Comments on the NOAA Scientific Panel Report by:

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The Scientific Panel did a thorough and impressive job in sifting through all data available to them. We agree with the panel's conclusion that food availability is critical to sustain SRKW populations. However, we believe several areas addressed in the report need further attention.

The report describes discrepancies between current and historical carrying capacity for the SRKW, but fails to discuss options for increasing current carrying capacity. The report also appears to be overwhelmingly focused on broad demographic associations between annual SRKW population growth and fish abundance that occur over a relatively long time scale. Too little attention is paid to changes occurring over shorter time scales, including key transitional pinch-points of salmon runs and availability facing the SRKW population over each year. The panel rightly recommended the need to focus on more sensitive tools to monitor nutritional and associated impacts on SRKW population growth. However, again, their only specific recommendation was for photogrammetric measures, which occur over a relatively long time scale. They dismissed the use of more short-term physiological analyses from scat, arguing that hormones are confounded because they have multiple functions. However, as argued below, the multiple functions of these hormones should instead be viewed as strengths; their measurement in properly designed studies allows investigators to observe how physiological levels change over the short-term with the various environmental pressures the SRKW are responding to. Such short-term temporal changes are particularly conducive for designing and evaluating mitigation.

Carrying capacity

Although SRKW may be at carrying capacity, it is reasonable to conclude this is only because their prey source cannot sustain more killer whales. Indeed, the report also indicates that ancestral killer whale populations may have been orders of magnitude greater than current numbers. Marked historic declines have also occurred in the SRKW salmonid prey, and particularly in their preferred Chinook salmon prey. What was not clearly articulated in the report was the need to raise the carrying capacity by restoring important diminished fish runs so that SRKW have the opportunity to increase their abundance. As acknowledged in the report, concern for this population is due to the current size of the population and "potential impacts of future, unforeseen events on a population that lacks the resilience created by a higher abundance." At this low population size, even a small, random event could extinguish the SRKW population.

Transitions throughout the year

The report spends a great deal of time dwelling on what we don't know, and correspondingly appears overly reluctant to make any strong inferences. While considerably more data are needed, it is clear that the SRKW undergo a number of key transitions in salmon runs and availability throughout the year, with each transition impacting the whale's ability to cope with whatever comes next. Very little is mentioned about the importance of these transitions in the panel report. Yet, these transitional pinch-points could represent areas of greatest vulnerability facing the SRKW population in any given year, and hence key focal areas for mitigation.

While we know very little about SRKW ranging and foraging behavior during winter, there is reasonably good evidence to indicate that winter is their harshest time of year. SRKW mortalities tend to be highest in winter and fish appear more dispersed, making large prey more difficult to find. Presumably, conditions start to change in the early spring as adults of some salmonid populations make their way to river mouths to begin their spawning migration. Many of these early spring Chinook tend to have very high fat to body ratios because they are the earliest to arrive at the rivers, having the furthest to swim upstream while fasting for most of the duration. Physiological data from Ayres et al. (2012), also presented by Wasser at workshop 1, suggest that the killer whales are consuming these early spring salmon runs just prior to arriving in the Salish Sea. This research accordingly concluded that specific early spring salmon runs are likely a key resource that allows SRKW to recover from the harshness of winter, as well as sustain them in early summer while the Fraser River Chinook runs are increasing (see also below). The Fraser River Chinook runs eventually peak in August and then decline to very low levels by October, when the whales undergo another transition, returning to their winter forage areas.

The above transitions indicate the need to identify the early spring salmon runs that the SRKW are feeding on, assess the current and historic abundances of those prey populations, and do all we can to restore them if historic levels greatly exceeded current levels. Ayres et al (2012) also indicated that early spring, interior races of Columbia River Chinook are the most likely source of this rich spring prey, as these runs are known for their excessively high fat to body ratios (Bret 1995, Mesa and Magie 2006). Unfortunately, the panel report largely ignored these conclusions.

