

abundance at that time, and consistent with the precipitous decline in T3 shortly following SRKW arrival [5].

2. Methods

2.1 Ethics statement

Fecal samples were collected in United States waters under National Marine Fisheries Service permits 532-1822-00, 532-1822, 10045 and 17344. Samples were collected in Canadian waters under Marine Mammal License numbers 2008-16, 2009-08, 2010-09 and 2012-08, as well as Species at Risk Act permits numbered 91, 102, 109 and 155. Sample collection methods were approved by the University of Washington's Institutional Animal Care and Use Committee (IACUC) under protocol 2850-08.

2.2 Scat (fecal) sampling using detection dogs

Scat sampling occurred in the Salish Sea between late May and October, from 2008-2014, coinciding with the time the SRKWs frequent the study area. Whenever possible, we aimed to evenly sample each pod by starting at the front of the pod's direction of travel, continuing to sample until the pod passes and then returning again to the front of the pod.

Scat samples are located by detection dogs trained to locate SRKW scat floating on the water's surface [5,13,14]. The use of detection dogs greatly increases sample size due to their remarkable ability to smell SRKW scats at distances up to one nautical mile away, even in fast moving currents. The detection dog rides on the bow of the boat, 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 emanating from the sample, the dog's behavior suddenly changes from resting to actively perched far over the bow of the boat, anticipating its reward for sample detection. As the boat passes through the center of the scent cone, where the odor is strongest, the dog leans heavily over the windward side of the boat, following the strongest scent, informing the handler to direct the boat driver to turn into the wind. Subtle cues by the dog, relative to wind direction, allow the driver to stay on the scent line until the sample is reached. The dog typically becomes restless, often whining at that point because the scent surrounds the boat and thus no longer has a clear direction. If at any time the boat travels out of the scent cone, the dog changes position and looks back to where the scent was strongest. The handler then directs the driver to circle back into the scent cone until the dog's change in behavior once again alerts the handler it has redetected the scent.

As soon as the sample is visually located, a 1-liter polypropylene beaker fastened to a 3-6 foot pole is used to scoop the sample by skimming the surface just under the sample. The first sample out of the water is presented to the dog, which is followed immediately by the toy reward and a few minutes of play. Meanwhile, the crew continues to scoop all remaining sample pieces from the water's surface. The majority of water is carefully poured off the sample, and the sample pieces are collected into a 50 mL polypropylene tube, centrifuged, and the remaining seawater is decanted. The sample is placed on dry ice until stored frozen at -20°C that evening and remains at that temperature until processed in the lab. Fecal samples range in size from 0.5 to 300 mls, but a typical sample collection volume is 2 mls. Fortunately, the consistency of SRKW scat makes the hormones fairly evenly distributed even in small samples (Ayres and Wasser, unpublished data).

2.3 Fecal DNA and hormone measures

Once thawed for hormone extraction, the homogenized sample is swabbed for DNA using a synthetic tip. The swab is then kept frozen at -20°C until being genotyped for species, sex, pod,