Completion Report

Period 2/1/2013 - 1/31/2014

Project R/LME-4 - Non-invasive Physiological Monitoring of Southern Resident Killer Whales

STUDENTS SUPPORTED

Lundin, Jessica, jlundin2@uw.edu, University of Washington, Biology, status cont, field of study Environmental Toxicology, advisor S. Wasser, degree type PhD, degree date 2014-09-01, degree completed this period No
Student Project Title Persistent Organic Pollutant Contamination in the Puget Sound Ecosystem A Novel Approach to Monitor Exposure and Evaluate Risk

Involvement with Sea Grant This Period phd student

Post-Graduation Plans Post-doc

Mastumoto-Hervol, Makie, quinchee@gmail.com, University of Wisconsin, Biology, status new, field of study Geography, no advisor, degree type BS, no degree date, degree completed this period No
Student Project Title none

Involvement with Sea Grant This Period We applied to have Makie on our research team as a JISAO intern. This is a competitive internship paid through the JISAO program and NOAA where students are matched with mentors. Makie worked in the field and in the lab for 9 weeks between June and August 2013. http //jisao.washington.edu/education/researchOps/matsumoto-hervol

Post-Graduation Plans Apply for graduate school.

Potter, Sara, saralouisepotter@gmail.com, The Evergreen State College, Environmental Studies, status cont, field of study Environmental microbes and antibiotic resistance, advisor Erin Ellis, degree type MS, degree date 2013-12-01, degree completed this period Yes
Student Project Title none

Involvement with Sea Grant This Period masters student

Post-Graduation Plans Sara is in the process of interviewing for a related PhD program building on the relationships she creating during her Master project on killer whale scat microbes.

CONFERENCES / PRESENTATIONS

Biology Tribeta Talk, public/profession presentation, 75 attendees, 2014-01-30
International Society of Wildlife Endocrinology, Key note talk, Chicago, public/profession

presentation, 150 attendees, 2013-10-14
The Aquarium of the Pacific, Long Beach, CA, invited talk, public/profession presentation, 150 attendees, 2013-10-09
9th International Conference on Behavior, Physiology and Genetics of Wildlife, Berlin Plenary talk “Stress and Disturbance", public/profession presentation, 200 attendees, 2013-09-18
Sampling for temporal patterns in toxicant exposure and physiological health in killer whales. PBDEs and Southern Resident Killer Whales in Puget Sound Technical Workgroup 3 Toxics Thresholds and Mixtures, meeting April 2013., public/profession presentation, 35 attendees, 2013-04-23
Human Impacts and Wildlife. Public presentation. University of Washington (UW) Club, Seattle, WA. March 2013., public/profession presentation, 35 attendees, 2013-03-15
Using scat to measure PBDEs and other toxicants in killer whales. Professional presentation. PBDE & Killer Whales in Puget Sound technical workgroup 2 PBDE Modeling Technical Workgroup. April 2013., public/profession presentation, 25 attendees, 2013-04-24
Pollutants and Poop. Professional presentation. 17th Annual Meeting of the Northwest Student Chapter of the Society for Marine Mammalogy, Seattle, WA. May 2013. Recipient of Best Presentation, PhD Student, Award., public/profession presentation, 75 attendees, 2013-05-15
How scientists use scat (poop) to help save endangered killer whales. Public presentation and interactive booth for kids and adults. Seattle Aquarium, Marine Mammal Mania. May 2013., public/profession presentation, 500 attendees, 2013-05-15
Toxins, scat, and dogs. Invited Speaker, Ocean Science Career Day at the Seattle Aquarium. February 1, 2014., public/profession presentation, 250 attendees, 2014-02-01
Persistent Organic Pollutants (POPs) in the Puget Sound Ecosystem An evaluation of POPs in fecal samples of Southern Resident killer whales. Professional presentation. Graduate Student Symposium, March 14 2014. Recipient of Faculty Selected Best Presentation Award., public/profession presentation, 50 attendees, 2014-03-14
Presenter, Seattle Expanding Your Horizons, “Find Willie’s Whale Pod.” Inspriing young girls, ages 11-14, to pursue Science, Technology, and Engineering., public/profession presentation, 36 attendees, 2014-03-15

ADDITIONAL METRICS

K-12 Students Reached	1000	Acres of degraded ecosystems restored as a result of Sea Grant activities	0
We have been told countless times that our research is used as an example to describe why the Southern Resident killer whales are endangered as well as Puget Sound stewardship to students K-12. We estimate 1000+ students are learning about our Sea Grant funded			

research in their classroom.			
Curricula Developed One interactive booth was developed for Marine Mammal Mania at the Seattle Aquarium. This included a "test YOUR nose" station where kids would smell three mystery scents to see if they could tell which was whale scat. We also had informational coloring sheets for younger kids, and a slideshow displaying our research. Jessica Lundin gave an Orca Expert talk during this same event.	1	Resource Managers who use Ecosystem-Based Approaches to Management	0
Volunteer Hours 72 hours time that citizens volunteered on our research boat without payment for their time or services.	72	HACCP - Number of people with new certifications	0
Cumulative Clean Marina Program - certifications	0		

PATENTS AND ECONOMIC BENEFITS

No Benefits Reported This Period

TOOLS, TECH, AND INFORMATION SERVICES

Description		Developed	Used	Names of Managers	Number of Managers
Evaluation and identification of antibiotic resistance genes in killer whale scat samples. R/LME-4	Actual (2/1/2013 - 1/31/2014)	1	1	NOAA	2
	Anticipated (2/1/2014 - 1/31/2015)	0	0		
Pregnancy	Actual (2/1/2013	1	1		0

test for killer whales using hormone measure in scat. R/LME-4	- 1/31/2014) Anticipated (2/1/2014 - 1/31/2015)	 0	 0		
Fecal DNA methods for analysis of Southern Resident killer whale prey. R/LME-4	Actual (2/1/2013 - 1/31/2014) Anticipated (2/1/2014 - 1/31/2015)	1 0	1 0	NMFS, NOAA, Office of Naval Research	3
Dataset of toxin levels in southern resident killer whale scat samples. R/LME-4	Actual (2/1/2013 - 1/31/2014) Anticipated (2/1/2014 - 1/31/2015)	1 0	1 0	NOAA	1
Nutritional and physiologic stress measures in killer whales for use by regulatory agencies to make management decisions based on salmon availability and stress from vessel traffic. R/LME-4	Actual (2/1/2013 - 1/31/2014) Anticipated (2/1/2014 - 1/31/2015)	1 0	1 0	NOAA, NMFS, and Canadian DFO	3