Noninvasive fecal hormone and toxicant analyses

Fecal samples contain host and prey DNA, a variety of biologically relevant hormones, and lipophilic toxicants. This, coupled with the relative ease of sample collection in nature allows investigators to observe how levels change in response to varying environmental pressures impacting killer whales. We currently measure five complementary hormones from killer whale feces, thyroid (T3), glucocorticoid (GC), aldosterone, progestagen and androgen hormones, all of which have been carefully validated for a wide range of species (Wasser et al. 1988, 1993, 1994, 1996, 2000, 2005, 2011; Ayres et al 2012). Detection dogs are used to enhance sample collection (Wasser et al 2004, Roland et al. 2006; Ayres et al. 2012), making these tools particularly effective. Last year alone, a single detection dog enabled us to collect 149 SRKW fecal samples over 5 months. Oddly, the panel also ignored the merits of the scat dog methodology, despite marked increases in sample size and its application to a number of ongoing NOAA projects.

Nutrition and stress measures

Glucocorticoid (GC), aldosterone and thyroid (T3) hormones provide key complementary indices of the physiological response to nutritional demands. GCs and aldosterone are secreted by the adrenal cortex. GCs mobilize glucose in response to nutritional emergencies, indicating immediate energetic demands on the animal (acute nutritional stress). Aldosterone also rises in response to acute nutritional stress. However, it serves as a brake, preventing GCs from over-metabolizing fat for glucose to meet nutritional demands (Devenport et al. 1985*)*. (Aldosterone also serves to retain water and sodium, and excrete potassium, in part to maintain blood pressure. These functions are especially important in marine mammals that get most of their water from prey. Marine mammals are constantly battling water loss to their highly saline environment and this challenge is likely exacerbated by lack of prey.) Thyroid hormone (T3) is indicative of long-term nutritional demands. Animals respond to persistent food shortages by lowering T3, reducing metabolism to more conservatively utilize the remaining food available. They do this by lowering T3. The T3 response is thus a longer-term response to nutritional demands, working by "resetting the thermostat". The three hormones together paint a comprehensive physiological picture of nutritional demands facing the animal on different temporal scales.

The panel report states: "Cortisol (also called GC) levels are indicative of activation of a stress response, which is a common physiological pathway that results from any stressor in addition to nutrition such as sound, boat traffic, or conspecific aggression. Thus, use of fecal cortisol as an indicator of nutritional stress is limited in an environment with considerable human activity."

To the contrary, Ayres et al. (2012) was able to take advantage of the GC rise in response to both nutritional stress from prey declines and psychological stress from other environmental stressors such as boat traffic. Both Fraser River Chinook and boat abundance parallel each other, being relatively low when the killer whales arrive in late May, peaking in mid- to late-August and declining thereafter until the killer whales depart in late October. Thus, if GCs are driven by boat stress, they should follow the same pattern as the boats, being lowest in early summer and late fall and highest during the August boat peak. By contrast, if fish abundance is driving GCs, they should show the opposite pattern. GCs should be highest in early summer and late fall and lowest in August. We found the latter pattern for four years in a row, clearly indicating that fish is much more important than boat stress in driving the physiological GC response. Moreover, in the year when Fraser River Chinook abundance was particularly low (2007), GCs also responded to boat stress, indicating that boat stress does matter in times of low fish availability (Ayres et al. 2012).

Similarly, the panel report states: "The fecal thyroid hormone data presented in Workshop 1 showed seasonal patterns consistent across years in batched feces. Such a pattern is hard to interpret as the number of individuals and their age and sex samples were not presented. Although nutritional status can influence fecal thyroid hormone levels, other factors such as age, activity, day length, reproductive status and contaminant exposure will also affect these fecal hormone levels (Oki and Atkinson 2004, Ciloglu et al. 2005, Ross et al. 2007). It is thus not clear whether the seasonal decrease reported in fecal thyroid hormone levels indicates nutritional stress or a seasonal endocrine shift for other reasons."

