hour dose exposure in Steller's eider. 100 µL of each diluted dose was added to the well. Doses consisted of: #1 20 µL 1:1 oil:DMSO with 2 mL media, #2 10 µL 1:1 oil:DMSO with 2 mL media, #4 5 µL 1:1 oil:DMSO with 2 mL media. Semiquantitative response grade 0-4 (0=none to 4=100 % effect).

Species	Dose	Dose Concen-tration	Sheet Confluency	Granular--ization	Vacuolar--ization	Rounded up (non-viable)	Cyto-plasmic Swelling	Debris	Asphalt-ing
Steller's eider	NS crude oil	#1	10-70%	0	0	3-4	0	2-3	0
Steller's eider	NS crude oil	#2	60-70%	0	0-1	1-3	0	1-2	0
Steller's eider	NS crude oil	#4	0-80%	0	0-1	1-2	0	1-2	0
Steller's eider	cells	none	30-90%	0	0-1	0-2	0	0-1	0

Table B6: CPE results to different amount of DMSO diluted Alaska North Slope crude oil doses and cell and media control with 24-hour dose exposure in Steller's eider. Semiquantitative response grade 0-4 (0=none to 4=100 % effect).

Species	Dose	Dose Concen-tration	Sheet Confluency	Granular--zation	Vacuolar--ization	Rounded up (non-viable)	Cyto-plasmic Swelling	Debris	Asphalt-ing
Steller's eider	NS crude oil	oil:DMSO 1:1	70-80%	0	0	0-1	0	2-4	0
Steller's eider	NS crude oil	oil:DMSO 3:1	10-50%	0	0	1	0	1	0
Steller's eider	NS crude oil	oil:DMSO 5:1	10-50%	0	0	1	0	1	0
Steller's eider	NS crude oil	oil:DMSO 10:1	10-50%	0	0	1	0	1	0
Steller's eider	cells	none	30-90%	0	0-1	0-2	0	0-1	0

Table B7: CPE results in Steller's eider to 1.0 µM chrysene dose with 12, 24, and 36-hour dose exposure. Semiquantitative response grade 0-4 (0=none to 4=100 % effect).

Species	Dose	Dose Concen-tration	Sheet Confluency	Granular--ization	Vacuolar--ization	Rounded up (non-viable)	Cyto-plasmic Swelling	Debris	Asphalt-ing
Steller's eider	12hr-cells	none	80-90%	0	0	1-2	0	0-1	0
Steller's eider	24hr-cells	none	60-90%	0	0-1	0	0-2	0-1	0
Steller's eider	36hr-cells	none	60-90%	0	0-1	0-2	0	0	0
Steller's eider	12hr-chrysene	1.0 µM	70-80%	0	0	1-2	0	0-1	0
Steller's eider	24hr-chrysene	1.0 µM	50-80%	0	0-1	1-2	0	0-1	0
Steller's eider	36hr-chrysene	1.0 µM	50-90%	0	0-2	0-2	0	0-1	0
Steller's eider	12hr-DMSO	1:3	70-80%	0	0	1-2	0	0-1	0
Steller's eider	24hr-DMSO	1:3	50-80%	0	0-1	0-2	0	0-1	0
Steller's eider	36hr-DMSO	1:3	50-90%	0	0-2	0	0-2	0-1	0

Table B8: CPE results in tufted puffin to different concentrations of chrysene doses with 24-hour dose exposure. Cell and carrier material (DMSO) controls are also included. Semiquantitative response grade 0-4 (0=none to 4=100 % effect).

Species	Dose	Dose Concen-tration	Sheet Confluency	Granular--ization	Vacuolar--ization	Rounded up (non-viable)	Cyto-plasmic Swelling	Debris	Asphalt-ing
Tufted puffin	chrysene	0.5 µM	70-80%	0	0	1	0	1	0
Tufted puffin	chrysene	1.0 µM	70-80%	0	0	1	0	1	0
Tufted puffin	chrysene	2.0 µM	70-80%	0	0	1	0	1	0
Tufted puffin	DMSO	1:3	70-80%	0	0	1	0	1	0
Tufted puffin	cells	none	70-80%	0	0	1	0	1	0

Table B9: CPE results in tufted puffin to different concentrations of BNF doses with 24-hour dose exposure. Cell and carrier material (ethanol) controls are also included. Semiquantitative response grade 0-4 (0=none to 4=100 % effect).