HAZARD RESILIENCE IN COASTAL COMMUNITIES

No Communities Reported This Period

ADDITIONAL MEASURES

Safe and sustainable seafood

Number of stakeholders modifying practices Actual (2/1/2013 - 1/31/2014) 0 Anticipated (2/1/2014 - 1/31/2015) 0	Number of fishers using new techniques Actual (2/1/2013 - 1/31/2014) 0 Anticipated (2/1/2014 - 1/31/2015) 0
<u>Sustainable Coastal Development</u> Actual (2/1/2013 - 1/31/2014) 0 Anticipated (2/1/2014 - 1/31/2015) 0	<u>Coastal Ecosystems</u> Actual (2/1/2013 - 1/31/2014) 0 Anticipated (2/1/2014 - 1/31/2015) 0 The U.S. and Canadian governments are mandated to protect the endangered SRKW population, which is also a sentinel population reflecting the health of Pacific Northwest marine and estuarine ecosystems. Our biomonitoring data and quantitative assessments contributed data to the workshop between US and Canadian scientists "Evaluating the Effects of Salmon Fisheries on Southern Resident Killer Whales." We will continue to contribute to the understanding of how best to allocate resources between salmon recovery, toxin clean-up, and regulation of ecotourism to promote recovery of SRKW and their marine environment. These data should also prove useful for monitoring the efficacy of mitigation efforts in an actionable time frame

PARTNERS

Partner Name Center for Whale Research
Partner Name National Marine Mammal Foundation
Partner Name Northwest Fisheries Science Center (NOAA NWFSC)
Partner Name Northwest Fisheries Science Center (US DOC
Partner Name Orca Network
Partner Name Soundwatch Boater Education Program, The Whale Museum
Partner Name University of California, Davis (UCD)
Partner Name University of Washington, Center for Conservation Biology, Department of Biology, College of Arts and Sciences (UW)
Partner Name University of Washington, Department of Environmental and Occupational

Health Sciences, School of Public Health (UW)
Partner Name University of Washington, Friday Harbor Laboratories, College of the Environment (UW)
Partner Name University of Washington, Joint Institute for the Study of the Atmosphere and Ocean (JISAO), College of the Environment (UW)

IMPACTS AND ACCOMPLISHMENTS

Title Washington Sea Grant research points to reduced prey as key to resident killer whales' reproductive losses and failed recovery

Type accomplishment

Description Relevance Endangered southern resident killer whales, the Salish Sea's peak predators, suffer low reproduction, forestalling their recovery. To understand and reverse their decline, researchers and managers must untangle a complex suite of environmental threats nutritional deficiency amidst declining and erratic salmon runs; vessel disturbance, especially by whale-watching boats; and the toxic exposures that afflict apex predators in impacted waters ringed by urban and industrial centers. Response Washington Sea Grant-supported researchers developed and validated an innovative, noninvasive, highly efficient system of gathering repeated whale scat samples using boat-borne detection dogs. They used these samples to measure and monitor, over the long term, individual whales' nutritional, physiological, and reproductive condition, including toxicant levels. Results These measurements point strongly to nutrition – specifically, spring Columbia and summer Fraser Chinook salmon runs – as key to the orcas' recovery. They reveal high rates of pregnancy failure, often mid-gestation when the physiological costs are especially high, and accelerated releases of persistent organic pollutants from fat tissue in malnourished whales. To save its cherished killer whales, the region must save its salmon. Toward this end, marine detection dogs – particularly the celebrated "Tucker" – proved an enormously successful if incidental outreach vehicle for raising media attention and public concern for the orcas' plight.

Recap Washington Sea Grant-sponsored research uses scat-detection dogs and long-term physiological, reproductive, and toxicant monitoring to unravel the mystery of the Salish Sea orcas' persistent decline.

Comments Primary Focus Area LME (HCE) State Goals Support conservation and sustainable use of living marine resources through effective and responsible approaches, tools, models and information for harvesting wild and cultured stocks and preserving protected species (HCE, Science).

Related Partners

PUBLICATIONS

Title Fecal finders. How poop sniffing dogs are helping killer whales.

Type Reprint from a Newsletter, Magazine, or Other Periodical (not peer reviewed; see RR for peer-reviewed reprints) Publication Year 2013 Uploaded File none URL http //www.theverge.com/2013/10/10/4793118/fecal-finders-how-poop-sniffing-dogs-are-helping-killer-whales

Abstract An article of our use of scat detection dogs to find whale scat, and how the scat is helping answer important questions about the recovery of the killer whales.

Citation Jarvus B. Fecal finders. How poop sniffing dogs are helping killer whales. The Verve (online), 10-10-2013.

Copyright Restrictions + Other Notes

Journal Title none

OTHER DOCUMENTS

No Documents Reported This Period

LEVERAGED FUNDS

Type influenced Period 2013-05-31 2013-10-18Amount $1152

Purpose Volunteer hours on research boat. 72 hours at estimated leveraged cost of $16 per hour.

Source Civilian, unpaid for their time or services.

Type influenced Period 2013-05-31 2013-10-18Amount $18000

Purpose Use of research boat donated in kind. 15 weeks at estimated leveraged cost of $1200 per week.

Source Civilian

COMPLETION NARRATIVE

Uploaded File Wasser_5767_completion....9.pdf

Final Report, Wasser: R/LME-4 - *Non-invasive Physiological Monitoring of Southern Resident Killer Whales.* **March 31, 2104.**

INTRODUCTION

The Southern Resident killer whale (SRKW; *Orcinus orca*) population is important to the ecology, culture and economy of the Pacific Northwest. These top-level carnivores are sentinels for the health of the Salish Sea, important in First Nations folklore, and the basis for a multi-million dollar tourist industry in Washington state and British Columbia. They are almost entirely fish eaters and represent the southern most population of this fish-eating ecotype in the northeast Pacific Ocean. The Southern Residents name derives from their late May to October presence in the inshore waters of Washington State and British Columbia, commonly known as the Salish Sea. The three SRKW pods, J, K and L, have slightly different winter distributions. J pod frequents the Salish Sea more than does K and L pods. While all three pods spend the majority of time during winter off the Pacific coast from CA to BC, K and L pods tend to spend more time further south than does J pod in winter.

