Location, weathering, bulk isotope, and 14C data for fossil seals from the western Ross Sea, Antarctica from from 2013-2014

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Data Type: Other Field Results
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Project
» Collaborative Research: Exploring the Vulnerability of Southern Ocean Pinnipeds to Climate Change - An Integrated Approach (Southern Ocean Pinnipeds)

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Abstract
This dataset includes location, weathering, bulk isotope, and 14C data for fossil seals from the western Ross Sea, Antarctica from from 2013-2014.

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Coverage

Spatial Extent: **N**:-76.67755 **E**:164.72143 **S**:-78.9066 **W**:160.58937
Temporal Extent: 2013-01-16 - 2014-01-16

Dataset Description

These data are published and discussed in:
Brault, E. (2017). An Examination of the Ecological and Oceanographic Effects of Mid-to-Late Holocene Climate Changes on the Ross Sea Ecosystem. UC Santa Cruz. ProQuest ID: Brault_ucsc_0036E_11435. Merritt ID: ark:/13030/m5dg1n5d. Retrieved from https://escholarship.org/uc/item/99s5j3fk

Methods & Sampling

Sample collection:
Fossil seal samples were collected during the austral summers of 2005/06, 2006/07, 2012/13, and 2013/14 in

Antarctica in the Dry Valleys and along the Victoria Land Coast (especially Inexpressible Island on Terra Nova Bay), Antarctica. Since this region experiences unusually dry and cold conditions, carcasses and bones are well-preserved, potentially for several thousand years, and therefore have unchanged isotopic compositions in most cases. The sampled species were crabeater, Weddell, leopard, and southern elephant seals. Latitude and longitude of each specimen was determined by GPS and recorded, and several photographs were taken of each specimen. Bone and carcass weathering states were determined. Several samples were gathered from the specimens. Most commonly, bone was collected, followed by skin and fur; nails, teeth, and whiskers were not frequently available for sampling, but taken when possible. Samples were collected with ethanol-cleaned tools, stored in Whirl-Pak bags, and refrigerated (~4 °C) when we returned from the field.

Carcass and Bone Weathering:
For each specimen, we assessed the extent to which the carcass had been degraded by exposure, as well as the weathering state of exposed bone.

We assigned each specimen to one of the following Carcass Weathering Stages. These stages are modified from Barwick and Balham (1967):
A = Complete.Fresh. Undamaged from wind erosion, no desiccation.
B = Complete. Slightly weathered. Partial erosion of hair on exposed surfaces, cracks in skin exposing deeper tissues or bones in the flippers or cranium.
C = Incomplete. Extensive erosion and exposure of bones of upward part of body (skull, ribs, limbs). Skeleton undamaged but soft tissues eroding between bony elements.
D = Incomplete. Extensive erosion with regional destruction of bone and tissues. Vertebral column completely exposed and 40-50% tissue is destroyed.
E = Very incomplete. >50% of carcass is eroded. Vertebral column and cranium incomplete; limbs and rib cage becoming remnants. Difficult or impossible to identify species, sex, size.
F = Fragments or isolated bones.

Bone Weathering Stages were determined on the most weathered bone from the carcass. Modified from Todd (1987).
0 = unweathered and fatty.
1 = unweathered, articular surfaces intact with no surface cracking.
2 = articular surfaces intact with some surface cracking.
3 = articular surfaces exhibit some deterioration, but >50% of articular surface remains intact.
4 = intact articular surface restricted to a few small "islands;" <50% of articular surface remains intact.
5 = no articular surface area remains intact.
6 = bone severely deteriorated; large areas of fibrous bone exposed.
7 = small weathered bone fragment or isolated tooth.

Species identification:
When possible, species identification of each specimen was conducted in the field via examination of teeth or bones with features unique to the four pinniped species. Additionally, the team at the University of Durham in the United Kingdom extracted and analyzed DNA from many specimens, described below, to confirm or establish the species identification

