

Update Report

Wasser, Sam

Period: 2/1/2012 - 1/31/2013

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 Pollutants in the Puget Sound Ecosystem: Temporal patterns in excretion of POPs and associated endocrine disruption in free-ranging killer whales

Involvement with Sea Grant This Period:

Lead on both the field and lab component of the project

Post-Graduation Plans:

Post-doc

Phillips, Amanda, amap22@gmail.com, University of Washington, School of Aquatic and Fisheries Sciences, status:new, field of study:Fish Biologist, *no advisor*, degree type:BS, degree date:2010-12-01, degree completed this period:No

Student Project Title: *none*

Involvement with Sea Grant This Period:

Amanda was a field assistant for the entire 2012 sampling season.

Post-Graduation Plans:

Amanda is in the process of applying for graduate school.

Potter, Sara, saralouisepotter@gmail.com, The Evergreen State College, Environmental Studies, status:new, field of study:Environmental microbes and antibiotic resistance, advisor:Erin Ellis, *no degree type, no degree date*, degree completed this period:No

Student Project Title:

Antimicrobial resistance in killer whale feces in relation to anthropogenic sources

Involvement with Sea Grant This Period:

Sara was a field assistant for the entire 2012 sampling season. Also, Sara's master's thesis will be using samples from this project to address the important question of antimicrobial resistance in killer whale fecal samples.

Post-Graduation Plans:

Sara is in the process of finishing her master degree and applying for PhD programs.

:: CONFERENCES / PRESENTATIONS

Wasser. University of Utah, Biology seminar., public/profession presentation, 200 attendees, 2012-10-25

Wasser. Presentation, University of Sherbrooke, MONTREAL., public/profession presentation, 200 attendees, 2012-10-19

(2/1/2013 -
1/31/2014) :

:: TOOLS, TECH, AND INFORMATION SERVICES

Description	Developed	Used	Names of Managers	Number of Managers
Fecal DNA methods for collection, analysis of southern resident killer whale genotyping data for NOAA. R/LME-4	Actual 1 (2/1/2012 - 1/31/2013) : Anticipated 0 (2/1/2013 - 1/31/2014) :	1	NMFS, Office of Naval Research, and the National Marine Mammal Foundation	3
Fecal genetic and physiologic methods to assess nutritional and physiologic stress in southern resident killer whales for use by regulatory agencies to make management decisions based on salmon availability and stress from vessel traffic. R/LME-4	Actual 1 (2/1/2012 - 1/31/2013) : Anticipated 0 (2/1/2013 - 1/31/2014) :	1	NMFS, and Canadian DFO. Count as 1, NMFS counted above	1
Dataset of toxin (Chlordanes, DDT, HCHs, PCBs and BDE) levels in southern resident killer whale scat samples. R/LME-4	Actual 1 (2/1/2012 - 1/31/2013) : Anticipated 0 (2/1/2013 - 1/31/2014) :	1	NMFS and University of Washington. RL: 0, NMFS counted above	0

:: HAZARD RESILIENCE IN COASTAL COMMUNITIES

No Communities Reported This Period

:: ADDITIONAL MEASURES

Safe and sustainable seafood

Number of stakeholders modifying practices
Actual (2/1/2012 - 1/31/2013) : 0
Anticipated (2/1/2013 - 1/31/2014) : 0

Number of fishers using new techniques
Actual (2/1/2012 - 1/31/2013) : 0
Anticipated (2/1/2013 - 1/31/2014) : 0

Sustainable Coastal Development

Actual (2/1/2012 - 1/31/2013) : 0
Anticipated (2/1/2013 - 1/31/2014) : 0

Coastal Ecosystems

Actual (2/1/2012 - 1/31/2013) :
Anticipated (2/1/2013 - 1/31/2014) :

:: PARTNERS

Partner Name: Center for Whale Research

Partner Name: Northwest Fisheries Science Center (NOAA NWFSC)

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, Friday Harbor Laboratories, College of the Environment (UW)

:: IMPACTS AND ACCOMPLISHMENTS

Title: Washington Sea Grant-supported research using scat detection dogs and hormonal analysis probes the effects of starvation and pollution on endangered killer whales

Type: accomplishment

Description:

Relevance: The Salish Sea's resident killer whales were listed as endangered in 2005. 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: Researchers supported by Washington Sea Grant employ a novel, noninvasive technique to collect timely physiological samples (scat) using detection dogs. In 2012 they collected 117 scat samples, which are now ready for hormonal, genetic, and toxin assays, and analyzed and genotyped samples collected in 2010 and 2011.