Our SRKW fecal samples were all genotyped by NOAA to determine the sex and individual identity by NOAA. This allowed us to adjust for individual, age and sex differences in our models (Ayres et al. 2012). More importantly, the adjusted hormone levels we have measured in scat samples over the past four years have indicated a highly consistent trend: Each year the SRKW arrive in the Salish Sea during late spring/early summer, their hormone levels reflect them to be in the best nutritional condition observed for that year. These results strongly suggest the importance of early spring salmon runs on SRKW overall recovery (see also above). We showed these data for 2007-2009 at workshop 1, also indicating that SRKW T3 concentrations at arrival in May/June tended to be highest in the years when salmon runs were highest (also published in Ayres et al, 2012). After that meeting, we added the 2010 data to the analysis, observing the same pattern (Figure 1).

We note, SRKW T3 levels at their late spring/early summer arrival in the Salish Sea in 2010 were higher than in any other year we recorded to date. Correspondingly, 2010 Columbia River early spring Chinook returns were more than double that of the three previous years. Factors such as change in T3 with day length are unlikely to account for these differences; day-length varies relatively little between June and October, and particularly on any given date between years.

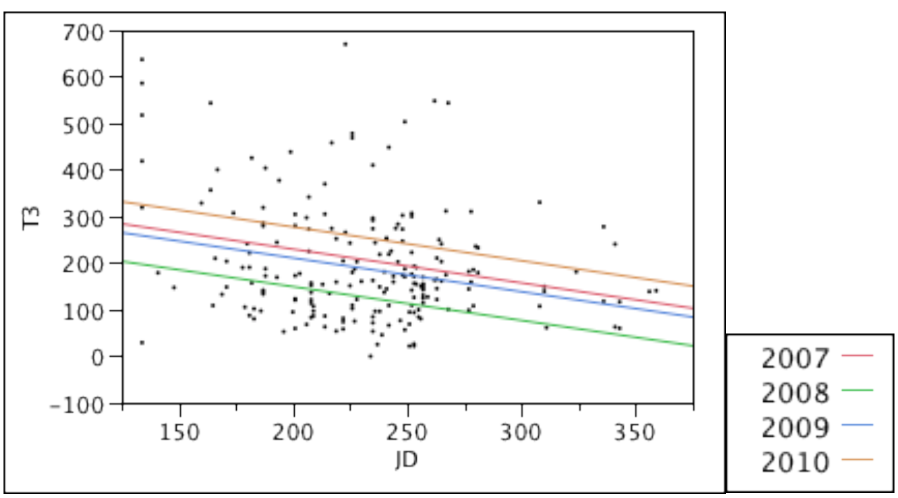

Figure 1. Change in SRKW thyroid (T3) hormone concentration by Julian date (JD) across years, adjusted for individual as a random effect.

Clearly, such methods allow managers to examine environmental impacts on SRKW health over a tight temporal time scale. These tools can help inform and assess the success of mitigation efforts. While the potential for endocrine disruption of T3 should also be considered, our ability to acquire toxicants (see below) and T3 from the same fecal samples in relation to changes in prey availability within and between years is again a key strength of this method. We can determine whether T3 levels are suppressed by endocrine disruption and thus whether toxins are more abundant and T3 suppression more extreme during times of low prey availability, within and between years. Such interactions should clarify the multiple physiological impacts that can result from low prey.

Fecal Toxicant Measures

Toxicant exposures from prey bioaccumulate in the SRKW adipose tissue. This raises the question whether immediate toxicant exposure in circulation is greatest at the time when toxicant-rich prey is being ingested (i.e., prey peaks) or during times of low prey because SRKW are releasing bioaccumulated toxins as they metabolize adipose tissue to meet energy demands. If the latter, toxicant exposure would be greatest when prey availability is lowest. Most available evidence supports the second assertion although such temporal specific data are difficult to acquire from invasive tissue biopsies. By contrast, collaborative work we have been conducting with Gina Ylitalo at NOAA (also part of the dissertation work of Jessica Lundin) indicates that we are successfully measuring PCB, PBDE and DDT congeners and metabolites in SRKW feces—known endocrine disruptors and reproductive toxicants—and these fecal profiles are comparable to those found in SRKW blubber biopsy samples (Figure 2). Fecal sampling may thus provide the ONLY reliable method to readily acquire toxicant samples from multiple SRKW that can be temporally tied to changes in their prey availability, associated nutritional pressures and endocrine disruption.

