Completion Report

Period: 2/1/2014 - 1/31/2015

Project: R/HCE/PD-2 - *Monitoring pregnancy impacts on Southern Resident Killer Whales*

STUDENTS SUPPORTED

Lundin, Jessica, jlundin2@uw.edu, University of Washington, Biology, status: cont, field of study: Biology, advisor: Samuel Wasser, degree type: PhD, degree date: 2015-05-01, degree completed this period: No

Student Project Title:

Persistent Organic Pollutants (POPs) in Southern Resident killer whale scat samples: a longitudinal evaluation of modulation by prey availability, contaminant accumulation and mobilization patterns, and deleterious biological effects including reproductive toxicity

Involvement with Sea Grant This Period:

PhD student conducting dissertation research

Post-Graduation Plans:

Post-Doc - NOAA

CONFERENCES / PRESENTATIONS

No Conferences / Presentations Reported This Period

ADDITIONAL METRICS

P-12 Students Reached:	**P-12 Educators Trained:**
Participants in Informal Education Programs:	**Volunteer Hours:**
Acres of coastal habitat protected, enhanced or restored:	**Resource Managers who use Ecosystem-Based Approaches to Management:**
Annual Clean Marina Program - certifications:	**HACCP - Number of people with new certifications:**

ECONOMIC IMPACTS

No Economic Impacts Reported This Period

SEA GRANT PRODUCTS

No Sea Grant Products Reported This Period

HAZARD RESILIENCE IN COASTAL COMMUNITIES

No Communities Reported This Period

ADDITIONAL MEASURES

Number of stakeholders modifying	Sustainable Coastal Development

practices:

# of coastal communities:

PARTNERS

No Partners Reported This Period

IMPACTS AND ACCOMPLISHMENTS

Title:
Type: accomplishment
Description:
Recap: *none*
Comments: *none*
Related Partners: ,

PUBLICATIONS

No Publications Reported This Period

OTHER DOCUMENTS

No Documents Reported This Period

LEVERAGED FUNDS

Type: influenced Period: 2014-08-01: : 2015-05-30Amount: $12000
Purpose:
Lab costs
Source: JISAO

Type: influenced Period: 2014-06-01: : 2014-10-30Amount: $9000
Purpose:
Field Costs
Source: Conservation, Research and Education Opportunities International (CREOi)

COMPLETION NARRATIVE

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Monitoring pregnancy impacts on Southern Resident Killer Whales (R/HCE/PD-2)

UW Principal Investigator:
Dr. Samuel Wasser, Endowed Chair and Director, Center for Conservation Biology**,** University of Washington.

2014 Project Overview:

The Southern Resident killer whale (SRKW; Orcinus orca) population is important to the ecology, culture and economy of the Pacific Northwest. The SRKW experienced an unexplained 20% decline in their population census in the late 1990s from which they have been unable to recover. The purpose of this study is to use noninvasive physiological and genetic measures to partition the relative impacts of the three major threats implicated in the ESA listing of the SRKW in 2005: precipitous declines in Chinook salmon (Oncorhynchus tshawytscha), their preferred prey; excessive exposures to environmental contaminants due to bio-accumulation of toxicants up the food chain; and disturbance from proximity and noise associated with whale watch boats.

Sampling was conducted from June 1 to October 20, 2014 in the Salish Sea, concentrating in the Haro Strait, the Strait of Georgia and the eastern portion of the Strait of Juan de Fuca.

We located a total of 60 southern resident killer whales (SRKWs) fecal samples, 56 of which were collected in US waters (see Figure 1) during approximately 100 hours of active sampling in the presence of the whales (see Figure 2). Our goal was to acquire samples from all whales (current population during survey was 78 animals). However, special emphasis was placed on sampling reproductive age females during the 2014 field season. Throughout the sampling period, we made every effort to follow different groups on different sampling days.

Non-invasive Survey Technique. SRKW fecal samples are collected from a boat using highly trained scat detection dogs to locate fresh killer whale scat floating on the water's surface. The remarkable olfactory ability of these highly reward-driven Conservation Canines (CK9) enable us to collect large sample sizes from over a quarter mile away from the whales. Previous work by our Center focused on measuring hormone levels in these samples to evaluate the effects of decreased prey and increased boat traffic on the health of the whales (Ayres et al. 2012). The present study extends this work to include the validation and application of reliable hormone-based pregnancy indicators (Wasser et al in review), the occurrence and causes of pregnancy failure and measurement of toxicants excreted in whale scat (Lundin et al in review).

The crew uses a range finder with a (750 m range) to maintain a 200 m distance from the whales whenever possible. The rare occasions when the team was unable to move out of the path of the whales were recorded in the take section under our NOAA permit (n=13). Only one close approach was associated with a change in whale behavior, a single tail slap from an individual whale. Changes of behaviors were defined as changes in overall behavior states such as travel, rest, forage, and/or surface active behaviors.