Results: Whale stress hormones tracked closely with nutritional but not with vessel stress, though boat density may further boost stress hormones when fish densities are low. Thyroid hormone levels, a metabolic indicator, showed that spring Chinook salmon runs at sea provide sustaining nutritional reserves until the Fraser River's summer Chinook arrive. These hormone levels appeared to correlate positively with pod births and negatively with deaths. Two pods that forage heavily off California showed significantly higher DDT levels, while a pod that forages more in Puget Sound showed higher PCB levels. The data can guide the allocation of management resources to best protect endangered killer whales.

Recap:

Washington Sea Grant-supported research using noninvasive scat sampling reveals significant relationships between killer whale health, prey availability, vessel stress, and toxic contaminants.

Comments:

Primary Focus Area – LME (HCE)

Associated 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:

Center for Whale Research

National Marine Mammal Foundation

Northwest Fisheries Science Center (US DOC, NOAA, NMFS, NWFSC)

Orca Network

Soundwatch Boater Education Program, The Whale Museum

University of California, Davis (UCD)

University of Washington, Center for Conservation Biology, Department of Biology, College of Arts and Sciences (UW)

University of Washington, Friday Harbor Laboratories, College of the Environment (UW)

University of Washington, Joint Institute for the Study of the Atmosphere and Ocean (JISAO), College of the Environment (UW)

:: PUBLICATIONS

Title: **Distinguishing the impacts of inadequate prey and vessel traffic on an endangered killer whale**

Type: Reprints from Peer-Reviewed Journals, Books, Proceedings and Other Documents Publication Year: 2012

Uploaded File: *none*

URL: *none*

Abstract:

Managing endangered species often involves evaluating the relative impacts of multiple anthropogenic and ecological pressures. This challenge is particularly formidable for cetaceans, which spend the majority of their time underwater. Noninvasive physiological approaches can be especially informative in this regard. We used a combination of fecal thyroid (T3) and glucocorticoid (GC) hormone measures to assess two threats influencing the endangered southern resident killer whales (SRKW; *Orcinus orca*) that frequent the inland waters of British Columbia, Canada and Washington, U.S.A. Glucocorticoids increase in response to nutritional and psychological stress, whereas thyroid hormone declines in response to nutritional stress but is unaffected by psychological stress. The inadequate prey hypothesis argues that the killer whales have become prey limited due to reductions of their dominant prey, Chinook salmon (*Oncorhynchus tshawytscha*). The vessel impact hypothesis argues that high numbers of vessels in close proximity to the whales cause disturbance via psychological stress and/or impaired foraging ability. The GC and T3 measures supported the inadequate prey hypothesis. In particular, GC concentrations were negatively correlated with short-term changes in prey availability. Whereas, T3 concentrations varied by date and year in a manner that corresponded with more long-term prey availability. Physiological correlations with prey overshadowed any impacts of vessels since GCs were lowest during the peak in vessel abundance, which also coincided with the peak in salmon availability. Our results suggest that identification and recovery of strategic salmon populations in the SRKW diet are important to effectively promote SRKW recovery.

Citation:

Ayres, K.L., R.K. Booth, J.A. Hempelmann, K.L. Koski, C.K. Emmons, R.W. Baird, K. Balcomb-Bartok, M.B. Hanson, M.J. Ford, S.K. Wasser. 2012. Distinguishing the impacts of inadequate prey and vessel traffic on an endangered killer whale. PLOS ONE, 7(6). doi:10.1371/journal.pone.0036842

Copyright Restrictions + Other Notes:

Journal Title: PLOS ONE

Title: **On a Whale's Tail: Orca Research**

Type: Publication Year: 2013

Uploaded File: *none*

URL: <http://www.seattlemet.com/news-and-profiles/best-places-to-work/articles/interns-on-internships-january-2013>

Abstract:

The work is hard, the hours are long, and best of all, there's no paycheck. Seattle Met's interns found the best free labor your sweat can buy.