Reproductive maturity in females occurs around 10 years of age, females calve approximately every 5.3 years and offspring tend to be born in autumn and winter (Olesiuk et al 1990). Gestation is 18 months, making the prior year's salmon particularly important to fecundity (Ford et al 2005). Maximum fecundity of SRKW occurs between ages 20-22, increasing quickly during the first four years after sexual maturity, slowly declining from age 22 to 39, and then precipitously declining thereafter (Ward et al. 2009).

The SRKW population was heavily harvested by the aquarium industry during much of the 1960's, with 47 animals removed out of a population that currently includes 80 individuals. The harvest significantly impacted the reproductive/age structure of the population along with their ability to reproductively recover from population declines following harsh years. The population then experienced an unexplained 20% decline in the late 1990s. This alarmed the public and scientists alike, suggesting that this ecosystem may be unable to sustain these apex predators. The SRKW population was listed as endangered under Canada's Species at Risk Act (SARA) in the late 1990's and under the U.S. Endangered Species Act (ESA) in 2005. The National Marine Fisheries Service published a recovery plan for the SRKW following their listing (NMFS, 2008), outlining the following three major proposed threats to this population:

- **Reduced prey availability** due to precipitous salmon declines, especially Chinook salmon (*Oncorhynchus tshawytscha)*. Many of these salmon populations near the Puget Sound region have been listed as threatened or endangered under the US Endangered Species Act. The most critical runs appear to include the WA and OR coast, Puget Sound, and the Columbia and Fraser Rivers, all measured by the West Coast Vancouver Island (WCVI) Index of the Pacific Salmon Commission (Ward et al 2009). The Fraser River run is the largest remaining Chinook migration in the SRKW population's home range and comprise up to 90% of the SRKW diet from June through September (Ford et al. 1998; Ford and Ellis, 2006; Hanson et al. 2010). The WCVI Chinook abundance estimates are positively correlated with SRKW fecundity (Ward et al. 2009), while other indices are more strongly correlated with survival (Ward et al. 2012).

- **Excessive exposures to environmental contaminants** due to bio-magnification of lipophilic toxicants up the food chain. Biopsy studies on the SRKWs have reported levels of polychlorinated-biphenyls (PCBs), polybrominated-diphenyl-ethers (PBDEs), and dichloro-diphenyl-trichloroethanes (DDTs) that exceed a health-effects threshold established for seals (no such threshold exists for whales) (Krahn et al. 2007, 2009; Kannan et al. 2000). Exposure to these persistent organic pollutants (POPs) has been linked to a range of deleterious biological impacts, including disruption and/or suppression of the reproductive system, thyroid dysfunction, and increased risk of infection (Beland et al. 1993; Kannan et al. 2000; Lahvis et al. 1995; Pierce et al. 2008; Subramanian et al. 1987; US EPA, 2002).

- **Disturbance from private and commercial whale watch boats (i.e. vessel effects)**. Vessel proximity and noise have been proposed to directly increase psychological stress and/or interfere with foraging. Vessel traffic grew enormously in the Salish Sea in the late 1990s (Koski et al. 2005). In response, NOAA introduced new vessel regulations to protect the SRKWs in April 2011 (NOAA, 2011), which the Washington Legislature used to update WA vessel regulations in RCW77.15.740 in 2012 (http://wdfw.wa.gov/conservation/orca/).

Nutritional demands are presumed to be greatest in winter when salmonid prey are more widely dispersed and smaller in size; SRKW calf survival also appears to be lowest in winter. SRKW then transition to spring and summer runs of adult Chinook salmon approaching river mouths unique to each run. Our published endocrine data (sampling years 2007-2009) suggest that prey consumed by SRKW during spring, prior to arriving in the Salish Sea, may be vital to replenishing nutritional reserves depleted during winter as well as sustaining the SRKW until the Fraser River Chinook salmon (FR) runs peaks in summer (Ayres et al 2012). We suggested that the interior race of Columbia River early spring Chinook run (CR) was particularly important in this transition. They have the longest freshwater migration, and thus have among the highest fat content of any adult Chinook (Brett 1995; Mesa and Maggie 2006). Consistent with the above, SRKW were detected twice as frequently at the Columbia River than would be expected by random chance based on acoustic recorder detections from 2006-2011 (Hanson et al., in review), with most detections occurring in early spring.

We have expanded our analyses of endocrine measures, prey availability, and vessel effects to include the four additional years of sample collection supported by Washington SeaGrant totaling an unprecedented 7-consecutive year longitudinal analysis (2007-2013). In addition, our more recent work evaluates how these combined pressures impact SRKW pregnancy occurrence and success. SRKW have surprisingly few births relative to the number of non-lactating females of reproductive age in this population; only one birth was observed during the last two years, and that calf died before it ever nursed (CWR 2013). As described below, we have validated reliable hormone-based pregnancy indicators in the SRKWs, and show these measures can reliably discriminate term pregnancies from apparent miscarriages.

Our more recent work also includes validated fecal toxicant measures of PCB and PBDE congeners, as well as DDT metabolites (namely, DDE) (in collaboration with NOAA NWFSC). Biopsy studies on SRKW toxicant exposure to Persistent Organic Pollutants (POPs; Krahn et al. 2007, 2009; Kannan et al. 2000) have offered a cross-sectional look at toxicant loads and demonstrated extremely high levels in this population. However, limitations on the ability to acquire frequent biopsy samples, or repeat samples from the same whale, have made it difficult to test hypotheses predicting increased lipophilic, bio-accumulated POPs in circulation from fat metabolism associated with reduced prey availability. We addressed this need by developing and validating measures of PCBs, PBDEs and DDTs in SRKW fecal samples collected across seasons, pods, and years.

