

Indication of two Pacific walrus stocks from whole tooth elemental analysis

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Abstract The Pacific walrus (*Odobenus rosmarus divergens*) is considered to be a single panmictic population for management purposes. However, studies on population structuring in this species are limited; in part, because portions of the population's range are often inaccessible. Therefore, alternative and complementary methods for investigating stock structure in the Pacific walrus are of particular interest. We used measures of elemental concentrations in whole tooth sections from ICP-MS in a discriminant analysis to investigate evidence of stock separation between walrus from two of three known breeding areas (S.E. Bering, St Lawrence, and Anadyr Gulf). Elemental compositions of teeth from female and male walrus from the S.E. Bering and St Lawrence breeding areas were significantly different, providing evidence of separate stocks. We also obtained insights into the potential relation of walrus from non-breeding areas to walrus from these breeding groups based on similarities in their dental elemental profiles.

Keywords Tooth · Trace elements · ICP-MS · Walrus · Subpopulations · Stocks

Introduction

The Pacific walrus (*Odobenus rosmarus divergens*) is an abundant, benthic-feeding pinniped, occurring in Arctic and sub-Arctic waters of the Chukchi and Bering Seas. The species is an important subsistence resource for Russian and Alaskan Natives. The Pacific walrus is one of two subspecies (Atlantic walrus, *O. r. rosmarus*) and comprises approximately 90% of the worldwide number of walrus (Fay 1985).

U.S. Fish and Wildlife Service manages the Pacific walrus and considers the species to be a single panmictic population for stock assessment purposes (U.S. Fish and Wildlife Service 2002). Understanding the structure of the Pacific walrus population is important to better understand mechanisms affecting their abundance and distribution and potential impacts to segments of the population from natural and human related agents. Because Pacific walrus are difficult to access, owing to their extreme range over remote environments, limited attention has been given to identifying potential stocks and it is necessary to consider alternate and complementary approaches to understanding their population structure.

Breeding occurs in winter in the Bering Sea in three general aggregations: a moderate concentration in southeastern Bering Sea (from Nunivak and the Pribilof Islands to Bristol Bay), a large concentration in northern Bering Sea (mostly southwest of St Lawrence Island), and a small concentration in western Bering Sea (Anadyr Gulf in Russia) (Fay 1982; Fig. 1). Routes of migration between the wintering and summering grounds are only generally known and annual fidelity of individuals to winter breeding areas is unknown. Most female and young walrus use sea ice year-round for hauling out, and migrate during fall and spring between the Bering and Chukchi Seas with the

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annual advance and retreat of sea ice. Some females and young use beaches in southern and northern Chukotka for hauling out during summer or fall (Smirnov et al. 2002). Most adult males remain in the Bering Sea year-round, using land haul-outs for much of the year when sea ice is unavailable. In summer, a substantial number of males occur at land haul-outs in northern Chukotka, and some are found with females on sea ice in the western and eastern Chukchi Sea.

Structure of the Pacific walrus population was provisionally explored in two genetic studies. One study found no difference in mitochondrial DNA (mtDNA) between walrus from two areas in the Chukchi Sea during the non-breeding period (Cronin et al. 1994), and another study found no substantive difference in mtDNA and nuclear multilocus minisatellites between walrus from four areas in the Bering Sea immediately after the breeding season (Scribner et al. 1997). Further population genetic studies with more contemporary techniques and directed sampling are warranted.

Marine mammal stock assessment guidelines in the United States indicate that, for purposes of management under the Marine Mammal Protection Act, “a stock is recognized as being a management unit that identifies a demographically isolated biological population”. These guidelines also recognize the utility of investigating population structure from sources beyond genetics, including variations among groups of animals in their distribution and movement patterns, population trends, morphologies, life histories, oceanographic habitats, and level of parasite, contaminant, and isotope loads (Dizon et al. 1992; Barlow et al. 1995; Wade and Angliss 1996; National Oceanic and Atmospheric Administration 2005).

Here, we examine elemental concentrations in Pacific walrus teeth to explore evidence of population structuring. Elements are incorporated into the structure of teeth and are not re-absorbed, and since teeth continue to grow during the lifetime of the animal, the elemental signature of teeth reflect the lifetime exposure of the animal to its geochemical environment (Outridge and Stewart 1999; Stewart et al. 2003). Walrus feed mainly on bivalve molluscs (Fay 1985), and thus, are likely to accumulate elements from bottom sediments through their sedentary filter and deposit feeding prey (Miles and Hills 1994; Thomas and Bendell-Young 1998). Groups of walrus that differ in their dental elemental composition might reflect geospatial differences in elements in surface sediments to which they were exposed during their lifetime, and hence, represent different stocks.