Sample Collection. Samples are scooped off the water surface using 1-liter wide-mouthed polypropylene beakers and transferred to 50-mL polypropylene graduated tubes. All samples are centrifuged and decanted immediately upon collection. The sample pellet is stored on dry-ice to minimize any potential post-excretion metabolism by fecal bacteria. At the end of each day, all samples are placed in a -20° C freezer.

DNA Collection Protocol. A portion of each SRKW sample is separated into a labeled 5-mL polypropylene tube for DNA-based sex determination and individual identification. DNA analyses on the SRKW samples are conducted at the Northwest Fisheries Science Center, NOAA, in Seattle, WA. Individual identification is based on analyses of ~90 single nucleotide polymorphisms (SNPs) while sex determination is based on the SRY/ZFX markers. Samples that do not yield killer whale DNA are excluded from any further analyses.

Hormone and toxicant analyses. All samples > 0.5 g in mass are analyzed for glucocorticoid, thyroid, aldosterone, progesterone and testosterone hormone metabolites using radioimmunoassay methods described by Wasser et al. (2000, 2010). Samples ≥ 15 ml of feces are also examined PCB, PBDE and DDT congeners using methods described by Lundin et al (in review).

All samples collected during 2014 are currently undergoing laboratory analyses.

Primary Objectives:

1) Understand the cumulative impacts of nutritional stress and toxicants on pregnancy success in the SRKW
2) Use these results to build an outreach program aimed at First Nation Communities that rely heavily on salmon for their sustenance in the Puget Sound region.

Recent Accomplishments:

Lab Validations. We developed and validated tools to measure glucocorticoid (GC), thyroid (T3), progesterone (P4) and testosterone (T) hormones from killer whale feces. Progesterone and testosterone collectively provide valuable indices of pregnancy occurrence and health in numerous species. Concentrations of both hormones typically increase following conception, increasing several-fold during gestation, and then rapidly decline to

pre-conception levels at or near parturition. By contrast, GC and T3 concentrations used to index physiological stress are much more responsive to changing environmental conditions. GCs rapidly rise in response to poor nutrition and psychological stressors, mobilizing glucose to provide energy to deal with the immediate emergency (Sapolsky et al 2000). Thyroid hormone (triiodothyronine, T3), on the other hand, has a more conservative response to nutritional stress by adjusting metabolism. When prey shortages are first encountered, T3 initially rises (along with GC) allowing the body to use all available fuel to search for food. However, T3 abruptly declines if poor food conditions persist, lowering metabolism to prevent the body from exhausting its remaining reserves (Douyon and Schteingart 2002; Holtorf et 2012). T3 also maintains a low metabolism under good food conditions when increased growth is required (e.g., the need to accumulate blubber stores in preparation for the relatively lean winter). The T3 response also tends to be slower and more enduring compared to GC. We also validated measures of PCB, PBDE and DDT congeners in scat by comparing levels to those in tissue samples from the same individual.

Potential Pregnancies. We validated pregnancy measures using fecal progesterone (P4) and testosterone (T) hormone metabolites in the SRKW and used this to estimate pregnancy success and failure. Figure 1 shows that pregnancy can be confirmed by fecal metabolites of progesterone (P4) above 2000 ng/g and testosterone (T) above 100 ng/g within 12 months of parturition in genotyped females. The one sample collected on a confirmed pregnant female in her first trimester had P4 levels below the 2000 ng/g threshold. Pregnancy was confirmed in all of these cases by subsequent observation of a newborn prior to the end of their 17-18 month gestation, with stage of gestation determined for each sample by backdating from the calf's estimated birth date.

Ten genotyped females were characterized as having 14 apparent miscarriages between 2007-2013, based on significantly elevated progesterone concentrations above 2000 ng/g that were not followed by a live birth. These females quantitatively separated into two distinct groups: one with T concentrations above 100 ng/g feces (high T) (7 females, 7 apparent miscarriages) and the other with T concentrations below 50ng/g feces (low T) (3 females, 7 apparent miscarriages). We respectively refer to these apparent miscarriages as high T and low T miscarriages. The 7 high T miscarriages out of 14 possible pregnancies (50%) likely occurred mid to late gestation, based on their relatively high progesterone concentrations. Although miscarriages are common among female mammals, most tend to occur in the early stages of pregnancy when they are less costly (Wasser and Barash 1983). The number of presumed late miscarriages in the SRKWs is thus particularly alarming owing to their considerable reproductive cost to the female. Indeed, autopsy of the most recent adult female that died in this population (J32) suggested that the female died as a result of infection from an incomplete abortion. *Data from 2014 field season are still being analyzed and therefore do not include the 3 new babies; J51, J52 and L121.*

To our knowledge, no other method is available to acquire such information and partition these pressures to address cumulative effects to this system. We are now using predictive models to help elucidate the primary risk

factors to this population, and evaluate interactions between these effects. Endocrine results suggest that the High T “Pg” females had significantly poorer nutrition (significantly higher GC and lower T3) than did either confirmed pregnant or Low T “Pg” females in our population.