Sample preparation for bulk isotope analyses:
Preparation of bone samples was based on established procedures and by preliminary method testing in the lab (DeNiro 1987, Ambrose 1990). Bone fragments (50 -100 mg) were weighed into a vial (BD Falcon 15 mL centrifuge tubes, polypropylene) and acidified (to remove bone mineral, sedimentary carbonates, and fulvic acids) by the addition of 5 mL HCl (0.5 M) at room temperature. The acid was removed approximately three days later. If the samples did not appear completely decalcified (i.e., remains inflexible), a fresh aliquot of acid was added every 24 hours until the bone was decalcified, leaving behind bone collagen. Upon decalcification, the collagen was rinsed five times with Milli-Q water (Thermo Fisher Scientific, Inc.), neutralizing the sample, and 5 mL NaOH (0.1 M) was added to the samples, which were then kept at room temperature, further purifying the bone collagen by removing humic acids. A fresh aliquot of 0.1 M NaOH was added to the sample every 24 hours until the sample was nearly white. Again, the material was rinsed five times with Milli-Q water; then, it was refrigerated until the lipid extraction procedure. Lipids were removed via an accelerated solvent extraction with 100% petroleum ether (Dionex ASE 200 Accelerated Solvent Extractor: 1500 psi; 60°C; 3 cycles).

Bone was the most common sample type available. As it integrates diet over the largest period among the sample types collected (i.e., much of the animal's lifespan), we analyzed bone from each specimen when available (n = 621). Hair (fur, whisker) samples were analyzed from specimens if no bone had been collected (n = 34). For some specimens (n = 18), both bone and fur were analyzed to determine the isotopic offset between these different tissues. We were unable to develop a protein purification method for skin samples to achieve reliable bulk isotope values, though skin values that yielded plausible C:N ratios are reported, some of

which are for comparison to bone values (n = 17).

Hair samples, between 10 and 20 hairs with their follicles removed, were prepared for isotopic analysis by first washing with Milli-Q water. Then, lipids were removed via three rinses with petroleum ether in an ultrasonic bath (Thermo Fisher Scientific, Inc.) for 15 minutes each time, and other exogenous material was eliminated with alternating treatments of acid (0.5 M HCl) and base (0.1 M NaOH) until the solution was clear. After each acid or base treatment, five rinses with Milli-Q water were performed. Skin samples were treated similarly.

Bulk stable isotope analysis:
For all bone and hair samples approximately 1 mg and 0.5 mg of sample, respectively, was weighed into tin cups (Costech, 3x5 mm) for elemental analysis-isotope ratio mass spectrometry (EA-IRMS). This analysis was performed at the Stable Isotope Lab (SIL) at the University of California (UC) Santa Cruz on a Carlo Erba EA 1108 elemental analyzer coupled to a Thermo-Finnigan DeltaPlus XP isotope ratio mass spectrometer. δ15N values were referenced to an AIR standard, and δ13C values were referenced to a V-PDB standard. On a day-to-day basis, we measured and calibrated analyses with a laboratory IU Acetanilide standard (δ15N = 1.18‰, δ13C = -29.52‰, %N = 10.36%, %C = 71.02%) and a laboratory gelatin standard (δ15N = 5.60‰, δ13C = -12.60‰, %N = 16.44‰, %C = 44.02‰). The isotopic and concentration value of these laboratory standards are known by calibration to international standards (IAEA601, IAEA-346, USGS24, USGS25, USGS26, USGS34, USGS35, USGS41). We applied mass and drift corrections during each instrument session using the gelatin standard. Standard deviations for standards were < 0.1 ‰ for both δ15N and δ13C and <0.05 for C/N (seven PUGel standards analyzed at the start of each session and a PUGel and an Acetanilide standard analyzed after every eight samples during the session).

Sample preparation for radiocarbon analysis:
When possible, skin samples from a specimen were analyzed for radiocarbon (14C), but when skin was unavailable, bone was analyzed instead. Prior to 14C analysis, skin samples were manually cleaned to remove visible sand and dried in an oven. Bone samples required more extensive preparation to isolate collagen and eliminate contaminants.

Bone samples were prepared for 14C according to an established laboratory protocol. First, between 150 and 200 mg of bone was weighed and ground into a coarse powder via a mortar and pestle (pre-cleaned with ethanol). Then, the material was transferred into a vial (BD Falcon 15 mL centrifuge tubes, polypropylene) for processing to isolate highly pure collagen. Ground bone was decalcified via the addition of 7 mL of HCl (0.5 M), agitation with a vortex, and storage at room temperature. Decalcifying samples were checked after 48 hours and if their decalcification was incomplete, the HCl was refreshed. Subsequently, the HCl was refreshed every 24 hours until decalcification appeared complete at which time the collagen was rinsed five times with Milli-Q water and 7 mL NaOH (0.1 M) was added at room temperature. After 24 hours, the NaOH was removed and the samples were rinsed five times with Milli-Q water. A final application of 7 mL of HCl (0.5 M) for 24 hours was performed to ensure complete decalcification, which was followed by five Milli-Q water rinses. Samples were kept frozen at -20 °C until the next preparatory step.