Citation:

On a Whale's Tail: Orca Research. Seattle Met, Dec 19, 2012

Copyright Restrictions + Other Notes:

Journal Title: *none*

Title: **Whale Watcher! These amazing dogs are trained to track orcas across Puget Sound**

Type: Publication Year: 2012

Uploaded File: *none*

URL: *none*

Abstract:

Our study was highlighted in Cesar Milan's magazine, including a centerfold of Tucker our whale scat sniffing dog.

Citation:

Whale Watcher! These amazing dogs are trained to track orcas across Puget Sound. Cesar's Way magazine, June 2012

Copyright Restrictions + Other Notes:

Journal Title: Cesar's Way

Title: **Similar titles from over 100 newspapers that picked up on the New York Times article, including Kansas, Kentucky, British Columbia, Miami, etc**

Type: Publication Year: 2012

Uploaded File: *none*

URL: *none*

Abstract:

Media placement in response to our PLoS ONE publication (Ayres et al. 2012)

Citation:

Print and online newspapers nationally and internationally, 2012

Copyright Restrictions + Other Notes:

Journal Title: *none*

Title: **Seattle dog tracks scent whale scat open ocean water**

Type: Publication Year: 2012

Uploaded File: *none*

URL: <http://www.fieldandstream.com/blogs/mans-best-friend/2012/09/seattle-dog-tracks-scent-whale-scat-open-ocean-water>

Abstract:

Media placement in response to our PLoS ONE publication (Ayres et al. 2012)

Citation:

Chad Love. Marine Biologists Use Dog to Track Whales. Field and Stream, September 5, 2012.
<http://www.fieldandstream.com/blogs/mans-best-friend/2012/09/seattle-dog-tracks-scent-whale-scat-open-ocean-water>

Copyright Restrictions + Other Notes:

Journal Title: *none*

Title: **Scat sniffing dogs help scientists studying stress**

Type: Publication Year: 2012

Uploaded File: *none*

URL: <http://www.kirotv.com/news/news/scat-sniffing-dogs-help-scientists-studying-stress/nPNSf/>

Abstract:

Media placement in response to our PLoS ONE publication (Ayres et al. 2012)

Citation:

Scat sniffing dogs help scientists studying stress. Kiro TV. June, 6 2012. <http://www.kirotv.com/news/news/scat-sniffing-dogs-help-scientists-studying-stress/nPNSf/>.

Copyright Restrictions + Other Notes:

Journal Title: *none*

Title: **Dwindling fish supply stresses**

Type: Publication Year: 2012

Uploaded File: *none*

URL: <http://www.miamiherald.com/2012/06/07/2837193/dwindling-fish-supply-stresses.html>

Abstract:

Media placement in response to our PLoS ONE publication (Ayres et al. 2012)

Citation:

Dwindling fish supply stresses. Miami Herald, 2012

Copyright Restrictions + Other Notes:

Journal Title: *none*

Title: **Dog sniff out whale poop for hormone study**

Type: Publication Year: 2012

Uploaded File: *none*

URL: <http://www.seattlepi.com/local/environment/article/Dog-sniff-out-whale-poop-for-hormone-study-3608532.php>

Abstract:

Media placement in response to our PLoS ONE publication (Ayres et al. 2012)

Citation:

Raechel Dawson. Dog sniffs out whale poop for hormone study. Seattle Post-Intelligencer, June 6, 2012.

<http://www.seattlepi.com/local/environment/article/Dog-sniff-out-whale-poop-for-hormone-study-3608532.php>.

