Isotopic Evidence of Salmon, *Oncorhynchus* spp., in the Diet of the Wolverine, *Gulo gulo,* on Princess Royal Island, British Columbia

THOMAS F. SHARDLOW

Fisheries and Oceans Canada, Pacific Biological Station, 3190 Hammond Bay Road, Nanaimo, British Columbia V9T 6N7 Canada; email: tomshardlow@shaw.ca

Shardlow, Thomas F. 2013. Isotopic evidence of salmon, *Oncorhynchus* spp., in the diet of the Wolverine, *Gulo gulo,* on Princess Royal Island, British Columbia. Canadian Field-Naturalist 127(4): 338–342.

Evidence from $\delta^{13}C$ and $\delta^{15}N$ isotope signatures from hair samples from a Wolverine, *Gulo gulo*, suggest the use of salmon, *Oncorhynchus* spp., in the diet of the Wolverine on Princess Royal Island on the north coast of British Columbia. This is also the first published record of Wolverines on that island.

Key Words: Wolverine; *Gulo gulo*; salmon; *Oncorhynchus* spp.; isotope; carbon-13; $\delta^{13}C$; nitrogen-15; $\delta^{15}N$; Princess Royal Island; British Columbia; camera station

Introduction

The Wolverine (*Gulo gulo*), the largest of the terrestrial mustelids, has a circumpolar distribution favouring northern latitudes and high elevations (Hatler *et al.* 2008). Wolverines are widely distributed in British Columbia, with an affinity for interior and snowy habitats. Wolverines are considered rare on the coast (Lofroth and Krebs 2007); however, they have been recorded in some coastal areas, such as Vancouver Island.

Wolverines found in coastal watersheds of British Columbia would be expected to encounter moribund salmon (*Oncorhynchus* spp.) from spawning runs in many of the streams. However, there are no records of salmon consumption by this scavenger. Wolverine diets have been reported as exclusively derived from terrestrial vertebrates (Magoun 1987; Lofroth *et al.* 2007; Dalerum *et al.* 2009), even in studies conducted in areas where spawning salmon were present.

This note presents evidence from carbon-13 isotopes ($\delta^{13}C$) and nitrogen-15 isotopes ($\delta^{15}N$) taken from Wolverine hair samples that suggest that salmon is present in the diet of a Wolverine on Princess Royal Island on the north coast of British Columbia. It is also the first published record of Wolverines on this island.

Methods

Study area and field sampling

Seventeen camera stations located in riparian and surrounding low-elevation habitats were established along Chapple Inlet on Princess Royal Island (Figure 1) during a study to monitor scavengers and predators of salmon (*Oncorhynchus* spp.) in riparian ecosystems (Shardlow 2013; Shardlow and Hyatt 2013).

Princess Royal Island (2250 km²) is the largest island on the north coast of British Columbia, and it remains mostly as mature forest. Chapple and Douglas creeks flow into the head of Chapple Inlet on the west side of the island. Pink Salmon (*Oncorhynchus gorbuscha*) are the predominant spawners, along with small numbers of Chum Salmon (*O. keta*) (DFO, 2013). Many small streams in the vicinity also support salmon. Columbian Black-tailed Deer (*Odocoieus hemionus columbianus*), Blue Grouse (*Dendragapus obscurus*), American Beaver (*Castor canadensis*), and Douglas' Squirrel (*Tamiasciuris douglassii*) were among some of the Wolverine's potential prey items recorded by the cameras during the study.

Wolverine sightings were recorded using a motion-activated infrared digital camera (PC-85 from Reconyx, Holmen, Wisconsin, USA) at each of the 17 trapping stations. Sampling techniques followed the methods detailed by Shardlow and Hyatt (2013). Untreated organic fish fertilizer was used as the primary scent lure. The fertilizer was poured onto the ground inside a barbed-wire "enclosure" used to snag hair samples. Additional scent lures (from Wildlife Control Supplies Ltd., East Granby, CT, USA), designed to attract a wide variety of predators and scavengers, were also placed inside the enclosures (e.g., beaver castor, anchovy extract, shellfish oil, anise seed oil, and essence of apple).

Camera stations were monitored from early August 2011 to early June 2012 (341 camera-weeks in total). A total of nine photographs of Wolverines were recorded at 3 of the 17 stations (Figure 1) between 8 August and 21 September 2011. The time period between individual photographs ranged from one to 38 days.

Hair snags and camera data were retrieved from the stations every 7 to 10 days. All barbs were cleaned of any residual hair using a propane torch. Camera images recorded Wolverine points of entry across the barbed wire, so that hair snags could be matched with the individuals in the photographs.

