and individual identification by NOAA NW Fisheries Science Center [34]. 76% of all individuals are currently genotyped to the individual, and 88% of all adult females. Fecal hormone metabolites of glucocorticoid (GC), thyroid (triiodothyronine, T3), testosterone (T) and progesterone (P4) are extracted using methods described in [21] and measured using assays in Wasser et al. [35] (P4), [36] (GC), [21] (T3)] and Vellosa et al. [37] (T). Briefly, each sample is thawed once and centrifuged (2,200 rpm for 20 minutes), allowing any excess salt-water to be decanted. Samples are lyophilized (48 hours in a Labconco FreeZone Freeze Dry System), thoroughly mixed and up to 0.1g weighed, transferred to a 50 ml polypropylene screw-top tube and extracted once in 15ml of 70% ethanol using a Multi-Tube Pulse Vortexer (Terre Haute, IN). Extracts are then stored at -20^0 C until assayed for hormone concentrations. Hormone concentrations are expressed per gram dry weight to control for inter-sample variation due to diet and variable moisture [38]. Wasser et al. [38] showed that expressing fecal hormones per gm dry weight controls for diet related changes in fecal bulk. Because fecal hormones are hydrophobic, removing all water from the sample removes the majority of variation in fecal bulk, significantly improving the blood-fecal hormone correspondence (see also [5] for killer whales). Samples smaller than 0.02 g dried weight were excluded from analysis to avoid inflation effects of low sample mass on hormone concentrations [39].

Radioimmunoassay was performed to measure fecal hormone metabolites using ^{125}I corticosterone RIA kits (#07–120103; MP Biomedicals, Costa Mesa, CA) and MP Biomedicals' Total T3 coated tube RIA kits (#06-B254216) for GC metabolites and T3, respectively. The T3 assay was previously validated for killer whales [21]. The GC assay [36] was validated for killer whales in Ayres et al [5]. Fecal pools as well as commercial controls from each assay kit were used to assess inter-assay coefficients of variation. Commercial T3 controls were prepared as previously described [21]. P4 and T were measured using an in house 3H progesterone RIA assay using antibody CL425 [35,40], and an in-house 3^H testosterone RIA assay using antibody #250 [37,40]. All other hormone assays were validated in the present study.

All five hormone assays exhibited parallelism; slopes of serially diluted SRKW fecal extracts were not significantly different from the slopes of the standard curves (GC: $F_{1,7} = 0.41$, $p = 0.54$; T3: $F_{1,9} = 2.89$, $p = 0.12$; P4: $F_{1,10} = 0.80$, $p = 0.3925$; T: $F_{1,9} = 3.65$, $p = 0.09$). Fifty percent binding of the radioactively labeled hormone occurred at target dilutions of 1:60 for GC, 1:30 for T3,1:60 for P4 and 1:10 for T metabolites. All five hormones also exhibited good accuracy at their target dilutions (GC: slope = 1.2, $r^2 = 0.98$; T3: 1.09, 1.00; P4: 1.07, 0.98; T: 0.68, 0.99), indicating that substances in SRKW fecal extract do not interfere with hormone binding. Inter-assay coefficients of variation were 7.8% for T3, 7.6% for GC; 17% for P4, and 19% for T. Intra-assay coefficients of variation (calculated as the percent of the mean divided by the standard deviation) were 1.9% for T3, 3% for GC, 3.1% for P4; and 3.2% for T. Antibody cross-reactivities are published in Wasser et al ([35], P4; [36], GC; [21], T3) and Velloso et al ([37], T).

2.4 Pregnancy assignment

All whales are photo-identified each day they are observed in the study area, making it unlikely that a newborn would be missed if present when the population is being observed [3]. This enabled us to establish temporal pregnancy profiles using fecal P4 and T concentrations for all pregnant females that subsequently gave birth, approximating gestational age at the time of sample collection based on the estimated birth date of the female's calf. All birth dates in our study (Table 1) were estimated by two independent observers from the Center for Whale Research, respectively with 40 and 30 years experience, using close range photographs taken of each calf at the time of first observation. Features used to assess calf age included: shape of