METHODS

Scat detection dogs. SRKW scat samples are located by detection dogs trained to locate SRKW scat floating on the water's surface (Wasser et al. 2004; Rolland et al. 2006; Ayres et al 2012). The dog method greatly increases our sample size due to their remarkable ability to locate SRKW scats at distances compatible with the "Be Whale Wise" guidelines (>200 yards away). The method also reduces sampling bias, and has enormous public appeal. Wind blowing over the scat causes it's scent to emanate from the samples in the shape of a cone. The detection dog rides on the bow of the boat, which is being driven perpendicular to the wind, beginning at least 200 yards downwind from an area where the whales have just traveled. As the boat approaches the edge of the scent cone, the dog's behavior suddenly changes from passively sitting or standing to alert while leaning way over the bow of the boat. This change informs the dog handler that the boat is entering the "scent cone" emanating from the sample. As the boat passes through the center of the scent cone, where the scent is strongest, the dog leans heavily over the windward side of the boat, following the strongest scent, informing the handler to direct the driver to turn into the wind. Subtle cues by the dog allow the driver to stay on the scent line until the sample is reached. If at any time the boat travels out of the scent cone, the dog begins to stand and look back to where the scent was strongest. The handler would then direct the driver to turn 180° and head back into the scent cone until the dog once again alerts the handler it has redetected the scent.

Fecal DNA and Hormone Measures. SRKW fecal samples were genotyped for species, sex, pod, and individual identification by NOAA NWFSC (Ford et al 2011). Fecal hormone metabolites measured include: glucocorticoid (GC), thyroid (triiodothyronine, T3), testosterone (T) and progesterone (P4). All hormone metabolites were extracted using methods described in Wasser et al (2000, 2010). The hormones are all measured in the same genotyped samples, allowing us to assess nutritional and disturbance stress (based on GC, and T3 concentrations) and pregnancy occurrence and loss (based on P4 and T concentrations) over time.

GCs rise quickly in response to poor nutrition and psychological stressors, mobilizing glucose to provide energy to deal with the immediate emergency (Sapolsky et al 2000). T3 responds oppositely and more slowly to nutritional stress but not to psychological stress; T3 declines in response to persistently poor nutritional conditions, lowering metabolism to protect the body from exhausting all its reserves (Douyon and Schteingart, 2002). We correlated T3 and GC with year, Julian date and Fraser River salmon abundance (based on Albion test fishery CPUE).

Pregnancy was diagnosed by temporal changes in P4 and T among adult females during their 16-18 month gestation. We validated these endocrine-based pregnancy indicators by demonstrating their unique and consistent elevation during the SRKW gestation period. Gestation was confirmed by backdating from the estimated birth date of the female's calf, assessed by approximate age of calf at first observation by the Center for Whale Research. Based on the consistency of these measures without exception, we identified apparent pregnancy miscarriages by samples with P4 and T concentrations in the pregnancy range from genotyped adult females that failed to give birth in the known gestation period. These pregnancy success indicators were then correlated with our physiological and temporal indices of nutritional stress.

Toxicant Measures. PCB and PBDE congeners, as well as metabolites of DDT, were measured in the scat samples using florisil and sulfuric acid clean methods modified from EPA methods 3620C and 1614, analyzed by GC-NCI-MS. The limit of quantification ranged from 0.008-0.900 ppb for all compounds. Fecal measures were validated by comparing cumulative concentrations of congeners of PCB, PBDE and DDE in blubber (ng/g lipid adjusted) and scat samples (ng/g dry weight) from the same whale ($p<0.01$ for all compound classes) (Blubber data provided by NOAA NWFSC). The fecal toxicant concentrations in scat were then correlated with temporal changes in nutritional condition using a mixed effects model incorporating individual whale as a random effect, where log-transformed toxicant level was the dependent variable and percent of total fish run binned into quartiles by year was the independent variable.

Fish and Boat Impact Analyses. We assessed FR Chinook abundance directly based on the Albion test fishery CPUE, lagged by 10 days after sample collection to account for the distance between the test fishery and geographic location of scat sample collection (Ayres et al 2012). Fish and boat abundance at any point in time was also assessed based on Julian date across years since annual Julian date correspond with the peaks in fish abundance (Fig 1, top left) and the proportional increase and decrease in whale watch boats, which peaks mid to late summer (Ayres et al 2012). These measures were directly compared with temporal changes in T3 and GC concentrations, across individuals and pod, and also by reproductive status for the subset of females with term pregnancies versus apparent miscarriages. We also constructed temporal indices of Columbia and Fraser River Chinook runs (using Chinook abundance changes starting several months before as predictor variables; Fig. 1) that could be correlated with transformed T3 and GC concentrations alternately as the response. Stepwise model selection was used to identify significant predictor variables at different temporal lags (using Akaike's Information Criterion, AIC).

MAJOR FINDINGS

Fish Impacts. Figure 1 shows the annual variation in CR and FR Chinook from 2003 -2012 (Ward et al 2012). The strength of these two respective run peaks and the interval length between them partly determine their annual nutritional impacts on SRKW. We use Julian date and year to index the timing and magnitude of these impacts, correlating these variables with SRKW stress and nutrition hormone concentrations. This also allows us to simultaneously examine boat impacts since Julian date also correlates with boat abundance in the Straits off the San Juan Islands.

Fraser River Chinook (FRC) salmon abundance (and whale watch boat activity) slowly begins to increase when the SRKW first arrive in the Salish Sea. For 6 years in a row, SRKW T3 concentrations show a significant, 5th order polynomial trend by Julian date. T3 concentrations are the highest when the whales first arrive in the Haro Strait in late spring/early summer and then sharply decline (Fig. 1 bottom left) to their lowest levels in the interim until the Fraser River run peaks. T3 concentrations then slightly increase again around the time of the FR peak. Given the slower T3 responsiveness, this overall pattern suggests that the SRKW arrive after having just fed on a very rich food source outside the Salish Sea (Ayres et al 2012), but then face the challenge of sustaining themselves until the FR run peaks. Mean T3 concentrations over this period also vary significantly across years in this model. Mean T3 concentrations are lowest (indicating nutritional stress) when the peak in FR fish is relatively late and/or low.   There was no corresponding effect of day-to-day FR CPUE (or pod) on T3, presumably due to the relatively slow responsiveness of T3.