Not all individuals could be identified; however, at least two individuals, i.e., individual no. 1 (Figure 2) and individual no. 2 (Figure 3) could be differentiated based on body size. Individual no. 2 appeared thinner than individual no. 1. A comparison of body depths, measured from the original photographs and corrected for the distance from the camera to the subject, confirmed individual no. 1 had a body depth at least 70%

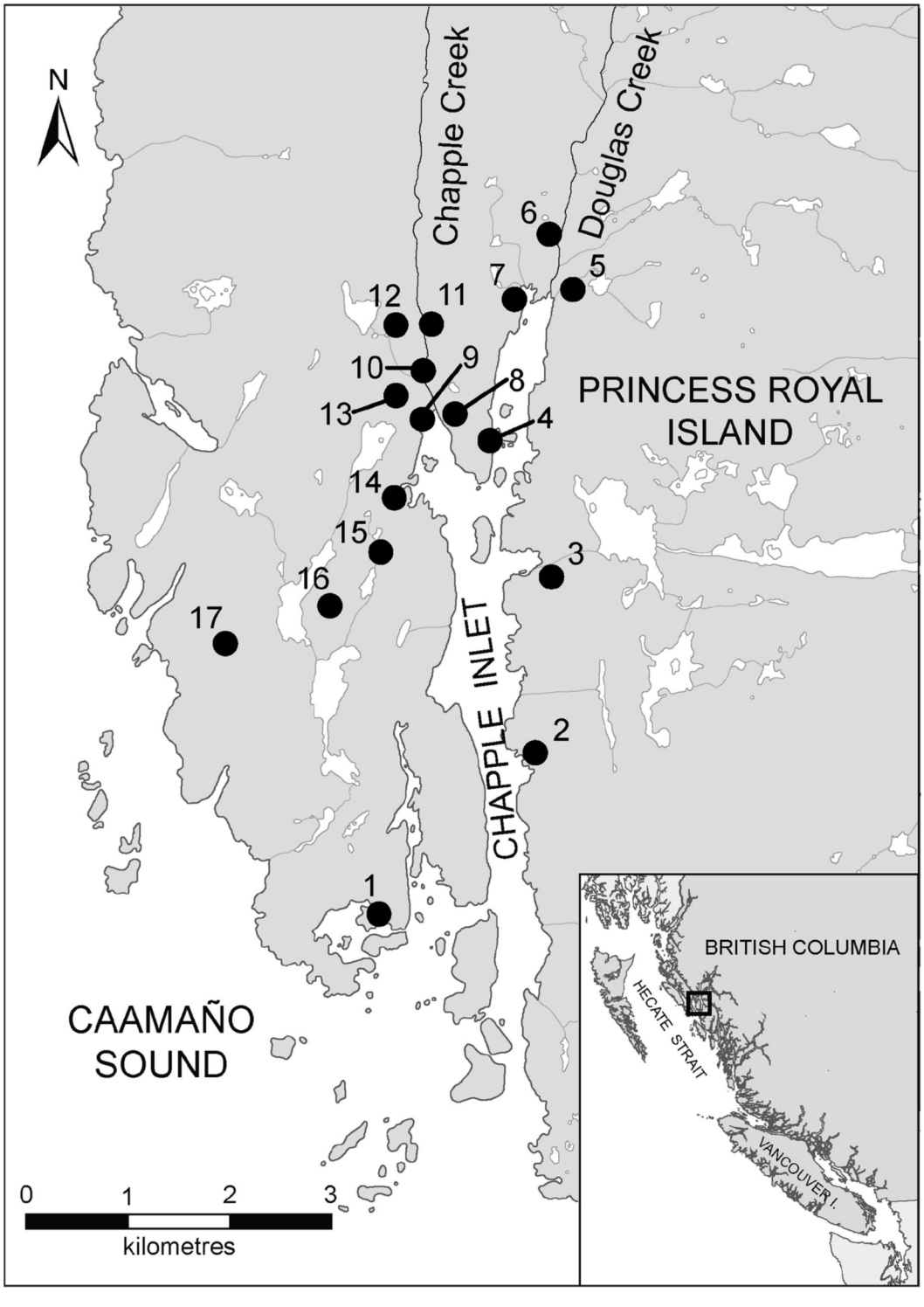

FIGURE 1. Study area showing location of camera stations. Wolverines (*Gulo gulo*) were recorded at 3 stations (stations 2, 3, and 5) between 8 August and 21 September 2011.

FIGURE 2. Wolverine (*Gulo gulo*) no. 1, photographed August 13, 2011 at station no. 5 near Douglas Creek on Princess Royal Island, British Columbia.