Copyright Restrictions + Other Notes:

Journal Title: *none*

Title: **Whats stressing orcas out think portion size**

Type: Publication Year: 2012

Uploaded File: *none*

URL: <http://earthfix.kuow.org/flora-and-fauna/article/whats-stressing-orcas-out-think-portion-size/>

Abstract:

Media coverage on our publication in PLoS ONE (Ayres et al. 2012).

Citation:

Ashley Ahearn. EarthFix:Whats stressing orcas out? Think portion size. KUOW-FM, broadcast June 6, 2012. <http://earthfix.kuow.org/flora-and-fauna/article/whats-stressing-orcas-out-think-portion-size>.

Copyright Restrictions + Other Notes:

Journal Title: KUOW NPR

Title: **Dog gets starring role in study on what stresses killer whales**

Type: Publication Year: 2012

Uploaded File: *none*

URL: http://usnews.msnbc.msn.com/_news/2012/06/06/12071751-dog-gets-starring-role-in-study-on-what-stresses-killer-whales

Abstract:

Media coverage on our publication in PLoS ONE (Ayres et al. 2012).

Citation:

Miguel Llanos. Dog gets starring role in study on what stresses killer whales. MSNBC, June, 6 2012.

Copyright Restrictions + Other Notes:

Journal Title: MSNBC

Title: **Sniffing out a subtle scent to help save some whales**

Type: Publication Year: 2012

Uploaded File: *none*

URL: <http://www.nytimes.com/2012/09/02/us/sniffing-out-a-subtle-scent-to-help-save-some-whales.html>

Abstract:

Media coverage on our publication in PLoS ONE (Ayres et al. 2012).

Citation:

Kirk Johnson. Sniffing out a subtle scent to help save some whales. The New York Times, September, 1 2012.

Copyright Restrictions + Other Notes:

Journal Title: New York Times

Title: Distinguishing the impacts of inadequate prey and vessel traffic on an endangered killer whale (*Orcinus orca*) population.

Type: Reprints from Peer-Reviewed Journals, Books, Proceedings and Other Documents Publication Year: 2012

Uploaded File: [Ayres et al. 2012.pdf](#), 408 kb

URL: *none*

Abstract:

Managing endangered species often involves evaluating the relative impacts of multiple anthropogenic and ecological pressures. This challenge is particularly formidable for cetaceans, which spend the majority of their time underwater. Noninvasive physiological approaches can be especially informative in this regard. We used a combination of fecal thyroid (T3) and glucocorticoid (GC) hormone measures to assess two threats influencing the endangered southern resident killer whales (SRKW; *Orcinus orca*) that frequent the inland waters of British Columbia, Canada and Washington, U.S.A. Glucocorticoids increase in response to nutritional and psychological stress, whereas thyroid hormone declines in response to nutritional stress but is unaffected by psychological stress. The inadequate prey hypothesis argues that the killer whales have become prey limited due to reductions of their dominant prey, Chinook salmon (*Oncorhynchus tshawytscha*). The vessel impact hypothesis argues that high numbers of vessels in close proximity to the whales cause disturbance via psychological stress and/or impaired foraging ability. The GC and T3 measures supported the inadequate prey hypothesis. In particular, GC concentrations were negatively correlated with short-term changes in prey availability. Whereas, T3 concentrations varied by date and year in a manner that corresponded with more long-term prey availability. Physiological correlations with prey overshadowed any impacts of vessels since GCs were lowest during the peak in vessel abundance, which also coincided with the peak in salmon availability. Our results suggest that identification and recovery of strategic salmon populations in the SRKW diet are important to effectively promote SRKW recovery.

Citation:

Ayres K.L., R. K. Booth, J.A. Hempelmann, K. L. Koski, C.K. Emmons, R.W. Baird, K. Balcomb-Bartok, M.B. Hanson, M.J. Ford, and S.K. Wasser. Distinguishing the impacts of inadequate prey and vessel traffic on an endangered killer whale (*Orcinus orca*) population. *PLoS One*. 2012;7(6):e36842.