Measures of elemental and lead isotope concentrations in the teeth of the Atlantic walrus have been used to estimate the relative contribution of contaminants from natural and anthropogenic sources (Outridge et al. 1997), infer movement patterns of individuals from reconstructed exposure histories identified from tooth growth layers

(Stern et al. 1999; Stewart et al. 2003), and identify stock structure from whole tooth sections (Outridge and Stewart 1999; Outridge et al. 2003). In the latter, the primary discriminators of stock structure were concentrations of several trace metals and the lead isotopes $^{206}\text{Pb}/^{207}\text{Pb}$ and $^{208}\text{Pb}/^{207}\text{Pb}$.

We analyzed elemental profiles of teeth collected from Pacific walrus in areas and times corresponding to two breeding groups and several non-breeding groups. Our purpose was to examine variation in elemental profiles between breeding groups, then classify walrus from non-breeding groups to a breeding group based on similarities in elemental profiles. From this, we hoped to gain further insight into the level of differences between walrus from the two breeding areas and an indication of the probable association of individuals to breeding and non-breeding areas.

Materials and methods

Sample selection

Samples came from tooth collections from U.S.-Russia joint pinniped research cruises conducted from 1981 to 1991 and walrus harvested during Russian and Alaskan Native subsistence hunts from 1997 to 2001 (Table 1). We selected teeth from walrus from prominent aggregations during, or shortly after, the January–February breeding period, and during the late summer to autumn non-breeding period (August–October), a time of maximal population range and sexual segregation (Fay 1982; Fay et al. 1984) (Table 1; Fig. 1). Samples obtained during the research cruises tended to come from a wider sampling area than those from subsistence hunts, because of their less restricted sampling range.

No samples were obtained from females along northern Chukotka, males on sea ice haul-outs in western Chukchi Sea, or from either sex in the Anadyr Gulf winter breeding aggregation (Fig. 1).

Tooth sectioning and ageing

The lower canines were obtained from each animal in most cases. Usually both, but sometimes only one, of the canines were available. When both teeth were available, one was sectioned and stored in a mixture of 35% ethanol, 5% glycerine and 60% water for subsequent determination of the animal's age (Garlich-Miller et al. 1993), and the other tooth was sectioned and further processed for elemental analysis. If only one tooth was available, it was sectioned, soaked in distilled water for age determination, and subsequently used for elemental analysis. Tooth sections were 0.4–0.6 mm thick and cut longitudinally from the central core of each

Table 1 Region, date, and source of walrus tooth samples used in discriminant analysis to assess differences between two winter breeding groups and potential breeding origin of non-breeding individuals

Group	Months	Source-year	Female	Male	Total
<i>Breeding</i>					
St Lawrence	Mar	ZK85	15	15	30
S.E. Bering	Feb, Mar, Apr	ZV81, ZS91	15	14	29
<i>Non-breeding</i>					
Koryak Coast	Apr	ZS91	0	24	24
S. Chukotka	Aug, Sep, Oct	EN01, ME99, ME01, SI01	11	25	36
E. Chukotka	Jul	LO01	0	12	12
N. Chukotka	Sep	IN01	0	10	10
W. Chukchi	Sep, Oct	ZK87	15	0	15
E. Chukchi	Sep	ZK87	14	14	28
S.E. Bering	Sep, Oct	RI97, RI98	0	15	15
		Total	70	129	199

EN Enmelen subsistence hunt, *IN* Inchoun subsistence hunt, *LO* Lorino subsistence hunt, *ME* Meechkykyn Spit subsistence hunt, *RI* Round Island subsistence hunt and salvaged carcasses, *SI* Sireniki subsistence hunt, *ZK* Zakharovo Russia-U.S. joint pinniped research cruises, *ZS* Zaslono-vo Russia-U.S. joint pinniped research cruise (Hills et al. 1991), *ZV* Zvyagino Russia-U.S. joint pinniped research cruise (Popov et al. 1981)

tooth using a lapidary saw with water-cooled, high concentration diamond wafering blades (Garlich-Miller 1997).

Ages were estimated by counting incremental growth layer groups (GLGs) in the tooth cementum under reflected light using a variable-power stereoscopic dissecting microscope (Fay 1982; Garlich-Miller et al. 1993). One cemental GLG was assumed to represent 1 year of growth (Mansfield 1958; Fay 1982). Cemental GLGs in each tooth section were counted three times in blind replicates. The final age estimate was the median of the three counts (Garlich-Miller 1997). Female and male walrus teeth selected for tooth elemental analysis averaged 20 (SD = 7, range 6–36) and 17 (SD = 7, range 6–34) years of age.

Determination of trace elements

Techniques for preparation and determination of trace and minor element concentrations in the walrus teeth closely followed those described by Outridge and Stewart (1999). Cementum sections, weighing <0.3 g and incorporating all growth layers, were removed with a rotary dremel cutting tool from whole tooth longitudinal sections (see above). These were briefly immersed in dilute nitric acid to remove contaminated surfaces, rinsed in distilled deionized water, and air-dried overnight. After weighing (sample weights were restricted to 0.2–0.5 g DW), samples were decomposed with 4 ml of 2:1 concentrated nitric and hydrochloric acids in screw-capped Teflon vials on a hot plate overnight. Procedural blanks and standard reference material (NIST 1400 Bone Ash; National Institutes for Standards and Technology, MD, USA) were also included.