The bone collagen was gelatinized via the addition of 7 mL HCl (0.05 M) after being transferred to 10 mL borosilicate culture tubes (Fisherbrand). Samples were heated in the HCl solution at 58 °C on a hot plate for 48 hours. Immediately after stopping the gelatinization the samples were filtered (Whatman glass microfiber filters, grade 934-AH circles, particle retention 1.5 µm), retaining the filtrate, which was transferred to borosilicate culture tubes. Lastly, samples were dried via a vacuum concentrator centrifugal evaporator system (Jouan RC 10-10) with liquid N2 and water traps. At this point, the nearly pure collagen from bone was obtained.

Radiocarbon analysis:
After pretreatment, about 20 mg of each bone or skin sample was sent to the National Ocean Sciences Accelerator Mass Spectrometry Facility (NOSAMS) at Woods Hole Oceanographic Institution (WHOI) for 14C analysis. Samples received further pretreatment to remove organic contaminants at NOASAMS (repeated acid-base rinse cycles until rinsing solutions were clear). NOSAMS used their organic combustion method to graphitize the samples, which were then pressed into target cartridge and loaded into the Accelerator Mass Spectrometer (AMS) for analysis. Samples were analyzed on either a 3 MV Tandetron or 500 kV Pelletron system. NBS Oxalic Acid I (NIST-SRM-4990) was the primary standard used for 14C measurements, and process blank materials were analyzed during each instrument session. The blank materials were the Fourth International Radiocarbon Intercomparison (FIRI) A and B woods, as well as acetanilide (CE Elantech). More details on analyses at NOSAMS are available at: http://www.whoi.edu/nosams/home

Upon receiving the data, 14C ages were calibrated with CALIB 7.0.1, the Marine13 calibration database (Reimer et al. 2013), using the delta-R value of 791 +/- 121 (Hall et al. 2010). Calendar ages were reported as the midpoint of the one standard deviation range. The correction for the applied reservoir effect was verified via

radiocarbon analysis of Weddell seal specimens from the Ross Sea that were of known age as they were collected during particular expeditions in the early 1900s. Additionally, an effect of contamination on the 14C data was disregarded since preparation and analysis of standards resulted in 14C measurements that had offsets from the known ages of the materials that were within instrument error. All all reported ages are relative to 1950. Not all samples could be calibrated and may immediately predate or postdate atmospheric nuclear testing. These samples have been assigned an age of 75.

Sample preparation for ancient DNA analysis:
All ancient DNA (aDNA) work was conducted in a dedicated aDNA laboratory at Durham University, using strict protocols to prevent and detect contamination. Briefly, this included one-way movement of reagents, samples, and personnel from the aDNA to the modern DNA lab, decontamination using 50% bleach and/or UV sterilization, use of filter tips, and inclusion of extraction and negative controls every 10 to 20 samples.

Ancient DNA was extracted from skin, bone, and hair samples. For bone and skin samples, an ~ 1 cm x 1 cm section of sample was removed using a rotary saw with disposable blades. These were homogenized in a Retsch MM 200 mill in stainless steel canisters cleaned with Virkon disinfectant, 50% bleach, and rinsed in 95% ethanol. Hair samples were cut into ~ 5 mm portions using a sterile blade.

aDNA extraction and amplification:
First, samples were digested overnight at 37 °C in 750 uL of solution of 0.5% SDS, 0.425 M EDTA (pH 8.0), 0.1 M Tris-HCl (pH 8.0), and 0.77 mg/mL Proteinase K. Following digestion, 3 mL of PB Buffer (QIAgen) was added to each sample, along with up to 50 uL of 3 M sodium acetate (pH 5.2) to adjust the pH for purification with the Qiaquick spin filter kit. Purification was conducted according to manufacturer directions; however, additional spins were required to bind the entire sample to the column. Elution was performed twice using 70 uL of EB buffer, which was incubated at 37 °C for 5 min.