Species	Dose	Dose Concen-tration	Sheet Confluency	Granular-ization	Vacuolar-ization	Rounded up (non-viable)	Cyto-plasmic Swelling	Debris	Asphalt-ing
Tufted puffin	BNF	0.5 µM	70-80%	0	0	1	0	1	0
Tufted puffin	BNF	1.0 µM	70-80%	0	0	1-2	0	1	0
Tufted puffin	BNF	2.0 µM	70-80%	0	0	1-2	0	1	0
Tufted puffin	ethanol	1:1	70-80%	0	0	1	0	1	0
Tufted puffin	cells	none	70-80%	0	0	1	0	1	0

Table B10: CPE results in ten marine bird species and mallard control to 1.0 µM chrysene dose with 24-hour dose exposure. Results from 2013-2016 for each species were combined. Semiquantitative response grade 0-4 (0=none to 4=100 % effect).

Species	Dose	Dose Concen-tration	Sheet Confluency	Granular-ization	Vacuolar-ization	Rounded up (non-viable)	Cyto-plasmic Swelling	Debris	Asphalt-ing
Brant	chrysene	1.0 µM	40%	1	0	0	0	1	0
Common eider	chrysene	1.0 µM	70-100%	0	0-3	1-2	0	0-1	0
Common murre	chrysene	1.0 µM	20-70%	0	0-1	1-3	0	0-2	0
Goose	chrysene	1.0 µM	20-80%	0-1	1-3	0-1	0	0-1	0
Greater white-fronted goose	chrysene	1.0 µM	10-25%	0	1	1	0	1	0
Horned puffin	chrysene	1.0 µM	40-50%	0	0	0	0	0	0
King eider	chrysene	1.0 µM	30%	0	0	2-3	0	1	0
Long-tailed duck	chrysene	1.0 µM	40-50%	0	0	1-2	0	0	0
Mallard	chrysene	1.0 µM	10-95%	0-2	0-3	0-3	0	0-1	0
Spectacled eider	chrysene	1.0 µM	15-50%	0	0	0-2	0	0-2	0
Steller's eider	chrysene	1.0 µM	40-80%	0	0-1	0-2	0	0-1	0
Tufted puffin	chrysene	1.0 µM	70-80%	0	0	1	0	1	0

Table B11: CPE results in six marine bird species and mallard control to 1.0 µM BNF dose with 24-hour dose exposure. Results from 2013-2016 for each species were combined. Semiquantitative response grade 0-4 (0=none to 4=100 % effect).

Species	Dose	Dose Concen-tration	Sheet Confluency	Granular-ization	Vacuolar-ization	Rounded up (non-viable)	Cyto-plasmic Swelling	Debris	Asphalt-ing
Common eider	BNF	1.0 µM	70-80%	0	1-3	1-2	0	0	0
Common murre	BNF	1.0 µM	20-80%	0	0	1-2	0	1-2	0
Goose	BNF	1.0 µM	15-80%	0-1	0	0-1	0	1	0
Greater white-fronted goose	BNF	1.0 µM	10%	0	0	1-2	0	1	0
Long-tailed duck	BNF	1.0 µM	40%	0	0	1-2	0	0	0
Mallard	BNF	1.0 µM	10-90%	0-2	0-3	1-3	0	0-1	0
Steller's eider	BNF	1.0 µM	20-80%	0	0-1	0-3	0	0-2	0
Tufted puffin	BNF	1.0 µM	70-80%	0	0	1-2	0	1	0

Table B12: CPE results to 1.0 µL neat Alaska North Slope crude oil dose with 24-hour dose exposure in four target species and one control species. Semiquantitative response grade 0-4 (0=none to 4=100 % effect).

Species	Dose	Dose Concentration	Sheet Confluency	Granular-ization	Vacuolar-ization	Rounded up (non-viable)	Cyto-plasmic Swelling	Debris	Asphalt-ing
Common eider	NS crude oil	1.0 µL	70-100%	0	1-3	1-2	0	0	0
Common murre	NS crude oil	1.0 µL	40-50%	0	0	3-4	0	0	0
Greater white-fronted goose	NS crude oil	1.0 µL	10%	0	0	1-2	0	1	0
Mallard	NS crude oil	1.0 µL	80-90%	0	0	1	0	1	0
Steller's eider	NS crude oil	1.0 µL	70-80%	0	0-1	0-2	0	0-1	0
Tufted puffin	NS crude oil	1.0 µL	70-80%	0	0	2	0	1	0

Table B13: CPE results to 5.0 µL neat Alaska North Slope crude oil dose with 24-hour dose exposure in four target species and one control species. Semiquantitative response grade 0-4 (0=none to 4=100 % effect).

