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# UNIVERSITY OF CALIFORNIA SAN DIEGO

Understanding early ontogeny and whisker growth dynamics of Weddell seal (*Leptonychotes weddellii*) pups through stable isotope analysis

A Thesis submitted in partial satisfaction of the requirements for the degree Master of Science

in

Biology

by

Danelle Angeline Baronia

Committee in charge:

Professor Carolyn Kurle, Chair Professor Elsa Cleland Professor Gerald Kooyman

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University of California San Diego

# **EPIGRAPH**

Yet, even amidst the hatred and carnage, life is still worth living. It is possible for wonderful encounters and beautiful things to exist.

— Hayao Miyazaki

Isn't it splendid to think of all the things there are to find out about? It just makes me feel glad to be alive – it's such an interesting world.

— L.M. Montgomery

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### **ABSTRACT OF THE THESIS**

Understanding early ontogeny and whisker growth dynamics of Weddell seal (*Leptonychotes weddellii*) pups through stable isotope analysis

by

Danelle Angeline Baronia

Master of Science in Biology

University of California San Diego, 2022

Professor Carolyn Kurle, Chair

Marine mammals spend much of their lives migrating across the ocean and are notoriously difficult to track over long periods of time. Stable isotope analysis (SIA) of animal tissues is an important tool used to determine foraging ecology and the timing of important developmental changes that occur throughout a marine mammal's lifetime. Stable isotope values from whisker segments provide long-term information of an animal's foraging, as whisker growth incorporates isotopic signals from their diet, such as dietary shifts that occur during birth and weaning events. We estimated the birth dates of 7–8-week-old Weddell seal (*Leptonychotes weddellii*) pups using the  $\delta^{15}$ N and  $\delta^{13}$ C values measured from sequentially sampled segments of their vibrissae to gain further insight into the utility of this technique to accurately predict important early ontogenetic events. Results showed that all pups shared similar stable carbon and nitrogen isotope trends. We designated a drop in nitrogen isotope values to pinpoint each individuals' birth event and, using prenatal and postnatal whisker growth rates, found that estimated birth dates aligned with the individuals' observed birth dates. Reconstructed timelines showed no evidence of a weaning signal. Post-birth, the  $\delta^{15}$ N values steadily increased, likely reflecting their dependency on their mothers' milk, and the  $\delta^{13}$ C values remained mostly constant, suggesting that mothers did not forage far from their breeding colonies.

#### **INTRODUCTION**

Marine mammals rarely spend their entire lifetime in one location (Boyd 2004; Stern et al. 2009), as many migrate between foraging and reproductive areas, sometimes traveling great distances under the surface of the ocean. These cryptic and extensive movements make marine mammals difficult to track throughout their lives (Boyd 2004; Norris et al. 2013). Stable carbon  $(\delta^{13}C)$  and nitrogen  $(\delta^{15}N)$  isotope analysis is a relatively recent ecological tool that can elucidate marine animal movement patterns, foraging habits, and ontogenetic shifts in resource or habitat use (Newsome et al. 2010). The  $\delta^{13}C$  and  $\delta^{15}N$  values obtained from animal tissues provide estimates of an animal's trophic position, foraging habitat, and consumed diet items (Rau et al. 1992; Hobson et al. 1996; Walker & Macko 1999; Kurle and Worthy 2001; Kurle 2002; Krause et al. 2020).

We can make different inferences about a consumer's foraging and life history through stable isotope analysis of specific tissues (Cerling et al. 2007; Kurle 2009). For example, tissues with quicker protein turnover rates, such as blood plasma, provide information about an animal's very recent foraging history (Kurle 2002, 2009; MacAvoy et al. 2006; Beltran et al. 2015), whereas consumer tissues that become inert after dietary isotopes are incorporated, such as teeth, bone, and vibrissae (whiskers), provide a broader picture of foraging behavior throughout a longer time span (Walker & Macko 1999; Hirons et al. 2001; Aurioles et al. 2006; Cherel et al. 2009; Ben-David & Flaherty 2012; Kernaléguen et al. 2016; McHuron et al. 2016; Lerner et al. 2018). By targeting isotope analyses to specific regions within these inert tissues, such as growth layers in teeth or sequential sections of vibrissae, we can estimate the timing of important ontogenetic shifts in diet or habitat utilization throughout an animal's lifetime (Hobson & Sease 1998; Walker & Macko 1999; Newsome et al. 2007, 2010; Mendes et al. 2007; Kernaléguen et

al. 2016; Turner Tomaszewicz et al. 2017, 2018). For example, analysis of the isotope values from sequential sections of a seal's whisker allows for the reconstruction of that seal's foraging habits over time and can be used to estimate the timing of their birth and weaning (Newsome et al. 2010; Rea et al. 2015; Vales et al. 2015; Kernaléguen et al. 2016; Lowther & Goldsworthy 2016).

An estimation of whisker growth rates for pinnipeds is required to assign the proper time frame to the stable isotope values collected along the length of a whisker (Cherel et al. 2009; Eder et al. 2010; Rea et al. 2015; McHuron et al. 2016). Whisker growth rates change throughout a pinniped's lifespan and are likely species- and family- (Otariidae vs. Phocidae) specific (Hirons et al. 2001; Beltran et al. 2015; Kernaléguen et al. 2015; Rea et al. 2015; McHuron et al. 2016, 2020). For example, otariids retain their whiskers throughout their lifetime and grow linearly, with the ends unpredictably breaking off with wear and age (Cherel et al. 2009; Kernaléguen et al. 2015), whereas phocids shed, then regrow, their entire whisker variably (Greaves et al. 2004; Zhao & Schell 2004; McHuron et al. 2020).

Methods used to estimate phocid vibrissae growth rates include the use of isotope tracers and isotope matching (Hirons et al. 2001; Zhao & Schell 2004; Hall-Aspland et al. 2005; Rogers et al. 2016), photogrammetry (Greaves et al. 2004; Beltran et al. 2015; McHuron et al. 2016, 2020), and clip and regrow strategies (Hindell et al. 2012). Two phocid species, Hawaiian monk seals (*Neomonachus schauinslandi*) and bearded seals (*Erignathus barbatus*), exhibit periods of fast and slow vibrissae growth that vary throughout their lifetime (McHuron et al. 2020). For most phocid species, however, vibrissae follow an asymptotic growth pattern in which a period of initial linear growth occurs, then growing slows or stops once the whisker reaches some length. The whisker remains in place until it breaks off annually and is replaced (Hall-Aspland et

al. 2005; McHuron et al. 2016, 2020). Age-specific whisker growth data in Phocidae is lacking for most species, where whisker growth rates in earlier phocid life stages have been reported only for nursing bearded seal pups and newly weaned southern elephant seal (*Mirounga leonina*) pups (Hindell et al. 2012).

Like most phocid species, the vibrissae from adult Weddell seals (*Leptonychotes weddellii*) exhibit asymptotic growth patterns in adulthood, with a growth rate of 0.6 mm/day and an asymptotic length of  $67 \pm 22$  mm (Beltran unpublished data). Weddell seals are one of the most well-studied phocids (e.g. Smith 1966; Plötz 1986; Burns & Castellini 1991; Burns et al. 1998; Thomas & Terhune 2009; Heerah et al. 2013; Weitzner et al. 2021), however no studies report whisker growth rates for earlier Weddell seal life stages. Knowing these growth rates would provide more reliable time estimates for interpreting temporal changes in stable isotope values from whiskers collected from younger animals. Further, understanding the patterns of isotopic change that may predictably occur along the length of vibrissae and that could reflect clear ontogenetic shifts would allow us to estimate the timing of these events when measuring isotope values from the whiskers of pups and young adults of unknown age (Rea et al. 2015).

Weddell seal vibrissae begin development in utero, thus the stable isotope values measured from the earliest segments of the pup whiskers should reflect those incorporated from their mothers' diets as they provide nutrients directly to their developing fetuses (Smith 1966). When pups are born, they switch from absorbing nutrients from maternal tissues to ingesting maternal milk. In otariid pups, this transition coincides with an abrupt decrease in the  $\delta^{15}$ N values measured from their sequentially sampled whiskers (Rea et al. 2015; Kelleher 2016; Jones et al. 2020, Howard et al., in review). Thus, birth dates for otariid pups can be estimated using the stable isotope values from their sequentially sampled vibrissae in combination with

calculated whisker growth rates. In addition, an increase in  $\delta^{15}$ N values from sequentially sampled whisker segments has been observed immediately after the  $\delta^{15}$ N minima described above in Steller sea lion pups (Rea et al. 2015), reflecting their dietary transition from catabolizing their mothers' tissue in utero to ingesting maternal milk during the nursing period, further clarifying the birth event for otariids as it is reflected in the stable isotope values of their whiskers. Neither of these patterns has been determined for phocid pups. Finally, studies examining stable isotope values along the lengths of vibrissae from otariid and phocid juveniles observed a pronounced decrease in whisker  $\delta^{15}$ N values that occurs just after weaning, reflecting a young animal's shift from ingesting maternal milk to independent foraging on marine prey (Walters et al. 2014; Vales et al. 2015; Kernaléguen et al. 2016).