We also examined toxin levels in these females. Our sample size of miscarrying females was limited to just two individuals due to the relatively large volume of sample that is required to accurately measure toxicants in feces. However, both of those samples had significantly higher levels of PCB congeners compared to those from confirmed pregnant females (Lundin et al in prep). Since the toxicant work using all available samples showed that toxicants were highest in feces when prey abundance was lowest (Lundin et al in review), it is possible that these miscarriages resulted from cumulative impacts of excessive toxicant exposure during times of nutritional stress. Continued longitudinal sampling should eventually allow us to temporally relate the physiologic measures reflecting, for example, variation in salmon abundance, toxicant levels and boat traffic to endpoint measures such as annual mortality and successful birth outcomes.

Figure 1. . *Pregnancy and miscarriage indices using (A) progesterone concentrations across gestation in confirmed births and (B) progesterone and testosterone concentrations relative to all reproductive classes.*

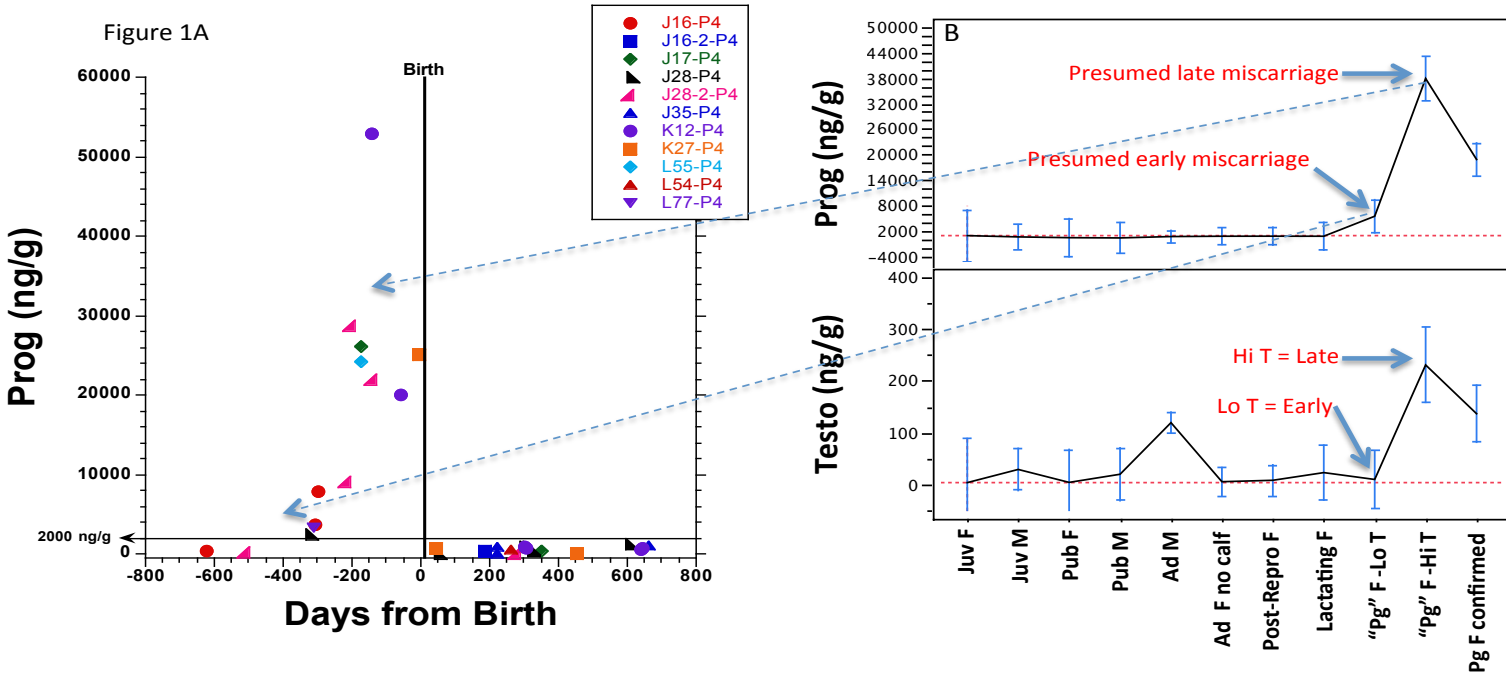

Figure 2. 2014 US killer whale fecal samples collected by Moja from June 1st thru October 22nd.