FIGURE 3. Wolverine (*Gulo gulo*) no. 2, photographed September 6, 2011 at station no. 2 near the shoreline of the east side of Chapple Inlet, Princess Royal Island, British Columbia.

larger than individual no. 2. Hair snag samples using whole hairs of approximately the same length from these two individuals were analyzed for isotopic signatures.

Laboratory procedures

Carbon and nitrogen isotopic signatures were analyzed by Aquatech Enviroscience Laboratories Inc., Victoria, British Columbia, using standard methods and instrumentations. Samples of Wolverine hair were cleaned of surface oils using repeated rinses of a 2:1 chloroform : methanol solution and then dried in an oven at 60°C for 12 hrs. Hair in the amount from 0.2 to 0.4 mg was cut and placed in a tin cup. The amount of sample or standard analyzed in the procedure was based on the criterion that the range of nitrogen must be within 0.02 to 0.2 mg.

Carbon and nitrogen isotope compositions were determined using thermal combustion elemental analyzer Costech ECS 4010 (Costech Analytical Technologies Inc., Valencia, California) coupled with a continuous flow elemental analysis isotope ratio mass spectrometer (EA-IRMS) (Thermo Finnigan DELTAplus Advantage, ThermoFinnigan Inc., Bremen, Germany). Each batch of samples included quality assurance and quality control (QA/QC) samples: three "working standards" analyzed before and after each batch of samples, a sample duplicate, and a procedural blank. The carbon and nitrogen contents of hair samples were determined based on the calibration created using EA-IRMS analysis of acetanilide used as a calibration standard.

Both carbon and nitrogen isotope data are reported in conventional δ-notation in units of per mil (‰) with respect to the Pee Dee Belemnite (PDB) standard and atmospheric nitrogen (air) for ^{13}C and ^{15}N, respectively:

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

where X is ^{13}C or ^{15}N and R is a corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. $R_{standard}$ for ^{13}C and ^{15}N corresponds to the ratio of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ in their respective international standards, PDB and Air.

All Wolverine hair samples were normalized against international reference standards: IAEA-N1 (ammonium sulphate reference material certified by the International Atomic Energy Agency) with a $\delta^{15}N$ value of 0.40‰, IAEA-N2 with a $\delta^{15}N$ value of 20.30‰, Sucrose ANU with a $\delta^{13}C$ value of −10.47‰, and NBS-22 OIL with a $\delta^{13}C$ value of −29.73‰. Long-term performance of the mass spectrometer was monitored by analysis of secondary reference material in every batch: acetanilide with carbon and nitrogen contents of 71.09% and 10.36%, respectively, DORM-2 dogfish muscle certified reference material from NRC with a $\delta^{15}N$ value of 14.33‰, and caffeine with a $\delta^{15}N$ value of −0.95‰. The long-term standard deviation of the values obtained from measurements of the secondary laboratory standards were within 0.1 and 0.25% for ^{13}C and ^{15}N, respectively.

Diet analysis

The processes of incorporating stable isotopes into animal tissues are outlined by Ben-David and Flaherty (2012). Isotope signatures in animal tissue are the consequence of the source (i.e., food or prey) and the fractionation of the metabolic steps into that tissue. Isotope signature values for potential prey items of Wolverine found on Princess Royal Island and correction factor values for $\delta^{13}C$ and $\delta^{15}N$ fractionation were taken from the literature. Salmon values were taken from Satterfield and Finney (2002), Mule Deer

(*Odocoileus hemionus*) from Darimont *et al.* (2009), microtines from Ben-David *et al.* (1997), and Blue Grouse from Dalerum *et al.* (2009).

Results

The isotope signatures from the two Wolverines on Princess Royal Island as well as those from Dalerum *et al.* (2009) are shown in Table 1 along with the values corrected for trophic fractionation (Δδ).

The average value and standard error for $\delta^{15}N$ presented by Dalerum *et al*. (2009) from Wolverine bone collagen was 7.2 (± 0.2) in an area of Alaska populated with spawning Chum Salmon. When corrected for fractionation, the $\delta^{15}N$ values ranged between approximately 1 and 4 (Figure 4). The data from Princess Royal Island are from hair samples; however, Hilderbrand *et al.* (1996) found little or no difference in $\delta^{13}C$ and $\delta^{15}N$ values between bone collagen and hair taken from House Mice (*Mus musculus*), European Rabbits (*Oryctolagus cuniculus*), and American Black Bears (*Ursus americanus*) fed a constant diet.