After complete dissolution, the solutions were made up to 50 ml final volume with high purity water and spiked

with Ru and Re as internal standards, giving final concentrations of 20 µg/l. Concentrations of the following elements in sample solutions were determined by inductively coupled plasma mass spectrometry (Elan 6100 ICP-MS, Perkin-Elmer Sciex) using multi-element standards also spiked with internal standards: Mn, Ni, Cu, Zn, Sr, Mo, Cd, Ba, La, Pb, and U. Element concentrations in the original tooth samples were calculated off-line after blank subtraction. Concurrent determinations of elements in the Bone Ash SRM replicates gave good agreement with certified or information values, with CVs ranging 3–24% in all but one element during the analytical run (Table 2).

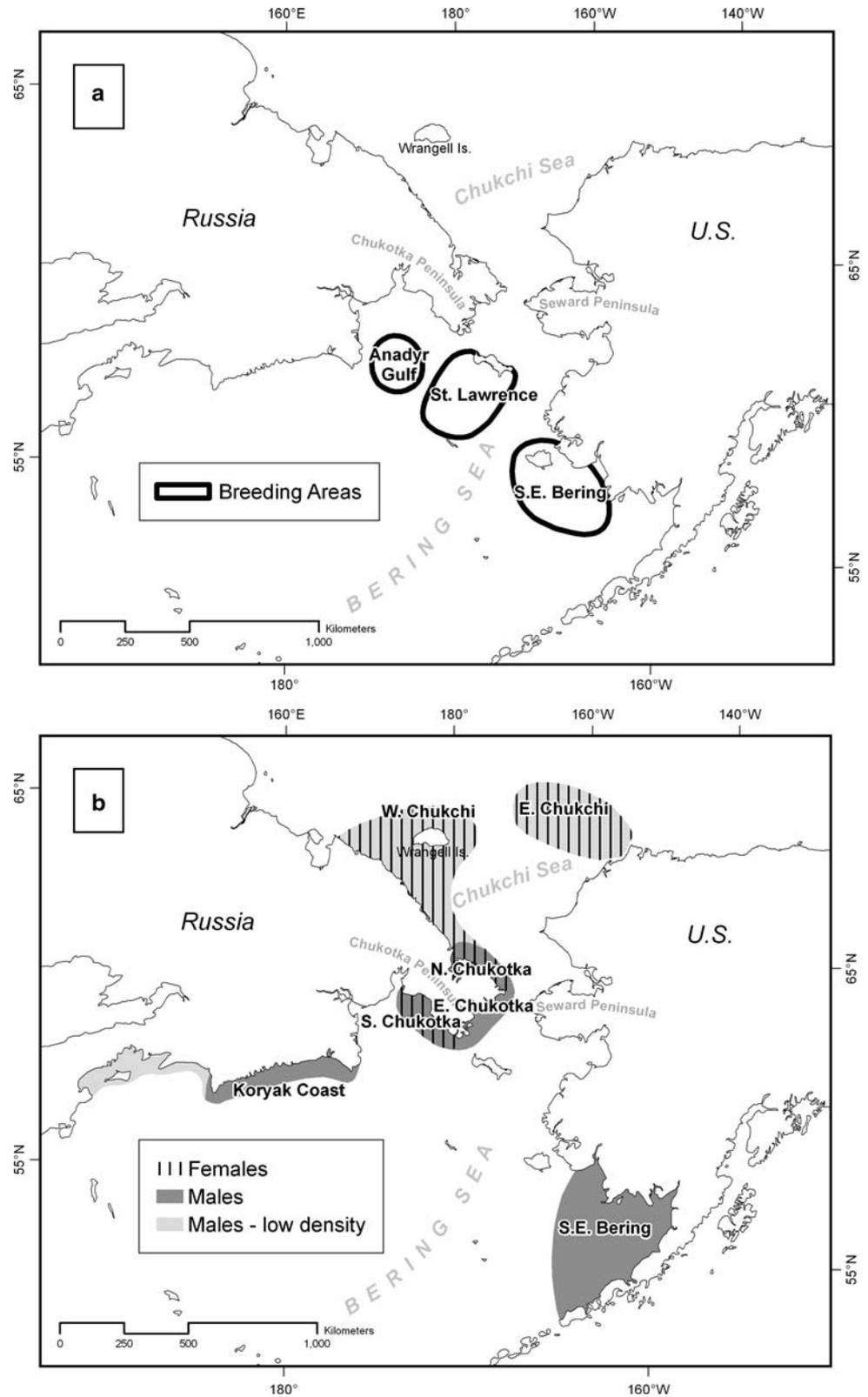
Statistical analyses

The frequency distribution of each of the eleven elemental concentrations were slightly to heavily right-skewed, so all elements were transformed to $\log(x + 1)$ to approximate normality. The log-transformed variables were screened for outliers by standardizing each variable within breeding groups to zero mean and unit variance (subtracting the mean and dividing by the standard deviation) and inspecting for observations that exceeded three absolute standard deviations from the mean.