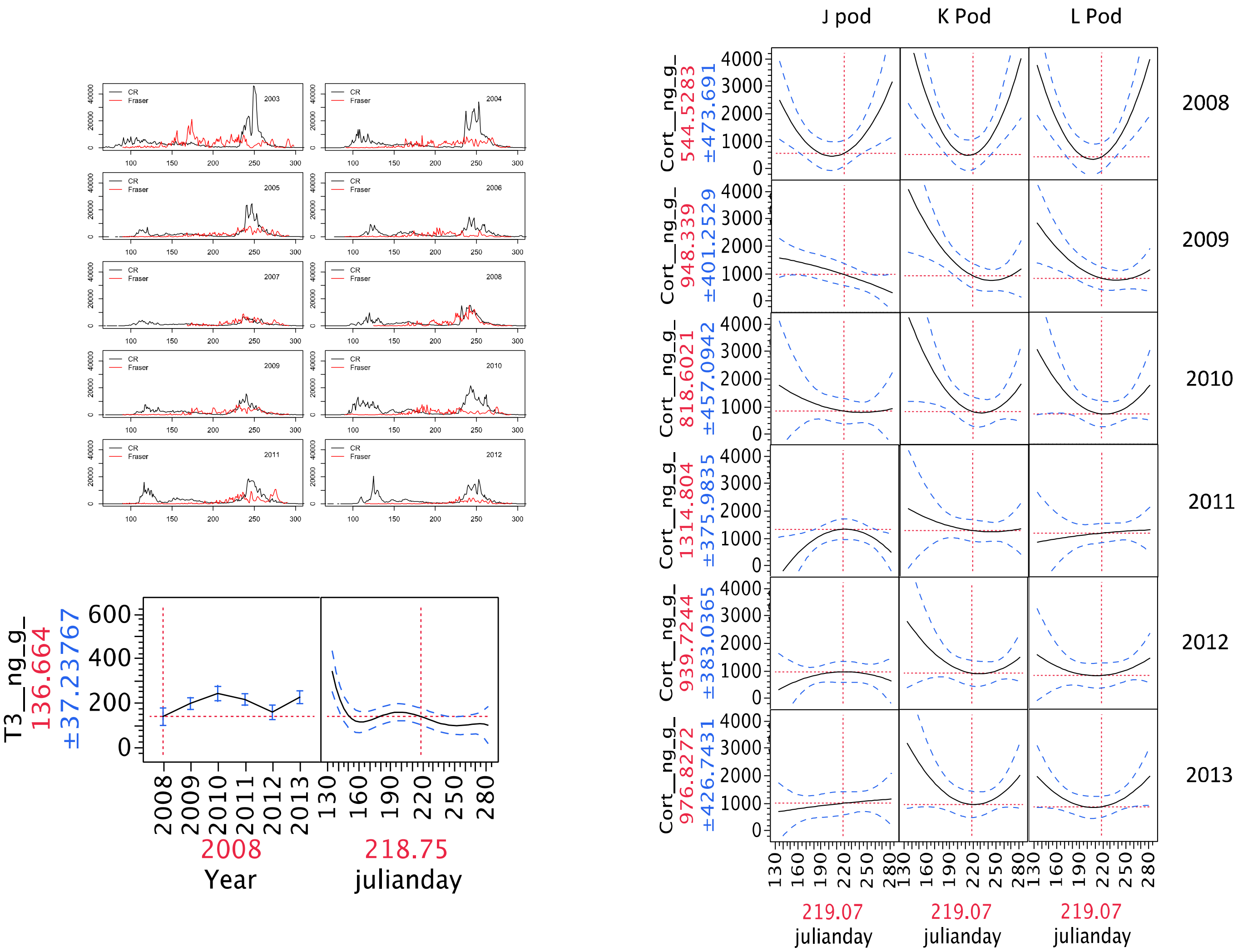


Figure 1. Columbia River (CR) and Fraser River Chinook Salmon Run abundances plotted by Julian day across years (top left, from Ward et al 2012). B. Changes in SRKW fecal thyroid hormone (tyriidothyronine, T3) by Julian date across years (lower left). C. Change in SRKW fecal glucocorticoid (Cort) hormone by Julian date across years and pods (right).

SRKW GC concentrations are correspondingly high across years and pods at the time of the late spring SRKW arrival in Haro Strait, reflecting the relatively low prey availability at that time. For six years in a row, concentrations of faster responding GCs reach their annual nadir around the same time as the peak in FR Chinook (and whale watch boat) abundances, increasing again as FR Chinook (and boat) abundance decline in fall (Fig 1 right and top left). This significant relation of GC concentrations to the quadratic of Julian date, as well as significant interactions with both year and pod by the Julian date quadratic suggests that GC are far more influenced by fish than boat abundance across years and pods. Interestingly, the GC pattern in 2011 seems to be the "exception that proves the rule" across all three pods. GCs are relatively flat across Julian date for K and L pod and reverse their shape altogether for J pod. These patterns are all consistent with expectation since Fraser River Chinook abundance was exceptionally high in 2011, based on the total area under the curve for annual Albion CPUE; the peak in Chinook abundance also occurred early and was sustained (see Fig 1, left, and Fig 3, bottom right).

Overall pod differences in GC variation by Julian date are also noteworthy in (Fig, right). With the exception of 2008, the U-shaped GC pattern observed across pods is more blunted in J pod compared to K and L, reverses shape altogether in 2011 and then progressively flattens in J pod only. The seasonal GC pattern observed in J pod during 2011 and slightly less so in 2012 and 2013 may, in fact, be more characteristic of a pattern expected from increased boat stress. GCs in J pod during 2011 (and somewhat in 2012) are highest, not lowest, around the time when boat abundance is highest and fish-related nutritional stress should be lowest. This pod difference might be expected since J pod also spends the greatest amount of time in Haro Straits where the majority of whale watch boat pressure occurs. It is also interesting that this effect is strongest in 2011, the year immediately prior to WA state's acceptance of the 2011 vessel regulation modifications enacted by NOAA, with the GC trend slightly diminishing in J pod during subsequent years.