Polymerase chain reaction (PCR) was carried out to amplify an approximately 180 bp fragment of the mitochondrial control region, using primers aSealCR1F (5’-GCATTAACGGTTTGCCCCAT-3’) and aSealCR1R (5’-GGTACACGTTTCACAAGGGT-3’), which were designed in the program Primer3 (Koressaar and Remm 2007, Untergasser et al. 2012) from 200 ancient southern elephant seal (sampled from the VLC; de Bruyn et al. 2009), 135 modern crabeater seal (Curtis et al. 2009), 83 modern Weddell seal (Curtis et al. 2009), and 2 modern leopard seal sequences from Genbank. This region carries several diagnostic sites that differ among the four species, thus facilitating species identification. PCR reactions were conducted in a total volume of 25 µl, which included 2 µl DNA, as well as 0.02 units of AmpliTaqGold, 1X AmpliTaq Gold buffer II, 2.5 mM MgCl2, 0.2 mM each dNTP, and 0.4 µM each primer. The thermocycler program used was 94 °C for 8 min, then 45 cycles of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 30 s, followed by a further extension step of 72 °C for 7 min. PCR products were visualized on a 2% agarose gel stained with ethidium bromide. In total, DNA extraction was attempted for 536 samples and was performed successfully for 468 samples.

Species identification from aDNA analysis:
We developed a restriction digest test to conduct an initial screen for species identification. Positive PCR products (20 uL) were cleaned via a standard ethanol precipitation procedure: 10% of 3 M sodium acetate (pH 5.2) and 1 mL of chilled 100% ethanol was added to each sample, which was then incubated overnight at -20 °C. Samples were spun for 15 min and then the ethanol was discarded and 1 mL of chilled 70% ethanol was added. Samples were spun for another 15 min, the ethanol was discarded, and the samples were dried for 45 min at 50 °C in a SpeedVac DNA concentrator. Next, 15 uL of EB buffer, preheated to 55 °C, was added to each sample and the samples were subsequently incubated at 55 °C for a further 10 min in order for elution.

Restriction digests were carried out using 1x Cutsmart Buffer (New England Biolabs), 7.5 uL of cleaned PCR product, and 0.5 uL BstE-II HF enzyme (New England Biolabs). Reactions were incubated at 37 °C for 30 min and terminated by the addition of 5 uL of gel loading buffer, following manufacturer instructions. The resulting products were run at 90 V for 3 hr on a 2.5% agarose gel stained with ethidium bromide. *In silico* tests via the program, Geneious v9.0 (Kearse et al. 2012), suggested that this restriction digest would result in the production of two fragments of 100 and 80 bp in length for approximately 80% of the crabeater seal sequences available on Genbank. However, up to 2% of ancient southern elephant seals shared this site and would also be cut since no perfect species-specific restriction site could be found within the sequences available on Genbank (for this site or any other mitochondrial region) that would be short enough to reliably amplify aDNA. Any cut PCR product was assumed to be a crabeater seal, and any uncut product was sent for Sanger sequencing in the forward direction at Durham University’s DBS Genomics facility.

Resulting sequences were visually inspected and manually edited in Geneious and then compared against the Genbank database using the BLAST tool to provide initial species assignments. The highest scoring hit was accepted as long as the e-value was below 1 x 10-20 and the sequence identity was greater than 95%. DNA sequences from Genbank, described above, were added to those obtained from our study. Sequences for

northern elephant seal (*Mirounga angustirostris*), Mediterranean monk seal (*Monachus monachus*), Ross seal (*Ommatophoca rossii*), and bearded seal (*Erignathus barbatus*) were included as outgroups. MAFFT v7 (Katoh et al. 2002) was used to align the sequences and the best-fit model of nucleotide evolution was investigated using jModelTest v2 (Darriba et al. 2012). A neighbor-joining tree was built in Geneious with 1,000 bootstraps and principle component analysis (PCA) was conducted in the program GenAlEx (Peakall and Smouse 2006, 2012). Results (not shown) displayed all putative and known crabeater seal individuals grouping together in a monophyletic group with a bootstrap support of 89. Similarly, all northern and southern elephant seals grouped together with bootstrap support of 67, and Ross seals grouped together with support of 96. Leopard seals and Weddell seals grouped together in a single clade with a bootstrap support of 76, but these species are known to have a close evolutionary history (Dasmahapatra et al. 2009, Fulton and Strobeck 2010). PCA was able to successfully separate all species – both individuals of known identity and those with putative assignments – thus confirming previous initial BLAST species assignments. These species assignments have also been largely confirmed through morphological identification of preserved remains (e.g. teeth, body size). Additionally, eight individuals were tested two to three times with the restriction digest test and Sanger sequencing, and the results were consistent in each case.