SAS (SAS Institute Inc., v. 8.01, Cary, NC, USA) was used for all statistical analyses and females and males were treated separately.

Discriminant analysis was used to describe differences between breeding groups and assign samples from non-breeding groups to a breeding group. Elements were the discriminating variables and group membership was the dependent variable. In general, sample size requirements for discriminant analysis increases with the number of

Fig. 1 Breeding (a) and non-breeding (b) areas of the Pacific walrus (After Fay 1982; National Oceanic and Atmospheric Administration 1987; Mimirin et al. 1990; Smirnov et al. 2002)



discriminating variables used in the analysis. Since sample sizes were fixed for each group, we restricted the number of variables to use in the analysis based on the following

criterion: $n \geq 3 * P$, where n = group sample size, and P = number of discriminating variables (McGarigal et al. 2000). Variables were selected using a stepwise discriminant

Table 2 Results of NIST SRM 1400 bone ash ($\mu\text{g/g}$ dry weight; $n = 14$)

Element	Mean	SD	CV of sample (%)	Certified values or Other	Duplicate analyses	Median difference (%)	Max difference (%)
Mn	18.8	1.3	6.9	17 ^a	Mn	8.3	18.9
Ni	5.09	0.68	13.4	5.75 ^b	⁶² Ni	27.1	173.3
Cu	2.59	0.45	17.3	2.3 ^a	⁶⁵ Cu	11.4	37.2
Zn	187	6	3.2	184	⁶⁶ Zn	8.7	19.3
Sr	263	21	8.0	249	Sr	3.8	29.1
Mo	0.27	0.19	68.0	–	Mo	9.7	22.6
Cd	0.034	0.008	23.6	0.03 ^a	¹¹⁴ Cd	12.9	66.7
Ba	228	13	5.5	240 ^b	Ba	6.1	13.3
La	0.334	0.029	8.7	0.386 ^b	La	50.0	175.0
Pb	8.61	0.36	4.1	9.07	²⁰⁸ Pb	7.1	34.1
U	0.078	0.015	18.6	0.066 ^b	U	14.3	40.0

^a Values from NIST^b Values from Hinners et al. (1998)

procedure (proc stepdisc in SAS, F -to-enter and F -to-remove set to 0.15). Samples with outliers within any variable were omitted from the selection process, but were admitted back into the data set for subsequent analyses if the outliers did not occur within the selected variables.

MANOVA was used to test for differences in tooth elemental concentrations between breeding groups. Group membership was the independent variable and the elements were the dependent variables.

Samples from the breeding groups were used to derive a separate function for females and males to classify non-breeding group samples. Before classifying samples from the non-breeding groups into one of the breeding groups, the expected effectiveness of the classification functions was assessed using a jackknife cross-validation procedure to resample observations from the breeding groups (SAS Institute Inc., v. 8.01). The procedure holds out a sample and derives a classification function, classifies the held out sample from the derived function, and tallies the classification result. The process is repeated until all samples from the original data are classified. A cross-validation procedure such as this gives a less biased estimate of the classification error rate than would be obtained by simply re-classifying the original samples from which the functions were derived (i.e. the apparent error rate, Tabachnick and Fidell 1996; McGarigal et al. 2000). We specified a 95% minimum posterior probability threshold for classification, whereby if a sample's largest posterior probability of group membership was less than 95%, it was assigned to the group "Other".

Results

There was considerable temporal overlap between the lives of walrus from the two breeding areas. The years spanned by the average birth date to average kill date of females from the S.E. Bering group (1971–1991) overlapped those

from the St Lawrence group (1963–1985) by 15 years. Similarly the years spanned by the lives of males (average birth date to average kill date) from the S.E. Bering group (1966–1985) overlapped those from the St Lawrence group (1967–1985) by 19 years.

Most elemental measurements were above the analytical detection limit of the element (Table 3). Only three elements had measurements that were below the detection limit (5, 1, and 16% of the measurements of Ni, Cu, and LA, respectively). The sample coefficient of variation for each element ranged 22–228%. Zn and Sr were orders of magnitude higher in concentration than the other elements. La occurred in very low concentrations. No pairs of elements were highly correlated ($r_{\text{max}} = 0.62$ Sr:Ba, Table 4). Four elements were linearly related to walrus age, but the

Table 3 Simple statistics of whole tooth elemental concentrations ($\mu\text{g/g}$ dry weight; $n = 198$)