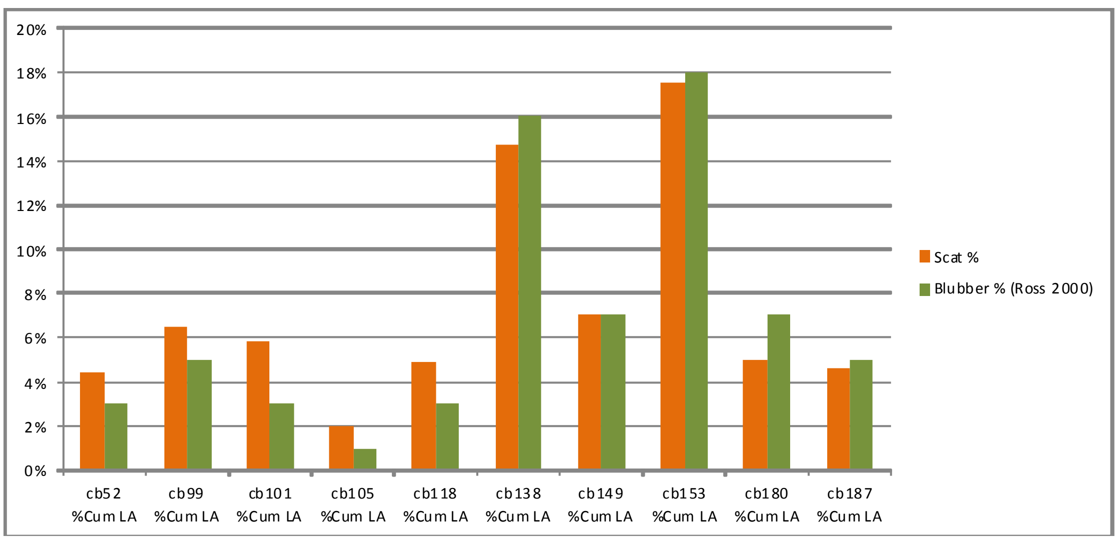

Figure 2. Mean PCB congener profiles in SRKW scat samples expressed as percent of sum PCB congener levels, by measures in scat and measures in SRKW blubber biopsies (Ross et al. 2000)

Measuring variance in population growth

The panel report relies on indices of population growth for a variety of analyses. Their growth estimates are largely restricted to mortality of known individuals. These are valuable measures, made possible by the diligence of the Center for Whale Research over the past four decades. However, these growth measures are limited in their ability to delineate more fine tuned impacts on offspring recruitment. Specifically, they are unable to ascertain pregnancy and neonatal failure, which provide some of the greatest variance in overall population growth. Monitoring fecal sampling of progestagens over time can greatly aid understanding of key determinants of recruitment.

As reported in workshop1, fecal progestagens provide a reliable index of pregnancy, which is especially important in SRKW because their gestation spans more than one year (gestation is approx. 18 months). In the absence of pregnancy measures, it becomes impossible to determine whether females abort their pregnancies or experience neonatal loss during winter. Since adult mortality is a relatively rare event, estimating the occurrence of pregnancy and neonatal failure could help identify an important, previously undescribed determinant of SRKW population growth. Dogs can sample adult females during early summer and late fall to determine the pregnancy status of individuals arriving and departing the Salish Sea. We have collected genetically confirmed fecal samples from 25 of the 49 known females (~50%) in the SRKW population and could increase this percentage by more directed sampling of adult females early and late in the field season. Known pregnancy indices on these females can help tie this highly valuable component of population growth to associated environmental and physiological measures, including endocrine disrupting toxicants, with considerable management implications.