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Data Processing Description

BCO-DMO Processing:
-modified parameter names (replaced spaces with underscores);
-changed date format from mm/dd/yyyy to yyyymmdd;
-there were two dates with a year of 3013; changed to 2013 (Samples "HGB-12-51,52,53 (ECB-12-01,-02,-03)" and "HGB-12-52").

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Data Files

File
fossil_seal_bulk_isotope_14C.csv (Comma Separated Values (.csv), 125.05 KB) MD5:d7d4bab309725e4a91bc94c25d8ff627 Primary data file for dataset ID 732524

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Related Publications

Brault, E. (2017). An Examination of the Ecological and Oceanographic Effects of Mid-to-Late Holocene Climate Changes on the Ross Sea Ecosystem. UC Santa Cruz. ProQuest ID: Brault_ucsc_0036E_11435. Merritt ID: ark:/13030/m5dg1n5d. Retrieved from https://escholarship.org/uc/item/99s5j3fk
Results

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Related Datasets

IsRelatedTo

Hall, B., Koch, P. L., Costa, D. P., Hoelzel, A. R. (2022) **C and N isotope data for individual amino acids from fossil seals from the western Ross Sea, Antarctica.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2018-03-26 doi:10.26008/1912/bco-dmo.732078.1 [view at BCO-DMO]

Hall, B., Koch, P. L., Costa, D. P., Hoelzel, A. R. (2022) **Skeletal measurements from fossil seals from the western Ross Sea, Antarctica.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2018-03-29 doi:10.26008/1912/bco-dmo.732661.1 [view at BCO-DMO]

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Parameters

Parameter	Description	Units

Sample_ID	Sample identification code	unitless
Collection_date	Date of sample collection formatted as yyyymmdd	unitless
General_area	General location of sampling	unitless
Sampling_region	More specific sampling location	unitless
Latitude	Latitude of sample collection (negative values = south)	decimal degrees
Longitude	Longitude of sample collection (negative values = west; postivie values = east)	decimal degrees
Altitude	Altitude	meters above sea level (mas)
Sample_types_collected	Type of tissues collected from specimen: B = bone, F = fur, N = nail, S = skin, T = tooth, W = whisker.	unitless
Brief_description	Narrative comments on the attributes of specimens	unitless
Age_class	Age class of specimen (adult, sub-adult, pup, unknown)	unitless
Carcass_erosion_stage	Measure of progressive carcass breakdown; Carcass Weathering Stage codes A-F modified from Barwick and Balham (1967). See Metadata Acquisition Description for definitions.	unitless
Bone_weathering_stage	Measure of progressive bone weathering and breakdown; Bone Weathering Stage codes 0-7 modified from Todd (1987). See Metadata Acquisition Description for definitions.	unitless
Taxon_field	Field identification of the specimen:Cr = crabeater seal,Lp = leopard seal,SES = southern elephant seal,Wd = Weddell seal,nd = not identifiable,? = tentative identification.	unitless
Taxon_aDNA	Identification through ancient DNA. See species codes above. Failed = aDNA attempt & failed; nd = aDNA not attempted.	unitless
Taxon_final	Final determination of species. See species codes above. U = unknown.	unitless
NOSAMS_lab_code	Identification code at the National Ocean Sciences Accelerator Mass Spectrometry facility	unitless
C14_age	Uncalibrated 14C age	years before present (1950)
C14_error	internal or external statistical error in 14C age (whichever is larger)	years
C14_d13C	?13C valued measured for 14C age estimate	permil (‰), V-PDB
Calendar_age	Calendar years before present determined by 14C age. Samples that can't be calibrated set to 75 years.	calendar years before present (1950)
one_sigma	1 sigma error on Calendar_age	years
Sample_type_for_C14	Type of sample used for 14C age determination:B = bone, F = fur, N = nail, S = skin, T = tooth, W = whisker.	unitless
C14_notes	Narrative comments on 14C	unitless
d13C	Stable carbon isotope value	permil (‰), V-PDB
d15N	Stable nitrogen isotope value	permil (‰), AIR
C_to_N_atomic_ratio	Atomic C to N ratio	unitless

Sample_type_for_isotopes	Type of sample used for isotopes:B = bone, F = fur, N = nail, S = skin, T = tooth, W = whisker.	unitless