Species	Dose	Dose Concen-tration	Sheet Confluency	Granular--ization	Vacuolar--ization	Rounded up (non-viable)	Cyto-plasmic Swelling	Debris	Asphalt-ing
Common eider	NS crude oil	5.0 µL	70-100%	0	1-3	1-2	0	0	0
Common murre	NS crude oil	5.0 µL	20-50%	0	0	2-4	0	1	0
Greater white-fronted goose	NS crude oil	5.0 µL	10%	0	0	1-2	0	1	0
Mallard	NS crude oil	5.0 µL	90%	0	1-2	1	0	1-2	0
Steller's eider	NS crude oil	5.0 µL	50-80%	0	0-1	0-3	0	0-1	0
Tufted puffin	NS crude oil	5.0 µL	70-80%	0	0	2-3	0	1	0

Appendix C: Statistical analysis of EROD results for DMSO and ethanol controls.

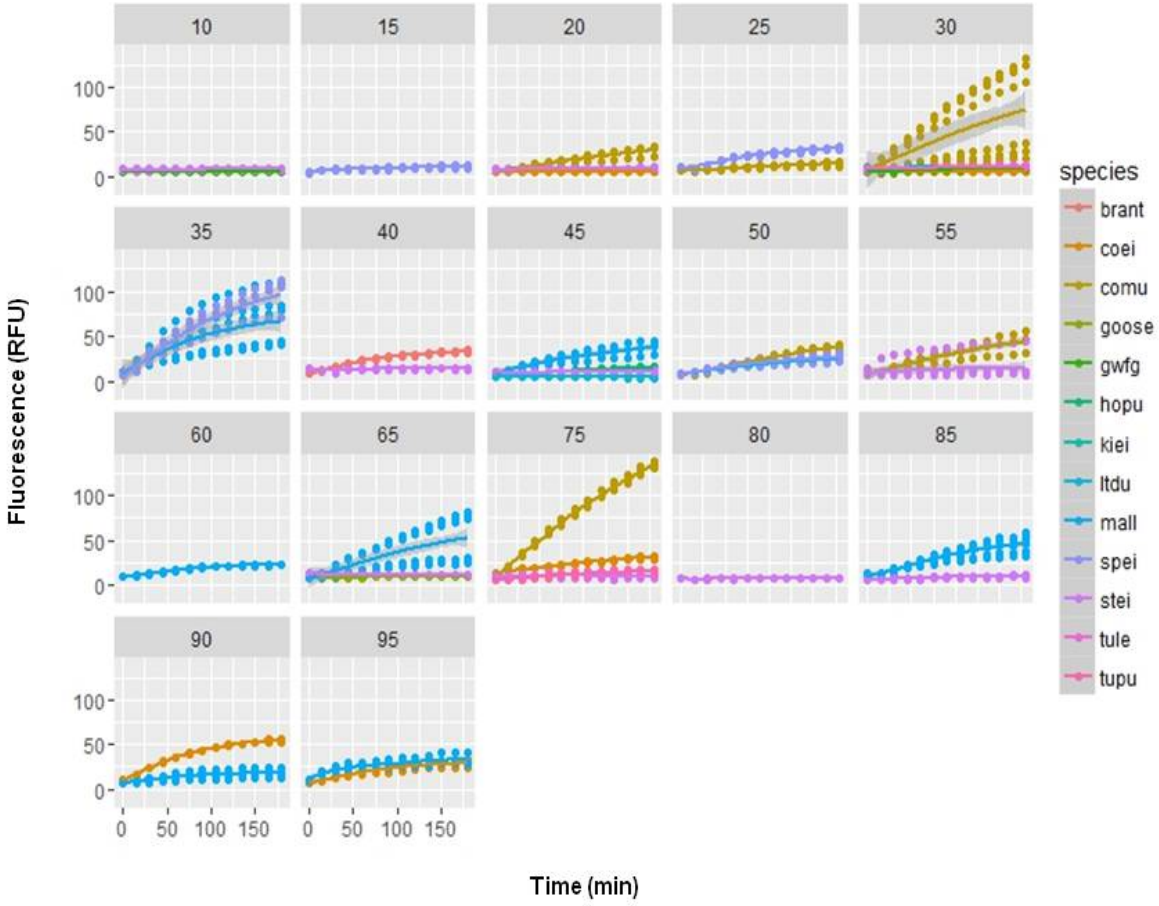