Finally, temporal indices of Columbia and Fraser River Chinook runs (using Chinook abundance changes starting several months before as predictor variables; Fig. 1, top left) were correlated with transformed T3 and GC concentrations alternately as the response. Stepwise model selection was used to identify significant predictor variables at different temporal lags (using Akaike's Information Criterion, AIC). Both the Columbia and Fraser River time series each indicated GCs to be responding positively to increased variation in salmon availability over approximately 50 days ($P < 0.02$). This positive relationship might be expected, as a more variable prey base translates to greater nutritional stress. For T3, the same patterns emerged across rivers, though the time scale is different. Variation in Chinook abundance appears to have the strongest effect on T3 over approximately 21 days for both the Columbia and Fraser runs ($P < 0.03$), and unlike the effect estimated for GC, the slope of the relationship is negative, also consistent with the hypotheses that more variable prey translate to greater nutritional stress.

Pregnancy Studies. P4 and T concentrations by age-sex class reveal a clear pattern of P4 concentrations significantly above 2000 ng with T also above 100 ng, without exception, in reproductive age females confirmed to be pregnant. Two other classes of reproductive age females also show significantly elevated P4 concentrations above 2000 ng, one with significantly higher P4 and T than confirmed pregnant females and the other with lower P4 and T compared to confirmed pregnant females. Since these elevations in the latter two groups of reproductive age females fall well within the otherwise exclusive pregnancy range but were not followed by a live birth, we assume that they represent miscarried pregnancies. Some of these may be explained as pseudo-pregnancies, although the temporal infertility effect is the same. From here on, we refer to these apparent miscarriages simply as miscarriages. The only other age sex class that shows significantly elevated T concentrations is adult males. However, P4 remains at baseline concentrations in all males (Fig. 2, top left).

Temporal changes in progesterone (P4) concentrations during gestation and subsequent lactation of 11 females confirmed to be pregnant by the presence of a newborn show a significant quadratic pattern of elevating P4 and T during gestation in SRKW feces, with a slight decline as parturition approaches, and a precipitous decline below 2000 ng P4 in lactation (Fig. 1, bottom left). T concentrations show the same temporal pattern, although the drop in T during early lactation is somewhat less precipitous than that observed for P4 (data not shown). Sixteen females were, in turn, characterized as having miscarriages, based on their significantly elevated progesterone concentrations, accompanied with either high or low T concentrations compared to confirmed pregnant females. It is noteworthy that the majority of these miscarriages likely occurred after the first trimester of pregnancy, based on their high progesterone concentrations. While miscarriages are common among female mammals, most tend to occur in the early stages of pregnancy (Wasser and Barash 1983) making later miscarriages fairly atypical.

Nutritional stress also appears to be lower during gestation of confirmed pregnant compared to miscarrying females. There was a significant change in T3 as a function of the interaction between pregnancy type (confirmed versus miscarriage) and Julian date as 3rd order quadratic. T3 concentrations increased with time in confirmed pregnant females, while decreasing in both high and low T miscarrying females (Fig. 2, top right). Thus, successful pregnancies likely entered winter in a comparatively good nutrition state compared to miscarrying pregnancies. Interestingly, this pattern of characteristic of increased nutrition status in later pregnancy stages is common among mammals, in preparation for the high nutritional demands of lactation (McNab, B. K. 1980). T3 was highest overall in the low T miscarriage females (Fig 2, top right).

By contrast, GC concentrations were significantly highest overall in the “high T” miscarriage females and also increased sharply among those females by Julian date compared to confirmed pregnant and low T miscarrying females. This further supports a low nutrition correlate to the “high T” miscarriage group.