Copyright Restrictions + Other Notes:

Journal Title: *PLoS*

:: OTHER DOCUMENTS

No Documents Reported This Period

:: LEVERAGED FUNDS

Type: influenced Period: 2012-05-25::2012-10-12 Amount: \$22705

Purpose:

Use of boat for research purposes

Source: Private boat, use donated in-kind, valued at \$1195 per week for the 19 weeks

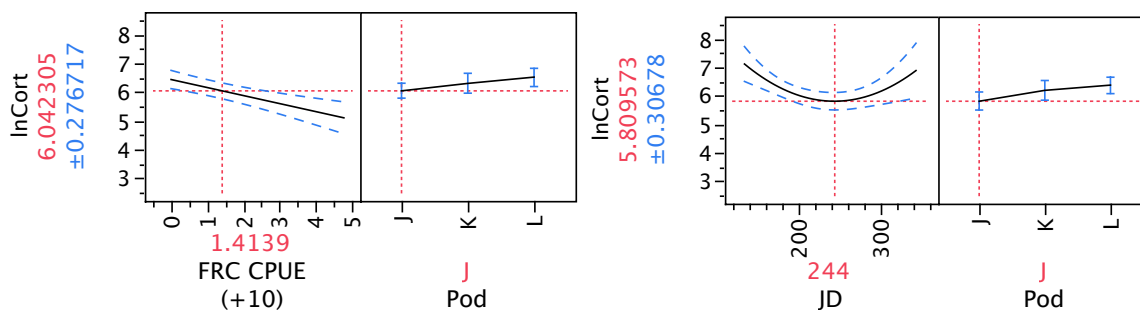
UPDATE (PROGRESS) REPORT NARRATIVE

Our first publication on the Southern Resident Killer Whales (SRKW) covered the period from 2006-2009, sampling May-October in 2008-2009 (Ayres et al 2012). We continued to longitudinally sample the SRKW between May and October in 2010-12. All samples since 2008 were collected using a detection dog, operating from the bow of a single vessel. The data from 2010-2011, but not 2012, have already been analyzed for most genetic and physiologic measures we are collecting. The 117 SRKW scat sample collections from 2012 occurred during 50 sampling days, ranging from 1-12 hours of effort per day. Of these samples, 97% were collected >200 meters, and 85% \geq 400 meters, away from the whales. The 2012 samples have all been freeze-dried and are ready to be extracted for hormone assays. All samples from 2010 and 2011 have been genotyped in collaboration with NOAA NWFSC. The 2012 samples should be genotyped within a few months.

Ayres et al (2012) demonstrated that the summer Fraser River Chinook salmon run periodically places the SRKW in nutritional deficit, during some years. Early spring Chinook runs may be particularly important to help the SRKW recover from a lean winter, simultaneously providing the buffer needed to sustain them until the Fraser River Chinook salmon run begins in earnest. Analyses of the 2010 and 2011 data replicate the findings of Ayres et al (see below).

Endocrine Results: GC concentrations were closely associated with nutritional, but not boat stress. GCs inversely track the abundance of Fraser River Chinook salmon (Fig 1, left), the predominant food source of SRKW during the study period. GCs mobilize glucose in response to nutritional or psychological emergencies. However, we only observed nutritional stress impacts on GC; GC concentrations did not appear to be strongly influenced by boat stress since GCs were lowest, not highest, when boat (and fish) densities were at their peak (~ Julian date 244). GCs then increased as boat (and fish) densities declined towards the end of the season (Figure 1 right). There was some indication that boat densities may elevate glucocorticoid hormones (GC) concentrations during years when fish densities are low (Ayres et al 2102). However, additional years with low fish densities are needed to provide sufficient statistical power to confirm this.

Figure 1. The relation of Glucocorticoids (InCort) with fish abundance (Fraser River Chinook Catch per Unit Effort (FRC CPUE); left figure) and Julian date (JD; right figure), by pod, from 2008-2011.