We sampled vibrissae from 18 Weddell seal pups with known birthdates, cut the vibrissae into sequential segments, and measured the  $\delta^{13}$ C and  $\delta^{15}$ N values from those longitudinally sampled whisker segments. We then determined the pre- and postnatal growth rates for the pups' vibrissae to align the stable isotope data from each segment with an estimated timeline to determine if patterns in the stable carbon and nitrogen isotope values from the whisker segments coincided with pup birth and weaning events. Based on the stable isotope patterns in whiskers described above for otariid pups, we hypothesized that we would observe distinct decreases followed by clear increases in the  $\delta^{15}$ N values measured from the sequentially sampled whiskers from the Weddell seal pups in this study that coincide with their known birth dates. We also expected to observe a second distinct decrease in the whisker  $\delta^{15}$ N values that would reflect weaning by the Weddell seal pups. To our knowledge, only one study has examined trends in the  $\delta^{13}$ C values in sequentially sampled whiskers from pinnipeds (otariids) in relation to birth events (Jones et al. 2020), so we also investigated those values for trends that could further illuminate

the degree to which stable isotope values from Weddell seal pup whiskers can be used to estimate ontogenetic events. Matching important ontogenetic events in a Weddell seal's early life with patterns of stable isotope values recorded in their whiskers will also help affirm the broad applicability of this technique for understanding other, lesser-studied pinnipeds' life histories and whisker growth dynamics. Given that we observed the birth dates of all wild pups in our study, we constructed more robust stable isotope profiles of Weddell seal ontogeny than has been previously available (Rea et al. 2015; Howard et al., in review). Aligning these known birth dates to  $\delta^{15}$ N minimums in individual Weddell seal pup whiskers also allowed us to measure the whisker growth rates for two different Weddell seal age classes (fetus and pup), to further our understanding of species- and age-specific phocid whisker growth dynamics (McHuron et al. 2016, 2020). Finally, we used the  $\delta^{15}$ N and  $\delta^{13}$ C values from the Weddell seal pup whiskers to explore the potential for illuminating patterns in the foraging ecology of the pups and their mothers during this important time of late gestation and lactation.

#### **MATERIALS AND METHODS**

### Weddell seal life history

Pupping season for Weddell seals in the Antarctic occurs in September as females near the end of parturition and haul out onto fast ice to give birth (Thomas & Terhune 2009). Weddell seals produce a single pup, and birth is followed by a 7–8-week nursing period when pups gain all their nutrition from their mother's milk (Thomas & Terhune 2009). Weaning marks the end of the pupping season in December when mothers leave their pups and the colony disperses (Thomas & Terhune 2009). The time Weddell seals spend on the ice during their four monthlong pupping season allowed us to access pups and collect samples (Tedman & Bryden 1979). *Sample collection* 

We collected all data during the Weddell seal pupping seasons of 2017 and 2019 as part of a larger study at Big Razorback Island and Turtle Rock, located off Ross Island, Antarctica (**Figure 1**). Observation of 18 Weddell seal pups spanned 7–8 weeks from birth to weaning. We recorded Weddell seal pups' birth dates within 24 hours through direct observation of the mothers and their pups (i.e. no pup yesterday, pup today). We estimated weaning dates based on either observation of a pup alone (without its mother) for three consecutive days as well as mass loss and rate recorded between 5 and 7 weeks old. We observed weaning in 8 of the 18 pups (**Table 1**). We pulled the longest mystacial (on the muzzle) vibrissae from each pup at 7 weeks old in December (**Table 1**). Following collection, we stored vibrissae in individually labeled coin envelopes and transported them first to the Liwanag Lab at the California Polytechnic State University--San Luis Obispo, then to the Kurle Lab at the University of California, San Diego to prepare them for stable isotope analysis.

### Sample processing

To remove lipids and contaminants, we scrubbed individual vibrissae with dish soap and rinsed them in DI water. After washing, we dried the vibrissae with Kimwipes and measured their mass and length. We stored individual vibrissae in centrifuge tubes with the thicker, proximal end at the bottom of the tube to prevent breakage. Centrifuge tubes were capped, labeled, and stored at room temperature until processing.

Using a 1 mm biopsy punch to cut sequential segments of the whisker, we started processing at the whisker's distal end (furthest from the face) and continued lengthwise toward the proximal end (the section of whisker plucked from the seal cheek) (**Figure 2**). We cut and grouped segments until they met the mass requirement (0.5–1.0 mg) for stable isotope ratio mass spectrometry (Rea et al. 2015; Scherer et al. 2015). Once the grouped segments met the mass requirement, we packaged the segments into 5x9 mm tin capsules and stored the completed samples in a 96-well plate. After we packaged each sample, we measured the remaining vibrissae length for precision. To determine the portion of the vibrissae represented by each sample, we found the difference in vibrissae length before and after each sample was taken. Because the whiskers tend to taper at the distal end, more of the whisker length at this end was needed to meet the mass required for a sufficient sample. Thus, temporal resolution of the isotopic signatures increases from the distal to the proximal end for each whisker. Further, due to the difficulty of cutting vibrissae segments with the biopsy punch, some segments were lost in processing, and this is accounted for as missing data.

### Stable isotope analysis

We sent prepared samples to the Stable Isotope Laboratory at the University of California Santa Cruz for analysis of their  $\delta^{13}$ C and  $\delta^{15}$ N values by a Carlo Erba 1108 elemental analyzer coupled to a Thermo-Finnigan Delta Plus XP isotope ratio spectrometer (EA-IRMS). The ratios of carbon and nitrogen stable isotopes are expressed in delta ( $\delta$ ) notation, such that

$$\delta = (R_{sample} / R_{standard} - 1) \times 1000$$

and R=  ${}^{13}$ C/ ${}^{12}$ C or  ${}^{15}$ N/ ${}^{14}$ N. The units are parts per thousand (per mil, ‰) deviations from the Vienna Pee Dee Belemnite standard for  $\delta^{13}$ C and atmospheric air standard for  $\delta^{15}$ N (Ben-David and Flaherty 2012). The precision for these data is the standard deviation (SD) of stable isotope values from a set of standards (acetanilide), and precision of the  $\delta^{15}$ N and  $\delta^{13}$ C values for our study was 0.03‰ and 0.07‰ for the 2017 samples and 0.21‰ and 0.05‰ for the 2019 samples, respectively.

### Birth Date and Vibrissae Growth Rate Determination

We estimated Weddell seal pup birth dates using predictable declines in the  $\delta^{15}$ N values measured along the length of their whiskers, as has been observed for otariids (Rea et al. 2015; Howard et al., in review). We defined the vibrissae's distal end as length = 0 mm, and the proximal end as the vibrissae's maximum length (**Figure 2**). We first visually examined the data and assigned estimated pup birth dates to an area of the whisker that exhibited an initial drop in its  $\delta^{15}$ N values. The  $\delta^{15}$ N minimums spanned across different lengths of each vibrissa, thus the section midpoint was uniquely calculated for each individual vibrissa and assigned as the estimated vibrissae length at birth (**Figure 2**). Whiskers with  $\delta^{15}$ N minimums that spanned short lengths have higher temporal resolution and thus are considered more precise. We defined the vibrissae length grown in utero as the length from the vibrissae's distal end (0 mm) to the point of the estimated birth date (**Figure 2**). For Weddell seals, Smith (1966) observed that vibrissae likely start development in utero at 120 days after conception. Weddell seals have a gestation period of 9–10 months (Stirling 1971). We used a gestation length of 285 days (9.5 months) and subtracted the 120 days after conception in which vibrissae began to grow in utero from this number to find that vibrissae grow in utero for 165 days. We divided the length grown in utero by 165 to determine the vibrissae's prenatal growth rate in mm/day:

$$prenatal growth rate = \frac{est. \ birth \ point \ - \ 0 \ mm}{165 \ days}$$

We calculated the vibrissae's postnatal length as the length from the vibrissae's proximal end to the estimated birth date point (**Figure 2**). The postnatal growth rate was calculated by dividing the postnatal length by the number of days that passed from the observed birth date until the sampling date:

$$postnatal growth rate = \frac{max \ length \ - \ est. \ birth \ point}{sampling \ date \ - \ act. \ birth \ date}$$

Using the prenatal and postnatal whisker growth rates, we constructed individual timelines for each pup with the stable isotope values measured longitudinally along each whisker from the distal (time in utero) to proximal (time at weaning) ends along the y axis and time on the x axis (**Figure 3**). Starting at 165 days before the observed birth date, we used the prenatal whisker growth rate to calculate the estimated length across time, matching the estimated length to the observed length at which samples were taken. Then we matched these sections to the  $\delta^{15}$ N and  $\delta^{13}$ C values they represented. We continued this method until we reached the designated  $\delta^{15}$ N minimum (birth point), at which point we began using the postnatal whisker growth rate to approximate the timeline. Using this method, we compared the estimated birth date at the  $\delta^{15}$ N

minimum for each individual to their actual observed birth date, allowing us to make inferences about the accuracy of the whisker growth rates.

#### RESULTS

We analyzed the  $\delta^{13}$ C and  $\delta^{15}$ N values from longitudinally segmented vibrissae from 18 individual Weddell seal pups collected 7–8 weeks after each pup's birth date. We also estimated two vibrissae growth rates for the pups, one reflecting the rate of growth for whiskers while pups are developing in utero and one reflecting the rate of whisker growth that occurs between the pup's birth to the sampling date. We combined these data to construct timelines of stable isotope values from the sequentially sampled vibrissae to determine if changes observed in the  $\delta^{13}$ C and  $\delta^{15}$ N values from whisker segments were coincident with expected shifts in nutrient utilization by the pups that could mark ontogenetic events such as birth and weaning. One pup, 1908-3, died and was sampled at 3 weeks, well before weaning. The length and weight of the vibrissa from individual 1908-3 was 77.8 mm and 13.0 mg, respectively. Although we did determine vibrissae growth rates and created stable nitrogen and carbon isotope profiles for this subject (**Figure 3Q**), we removed 1908-3 from collective analysis. The mean (±SD) vibrissae length for the remaining 17 Weddell seal pups was 88.8 ± 9.3 mm (range 75.9 – 107.8 mm) (**n = 17; Table 1**) and the mean (±SD) weight was 21.1 ± 4.1 mg (range 15.8 – 29.3 mg) (**Table 1**).