Element	Mean	SD	Sample CV (%)	Min	Max	Detection limit
Mn	0.77	0.19	25	0.42	1.45	0.03
Ni	0.26	0.20	77	* <0.07	1.77	0.07
Cu	1.61	3.66	228	* <0.07	37.03	0.07
Zn	181	42	23	102	340	2
Sr	218	47	22	134	421	1
Mo	0.08	0.08	101	0.03	0.86	0.01
Cd	0.355	0.601	169	0.038	6.926	0.012
Ba	3.11	0.94	30	1.76	7.28	0.07
La	0.006	0.005	86	* <0.002	0.031	0.002
Pb	1.15	0.54	47	0.29	4.6	0.09
U	0.023	0.010	44	0.007	0.054	0.001

Detection limits were calculated from 3SD of blank concentrations and assuming 25 ml final volume and 200 mg dry weight of digest

*The following number of teeth had concentrations below the element's detection limit: Ni = 9, Cu = 1, and La = 32 (note though, these elements were not ultimately used in the discrimination of walrus stocks)

Table 4 Total sample Pearson correlation coefficients (*r*) and probability of $|r| = 0$ ($n = 198$, $P \leq 0.05$ in bold)

	Mn	Ni	Cu	Zn	Sr	Mo	Cd	Ba	La	Pb	U
Mn	1.00000	0.03897	0.06963	0.45660	0.34483	0.12098	0.04377	0.24269	0.28141	0.30009	0.38717
		0.5857	0.3297	<0.0001	<0.0001	0.0895	0.5403	0.0006	<0.0001	<0.0001	<0.0001
Ni		1.00000	-0.00082	-0.01198	-0.00694	0.36402	0.02020	0.01415	0.08329	-0.05082	-0.12099
			0.9908	0.8670	0.9227	<0.0001	0.7776	0.8432	0.2434	0.4771	0.0895
Cu			1.00000	0.00675	-0.01957	0.04737	0.07307	-0.10913	-0.03621	0.02039	-0.01666
				0.9248	0.7843	0.5075	0.3063	0.1259	0.6125	0.7755	0.8158
Zn				1.00000	0.02001	0.01547	0.09494	0.08106	0.48200	0.53515	0.28574
					0.7796	0.8287	0.1834	0.2563	<0.0001	<0.0001	<0.0001
Sr					1.00000	0.06769	0.15189	0.62464	-0.20174	0.13346	0.13743
						0.3434	0.0327	<0.0001	0.0044	0.0609	0.0535
Mo						1.00000	0.07574	0.10933	-0.01048	0.13614	-0.03710
							0.2889	0.1252	0.8835	0.0558	0.6038
Cd							1.00000	0.04055	0.01833	0.12865	-0.09523
								0.5706	0.7977	0.0709	0.1820
Ba								1.00000	-0.04985	0.15436	0.01535
									0.4855	0.0299	0.8300
La									1.00000	0.29376	0.23239
										<0.0001	0.0010
Pb										1.00000	0.17858
											0.0118
U											1.00000

relationships were very weak (+Zn: $P < 0.0001$, $r^2 = 0.1998$; -Sr: $P = 0.0010$, $r^2 = 0.0540$; -Ba: $P = 0.0001$, $r^2 = 0.0809$; +La: $P = 0.0002$, $r^2 = 0.0691$; $df = 196$).

Since sample sizes were mostly ≥ 14 observations per group (Table 1), we limited our analysis to four discriminating variables. Two teeth had a measurement outlier in Ni, Cu, or Mo, so were excluded during the variable selection procedure. The selection procedure indicated that Pb, U, Sr, and Cu contributed highest to group separation among five significant variables in females, and U, Mo, Sr, and Ni contributed highest to group separation among six significant variables in males. One male tooth had an extreme outlier measurement for Ni and Mo and was excluded from further analyses.

Discriminant analysis assumes equal variances and covariances between groups within each variable (McGarigal et al. 2000). This assumption was met for samples from females ($X^2 = 14.40$, $df = 10$, $P = 0.156$), but not males ($X^2 = 17.95$, $df = 10$, $P = 0.056$). Therefore a pooled covariance matrix and a linear discriminant function were used for females, whereas within group covariance matrices and a quadratic discriminant function were used for males.

The degree of discrimination possible between the breeding groups, and its relation to the original discriminating variables (elements), is demonstrated by deriving a canonical function and plotting its canonical scores (Fig. 2), and determining a correlation of these scores with values of

the original variables. Greater group separation was achieved for females than males. In females, canonical scores along the canonical axis from the S.E. Bering group to the St Lawrence group were positively correlated with levels of Pb and Sr, negatively correlated with U, and had a very low correlation with Cu (Table 5). In males, canonical scores were positively correlated with Mo and Sr, and negatively correlated with U and Ni.

MANOVA indicated a significant difference in dental elemental composition between the St Lawrence and S.E. Bering breeding groups (Wilks' Lambda statistic, females $P < 0.0001$, males $P < 0.0001$); however, the test was not strictly valid for males because they had unequal group dispersions.

