Species and stock identification of scale/tissue samples from southern resident killer whale predation events collected off the Washington coast during PODs 2009 cruise on the McArthur II

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Introduction
In order to improve the Critical Habitat designation for SRKW and determine prey selection during the winter and spring, the NWFSC has undertaken 5 survey cruises on the McArthur II in the coastal waters of Washington Oregon and British Columbia between 2004 and 2009 to locate pods from this population. Despite locating SRKWs on three of the four previous cruises, we had not been successful in collecting any predation event samples or feces.

Field Methods
Field activities were based off the McArthur II during a survey for southern resident killer whales in the coastal waters of Washington and Oregon from 23 March to 6 April 2009. We located southern resident killer whales twice during the survey (26 and 27 March) and were able to conduct small boat operations on both days. We used a 7-m rigid-hulled inflatable boat to follow the whales at close range and for each encounter we recorded location, pod(s) present, the approximate area covered by the group of whales (an indicator of how spread out the group was), and the focal animal or group (sensu Altmann 1974)). Photographs were taken with digital SLR cameras with lenses ranging from 100 to 300 mm in focal length and identities of individual whales in the focal group were verified as members of L pod using a published catalog (Center for Whale Research 2008). Following Ford and Ellis (2006), whenever possible we closely followed one or more focal whales and attempted to obtain predation event samples associated with just those whales. Alternatively, if the number of whales near the boat was too great to keep track of individual whales, we collected samples opportunistically.
The method we used for predation event detection was similar to Ford and Ellis (2006) and the sample collection method was based on the approach developed by Ford et al. (1998). We used several cues (e.g., fast directional or non-directional, moderate non-directional, first surface after a long dive, or whales converging) to trigger close approaches to look for fish parts. Whenever any whale surfaced less than ~20 m from the boat, we would also watch the mouth-line of the whale to try to assess whether it was carrying prey. Upon observing one or more cues we would approach the “fluke print” (glassy areas of water caused by upwelling from the whales’ tail as it dives) of the focal whale, noting the presence of other whales nearby and recording information on any interactions between the focal whale and other whales (e.g., change in distance among whales). Approaches were always made in a way to avoid or minimize disturbance to the whale(s) present, by slowing the vessel speed either to a stop in the fluke print if the whale was still actively non-directionally surfacing, or matching the speed and direction of the whale upon arrival at the fluke print.

Once at the fluke print, we recorded whether we observed any fish, fish scales, fish parts, fecal material or other types of material discharged by the whales. If material was observed, we recorded the estimated number of prey parts or other material visible in the water column. When no material was observed in the first fluke print, we would proceed to subsequent fluke prints. We also attempted to collect feces from or between fluke prints. We used a long-handled (4-m) fine-mesh net to collect any material observed in the water. Samples collected were stored in plastic bags in a cooler while in the field. Prey samples (comprised of one or more prey parts) were later stored at -80°C prior to analyses, except for the fish scales which were removed from the initial sample bag and dried at room temperature.

**Genetic methods**

Scales were dried and DNA was extracted from each individual scale using standard methods (QIAamp® DNA Mini Kit) on May 14th 2009. Species was determined by PCR amplification and sequencing the COIII/ND3 region of the mitochondrial genome using the primers and PCR reaction conditions described in (Purcell et al. 2004). DNA
that was positively identified as Chinook salmon were then subjected to PCR amplification for the 13 microsatellite loci used in a coast-wide data set of genotypes developed by a consortium of laboratories (Seeb et al. 2007). PCR amplification products were then analyzed using an Applied Biosystems 3100 capillary electrophoresis system. GeneScan and Genotyper software (Applied Biosystems) were used to determine the genotype of every sample at each locus. We used the program ONCOR (Kalinowski 2003) to compare the resulting genotypes to the coast-wide baseline of genotypes from samples of known origin, and estimated the most likely region of origin for each sample.

Results

Field sampling

Following an acoustic detection of SRKW calls at approximately 0400 on 26 March 2010 near Gray’s Canyon, L pod was sighted traveling northeast just offshore of Ocean Shores, Washington, shortly after daybreak. The whales turned south approximately a mile offshore and we were able to conduct small boat operations for most of the day with the whales. Two predation event samples were collected as the whales travel parallel to the coast between the entrances to Gray’s Harbor and Willapa Bay. We also observed two fish chases but did not recover scales or tissue later in the day. We relocated the whales on 27 March 2010 a couple of miles west of Cape Disappointment heading north. No samples were collected.

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As shown below, both of the samples were Chinook salmon, and the most likely regions of origin for these samples were in the Columbia River; one from the Upper Columbia, the other from the Snake River. Both assignments had a high degree of probability ($P = 1.000$) compared to their possible origin from a different region.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Estimated Region of Origin</th>
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<tr>
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<td>2009MAR26-02B</td>
<td>Snake R. Spring/Summer Run</td>
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References