We observed the predicted decrease in the  $\delta^{15}$ N values from the segments of whiskers that likely coincided with timing of birth for all but one pup, 1910-7. This was most likely due to poor temporal resolution at the whisker's proximal end. We were unable to pinpoint a birth point from which to calculate whisker growth rates and create temporal stable isotope profiles for this pup, but for the remaining 16 pups, the mean prenatal and postnatal vibrissae growth rates were  $0.24 \pm 0.06$  mm/day and  $1.00 \pm 0.19$  mm/day, respectively (**n=16; Table 1**). Applying these growth rates, we found that vibrissae growth in utero accounted for  $42 \pm 6\%$  of total vibrissae length, ranging from 29–56% of the total vibrissae length, and postnatal vibrissae growth

accounted for  $56 \pm 8\%$ , ranging from 38-71% of the total vibrissae length (**Table 1**). Processing of each vibrissa resulted in an average of  $33 \pm 5$  (**Table 1**) samples per animal, with prenatal and postnatal vibrissae growth accounting for  $4 \pm 2$  and  $28 \pm 5$  of those samples, respectively (**Table 1**).

We observed similar patterns in the individual Weddell seal pups' stable carbon and nitrogen isotope profiles (**n=16; Figure 3**). The beginning of the timeline we created starts in May and the stable isotope values from those whisker segments reflect nutrition assimilated during vibrissae growth in utero, beginning an estimated 165 days before birth (Smith 1966). Estimated birth dates were consistently 1 day before the actual birth date (**Table 1**). Weaning was observed in 8 of the 18 pups (**Table 1**). The timeline ends at the early-December sampling date.

To better illustrate how the  $\delta^{15}$ N and  $\delta^{13}$ C values measured along the lengths of vibrissae reflect the seal pups' nutrient use from in utero until weaning, we chose a single individual, 1907-7 (Twix) to represent all seals (**Figure 3O**).

Three vibrissae segments reflected Twix's time in utero from May until October, and the mean  $\delta^{15}$ N value from these prenatal samples was  $13.5 \pm 0.2\%$  (**Figure 3O**). Twix then exhibited a drop in  $\delta^{15}$ N values of 0.3‰, from 13.6‰ to 13.3‰. We marked the midpoint of this drop, which spanned 2.0 mm of the total 92.0 mm vibrissae length, as the October birth event. Our constructed timeline estimated the birth date on October 20, 2019, one day before the actual observed birth date (**Table 1**). Following birth, the  $\delta^{15}$ N values increased, rising to 15.5‰ just before sampling on December 7th, 2019. This period from birth to sampling represents the first 7 weeks of the pup's life and constitutes 38.2% of the entire vibrissa. Although we observed

Twix's weaning by November 26th, the  $\delta^{15}$ N value profile showed no evidence of a weaning event. Overall, the trends in  $\delta^{15}$ N values were consistent across all 16 pups' vibrissae (**Figure 3**).

Likewise, we observed similar patterns across all individuals in the  $\delta^{13}$ C values measured along the lengths of the Weddell seal whiskers (**Figure 3**). From May to October, while the most distal end of Twix's vibrissae was growing in utero, the mean (±SD)  $\delta^{13}$ C values across the 3 prenatal samples was -23.9 ± 0.03‰ (**Figure 3O**). Following birth, the  $\delta^{13}$ C values dropped to around -24.0 to -24.5‰ until mid-November (November 16th), and then steadily increased back to  $\delta^{13}$ C values similar to those from the original in utero period (~-23.4‰) that reflect the time just before the December sampling date.

While this pattern was consistent across  $\delta^{13}$ C value profiles, the overall changes in  $\delta^{13}$ C values across pup vibrissae were minimal with a mean  $\delta^{13}$ C (±SD) range of only  $0.9 \pm 0.5$ ‰. Overall, the trends in the  $\delta^{15}$ N and  $\delta^{13}$ C values we measured from the sequentially sampled whiskers were very similar across all individuals, providing reliable insights toward a finer scale understanding of the relationship between the stable isotope values measured along Weddell seal pup vibrissae and dietary and ontogenetic shifts.

#### DISCUSSION

We constructed timelines that track Weddell seal pups' changing stable nitrogen and carbon isotope values along their vibrissae and reflect their development in the womb to the days near their weaning, spanning seven months from May to December. Stable isotope profiles from pup vibrissae followed similar trends over the observed time period in both  $\delta^{15}$ N and  $\delta^{13}$ C values (**Figure 3**).

#### Using SIA values as indicators of ontogenetic events

We observed a notable drop in the  $\delta^{15}$ N values measured from sequentially sampled vibrissae in 16 of the 17 pups we sampled. This drop was followed immediately by a sharp increase in  $\delta^{15}$ N values, allowing us to denote the mid-October birthing event in relation to a specific section of each pup's whisker. Past studies used this decrease in  $\delta^{15}$ N values along pup vibrissae to indicate birth, but those studies used estimated birth dates, introducing a potential source of error when matching stable isotope values measured along whisker lengths to time periods across a pinniped's life (Rea et al. 2015; Kelleher 2016; Jones et al. 2020; Howard et al., in review). We observed the birth dates of the pups in this study which allowed us to build more robust timelines associated with the stable isotope profiles we measured from the sequentially sampled whiskers. We established both a linear prenatal and postnatal growth rate for each individual vibrissa (**Table 1**), which we used to temporally track the changes in each seal's  $\delta^{15}$ N and  $\delta^{13}$ C values throughout the growth of their vibrissa (**Figure 3**). The birth dates we estimated from the whisker growth rates were consistently one day earlier than the observed birth event. Our slight underestimation of the birth event may have resulted because the  $\delta^{15}$ N drop for every individual spanned 2 or more millimeters and we used the isotope value from the midpoint of

this span to estimate the growth that had occurred up until birth. Alternatively, it is possible that a time lag exists in the incorporation of stable isotopes reflecting the nutrients absorbed by the pup while in utero to those reflected in the ingestion of their mother's milk after they are born (Ayliffe et al. 2004).

Many pups also experienced a drop in the  $\delta^{13}$ C values measured from a section of their whiskers that also coincided with the estimated birth date. This is consistent with a similar trend found in South American fur seal (*Arctocephalus australis australis*) pups, suggesting a change of maternal foraging strategies post-birth (Jones et al. 2020). While the pattern was clear, these  $\delta^{13}$ C value drops were considerably smaller than the  $\delta^{15}$ N minima, and thus using a  $\delta^{15}$ N minima as a birth indicator is likely still ideal. However, future studies seeking to use temporal SIA analysis on vibrissae can use this pattern in  $\delta^{13}$ C values in conjunction with patterns in  $\delta^{15}$ N value to more fully understand the temporal relationships between stable isotope values measured along pup vibrissae and ontogenetic shifts such as the birth event.

Past studies examining stable isotope values along the lengths of vibrissae from otariid and phocid juveniles observed a drop in their  $\delta^{15}$ N values following weaning, reflecting a diet shift from mother's milk to independent foraging (Walters et al. 2014; Vales et al. 2015; Lübcker et al. 2016; Kernaléguen et al. 2016). We also expected to observe a drop in the  $\delta^{15}$ N values 7 weeks post birth in the reconstructed timeline because we observed that weaning occurred for some of the pups in our study (n = 8; **Table 1**) an average of 6 ± 3 days before their whiskers were plucked. Despite this, we did not detect any evidence of a weaning event in the stable isotope profiles of the whiskers we measured (**Figure 1**). There are two potential reasons for this. First, not enough time may have passed following weaning for the stable isotopes reflecting their diet change from maternal milk to marine prey to become incorporated into vibrissae tissue.

Future studies can attempt to gather vibrissae samples later than 7–8 weeks post-birth to investigate if a stable isotope weaning signature is present, but this could be problematic for Weddell seals as they leave the ice and go to sea shortly after weaning (Thomas & Terhune 2009). Second, as mentioned above, whisker growth in some phocid seals is asymptotic, meaning growth stops when the whisker reaches a certain length (Hall-Aspland et al. 2005; McHuron et al. 2016, 2020). The pups sampled in this study may have all reached their final whisker lengths before the weaning event, and thus the stable isotope values that would reflect a change in diet from maternal milk to their post-weaning diets would not be incorporated into the vibrissae (see further discussion below).

Walters et al. (2014) found a weaning signature for southern elephant seals despite their vibrissae also exhibiting asymptotic growth. This may have been possible because southern elephant seals have a lactation period of only 24 days compared to the 7-week lactation period for Weddell seals (McMahon & Bradshaw 2004). Thus, vibrissae from southern elephant seals may not reach their asymptotic length until well after weaning occurs. While we did not detect a weaning signature, it is possible that studies on other phocid species with shorter nursing periods may be able to.

#### Prenatal and postnatal vibrissae growth rates

To our knowledge, ours is the first study to establish vibrissae growth rates for fetal and nursing Weddell seal pups. Otariid vibrissae growth is age-specific, and they grow fastest as pups, slowest as adults, and at an intermediate rate as fetuses (Rea et al. 2015). Our findings found that a different pattern is experienced by Weddell seals. Prenatal vibrissae growth rates were similar between each seal pup, and notably slower than postnatal vibrissae growth rates. In

addition, using clip and regrowth methods, Beltran (unpublished data) found that adult Weddell seal vibrissae experience asymptotic growth, with a growth rate of 0.6 mm/day, which is slower than both our postnatal growth but faster than our mean prenatal growth rate. This may have resulted because Weddell seal vibrissae grows asymptotically while Steller sea lions grow their vibrissae linearly.

Whisker growth rates for phocid pups are likely species-specific as well. For example, Hindell et al. (2012) reported mean whisker growth rates of 0.87 mm/day for nursing bearded seal pups', which are similar to our findings for Weddell seals. However, newly weaned southern elephant seal pups have a reported whisker growth rate of 0.22 mm/day (Hindell et al. 2012), which are considerably slower than our mean postnatal whisker growth rate.