Thyroid hormone (T3) provided an index of more persistent nutritional conditions. T3 responds to nutritional pressures by lowering metabolism, allowing resources to be more conservatively utilized when limited. The slower responsiveness of T3 means that when the SRKW first arrive in May/June, their T3 levels more closely reflect the nutritional conditions from where they just came from. Temporal patterns in T3 concentrations were consistently highest at the time of SRKW arrival in the Salish Sea in late spring, suggesting that the SRKW arrive after feeding on

a very rich food resource, presumed to be the fat-rich early spring Chinook salmon runs. T3 levels begin to decline shortly after the SRKW spring arrival throughout the fall (Fig 2, left). When analyzed one year at a time, T3 reaches a plateau in the interim coincident with the summer peak in Fraser River Chinook salmon abundance each year (Ayres et al 2011). Mean annual T3 concentrations also appear to correspond positively to killer whale births, and early Columbia and Fraser River Chinook abundances, and negatively with killer whales mortalities in the same year (Figure 3).

Figure 2. The relation between thyroid hormone (LnT3) and Julian Date from 2008-2011.

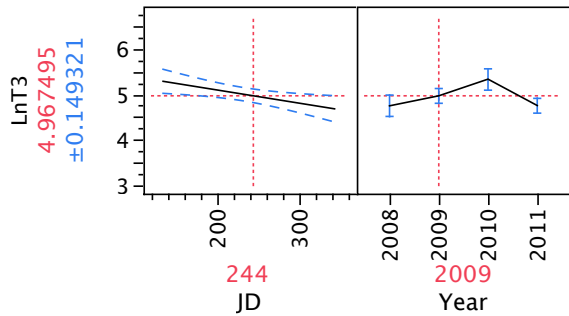
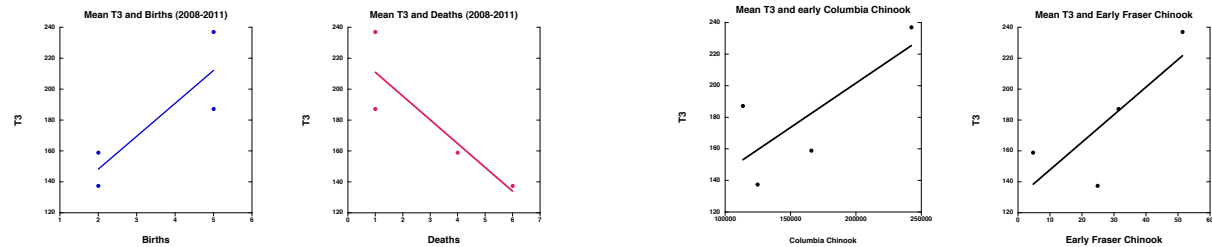


Figure 3. Mean annual T3 concentrations (May-Oct) in relation to that year's killer whale births and deaths (left two graphs), and early Chinook abundances in Columbia and Fraser Rivers, from 2008-2011 (right two graphs). NOTE: each point is one year.



Toxicant Results: Our most recent work aims to examine the toxicant pressures on the southern resident killer whales and whether this is exacerbated by the nutritional stress we are measuring. Fine-grained toxicant analyses are being conducted in collaboration with NOAA's Northwest Fisheries Science Center, under the supervision of Gina Ylitalo, using the same analytic protocol previously used to analyze killer whale blubber biopsy samples. Ylitalo is able to analyze approximately 20 samples per year in these fine-grained analyses of our largest SRKW fecal samples. Thus far, we have measured toxicant levels of 21 samples collected in 2010. The 2011 and 2012 samples will be completed this year.

Figure 4 shows a close correspondence between the percent of each PCB congener identified in 21 SRKW scat samples (Ylitalo method) and those acquired from blubber biopsy samples (Ross et al 2000). However, scat may have disproportionately more lower chlorinated congeners and blubber may have more higher chlorinated congeners (Figure 4). Figure 5 shows results of POP analyses on 14 genetically confirmed SRKW fecal samples collected in 2010. Consistent with previous work on blubber biopsy samples, scat toxicant profiles demonstrate significantly higher $\sum\text{DDT}/\sum\text{POP}$ ratios in K and L pods, after controlling for sex and sample collection date. This finding is consistent with forage patterns of K and L pods off the California

coast compared to J pod ($p < 0.05$). Likewise, $\Sigma\text{PCB}/\Sigma\text{POP}$ were statistically higher ($p < 0.05$) in J pod, known to spend far more time foraging in Puget Sound during winter. Additionally, ΣPCB and ΣPBDE levels are lower in adult females compared to the level in the one genetically confirmed adult male (J pod, age 19) for the samples analyzed thus far (data not shown).