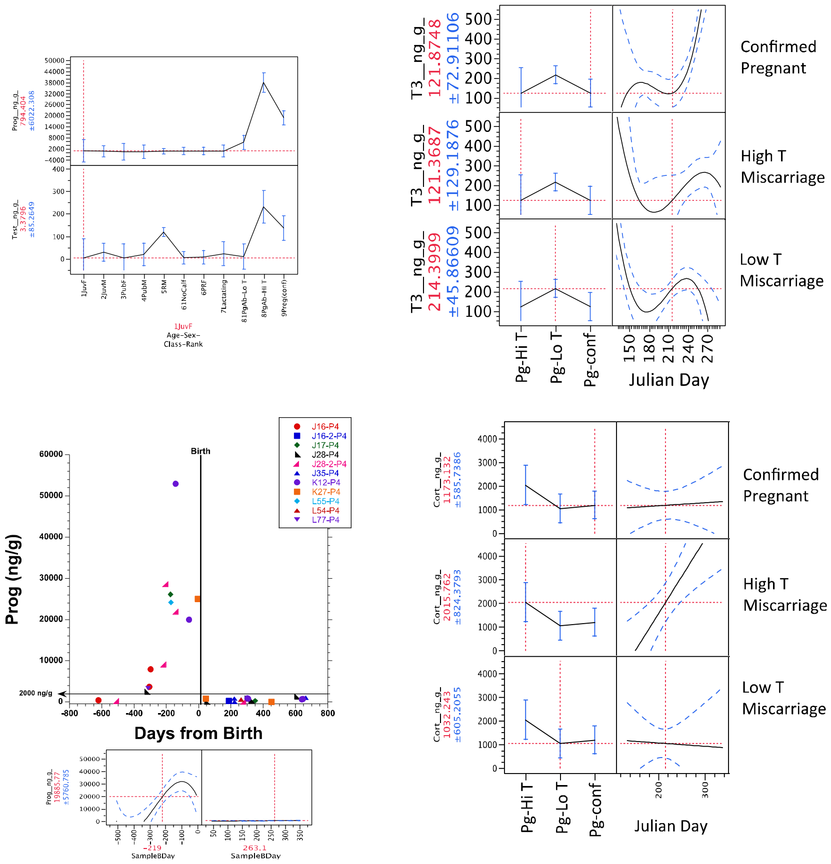


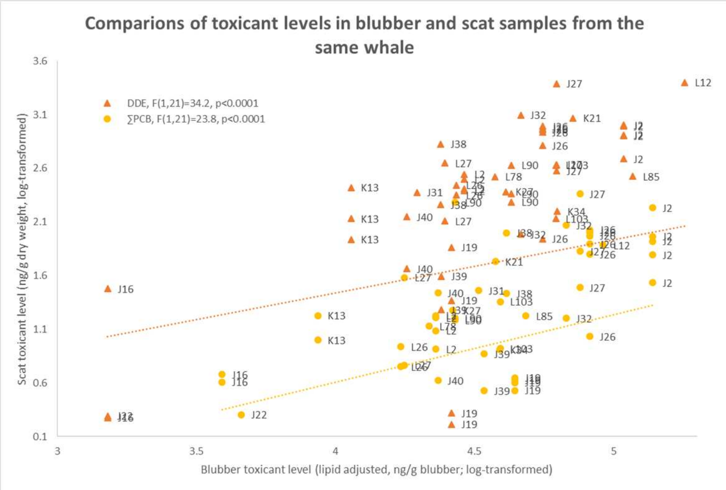
Figure 2. Pregnancy thresholds based on progesterone (Prog) and testosterone (Test) metabolite concentrations in SRKW killer whale feces by age-sex class (top left) and across gestation and lactation for females confirmed to be pregnant by a subsequent live birth (bottom left). Trends in T3 and GC (Cort) nutrition indices across Julian date for the confirmed pregnant females (Pg-conf) versus the females presumed to eventually miscarry [High Prog females with high or low T (Pg-Hi T and Pg-Lo T), respectively] are also shown in the right hand figure (T3 on top and GC on bottom).

Toxicant Impacts. We can now reliably measure Persistent Organic Pollutants (POPs) in SRKW fecal samples at levels that correspond to those from blubber biopsies (Fig 3; Lundin, Ylitalo and Wasser, in prep): Summed PCB congeners and DDE show significant correspondence in SRKW tissue and blubber ($p<0.01$; Fig 3).

DDE in scat (Fig 4, top right) are most abundant in the more southern ranging K and L pods (compared to J-pod: $p<0.01$ and $p<0.01$, respectively) whereas summed PCB concentrations ($p<0.01$ and $p<0.01$, respectively) and

PBDE47 are most abundant in the more urban foraging J pod compared to K and L pod. Consistent with blubber data (Ross et al. 2000), POPs in SRKW scat are highest in adult males and nulliparous adult females and increase with their respective ages. POPs are lowest in females with at least one calf but do not increase with multiparous female age until these females approach reproductive senescence (Fig 4, top left).

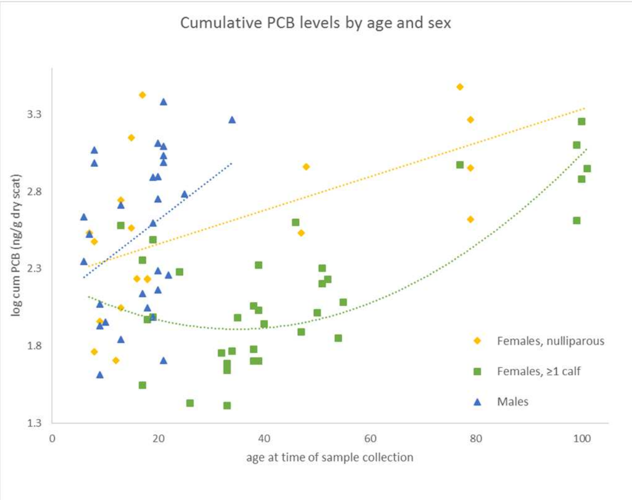
Figure 3. Cumulative PCB congeners and DDE in blubber (ng/g lipid adjusted) and scat samples (ng/g dry weight) from the same whale. (Blubber toxicant levels provided by NOAA NWFSC)

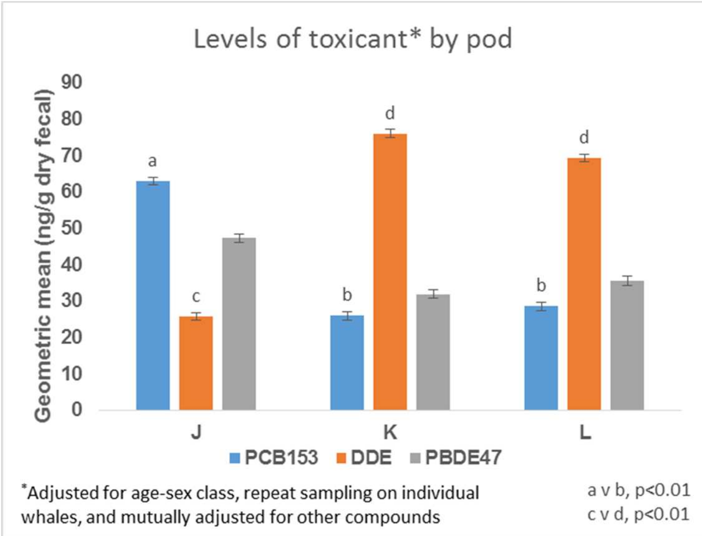


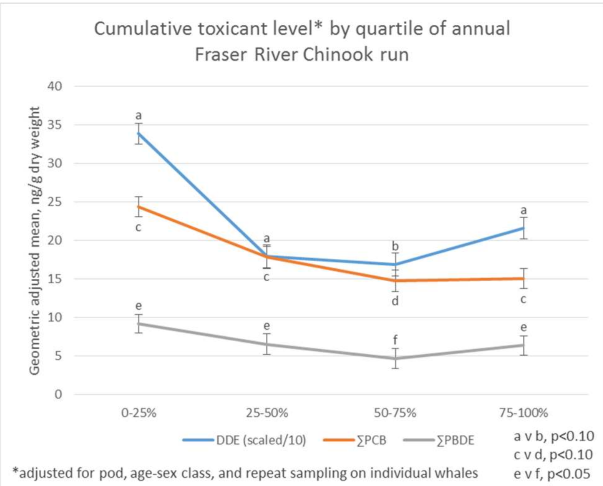
Toxicant levels in scat are expected to reflect a combination of immediate ingestion through prey, normal excretion of body burden from fat stores, and/or increased excretion from metabolizing fat stores of these bio-accumulated, lipophilic toxicants. If prey ingestion is the greatest source of excreted toxicants, toxicants in scat would be highest when fish abundance is greatest and would not vary between whales with the same prey base. If the greatest source of excreted toxicants is a reflection of expected excretion due to body burden the toxicant pattern in scat would directly reflect the blubber biopsy levels. If the greatest source of excreted toxicants is from increased excretion of bio-accumulated toxicants during fat metabolism, toxicant levels in scat would be predicted to increase when prey availability is low. Our evaluation of 84 scat samples collected from 44 unique whales during 2010, 2011, and 2012 demonstrate that measured toxicant level in the scat statistically correlate to blubber biopsy levels ($p<0.01$) and demonstrate an increase in toxicant excretion during the period just after the late spring arrival of whales in Haro Strait, when Fraser River Chinook abundance is lowest (Fig 4, bottom). Correspondingly, the excretion levels of POPs in scat decline as fish abundance increases. This also corresponds to the sharp decline in T3 and rise in GC immediately following SRKW arrival in Haro Strait (Fig. 1 bottom left, and right).