It is possible that post-birth vibrissae growth was slower than our reported growth rate. One pup, 1908-3, died prematurely and we plucked her whisker at 3 weeks old. This whisker was 77.8 mm, which is reasonably close to our mean length of vibrissae from the 7-week old pups ( $88.8 \pm 9.3 \text{ mm}$ ). For 1908-3, we estimated a linear postnatal growth rate of 1.49 mm/day. Under these assumptions, if 1908-3 lived to 7 weeks, their vibrissae length would be 118.06 mm, a value much greater than the mean vibrissae length we measured for all pups at 7 weeks old. This leads us to believe that, consistent with Beltran's unpublished data, Weddell seal vibrissae likely grow asymptotically. Thus, the vibrissae sampled for our study may have reached their asymptotic length well before the 7-week post-birth sampling period.

Using photogrammetry on captive phocids that exhibit asymptotic whisker growth, Beltran et al. (2015) and McHuron et al. (2016, 2020) found that constant linear growth occurred in only 75% of the whisker length (starting at the distal end of the whisker). This suggests that timelines created using stable isotope values measured from sequentially sampled vibrissae will

only be accurate for the first 75% of a whisker's growth. If the asymptotic vibrissae length for pups measured in our study was reached before the 7-week sampling period, then our estimated linear postnatal whisker growth rate is likely faster than their actual growth rates. The prenatal portion of each whisker was less than 75% of the total length,  $42 \pm 6\%$ , thus it is highly likely that this portion grew linearly and our prenatal whisker growth rates are reliable.

### Postnatal $\delta^{15}N$ and $\delta^{13}C$ values

For 16 of the pups measured in this study, there was a noticeable increase in the  $\delta^{15}N$  values from the sequentially sampled whiskers that occurred just after the minimal  $\delta^{15}N$  values we observed that were used to indicate the birthing event. This increase likely reflects the dietary shift pups experience when they transition from absorbing nutrients from their mothers while in utero to nursing from their mothers after they are born (**Figure 3**) (Smith et al. 1966). Stable nitrogen isotope values from pups nursing from their mothers should be higher than those from the mothers due to isotopic fractionation (Kurle et al. 2014), or the increase in  $\delta^{15}N$  values that occurs predictably with increasing trophic level, and our data support that. The mean  $\delta^{15}N$  value we measured in postnatal pups (14.5 ± 0.7‰), was greater than the mean  $\delta^{15}N$  value of adult Weddell seal vibrissae (13.0 ± 0.4‰) as reported by Goetz et al. (2017).

Based on other studies examining stable isotope differences between blood from offspring and whole milk of their mothers, we expected that our reported  $\delta^{15}$ N vibrissae values would be higher relative to their mothers' whole milk by about 1-3‰ (Jenkins et al. 2001; Polischuk et al. 2001; Stegall et al. 2008; Cherel et al. 2015; Stricker et al. 2015; Habran et al. 2018, 2019). Palozzi (2010) reported that the  $\delta^{15}$ N values from separated protein and aqueous portions of Weddell seal milk were  $14.8 \pm 0.1\%$  and  $6.0 \pm 0.2\%$ , respectively. The mean

differences between the  $\delta^{15}$ N values from the postnatal vibrissae sections (14.5 ± 0.7‰) in our study and these milk values were 0.8‰ (milk protein) and 9.5‰ (milk aqueous). The difference between our mean reported pup vibrissae and Palozzi's mean aqueous milk  $\delta^{15}$ N value is greater than expected. Past studies found little discrimination between  $\delta^{15}$ N values of Stellar sea lion pup vibrissae and their mothers' milk (Stegall et al. 2008; Stricker et al. 2015), which reflects the similarity between the average  $\delta^{15}$ N values of milk protein reported by Palozzi and this study's pup vibrissae (Palozzi 2010). Without the  $\delta^{15}$ N values from whole, intact milk, however, we lack the basis for adequate comparisons between the isotope values from Weddell seal milk and pup whiskers. Further, because diets vary between individual Weddell seal mothers (Shero & Burns 2022), without direct comparison of the  $\delta^{15}$ N values between mother and pup pairs, we do not know the degree to which pup  $\delta^{15}$ N values were higher in comparison to those from their mothers' tissues.

Apart from consuming their mother's milk, Weddell seal pups have been known to begin diving at 2 weeks old (Castellini et al. 1994; Sato et al. 2003). Burns et al. (1998) observed newly weaned pups foraging on Antarctic silverfish and squid during their early dives, but it is unclear if this foraging starts before or after weaning. If pups were foraging on marine prey, we would expect their  $\delta^{15}$ N values to decline as common prey would have lower  $\delta^{15}$ N values than those from seal milk which is at a much higher trophic level. Our postnatal  $\delta^{15}$ N values steadily increased from birth until the sampling date, and thus do not indicate that our pups were foraging upon prey items aside from their mother's milk, supporting the likelihood that pre-weaned Weddell seal pups do not actively ingest prey during their dives (Sato et al. 2003; Weitzner et al. 2021). It is also possible that if our Weddell seal pups did forage on these dives, the contribution

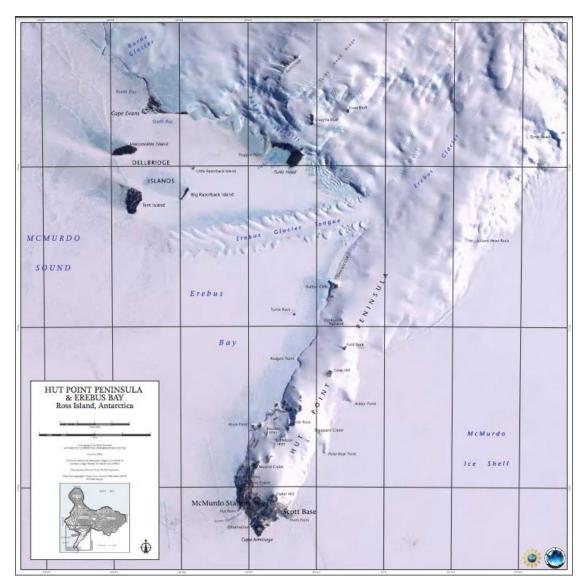
of their prey items was so low that it did not make an impact on their vibrissae's overall  $\delta^{15}$ N values.

We lack stable isotope or other dietary data from the mothers of the pups sampled in this study, but previous studies have demonstrated that the stable isotope values of pups that are reflective of their time in utero or nursing correlate with stable isotope values from their mothers across the same time frame (Aurioles et al. 2006; Hindell et al. 2012; Scherer et al. 2015; Dannecker 2016; Baylis et al. 2016; Eder et al. 2019; Urquía & Páez-Rosas 2019; Jones et al. 2020; Lubcker et al. 2020; Howard et al., in review). If the isotope values from pup whiskers do correspond with isotope values from their mothers, then we can make some broad inferences about their foraging ecology during the last stages of pregnancy and the lactation period. Across all individuals, the  $\delta^{13}$ C values were consistent across time, with a range of  $0.9 \pm 0.5\%$  (Table 1). This suggests that during lactation, mothers likely had a narrow habitat range within which they were foraging as  $\delta^{13}$ C values in marine animal tissues reflect foraging location (Kurle & McWhorter 2017). Past studies have shown that Weddell seal mothers limit their movement during lactation to readily provide milk for their nursing pups (Hindell et al. 2002). Weddell seal mothers, however, have a longer lactation period than most phocids, weaning their pups at 5–7 weeks (Shero & Burns 2022). Lactating Weddell seal mothers also spend a greater time foraging and diving than other phocids (Hindell et al. 2002). While Weddell seal mothers have vertical variability in foraging, they are known to use ice holes close to their breeding colonies (Hindell et al. 2002), which could be why we see little variability in  $\delta^{13}$ C values across pup vibrissae length.

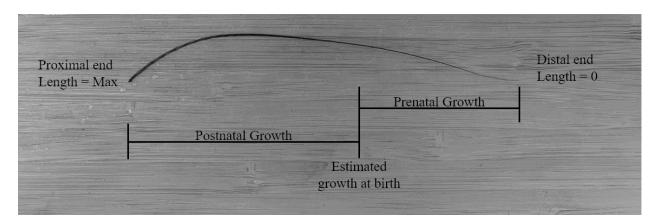
### Conclusion

Knowing the timing of ontogenetic events for phocids is important because the timing can affect a pup's chance of survival to weaning. For Weddell seals, both early and late birth has indicated a lower chance of survival to weaning (Thomas & DeMaster 1983; Proffitt et al. 2010). Early ice breakup influenced by climate change can also lead to premature weaning, further decreasing a pup's rate of survival (Thomas & DeMaster 1983; Proffitt et al. 2010). We estimated the birth dates of 7–8-week-old Weddell seal pups using the  $\delta^{15}$ N and  $\delta^{13}$ C values measured from sequentially sampled segments of their vibrissae to gain further insight into the utility of this technique to accurately predict important early ontogenetic events. We offer promising evidence that trends in both  $\delta^{13}$ C and  $\delta^{15}$ N values coincide with a "birth signature" and these data can potentially be applied more widely to other, lesser-studied phocid species to gain insights into early life ontogeny. While we did not capture a weaning signal, it is still possible that studies on other phocid species with shorter lactation periods can detect a signal through temporal stable isotope analysis. We are the first study to calculate estimated vibrissae growth rates for Weddell seals in utero and post-birth until the time of weaning. Finally, our data indicate that, though Weddell seal pups start diving as early as two weeks post birth, it is unlikely that they are foraging during this period and remain dependent on their mothers' milk. The mothers themselves likely do not forage far from their breeding colonies.