Figure C1: Statistical analysis of tested species DMSO control EROD results by estimated percent of cell coverage from 10-95% (i.e., 10=10% cell coverage in the assay well). Assay time in minutes is on the x-axis and fluorescence on the y-axis. Real data is represented in dots, the solid line is the estimated response trend over time, and the shaded area is the 95% confidence interval. Species abbreviations: brant-brant, coei-common eider, comu-common eider, goose-domestic goose, gwfg-greater white-fronted goose, hopu-horned puffin, kiei-king eider, ltdu-long-tailed duck, mall-mallard, spei-spectacled eider, stei-Steller's eider, tule-domestic tule goose, tupu-tufted puffin.

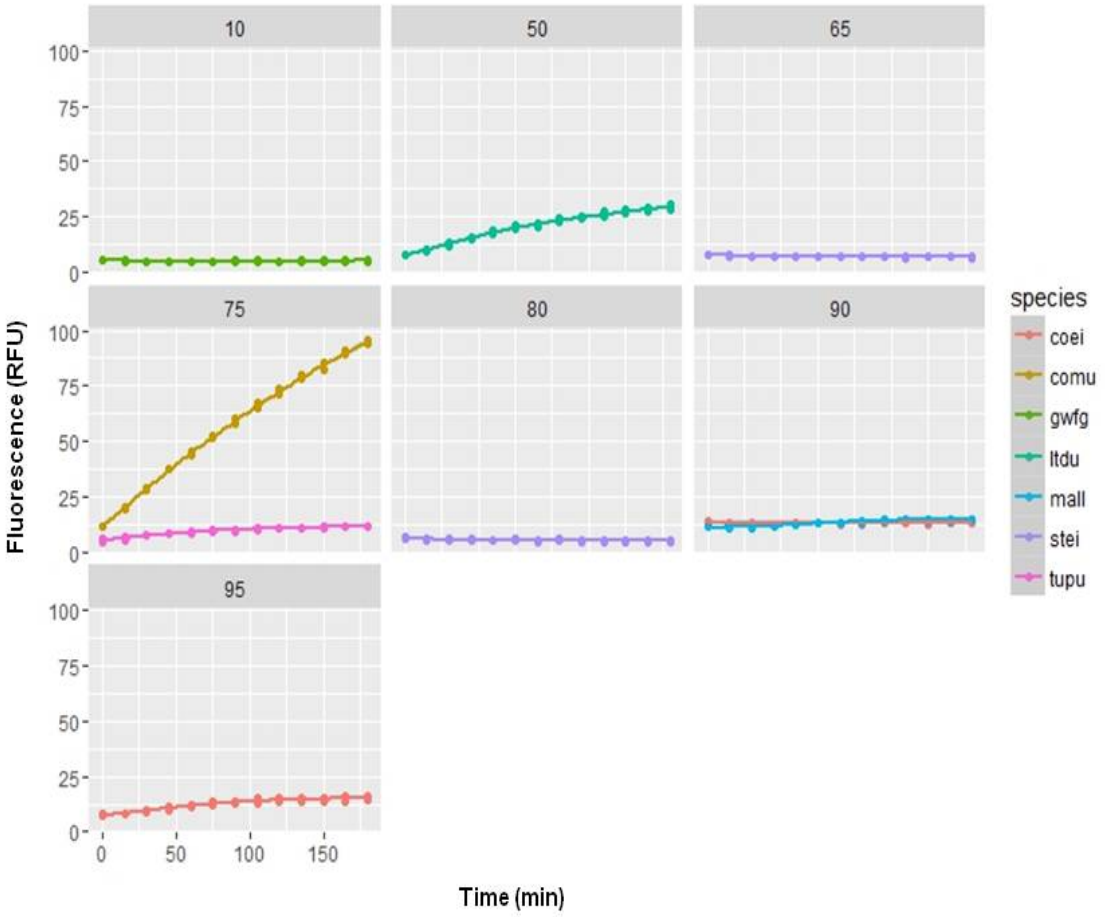
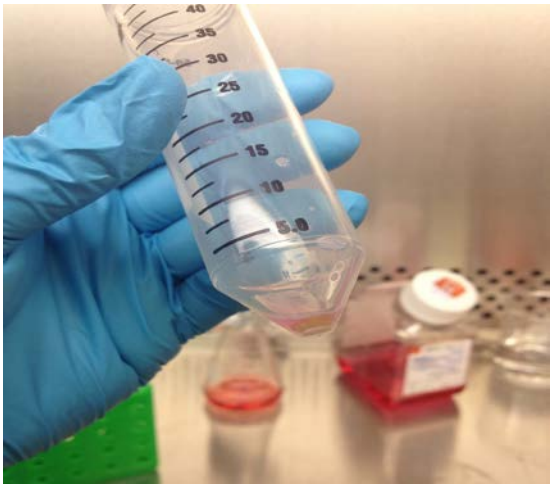