The cross-validation procedures classified 28/30 (93%) and 18/29 (62%) of the female and male breeding group samples correctly (Table 6). This suggests that a classification function derived for females may be fairly accurate in classifying non-breeding female samples into breeding groups. The poorer classification success of male samples partially reflects the greater degree of overlap in elemental profiles of male walrus from the breeding groups than was observed for female walrus (Fig. 2). This, small sample sizes, and unequal group dispersions of male samples, suggest that a classification function derived for males may not be very accurate in classifying non-breeding male samples into breeding groups.

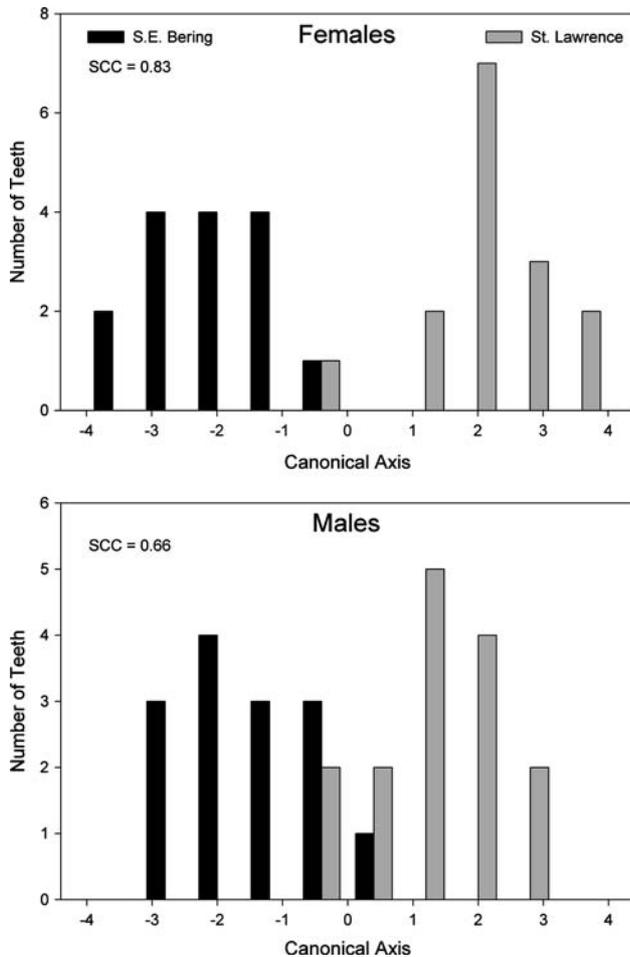


Fig. 2 Frequency histogram of canonical scores for teeth samples from the S.E. Bering and St Lawrence breeding groups. The squared canonical correlation (*SCC*) indicates the amount of variation in canonical scores explained by the groups

Table 5 Total canonical structure indicating the level of correlation between the discriminating variables and canonical scores along the canonical axis separating the S.E. Bering and St Lawrence breeding groups

	Variable	Correlation
Females	Pb	0.86
	U	-0.66
	Sr	0.77
	Cu	0.27
Males	U	-0.67
	Mo	0.52
	Sr	0.57
	Ni	-0.60

The classification function derived for females assigned samples from each of the three non-breeding groups (S. Chukotka, W. Chukchi, and E. Chukchi) more frequently to the St Lawrence breeding group than to the

Table 6 Number and proportion of resampled original breeding group samples classified into one of the two breeding groups or into the group “Other” using a jackknife cross-validation procedure

From	Into			Total	Error
	S.E. Bering	St Lawrence	Other		
Females					
S.E. Bering	14	0	1	15	1
	0.93	0.00	0.07	1.00	0.07
St Lawrence	1	14	0	15	1
	0.07	0.93	0.00	1.00	0.07
Males					
S.E. Bering	8	2	4	14	6
	0.57	0.14	0.29	1.00	0.43
St Lawrence	1	10	4	15	5
	0.07	0.66	0.27	1.00	0.33

A sample was assigned to the Other group if the posterior probability of membership into one of the breeding groups was <95%

S.E. Bering breeding group (Table 7). Twenty-five percent (10/40) of all female non-breeding group samples could not be classified into one of the two breeding groups and were assigned to the Other group.

The high classification error rates estimated for males ($\geq 33\%$, Table 6) limits our interpretation of the classification results of non-breeding group male samples (Table 7). We can only suggest that a significant proportion of samples from the S. Chukotka, E. Chukotka, and N. Chukotka non-breeding groups were assigned to the St Lawrence breeding group. It is also notable however, that a high proportion (54%) of samples from the Koryak group was assigned to the other group. Thirty-nine percent (39/100) of all male non-breeding group samples were assigned to the other group.