# **FIGURES**

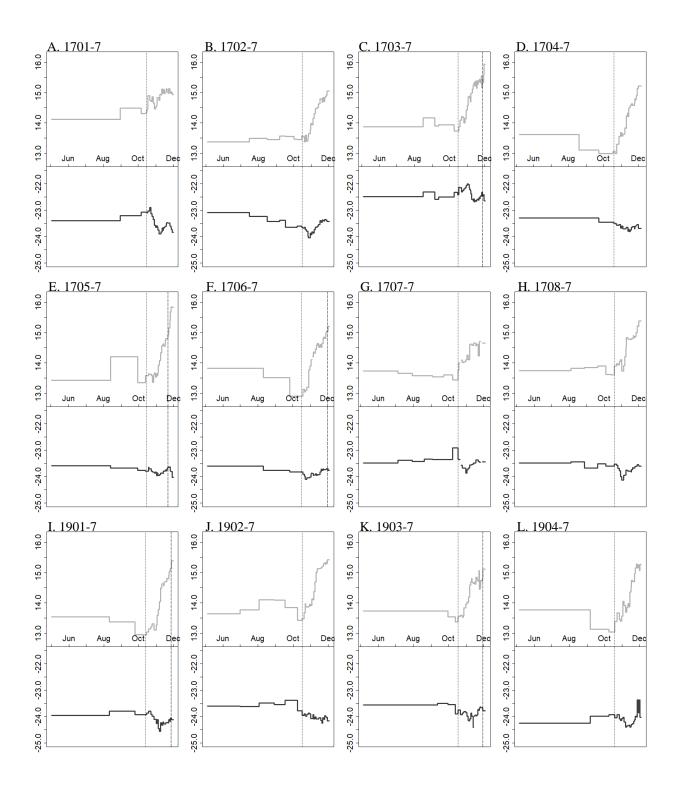


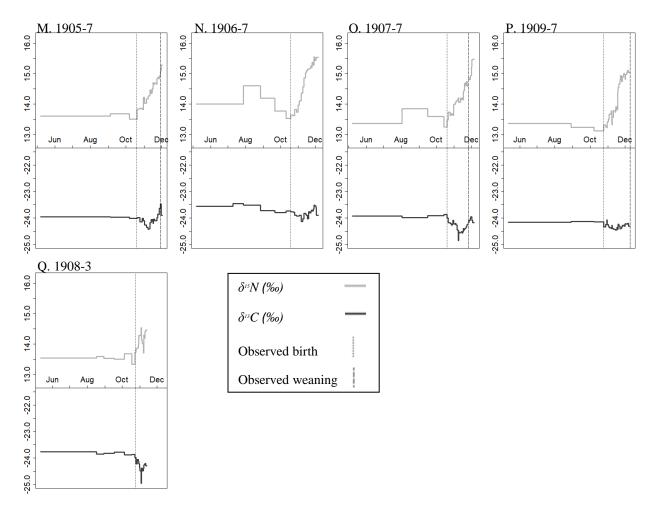
**Figure 1.** Study sites where we collected vibrissae from Weddell seal pups at Turtle Rock (2017 & 2019), Big Razorback Island (2017 only), and Hutton Cliffs (2019 only) off the Hut Point Peninsula. CREDIT TO: Brad Herried, Polar Geospatial Center



**Figure 2.** Photo of a Weddell seal pup whisker, indicating the proximal and distal ends. We defined the distal end as length = 0 and the proximal end as the maximum length. The vertical line indicates the estimated point of birth designated by the midpoint of an initial drop in the  $\delta^{15}$ N values which was observed in sequentially sampled segments of each vibrissa. The section of whisker to the right of the vertical line represents vibrissa growth that occurred in utero from 165 days before birth (when whiskers start development) until the birth date. The left of the vertical line represents vibrissa growth that occurred after birth until the sampling date.

**Figure 3.** Profiles of the  $\delta^{15}$ N (top) and  $\delta^{13}$ C (bottom) values from sequentially sampled vibrissae collected from 16 individual Weddell seal pups aligned along an estimated timeline from development in utero to 7 weeks post-birth. Two vertical lines mark the observed birth event (dotted line; left) and observed weaning event (dashed line; right). The area to the left of the birth event line represents time in utero and the area to the right of the birth event line represents time after birth. Weaning was observed in only 8 of the pups. Gaps in data show lengthwise sections of vibrissae lost either during processing or analysis. Reconstructed timelines are specific to each individual pup.





**Figure 3, cont.** Profiles of the  $\delta^{15}$ N (top) and  $\delta^{13}$ C (bottom) values from sequentially sampled vibrissae collected from 16 individual Weddell seal pups aligned along an estimated timeline from development in utero to 7 weeks post-birth.

## TABLES

**Table 1.** Weddell seal sample ID numbers, sex, their estimated (inferred from the midpoint of the initial  $\delta^{15}$ N minima observed in the sequentially sampled vibrissa from each animal) and observed birth dates, observed weaning dates, the date we sampled each vibrissa, the age of each seal, the length and mass of each vibrissa, the total number of samples analyzed for each vibrissa, and the number of samples, percent of vibrissa growth, and growth rates for prenatal (grown by the seal while in utero) and postnatal (grown after birth) sections of each vibrissa. Because animal 1910-7 did not exhibit a clear drop in the  $\delta^{15}$ N values at any point along their whisker, we were unable to establish a birth point to calculate whisker growth rates for this pup.

\*1908-3 died and was sampled at 3 weeks.

th (%) Growth Rate (mm/day)	Postnatal Prenatal Postnatal	56.75 0.22 1.22	43.63 0.32 0.85	54.59 0.35 1.14	56.3 0.23 0.98	61.87 0.23 1.29	56.57 0.2 1.18	37.87 0.23 0.79	47.45 0.19 1	61.65 0.26 0.97	60.39 0.22 1.05	52.04 0.24 1.19	58.04 0.18 0.78	59.06 0.21 1.18	56.69 0.18 0.78	61.78 0.21 1.18	70.68 0.14 1.19	N/A N/A N/A	
Percent Growth (%)	Prenatal P.	40.29	56.37	45.69	43.7	38.13	38.58	50.11	46.31	38.35	39.61	47.96	41.96	40.94	43.31	38.22	29.32	N/A	
	Postnatal	33	25	36	24	30	28	19	34	25	27	30	24	24	23	37	30	N/A	
Samp les	Prenatal	3	7	S	3	3	3	٢	9	3	9	4	3	3	9	4	3	N/A	
	Total	36	32	41	27	33	31	26	40	28	33	34	27	27	29	41	33	36	
Vibrissae Mass (mg)		22.5	22.3	28.3	17.5	21.1	17.3	19.8	19.1	17.3	22.2	23	15.8	18.2	16.8	29.3	21.2	23.2	
Vibrissae Length	(mm)	95.05	93.67	100.51	85.26	100.34	92.11	75.91	88.38	81.31	76.02	89.21	86.86	96.36	67.32	92.02	81.35	107.8	
Age (day s)		48	48	48	49	48	48	48	48	50	48	48	48	48	49	48	47	48	
Sampling Date (mm-dd- yy)		12/2/2017	12/3/2017	12/3/2017	12/7/2017	12/1/2017	12/4/2017	12/5/2017	12/6/2017	12/2/2019	12/4/2019	12/5/2019	12/5/2019	12/6/2019	12/7/2019	12/7/2019	12/11/2019	12/13/2019	
Observed Weaning Date	( y y - u - u - u - u - u - u - u - u - u		,	11/27/2017	ı	11/21/2017	12/1/2017	1		11/27/2019		11/30/2019		11/29/2019	-	11/26/2019	12/10/2019	12/9/2019	
-yy)	Est. – A.ct. (day s)	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	N/A	
Birth Date (mm-dd-yy)	Actual	10/14/2017 10/15/2017	10/15/2017 10/16/2017	10/15/2017 10/16/2017	10/18/2017 10/19/2017	10/13/2017 10/14/2017	10/16/2017 10/17/2017	10/17/2017 10/18/2017	10/18/2017 10/19/2017	10/12/2019 10/13/2019	10/16/2019 10/17/2019	10/17/2019 10/18/2019	10/17/2019 10/18/2019	10/18/2019 10/19/2019	10/18/2019 10/19/2019	10/19/2019 10/20/2019	10/24/2019 10/25/2019	10/26/2019	
Birth	Estimated	10/14/2017	10/15/2017	10/15/2017	10/18/2017	10/13/2017	10/16/2017	10/17/2017	10/18/2017	10/12/2019	10/16/2019	10/17/2019	10/17/2019	10/18/2019	10/18/2019	10/19/2019	10/24/2019	N/A	
Sex		М	ц	ц	М	F	М	М	М	М	М	ц	ц	Г	М	М	М	Μ	
Samp le ID		1701-7	1702-7	1703-7	1704-7	1705-7	1706-7	1707-7	1708-7	1901-7	1902-7	1903-7	1904-7	1905-7	1906-7	1907-7	1909-7	1910-7	

## APPENDIX

**Table 2.** The  $\delta^{15}$ N and  $\delta^{13}$ C values of the whisker segments cut sequentially across the whisker lengths of 17 Weddell seal pups, along with the whisker length we estimated across time using prenatal and postnatal growth rates unique to each pup. The timeline begins at the beginning of the whisker's growth while the pup was in utero to the sampling date. The estimated birth dates, inferred from the midpoint of the initial  $\delta^{15}$ N minima observed, are highlighted.