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Instruments

Dataset-specific Instrument Name	Accelerator Mass Spectrometer (AMS)
Generic Instrument Name	Accelerator Mass Spectrometer
Dataset-specific Description	NOSAMS used their organic combustion method to graphitize the samples, which were then pressed into target cartridge and loaded into the Accelerator Mass Spectrometer (AMS) for analysis.
Generic Instrument Description	An AMS measures "long-lived radionuclides that occur naturally in our environment. AMS uses a particle accelerator in conjunction with ion sources, large magnets, and detectors to separate out interferences and count single atoms in the presence of 1x1015 (a thousand million million) stable atoms, measuring the mass-to-charge ratio of the products of sample molecule disassociation, atom ionization and ion acceleration." AMS permits ultra low-level measurement of compound concentrations and isotope ratios that traditional alpha-spectrometry cannot provide. More from Purdue University: http://www.physics.purdue.edu/primelab/introduction/ams.html

Dataset-specific Instrument Name	EA-IRMS
Generic Instrument Name	Elemental Analyzer
Dataset-specific Description	This analysis was performed at the Stable Isotope Lab (SIL) at the University of California (UC) Santa Cruz on a Carlo Erba EA 1108 elemental analyzer coupled to a Thermo-Finnigan DeltaPlus XP isotope ratio mass spectrometer.
Generic Instrument Description	Instruments that quantify carbon, nitrogen and sometimes other elements by combusting the sample at very high temperature and assaying the resulting gaseous oxides. Usually used for samples including organic material.

Dataset-specific Instrument Name	EA-IRMS
Generic Instrument Name	Isotope-ratio Mass Spectrometer
Dataset-specific Description	This analysis was performed at the Stable Isotope Lab (SIL) at the University of California (UC) Santa Cruz on a Carlo Erba EA 1108 elemental analyzer coupled to a Thermo-Finnigan DeltaPlus XP isotope ratio mass spectrometer.
Generic Instrument Description	The Isotope-ratio Mass Spectrometer is a particular type of mass spectrometer used to measure the relative abundance of isotopes in a given sample (e.g. VG Prism II Isotope Ratio Mass-Spectrometer).

Dataset-specific Instrument Name	
Generic Instrument Name	Thermal Cycler
Generic Instrument Description	A thermal cycler or "thermocycler" is a general term for a type of laboratory apparatus, commonly used for performing polymerase chain reaction (PCR), that is capable of repeatedly altering and maintaining specific temperatures for defined periods of time. The device has a thermal block with holes where tubes with the PCR reaction mixtures can be inserted. The cycler then raises and lowers the temperature of the block in discrete, pre-programmed steps. They can also be used to facilitate other temperature-sensitive reactions, including restriction enzyme digestion or rapid diagnostics. (adapted from http://serc.carleton.edu/microbelife/research_methods/genomics/pcr.html)

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Project Information

Collaborative Research: Exploring the Vulnerability of Southern Ocean Pinnipeds to Climate Change - An Integrated Approach (Southern Ocean Pinnipeds)

Coverage: McMurdo Dry Valleys Region; Royal Society Range, Victoria Land Coast , Antarctic Peninsula, Amundsen Sea, Ross Sea

NSF abstract:
Building on previously funded NSF research, the use of paleobiological and paleogenetic data from mummified elephant seal carcasses found along the Dry Valleys and Victoria Land Coast in areas that today are too cold to support seal colonies (Mirougina leonina; southern elephant seals; SES) supports the former existence of these seals in this region. The occurrence and then subsequent disappearance of these SES colonies is consistent with major shifts in the Holocene climate to much colder conditions at the last ~1000 years BCE).

Further analysis of the preserved remains of three other abundant pinnipeds ? crabeater (Lobodon carciophagus), Weddell (Leptonychotes weddelli) and leopard (Hydrurga leptonyx) will be studied to track changes in their population size (revealed by DNA analysis) and their diet (studied via stable isotope analysis). Combined with known differences in life history, preferred ice habitat and ecosystem sensitivity among these species, this paleoclimate proxy data will be used to assess their exposure and sensitivity to climate change in the Ross Sea region during the past ~1-2,000 years

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Funding

Funding Source	Award
NSF Office of Polar Programs (formerly NSF PLR) (NSF OPP)	OPP-1141849
NSF Office of Polar Programs (formerly NSF PLR) (NSF OPP)	OPP-1142108

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