Figure 4. Comparison of percent PCB congener (Individual PCB/ ΣPCB) in killer whale scat samples vs published blubber biopsy levels

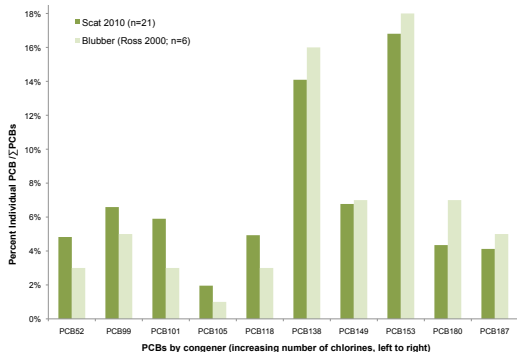
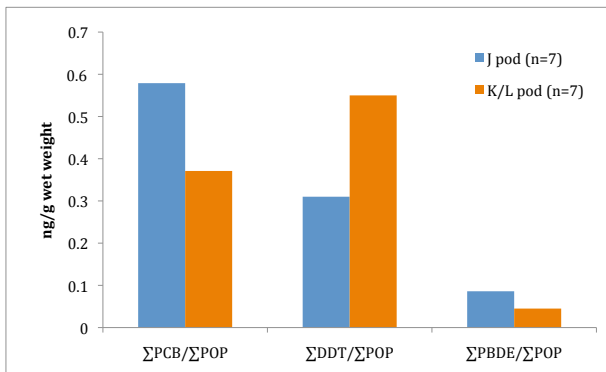


Figure 5. Comparison of mean toxicant level of genetically confirmed samples between pods, adjusted for sex and sample collection date



We have also initiated more coarse-grained toxicant analyses in collaboration with the University of Washington Environmental Health Trace Analytics Lab. These latter analyses focus on the more abundant congeners that NOAA previously identified in our SRKW scat samples. We hope that the smaller sample volume and greater efficiency of this new method will increase opportunities for parallel measures of hormones and toxicants from the sample. The latter method will be validated by comparisons with the fine-grained analyses conducted in collaboration with Ylitalo.

Concluding Remarks: A broad goal of our work is to promote recovery of SRKW and their marine environment. More specifically, we are applying bio-monitoring tools from our longitudinal study to guide allocation of management resources between salmon recovery, toxin clean-up and ecotourism regulation. Our collective tools evaluate killer whale temporal physiologic response measurements to environmental influences. Upcoming work includes evaluating how contaminant levels vary with nutrition, prey availability, endocrine disruption, and reproductive health outcomes. We plan to test whether toxicants in scat increase directly from consumption of contaminated prey, or from release of accumulated toxicants liberated during lipid metabolism during lean times. We also plan to link these impacts to the occurrence, health and success of pregnancies using fecal progestins and associated GCs, already validated for killer whales (Ayres and Wasser, *in prep*). The results described in this report indicate that we have the needed tools and precision to achieve these important goals.

Ayres KL RK Booth, JA Hempelmann, KL Koski, CK Emmons, RW Baird, K Balcomb-Bartok, MB Hanson, MJ Ford, SK Wasser. 2012. Distinguishing the Impacts of Inadequate Prey and Vessel Traffic on an Endangered Killer Whale (*Orcinus orca*) Population. *PLoS One* 7(6): e36842

Ross, P.S., Ellis, G.M., Ikonomidou, M.G., Barrett-Lennard, L.G., and Addison, R.F. 2000. High PCB concentrations in free-ranging Pacific killer whales, *Orcinus orca*: effects of age, sex and dietary preference. *Mar. Pollut. Bull.* 40: 504–515.