1701-7							
Date	Estimated Length	$\delta^{15}$ N (‰)	δ <sup>13</sup> C (‰)				
(mm/dd/yyyy)	(mm)						
5/3/2017	0.22	14.12	-23.4				
8/31/2017	26.78	14.12	-23.4				
10/6/2017	34.75	14.49	-23.21				
10/14/2017	36.52	14.31	-23.07				
10/15/2017	37.74	14.31	-23.07				
10/18/2017	41.40	14.49	-23.11				
10/21/2017	45.06	14.9	-23.03				
10/23/2017	47.49	14.74	-22.9				
10/25/2017	49.93	14.71	-23.13				
10/27/2017	52.37	14.86	-23.23				
10/29/2017	54.81	14.66	-23.35				
10/31/2017	57.25	14.47	-23.58				
11/2/2017	59.69	14.53	-23.65				
11/3/2017	60.91	14.59	-23.59				
11/4/2017	62.13	14.83	-23.63				
11/6/2017	64.57	14.83	-23.75				
11/8/2017	67.00	14.75	-23.9				
11/10/2017	69.44	14.94	-23.9				
11/11/2017	70.66	14.93	-23.82				
11/12/2017	71.88	15.11	-23.83				
11/13/2017	73.10	15.12	-23.67				
11/14/2017	74.32	14.97	-23.76				
11/15/2017	75.54	14.99	-23.66				
11/16/2017	76.76	15.1	-23.68				
11/17/2017	77.98	14.96	-23.63				
11/18/2017	79.20						
11/19/2017	80.42	15.01	-23.61				
11/20/2017	81.64	15.13	-23.48				
11/21/2017	82.86	15.09	-23.47				
11/22/2017	84.08	15.09	-23.49				
11/23/2017	85.29	14.98	-23.49				
11/25/2017	87.73	15.13	-23.48				
11/26/2017	88.95	15.04	-23.59				
11/27/2017	90.17	14.97	-23.61				
11/28/2017	91.39	15.02	-23.67				
11/29/2017	92.61	15	-23.73				
12/1/2017	95.05	14.94	-23.84				

1702-7							
Date	Estimated Length	$\delta^{15}$ N (‰)	δ <sup>13</sup> C (‰)				
(mm/dd/yyyy)	(mm)						
5/4/2017	0.32	13.38	-23.09				
7/16/2017	23.68	13.38	-23.09				
8/16/2017	33.60	13.49	-23.23				
9/7/2017	40.64	13.46	-23.42				
9/17/2017	43.84	13.57	-23.39				
10/2/2017	48.64	13.56	-23.65				
10/7/2017	50.24	13.47	-23.65				
10/15/2017	52.80	13.45	-23.61				
10/17/2017	54.50	13.57	-23.65				
10/20/2017	57.06	13.54	-23.68				
10/21/2017	57.91	13.38	-23.64				
10/23/2017	59.61	13.59	-23.73				
10/25/2017	61.31	13.4	-23.8				
10/26/2017	62.17	13.4	-23.8				
10/27/2017	63.02	13.51	-23.98				
10/30/2017	65.57	13.64	-24.04				
10/31/2017	66.42	13.77	-23.87				
11/2/2017	68.13	13.98	-23.92				
11/4/2017	69.83	13.94	-23.82				
11/6/2017	71.53	14.16	-23.86				
11/8/2017	73.23	14.31	-23.74				
11/11/2017	75.79	14.48	-23.64				
11/13/2017	77.49	14.51	-23.6				
11/16/2017	80.05	14.63	-23.54				
11/18/2017	81.75	14.79	-23.4				
11/20/2017	83.45	14.78	-23.46				
11/21/2017	84.30	14.68	-23.37				
11/23/2017	86.01	14.84	-23.38				
11/24/2017	86.86	14.71	-23.41				
11/25/2017	87.71	14.79	-23.36				
11/27/2017	89.41	14.86	-23.36				
11/28/2017	90.26	14.91	-23.39				
12/2/2017	93.67	15.05	-23.42				

	1703-7		
Date	Estimated Length	$\delta^{15}$ N (‰)	δ <sup>13</sup> C (‰)
(mm/dd/yyyy)	(mm)		
5/4/2017	0.28	13.88	-22.49
8/16/2017	29.22	13.88	-22.49
9/5/2017	34.79	14.17	-22.31
9/12/2017	36.74	13.9	-22.59
10/9/2017	44.25	13.94	-22.5
10/15/2017	45.92	13.74	-22.33
10/17/2017	48.19	13.74	-22.41
10/19/2017	50.47	13.88	-22.13
10/22/2017	53.88	14.09	-22.15
10/23/2017	55.02	14.21	-22.32
10/24/2017	56.16	14.18	-22.3
10/26/2017	58.43	14.2	-22.26
10/28/2017	60.70	14.01	-22.2
10/30/2017	62.98	14.14	-22.15
11/1/2017	65.25	14.42	-22.06
11/2/2017	66.39	14.58	-22.01
11/4/2017	68.67	14.86	-22.03
11/5/2017	69.80	14.89	-22.15
11/6/2017	70.94	14.82	-22.23
11/7/2017	72.08	14.69	-22.27
11/8/2017	73.22	14.98	-22.39
11/10/2017	75.49	14.99	-22.59
11/11/2017	76.63	15.11	-22.59
11/12/2017	77.76	15.25	-22.65
11/13/2017	78.90	15.28	-22.58
11/14/2017	80.04	15.35	-22.69
11/16/2017	82.31	15.22	-22.64
11/17/2017	83.45	15.39	-22.67
11/18/2017	84.59	15.14	-22.61
11/19/2017	85.73	15.38	-22.61
11/20/2017	86.86	15.27	-22.58
11/21/2017	88.00	15.39	-22.62
11/22/2017	89.14	15.35	-22.53
11/23/2017	90.27	15.43	-22.56
11/24/2017	91.41	15.38	-22.47
11/25/2017	92.55	15.32	-22.46
11/26/2017	93.69	15.55	-22.48
11/27/2017	94.82	15.17	-22.33
11/28/2017	95.96	15.43	-22.48
11/29/2017	97.10	15.33	-22.43
11/30/2017	98.24	15.59	-22.41
12/2/2017	100.51	15.93	-22.65

1704-7							
Date	Estimated Length	$\delta^{15}$ N (‰)	$\delta^{13}$ C (‰)				
(mm/dd/yyyy)	(mm)						
5/7/2017	0.23	13.62	-23.3				
8/19/2017	23.71	13.62	-23.3				
9/23/2017	31.61	13.11	-23.29				
10/18/2017	37.26	12.99	-23.46				
10/21/2017	40.20	13.07	-23.48				
10/24/2017	43.14	13	-23.51				
10/28/2017	47.06	13.3	-23.57				
10/31/2017	49.99	13.56	-23.55				
11/2/2017	51.95	13.68	-23.67				
11/4/2017	53.91	13.57	-23.61				
11/7/2017	56.85	13.63	-23.71				
11/9/2017	58.81	13.84	-23.71				
11/10/2017	59.79	13.82	-23.68				
11/12/2017	61.75	14.24	-23.64				
11/13/2017	62.73	14.32	-23.64				
11/14/2017	63.71	14.21	-23.8				
11/15/2017	64.69	14.46	-23.72				
11/17/2017	66.65	14.51	-23.81				
11/19/2017	68.61	14.58	-23.75				
11/21/2017	70.57	14.63	-23.64				
11/24/2017	73.50	14.58	-23.62				
11/25/2017	74.48	14.66	-23.7				
11/26/2017	75.46	14.79	-23.62				
11/27/2017	76.44	14.76	-23.6				
11/28/2017	77.42	14.84	-23.57				
11/29/2017	78.40	14.9	-23.56				
12/1/2017	80.36	15.14	-23.54				
12/6/2017	85.26	15.22	-23.69				

	1705-7		
Date	Estimated Length	$\delta^{15}$ N (‰)	δ <sup>13</sup> C (‰)
(mm/dd/yyyy)	(mm)		
5/2/2017	0.23	13.43	-23.59
8/13/2017	24.12	13.43	-23.59
9/29/2017	35.01	14.21	-23.68
10/13/2017	38.26	13.35	-23.77
10/18/2017	44.73	13.58	-23.79
10/19/2017	46.02	13.64	-23.65
10/23/2017	51.19	13.63	-23.7
10/25/2017	53.78	13.58	-23.79
10/26/2017	55.07	13.36	-23.82
10/27/2017	56.37	13.36	-23.82
10/28/2017	57.66	13.62	-23.84
10/30/2017	60.25	13.67	-23.86
10/31/2017	61.54	13.53	-23.83
11/1/2017	62.83	13.54	-23.84
11/2/2017	64.13	13.64	-23.97
11/3/2017	65.42	13.6	-23.87
11/4/2017	66.71	13.67	-23.96
11/6/2017	69.30	13.93	-23.95
11/8/2017	71.89	14.08	-23.93
11/10/2017	74.47	14.34	-23.87
11/12/2017	77.06	14.56	-23.86
11/13/2017	78.35	14.63	-23.88
11/14/2017	79.65	14.59	-23.79
11/16/2017	82.23	14.55	-23.8
11/17/2017	83.53	14.75	-23.75
11/19/2017	86.11	14.8	-23.78
11/20/2017	87.41	14.81	-23.7
11/21/2017	88.70	14.82	-23.67
11/22/2017	89.99	14.94	-23.63
11/24/2017	92.58	15.15	-23.65
11/25/2017	93.87	15.38	-23.63
11/26/2017	95.17	15.68	-23.8
11/28/2017	97.75	15.84	-23.81
11/30/2017	100.34	15.83	-24.03