Assuming that collagen and hair have similar trophic fractionation values, a $\delta^{15}N$ value of 12.4 (or 7.7 corrected for trophic fractionation) found in Wolverine hair from Princess Royal Island would indicate a substantial level of marine protein, most likely from salmon, in the diet of Wolverine no. 1. The isotope signature for Wolverine no. 2, on the other hand, indicates a diet of Columbian Black-tailed Deer and microtines (Figure 4).

TABLE 1. Wolverine (*Gulo gulo*) hair isotope values, corrected for fractionation (Δδ), for two individuals on Princess Royal Island, British Columbia, and for Wolverine collagen (average value) from Alaska and isotope signatures for prey items from Princess Royal Island.

Isotope signatures	$\delta^{13}C$	$\Delta\delta^{13}C$	$\delta^{13}C$ corrected	$\delta^{15}N$	$\Delta\delta^{15}N$	$\delta^{15}N$ corrected
Wolverine						
Princess Royal Island, no. 1	−21.3	3.2[1]	−24.5	12.4	4.7[1]	7.7
Princess Royal Island, no. 2	−24.4	3.2[1]	−27.6	6.9	4.7[1]	2.2
Collagen (Alaska average)[2]	−20.5	2.9	−23.4	6.7	4.6	2.1
Prey						
Salmon[3]	−19.9	−0.8		12.5	3.8	
Microtines[3]	−26.5	2.9		2.9	4.6	
Mule Deer[2]	−27.4	3.5		3	4.9	
Blue Grouse[2]	−23.7	2.9		2.8	4.6	

[1]Mule Deer, Blue Grouse, and microtine average.
[2]Values from Dalerum *et al*. (2009) and Mule Deer from Darimont *et al*. (2009).
[3]Salmon from Satterfield and Finney (2002) and microtines from Ben-David *et al*. (1997).

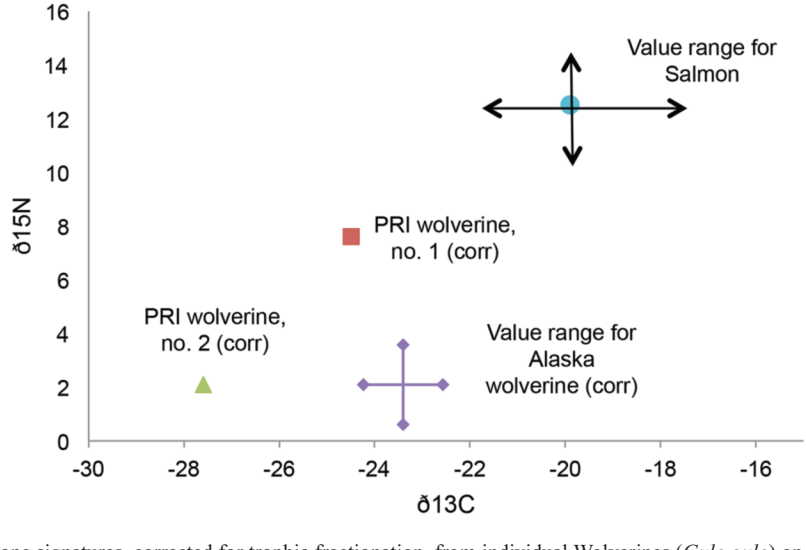

FIGURE 4. Isotope signatures, corrected for trophic fractionation, from individual Wolverines (*Gulo gulo*) on Princess Royal Island (PRI) and a range of values from femur collagen of Wolverines from Alaska (n = 37) (taken from Dalerum *et al*. 2009) corrected for trophic fractionation. The range of values for salmon (*Oncorhynchus* spp.) (n = 47) from Alaska are taken from Satterfield and Finney (2002).

Discussion

While the evidence for the consumption of salmon by Wolverine comes from just one individual, the finding is noteworthy for two reasons. First, the isotopic signature is well outside the range that can be explained by a diet consisting exclusively of terrestrial vertebrates. Second, it is the first isotopic evidence of the consumption of salmon by this species.

It seems reasonable that Wolverines, now known to be on this coastal island, would exploit salmon carcasses. Ungulates, such as Moose (*Alces americanus*) and Caribou (*Rangifer tarandus*), which are commonly found in the diet of Wolverines elsewhere (Lofroth *et al*. 2007), are most likely not present on the island (Darimont *et al*. 2005, Shackleton 1999).

Further samples from individuals on Princess Royal Island or other coastal islands may reveal that salmon are part of the diet of some other coastal Wolverines.

Acknowledgements

S. Verenitch kindly reviewed the manuscript and P. Sinclair provided valuable assistance in the field.

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Received 23 May 2013
Accepted 10 September 2013