Discussion

Our study provides evidence of stock structure within the Pacific walrus and some insight on the possible breeding group origin of walrus samples from non-breeding aggregations. However, because the sampled animals were of mixed ages and not always from the same cohort (i.e. the lives of all individuals did not overlap completely), a couple caveats should be considered. Firstly, our interpretations could be partially confounded if some walrus encountered a period of atypical environmental chemistry (Evans et al. 1995). Outridge and Stewart (1999) found declines in some trace elements in the whole teeth of Atlantic walrus from a single region over an 8-year period. The reasons were unclear, but they suggest that the declines may have reflected a decrease in storm frequency and

Table 7 Number and proportion of samples from non-breeding groups classified into one of the two breeding groups or into the group “Other”

From	Into			Total
	S.E. Bering	St Lawrence	Other	
Females				
S. Chukotka	2	7	2	11
	0.18	0.64	0.18	1.00
W. Chukchi	4	7	4	15
	0.27	0.46	0.27	1.00
E. Chukchi	3	7	4	14
	0.21	0.50	0.29	1.00
Total	9	21	10	40
Males				
Koryak	4	7	13	24
	0.17	0.29	0.54	1.00
S. Chukotka	3	12	10	25
	0.12	0.48	0.40	1.00
E. Chukotka	4	6	2	12
	0.33	0.50	0.17	1.00
N. Chukotka	2	5	3	10
	0.20	0.50	0.30	1.00
E. Chukchi	5	4	5	14
	0.36	0.28	0.36	1.00
S.E. Bering	6	3	6	15
	0.40	0.20	0.40	1.00
Total	24	37	39	100

A sample was assigned to the Other group if the posterior probability of membership into one of the breeding groups was <95%. Classifications of a proportion above the estimated classification error rate for that category (Table 6) are in bold

resuspended metal-bearing particulates. This was not likely to be a large problem in our study, because the average years of life of males between the S.E. Bering and St Lawrence groups overlapped almost exactly and the lives of females overlapped by an average of about 15 years. Still, it is possible that a particularly large regional signal could have occurred during years of non-overlap in females to bring about the observed difference between the two female groups. However, the signal would have had to be strong enough to bring about a significant change in mean elemental concentrations of the whole tooth.

Secondly, adult walrus begin periods of fasting at roughly 10 years of age, which could potentially cause differences in whole tooth elemental signatures between younger and older animals, even if they occupied the same geographic range throughout their lifetimes. Females reach full physical development and peak reproductive performance at 9–10 years old (Fay 1982), and thereafter may fast for several days at estrus in January–February in the

Bering Sea, and for a week or more at parturition (Gehrich 1984; Fay et al. 1984) between mid-April to mid-June (Fay 1985) while in the Bering Sea and migrating to the Chukchi Sea. Males become sexually mature at about 10 years old (Fay 1982), and thereafter fast for 3–5 months starting in about January (Gehrich 1984; Fay et al. 1984) during the breeding period in the Bering Sea. Because female fasting periods are brief, females may be less likely to differentially accumulate elements across their geographic range than males.

The potential effect of fasting on whole tooth elemental concentrations between young and old animals may be lessened somewhat by the rate of growth layer formation in the tooth. The thickness of successive layers of cementum in the walrus tooth decreases with age. The thickest layer is produced in the first year of life and the greatest decrease in thickness occurs within the first 10 years of life (Fay 1982). Therefore, the greatest rate of accumulation of elements in the teeth would likely occur during the first tens years of life. Studies using whole tooth analyses would benefit from sampling animals of the same age and cohort to lessen potential biases associated with fasting behaviors and potential changes in environmental chemistries.

Female and male breeding groups

We found a significant difference in the elemental composition of teeth between walrus from the S.E. Bering and St Lawrence breeding groups, in both sexes, suggesting that these two breeding areas may comprise different walrus stocks. Our evidence of separate stocks is consistent with general descriptions of the timing of the northward migration of walrus in spring. Fay (1982) postulates that walrus originating from the St Lawrence breeding area are the first to pass through the Bering Strait and into the Chukchi Sea in April–May; while those originating from the S.E. Bering breeding area travel northward along the Alaskan coast and through the Bering Strait as a second wave of migrants mainly in June. The difference we found in the elemental composition of teeth from walrus in the S.E. Bering and St Lawrence breeding groups suggests that walrus from these areas occupied different geographic areas during their lifetime and is consistent with temporally and spatially segregated migrations from these areas.

Scribner et al. (1997) discuss unpublished data from their analysis of nuclear multilocus minisatellites and mtDNA from walrus sampled immediately after the breeding season in four areas of the Bering Sea: Anadyr Gulf and the Koryak coast in Russia and the S.E. Bering and St Lawrence areas in the USA. Their analysis indicated high levels of genetic variability, extensive gene flow, and an admixed population, which is counter to our findings. However, they also indicate the need for further studies that

employ highly polymorphic genetic markers to better assess population structuring. Even still, failure to detect genetic differences should not preclude consideration of separate stocks. Dispersal rates may be sufficient to maintain genetic homogeneity within a population, but insufficient to counter high rates of localized mortality (National Oceanic and Atmospheric Administration 2005). Differences in habitat within a species' range or high fidelity to certain ranges may also be reasons for considering separate stock boundaries (e.g. Amstrup et al. 2000; Cronin et al. 2006).