1706-7							
Date	Estimated Length	$\delta^{15}$ N (‰)	$\delta^{13}$ C (‰)				
(mm/dd/yyyy)	(mm)						
5/5/2017	0.20	13.83	-23.6				
8/11/2017	20.28	13.83	-23.6				
9/26/2017	29.70	13.52	-23.76				
10/16/2017	33.80	12.9	-23.83				
10/19/2017	37.34	12.9	-23.83				
10/22/2017	40.87	13.13	-23.91				
10/23/2017	42.05	13.05	-23.98				
10/26/2017	45.59	13.1	-24.1				
10/29/2017	49.12	13.2	-24.04				
10/31/2017	51.48	13.77	-24.05				
11/2/2017	53.84	13.9	-24.02				
11/3/2017	55.01	14.1	-23.9				
11/4/2017	56.19	14.11	-23.87				
11/5/2017	57.37						
11/6/2017	58.55	14.21	-23.88				
11/7/2017	59.73	14.36	-23.95				
11/10/2017	63.26	14.32	-23.94				
11/12/2017	65.62	14.45	-23.89				
11/13/2017	66.80	14.58	-23.95				
11/15/2017	69.16	14.64	-23.93				
11/16/2017	70.33	14.59	-23.92				
11/18/2017	72.69	14.52	-23.92				
11/20/2017	75.05	14.61	-23.82				
11/21/2017	76.23	14.51	-23.72				
11/23/2017	78.58	14.64	-23.77				
11/24/2017	79.76	14.8	-23.7				
11/26/2017	82.12	14.85	-23.74				
11/28/2017	84.48	14.81	-23.73				
11/29/2017	85.66	14.98	-23.74				
11/30/2017	86.83	15.04	-23.7				
12/2/2017	89.19	15.05	-23.7				
12/4/2017	91.55	15.21	-23.76				

1707-7							
Date	Estimated Length	$\delta^{15}$ N (‰)	δ <sup>13</sup> C (‰)				
(mm/dd/yyyy)	(mm)						
5/6/2017	0.23	13.74	-23.49				
7/5/2017	14.06	13.74	-23.49				
7/30/2017	19.83	13.66	-23.38				
8/20/2017	24.67	13.58	-23.42				
9/3/2017	27.90	13.58	-23.34				
9/23/2017	32.51	13.54	-23.35				
10/8/2017	35.97	13.61	-23.36				
10/17/2017	38.04	13.44	-22.92				
10/18/2017	38.83	13.44	-22.92				
10/19/2017	39.62	13.76	-23.34				
10/21/2017	41.20	14	-23.36				
10/23/2017	42.77						
10/25/2017	44.35	14.09	-23.58				
10/28/2017	46.72	14.02	-23.68				
10/31/2017	49.09	14.04	-23.68				
11/2/2017	50.66	14.11	-23.86				
11/4/2017	52.24	14.26	-23.73				
11/7/2017	54.61	14.11	-23.68				
11/11/2017	57.76	14.62	-23.56				
11/14/2017	60.13	14.58	-23.53				
11/18/2017	63.29	14.65	-23.48				
11/19/2017	64.08	14.57	-23.37				
11/22/2017	66.44	14.62	-23.37				
11/24/2017	68.02	14.23	-23.42				
11/26/2017	69.60	14.71	-23.45				
11/29/2017	71.97						
12/4/2017	75.91	14.65	-23.44				

1708-7							
Date	Estimated Length	$\delta^{15}$ N (‰)	$\delta^{13}$ C (‰)				
(mm/dd/yyyy)	(mm)						
5/6/2017	0.25	13.75	-23.48				
8/4/2017	22.95	13.75	-23.48				
8/28/2017	29.07	13.82	-23.44				
9/20/2017	34.93	13.85	-23.68				
9/20/2017	34.93	13.9	-23.52				
10/4/2017	38.50	13.62	-23.61				
10/18/2017	42.07	13.61	-23.6				
10/19/2017	43.03	13.9	-23.53				
10/23/2017	46.89	13.98	-23.58				
10/25/2017	48.82	13.94	-23.69				
10/28/2017	51.72	14.05	-23.78				
10/30/2017	53.65	14.1	-24				
11/1/2017	55.58	13.73	-24.13				
11/4/2017	58.47	13.91	-23.95				
11/6/2017	60.40	14.21	-23.94				
11/8/2017	62.33	14.54	-23.75				
11/9/2017	63.30	14.83	-23.76				
11/12/2017	66.19	14.78	-23.79				
11/18/2017	71.98	14.77	-23.83				
11/20/2017	73.91	14.8	-23.73				
11/21/2017	74.87	14.81	-23.66				
11/22/2017	75.84	14.9	-23.67				
11/24/2017	77.77	14.84	-23.67				
11/26/2017	79.70	14.87	-23.62				
11/28/2017	81.63	15	-23.61				
11/30/2017	83.56	15.22	-23.55				
12/1/2017	84.52	15.21	-23.56				
12/5/2017	88.38	15.39	-23.61				

1901-7							
Date	Estimated Length	$\delta^{15}$ N (‰)	$\delta^{13}$ C (‰)				
(mm/dd/yyyy)	(mm)						
5/1/2019	0.19	13.54	-23.95				
8/11/2019	19.46	13.54	-23.95				
9/25/2019	27.97	13.38	-23.8				
10/12/2019	31.18	12.96	-23.92				
10/15/2019	34.19	12.96	-23.92				
10/19/2019	38.20	13.05	-23.87				
10/23/2019	42.21	13.15	-23.79				
10/25/2019	44.21	13.22	-23.91				
10/28/2019	47.22	13.32	-23.99				
11/1/2019	51.23	13.11	-24.1				
11/3/2019	53.24	13.28	-24.27				
11/5/2019	55.24	13.6	-24.11				
11/7/2019	57.25	13.79	-24.46				
11/9/2019	59.25	14.04	-24.55				
11/11/2019	61.26	14.49	-24.24				
11/13/2019	63.26	14.63	-24.32				
11/14/2019	64.27	14.63	-24.21				
11/15/2019	65.27	14.56	-24.26				
11/16/2019	66.27	14.69	-24.28				
11/18/2019	68.28	14.69	-24.28				
11/20/2019	70.28	14.71	-24.24				
11/21/2019	71.28	14.81	-24.22				
11/23/2019	73.29	14.8	-24.24				
11/24/2019	74.29	14.92	-24.11				
11/25/2019	75.29	15.03	-24.11				
11/26/2019	76.30	15.07	-24.17				
11/28/2019	78.30	15.13	-24.06				
11/29/2019	79.30	15.39	-24.11				
12/1/2019	81.31						

	1902-7		
Date	Estimated Length	$\delta^{15}$ N (‰)	$\delta^{13}\mathrm{C}$ (‰)
(mm/dd/yyyy)	(mm)		
5/5/2019	0.18	13.65	-23.61
7/1/2019	10.59	13.65	-23.61
8/3/2019	16.61	13.77	-23.62
8/29/2019	21.35	14.11	-23.49
9/17/2019	24.82	14.09	-23.54
10/9/2019	28.84	13.85	-23.38
10/16/2019	30.11	13.43	-23.78
10/17/2019	31.07	13.43	-23.78
10/20/2019	33.94	13.48	-23.96
10/22/2019	35.85	13.69	-23.9
10/23/2019	36.81	13.98	-23.97
10/25/2019	38.72	13.88	-23.98
10/28/2019	41.59	13.86	-23.91
10/30/2019	43.50	13.89	-23.87
11/1/2019	45.42	13.95	-23.89
11/3/2019	47.33	13.91	-24.07
11/5/2019	49.24	14.13	-24.07
11/6/2019	50.20	14.11	-23.93
11/8/2019	52.11	14.37	-24.08
11/10/2019	54.02	14.7	-24.02
11/12/2019	55.94	15.01	-24.09
11/15/2019	58.81	15.13	-24.09
11/16/2019	59.76	15.13	-24.05
11/18/2019	61.67	15.14	-24.04
11/20/2019	63.59	15.16	-24.15
11/22/2019	65.50	15.19	-24.25
11/23/2019	66.46	15.21	-24.04
11/24/2019	67.41	15.29	-24.09
11/25/2019	68.37	15.29	-24.1
11/27/2019	70.28	15.32	-23.97
11/27/2019	70.76	15.26	-24.01
11/29/2019	72.19	15.37	-24.05
11/30/2019	73.15	15.31	-24.03
12/3/2019	76.02	15.42	-24.16

1903-7							
Date	Estimated Length	$\delta^{15}$ N (‰)	$\delta^{13}\mathrm{C}$ (‰)				
(mm/dd/yyyy)	( <b>mm</b> )						
5/6/2019	0.26	13.74	-23.56				
9/12/2019	33.71	13.74	-23.56				
9/30/2019	38.38	13.73	-23.5				
10/13/2019	41.75	13.55	-23.55				
10/17/2019	42.78	13.38	-23.9				
10/22/2019	47.62	13.56	-23.75				
10/25/2019	50.52	13.59	-23.92				
10/27/2019	52.46	13.53	-23.86				
10/28/2019	53.42	13.58	-23.84				
10/31/2019	56.33	13.84	-23.81				
11/2/2019	58.26	13.97	-23.85				
11/4/2019	60.19	14.03	-23.99				
11/5/2019	61.14	14.02	-24.15				
11/7/2019	63.07	14.2	-24.16				
11/9/2019	65.03	14.59	-24.09				
11/10/2019	66	14.71	-23.86				
11/11/2019	66.96	14.81	-23.99				
11/13/2019	68.9	14.68	-23.99				
11/14/2019	69.87	14.64	-24.41				
11/15/2019	71.8	14.63	-23.98				
11/16/2019	72.77	14.7	-23.95				
11/17/2019	73.74	14.62	-23.94				
11/18/2019	75.67	14.85	-23.97				
11/20/2019	76.64	14.76	-23.93				
11/21/2019	78.57	14.72	-23.74				
11/23/2019	79.54	15.07	-23.77				
11/24/2019	80.51	14.45	-23.68				
11/25/2019	81.47	14.52	-23.63				
11/26/2019	82.44	14.74	-23.65				
11/27/2019	82.44	14.74	-23.65				
11/28/2019	83.41						
11/29/2019	84.37	14.72	-23.66				
11/30/2019	85.34	14.84	-23.71				
12/4/2019	89.21	15.11	-23.78				