SIGNIFICANCE OF THE RESULTS

The SRKW are still not recovering from their decline in the late 1990's. Their reproduction remains low, severely impacting recruitment and potential for recovery. Some of the greatest challenges facing recovery efforts have been delineating the relative importance of prey availability, toxicant exposures, and vessel abundance to SRKW population declines and particularly to SRKW reproductive success. Our initial work addressed these issues by using physiological measures to characterize changes in nutritional and psychological stress respectively associated with fish and boat abundance (Ayres et al 2012). Since that time, our work continues to show that declining prey abundance most significantly impacts SKRW recovery. More recently, we developed reliable pregnancy indicators and toxicant measures from SRKW scat, allowing us to better test existing hypotheses and to determine their direct impacts on reproductive success. Our results suggest SRKW are experiencing high rates of pregnancy failure, much of which is occurring around mid-gestation when the physiological cost of failure is particularly high. Poor nutrition appears to be one important cause of the pregnancy failure. Nutrition impacts from reduced prey abundance also appear to be increasing release of bioaccumulated, lipophilic persistent organic pollutants into SRKW circulation. The combination of poor nutrition and associated increased exposure to toxicants released into circulation as adipose tissue is metabolized may be resulting in cumulative impacts on SRKW population declines, especially through lowered recruitment realized by reduced pregnancy success. The toxicant component of our study may, in fact, serve as a model for the enigmatic high rate of reproductive failure and molar pregnancies reported in the coastal Shoalwater Bay tribe; the Shoalwater tribe also depends heavily on marine resources in Puget Sound, are exposed to toxicants and subject to periodic food shortages (Shukovsky 1999). Finally, for the first time our data also provide physiological evidence suggesting that whale watch boats may be adding to the cumulative impacts on SRKW recovery, with the greatest impacts on J pod, the pod most heavily exposed to whale watch boats during summer.







Julian dates for each quartile, by year				
	2010	**2011**	**2012**	**2013**
0-25%	90-187	90-229	115-227	110-219
25-50%	187-217	229-245.5	227-240	219-231
50-75%	217-249	245.5-270	240-252	231-245.5
75-100%	249-292	270-290	252-293	245.5-292

Figure 4. Cumulative PCB levels by sex and female reproductive status (top left). Level of toxicant class by pod (top right). Cumulative toxicant level by quartile of annual Fraser River Chinook Run, followed by annual Fraser River Chinook abundance (Albion CPUE) by quartile (bottom). All data are from 2010-2012 (2013 toxicant samples are still being run).

STUDENTS SUPPORTED, OUTREACH EFFORTS, AND INFORMATION TRANSFER ACTIVITIES

The funding made available through Washington Sea Grant supported the dissertation research for Jessica Lundin, a doctoral candidate in the Center for Conservation Biology at the University of Washington (Expected graduation, fall 2014). This study also supported Katherine Ayres' doctoral dissertation (PhD, University of

Washington, 2011) and Sara Potter's Master thesis project (MS, Evergreen State College, 2013). We have been actively involved in NOAA's JISAO program, including mentoring a JISAO Undergraduate Intern for 9 weeks in 2010, 2011, and 2013. These students were Kelsey Powers, Rachel Pausch, and Makie Matsumoto-Hervol, respectively, each with a full page profile including individual research poster and 3-minute internship video at http://www.jisao.washington.edu/education/researchOps. Other student interns and team members include Amanda Phillips, Deborah Giles, Charles Rolsky, and Katrina van Raay.

This project greatly benefits from the numerous partnerships that collaborate with our research efforts. Our Center has a long and respected history of collaboration with NOAA NWFSC, WA Department of Fish and Wildlife, US Fish and Wildlife Service, USGS, Center for Whale Research, The Whale Museum, Salish Sea Marine Naturalists, Canadian Consulate General, the Canadian Department of Fisheries and Oceans, Northwest Science Association, JISAO, the Conservation Canine Program, the University of Washington Department of Environmental and Occupational Health Sciences, Orca Network, and the support of the whale community on San Juan Island. We understand that our science can only inform policy decisions if it is communicated widely and in a timely manner. We actively communicate our research findings with these groups and organizations, and other government agencies and legislators involved in the protection of the SRKW population, their prey, and the restoration of the Salish Sea.

We have a very strong publication record in high impact, peer-reviewed journals. This information is accessible for policy makers to incorporate our findings in their management plans. The longitudinal data describing our SRKW endocrine work up through 2009 has already been published (Ayres et al 2012) and results through 2013 are in preparation and are being made available to policy makers. For the past seven years we have given regular presentations and attended meetings conducted by the Whale Museum, The Whale Watch Operators Association, the Seattle Aquarium, Pacific Science Center, NOAA, EPA, and many more. We have also established important communications with the Puget Sound Partnership. Our Center's website and Facebook page receive many visitors and is updated regularly with current data. Lastly, we capitalize heavily on the newsworthiness of our work. Media outlets are a great way to communicate important findings to the public and the media makes frequent use of our work. In fact, our work with our detection dog, Tucker, is now arguably world renown due to the considerable media attention this program draws for marine stewardship.

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