Figure C2: Statistical analysis of tested species ethanol control EROD results by estimated percent of cell coverage from 10-95% (i.e., 10=10% cell coverage in the assay well). Assay time in minutes is on the x-axis and fluorescence on the y-axis. Real data is represented in dots, the solid line is the estimated response trend over time. Species abbreviations: coei-common eider, comu-common eider, gwfg-greater white-fronted goose, ltdu-long-tailed duck, mall-mallard, stei-Steller's eider, tupu-tufted puffin.

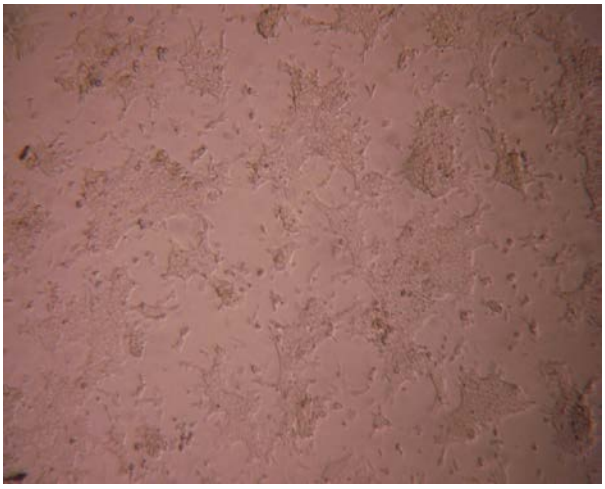
Appendix D: Project images.

Eggs from various species incubating.

Common murre egg shells.



Pellet of liver cells during the extraction process.



Culture of Steller's eider liver cells.

Snow machine with sled to haul gear to the snow blind. Barrow, AK, May 2015.

Ann Riddle-Berntsen (UAF CFOS Graduate Student) and Robert Sarren (NSB-DWM) in a snow blind near Barrow, AK, May 2015.

Goose decoys used during spring hunts. Barrow, AK, May 2015.

Robert Sarren (NSB-DWM) weighing a common eider post mortem. Barrow, AK, October 2014.

Hunters wait in their cars for flocks of eiders. Barrow, AK, August 2015.

Field truck loaded with supplies including a 20 L liquid nitrogen dewar, tote with sampling supplies, and a kayak to retrieve birds that fall into the water. Barrow August 2015.

5 L liquid nitrogen dewar attached to the back of the 4-wheeler. Barrow, AK, August 2014.

Robert Sarren and Ryan Klimstra (both from NSB-DWM) looking for greater white-fronted geese near Barrow, AK, August 2014.

A flock of greater white-fronted geese near Barrow, AK, August 2015.

Laboratory station in NSB-DWM bunk house. We used this space to process blood and organize samples and supplies post hunts. Barrow, AK, August 2014.

Looking for flocks of eiders near Barrow, AK, August 2015.

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The Department of the Interior Mission

As the Nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This includes fostering the sound use of our land and water resources, protecting our fish, wildlife and biological diversity; preserving the environmental and cultural values of our national parks and historical places; and providing for the enjoyment of life through outdoor recreation.The Department assesses our energy and mineral resources andworks to ensure that their development is in the best interests of all our people by encouraging stewardship and citizen participation in their care. The Department also has a major responsibility for American Indian reservation communities and for people who live in island communities.

The Bureau of Ocean Energy Management

The Bureau of Ocean Energy Management (BOEM) works to manage the exploration and development of the nation's offshore resources in a way that appropriately balances economic development, energy independence, and environmental protection through oil and gas leases, renewable energy development and environmental reviews and studies.