1904-7			
Date	Estimated Length	$\delta^{15}$ N (‰)	δ <sup>13</sup> C (‰)
(mm/dd/yyyy)	(mm)		
5/6/2019	0.22	13.77	-24.25
9/6/2019	27.39	13.77	-24.25
10/9/2019	34.68	13.14	-23.99
10/17/2019	36.45	13.05	-23.92
10/19/2019	38.55	13.05	-23.92
10/23/2019	42.75	13.39	-24.05
10/27/2019	46.95	13.67	-23.92
10/29/2019	49.05	13.46	-24.08
10/31/2019	51.15	13.4	-24.15
11/2/2019	53.25	13.57	-24.07
11/4/2019	55.35	14.05	-24.03
11/7/2019	58.50	13.92	-24.2
11/9/2019	60.60	13.7	-24.4
11/11/2019	62.70	13.85	-24.35
11/13/2019	64.80	13.98	-24.33
11/14/2019	65.85	13.88	-24.32
11/16/2019	67.95	14.36	-24.39
11/17/2019	69.00	14.54	-24.34
11/19/2019	71.11	14.74	-24.29
11/21/2019	73.21	14.71	-24.26
11/22/2019	74.26	14.7	-24.17
11/24/2019	76.36	14.96	-24.12
11/26/2019	78.46	15.15	-23.98
11/27/2019	79.51	15.28	-24.02
11/29/2019	81.61	15.15	-23.37
12/1/2019	83.71	15.25	-23.85
12/2/2019	84.76	15.07	-23.37
12/4/2019	86.86	15.27	-24.03

1905-7			
Date	Estimated Length	$\delta^{15}$ N (‰)	$\delta^{13}$ C (‰)
(mm/dd/yyyy)	(mm)		
5/7/2019	0.24	13.61	-23.96
9/4/2019	28.93	13.61	-23.96
10/7/2019	36.82	13.69	-23.97
10/18/2019	39.45	13.51	-24.02
10/20/2019	41.83	13.51	-24.02
10/25/2019	47.75	13.84	-23.99
10/29/2019	52.50	13.86	-24.14
10/31/2019	54.87	13.83	-24.02
11/2/2019	57.24	14.22	-24.1
11/5/2019	60.79	14.03	-24.26
11/8/2019	64.35	14.17	-24.35
11/9/2019	65.54	14.25	-24.43
11/11/2019	67.91	14.28	-24.41
11/13/2019	70.28	14.46	-24.18
11/14/2019	71.46	14.35	-24.06
11/15/2019	72.65	14.39	-24.11
11/16/2019	73.84	14.52	-24.06
11/17/2019	75.02	14.71	-24.2
11/19/2019	77.39	14.66	-24.04
11/22/2019	80.95	14.69	-24.1
11/23/2019	82.13	14.66	-24.1
11/24/2019	83.32	14.83	-23.98
11/25/2019	84.50	14.87	-23.85
11/26/2019	85.69	14.89	-23.87
11/27/2019	86.88	14.83	-23.87
11/29/2019	89.25	14.93	-23.63
11/30/2019	90.43	15.09	-23.47
12/2/2019	92.80	15.28	-23.89

1906-7			
Date	<b>Estimated Length</b>	$\delta^{15}$ N (‰)	$\delta^{13}$ C (‰)
(mm/dd/yyyy)	(mm)		
5/7/2019	0.18	14	-23.56
7/10/2019	11.49	14	-23.56
7/28/2019	14.67	14.01	-23.45
8/27/2019	19.97	14.61	-23.52
9/21/2019	24.38	14.2	-23.72
10/11/2019	27.92	13.77	-23.79
10/18/2019	29.16	13.53	-23.74
10/19/2019	29.93	13.53	-23.74
10/23/2019	33.05	13.64	-23.77
10/26/2019	35.39	13.62	-23.78
10/29/2019	37.72	13.82	-23.89
11/1/2019	40.06	13.74	-23.92
11/3/2019	41.62	13.96	-23.92
11/6/2019	43.95	14.1	-23.9
11/9/2019	46.29	14.36	-24.13
11/11/2019	47.85	14.57	-24.03
11/14/2019	50.18	14.86	-23.83
11/15/2019	50.96	15.02	-23.85
11/17/2019	52.52	15.09	-23.9
11/19/2019	54.08	15.14	-24.03
11/21/2019	55.64	15.15	-23.77
11/23/2019	57.19	15.18	-23.81
11/24/2019	57.97	15.26	-23.75
11/25/2019	58.75	15.2	-23.7
11/27/2019	60.31	15.37	-23.73
11/29/2019	61.87	15.28	-23.66
11/30/2019	62.65	15.55	-23.52
12/2/2019	64.20	15.44	-23.55
12/3/2019	64.98	15.53	-23.61
12/6/2019	67.32	15.54	-23.89

1907-7			
Date	Estimated Length	$\delta^{15}$ N (‰)	$\delta^{13}$ C (‰)
(mm/dd/yyyy)	( <b>mm</b> )		
5/8/2019	0.21	13.36	-23.93
8/2/2019	18.54	13.36	-23.93
9/16/2019	28.14	13.85	-23.98
10/14/2019	34.10	13.6	-23.91
10/19/2019	35.17	13.25	-23.86
10/20/2019	36.35	13.25	-23.86
10/22/2019	38.72	13.48	-24
10/25/2019	42.28	13.74	-24.18
10/27/2019	44.65	13.63	-24.2
10/29/2019	47.01	13.69	-24.26
10/30/2019	48.20	13.64	-24.24
10/31/2019	49.38	13.63	-24.08
11/2/2019	51.75	13.99	-24.22
11/3/2019	52.94	13.99	-24.24
11/4/2019	54.12	14.04	-24.21
11/6/2019	56.49	14.09	-24.27
11/8/2019	58.86	14.14	-24.45
11/9/2019	60.04	14.21	-24.85
11/10/2019	61.23	14.06	-24.53
11/12/2019	63.60	14.07	-24.56
11/13/2019	64.78	14.2	-24.54
11/14/2019	65.96	14.17	-24.52
11/15/2019	67.15	14.16	-24.51
11/16/2019	68.33	14.21	-24.57
11/17/2019	69.52	14.15	-24.53
11/18/2019	70.70	14.39	-24.4
11/19/2019	71.89	14.45	-24.46
11/20/2019	73.07	14.67	-24.43
11/21/2019	74.25	14.69	-24.39
11/22/2019	75.44	14.6	-24.38
11/23/2019	76.62	14.72	-24.33
11/24/2019	77.81	14.74	-24.28
11/25/2019	78.99	14.59	-24.23
11/26/2019	80.18		
11/27/2019	81.36	14.86	-24.16
11/28/2019	82.55	14.81	-24.08
11/29/2019	83.73	14.81	-24.08
11/30/2019	84.91	14.92	-24
12/1/2019	86.10	14.94	-23.95
12/2/2019	87.28	15.22	-23.96
12/3/2019	88.47	15.46	-24.08
12/6/2019	92.02	15.47	-24.18

1908-3			
Date	Estimated Length	$\delta^{15}$ N (‰)	δ <sup>13</sup> C (‰)
(mm/dd/yyyy)	(mm)		
5/11/2019	0.27	13.55	-23.77
8/17/2019	27.05	13.55	-23.77
8/30/2019	30.61	13.59	-23.85
9/17/2019	35.53	13.53	-23.83
10/5/2019	40.44	13.51	-23.78
10/18/2019	44.00	13.68	-23.88
10/22/2019	45.09	13.34	-23.86
10/23/2019	46.58	13.34	-23.86
10/25/2019	49.55	13.74	-23.98
10/27/2019	52.53	13.85	-24.22
10/29/2019	55.51	13.88	-24.06
10/30/2019	57.00	14.01	-24.11
10/31/2019	58.48	14.28	-24.22
11/1/2019	59.97	14.27	-24.37
11/3/2019	62.95	14.29	-24.48
11/4/2019	64.44	14.54	-24.95
11/5/2019	65.92	14.13	-24.38
11/7/2019	68.90	14.02	-24.49
11/8/2019	70.39	13.71	-24.4
11/9/2019	71.88	14.3	-24.27
11/10/2019	73.37	14.2	-24.23
11/11/2019	74.85	14.41	-24.21
11/13/2019	77.83	14.47	-24.29

1909-7			
Date	Estimated Length	$\delta^{15}$ N (‰)	$\delta^{13}$ C (‰)
(mm/dd/yyyy)	(mm)		, í
5/12/2019	0.14	13.37	-24.16
8/29/2019	15.90	13.37	-24.16
10/8/2019	21.68	13.24	-24.13
10/23/2019	23.85	13.12	-24.15
10/24/2019	23.99	13.12	-24.15
10/25/2019	26.25	13.12	-24.15
10/28/2019	29.84	13.32	-24.33
10/30/2019	32.24	13.25	-24.25
10/31/2019	33.43	13.22	-24.07
11/2/2019	35.83	13.41	-24.25
11/3/2019	37.03	13.5	-24.31
11/7/2019	41.82	13.67	-24.37
11/8/2019	43.02	13.78	-24.4
11/9/2019	44.21	13.33	-24.28
11/11/2019	46.61	13.57	-24.4
11/14/2019	50.20	13.79	-24.43
11/15/2019	51.40	13.74	-24.45
11/17/2019	53.80	13.91	-24.45
11/18/2019	55.00	13.81	-24.35
11/19/2019	56.19	14.41	-24.43
11/20/2019	57.39	14.54	-24.23
11/22/2019	59.79	14.67	-24.31
11/23/2019	60.99	14.79	-24.41
11/25/2019	63.38	14.94	-24.4

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