Concluding Remarks

In our opinion, the greatest weakness of the scientific panel report is its nearly entire focus on long time scale methods. While important, such methods fail to capture immediate responses to changing environmental pressures. Tools that can temporally measure SRKW health in relation to changing environmental conditions offer the best method for guiding mitigation, as well as quantifying its success. Physiological measures of biologically relevant hormones and lipophilic toxicants from genotyped SRKW fecal samples collected by detection dogs should be particularly useful in this regard.

References:

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. doi:10.1371/journal.pone.0036842.

Brett JR (1995) Energetics. In: C. Groot, L. Margolis, and W.C. Clarke, editors. Physiological Ecology of Pacific Salmon. Vancouver: UBC Press, 3–63.

Devenport, LD, KG Goodwin, PM Hopkins. 1985. Continuous infusion of aldosterone: Correlates of body weight gain. *Pharmacology, Biochemistry and Behavior* 22: 707-709.

Mesa MG, Magie CD (2006) Evaluation of energy expenditure in adult spring Chinook salmon migrating upstream in the Columbia River basin: an assessment based on sequential proximate analysis. River Res Appl 22: 1085–1095.

Rolland, RM, PK Hamilton, SD Kraus, B Davenport, RM Bower, SK Wasser. 2006. Use of scat detection dogs to study reproduction and health in North Atlantic right whales. *Journal of Cetacean Research and Management* 8(2):121–125.

Ross, PS, GM Ellis, MG Ikonomou, LG Barret-Lennard, RK Addison. 2000. High PCB concentrations in free-ranging Pacific killer whales, *Orcinius orca*: Effects of age, sex and dietary preference. Marine Pollution Bulletin 40: 504-515.

Wasser, S.K., L. Risler, and R.A. Steiner. 1988. Excreted steroids in primate feces over the menstrual cycle and pregnancy. *Biology of Reproduction* 39: 862-872.

Wasser, S.K., R. Thomas, P.P. Nair, C. Guidry, J. Southers, J. Lucas, D.E. Wildt and S.L. Monfort. 1993. Effects of dietary fiber on faecal steroid measurements. *Journal of Reproduction and Fertility* 97: 569-574.

Wasser, S.K., S.L. Monfort, J. Southers and D.E. Wildt 1994. Excretion rates and metabolites of oestradiol and progesterone in baboon (*Papio cynocephalus*) faeces. *Journal of Reproduction and Fertility* 101: 213-220.

Wasser, S.K., S. Papageorge, C. Foley, J.L. Brown. 1996. Excretory fate of estradiol and progesterone in the African elephant (*Loxodonta africana*) and patterns of fecal steroid concentrations throughout the estrous cycle. *General and Comparative Endocrinology* 102: 255-262.

Wasser, SK, KE Hunt, JL Brown, K Cooper, CM Crockett, U Bechert, JJ Millspaugh, S Larson, SL Monfort. 2000. A generalized fecal glucocorticoid assay for use in a diverse array of non-domestic mammalian and avian species. *General and Comparative Endocrinology* 120: 260-275.

Wasser, SK, B. Davenport, ER Ramage, KE Hunt, M Parker, C Clarke, G Stenhouse. 2004. Scat detection dogs in wildlife research and management: Applications to grizzly and black bears in the Yellowhead Ecosystem, Alberta, Canada. *Canadian Journal of Zoology* 82: 475-492.

Wasser, SK, KE Hunt. 2005. Noninvasive measures of reproductive function and disturbance in the barred owl, great horned owl, and northern spotted owl. *Annals of the New York Academy of Sciences* 1046: 109-137.

Wasser, SK, J Cristòbal-Azkarate, RK Booth, L Hayward, K Hunt, K Ayres, C Vynne, K Gobush, D Canales-Espinosa, E. Rodríguez-Luna. 2010. Non-invasive Measurement of Thyroid Hormone in feces of a Diverse Array of Avian and Mammalian Species. *General and Comparative Endocrinology* 168: 1-7. Doi: 10.1016/j.ygcen.2010.04.004

Wasser, SK, JL Keim, ML Taper, SR Lele. 2011. The influences of wolf predation, habitat loss and human activity on caribou and moose in the Alberta oil sands. *Frontiers in Ecology and the Environment* 9: 546-551.