Female non-breeding groups

The currently held understanding of walrus migrations and distributions were primarily established from a compilation of walrus sightings from land, ship, and aircraft from published and unpublished historical accounts (Fay 1982; Fay et al. 1984) and various walrus abundance surveys (Gilbert 1989, 1999). Few walruses have been tracked over long enough distances and time periods to determine levels of interannual fidelity to breeding and non-breeding areas.

In general, as walruses reach the Chukchi Sea during their northward spring migration, they travel either north-westward towards Wrangell and Herald Islands or north-eastward towards Point Barrow (Fay 1982). Our study suggests that females in the Chukchi Sea in autumn were comprised of animals with lifetime geographic ranges similar to walruses from both the St Lawrence and S.E. Bering breeding areas (Table 7). This is consistent with findings by Cronin et al. (1994) in a lack of difference in mtDNA between walruses from two areas in the Chukchi Sea (eastern and southwestern) during the non-breeding period. The relatively high proportion of samples from walruses in the Chukchi Sea that was classified into the Other group, compared to the proportion classified into the Other group from the cross-validation procedure (Table 6), suggests that another walrus breeding group (or groups) may not have been sampled (Fig. 1).

Elemental profiles in teeth from non-breeding females in southern Chukotka suggest that most of these females had a lifetime geographic range similar to ranges of walruses from the St Lawrence breeding group (Table 7). The smaller number of females from southern Chukotka that were classified into the Other group may have had a range more closely associated with walruses from an un-sampled breeding group, such as from the Anadyr Gulf area. Fay (1982) suggested that females from the Anadyr breeding group migrate in spring toward coastal areas of Anadyr Gulf, where they are joined by walruses from the St Lawrence breeding group. Details of subsequent migration of Anadyr Gulf females are complicated. Some apparently travel northward in summer and through the western part of

the Bering Strait by September, then occupy the northern coast of Chukotka. A small group of adult females, young, and adult males remain within Anadyr Gulf and utilize coastal haul-outs along the southern coast of Chukotka throughout summer (Mimrin et al. 1990). Given Fay's (1982) account of spring migrants from the S.E. Bering area traveling along the Alaska coast, and radio-tracking data from a small number of females tagged in the S.E. Bering in spring that similarly showed northward migration along the Alaska coast (C. Jay, unpublished data), it seems unlikely that a substantial number of females in southern Chukotka come from the S.E. Bering breeding area.

Male non-breeding groups

The probable greater roaming tendency of adult males compared to females may partially explain the lower level of discrimination we achieved between breeding groups in males relative to females (Fig. 2). Large variations in the range of movements among male walruses have been demonstrated from radio-tracking studies of Atlantic and Pacific walruses (Wiig et al. 1996; Jay and Hills 2005), and from links between Pb isotope levels in geological provinces with levels in the annual growth layers of teeth from the Atlantic walrus (Stern et al. 1999; Stewart et al. 2003).

In spring and early summer, most adult males move from their winter breeding groups to haul-outs along the coasts of Russia and Alaska in the Bering Sea, and along the north coast of Chukotka, while a smaller number of males move with the sea ice into areas farther north into the Chukchi Sea (Fig. 1). Adult males at coastal haul-outs in Russia during summer and autumn are likely to be derived mostly from the St Lawrence and Anadyr Gulf breeding groups. Our results suggest that some males from Chukotka haul-outs (S. Chukotka, E. Chukotka, and N. Chukotka) do indeed come from the St Lawrence breeding group. The relatively high proportion of Koryak samples assigned to the Other group suggests that a substantial number of these males may have come from the Anadyr Gulf breeding area.

Future studies

Pb isotopes may provide a better tool for stock discrimination than whole tooth elemental analyses. Outridge and Stewart (1999) and Outridge et al. (2003) suggest that measures of Pb isotopes are more robust to group discrimination than elemental signatures, because Pb isotope ratios are stable over time since they are controlled solely by geological processes and are not measurably influenced by climate, nor apparently by variations in animal diet. $^{208}\text{Pb}/^{204}\text{Pb}$ ratios were particularly useful in group discrimination of the Atlantic walrus (Outridge et al. 2003). Analysis of elements in the whole tooth does not provide information on

the amount of segregation between groups of walruses; whereas analysis of Pb isotope ratios along tooth layers, such as annual growth layers, can provide insights of animal movements at a finer temporal resolution (see Stewart et al. 2003).

Our study provides a preliminary look at stock structure in the Pacific walrus using natural marks. Complimentary methods of stock investigations are useful, because each offers a slightly different view of stock structure, and together, provide a more complete picture to resource management (Cadrin et al. 2005). For example, dispersal rates between groups of individuals may be sufficient to homogenize genetic differences, but insufficient to provide the necessary recruitment to adjacent populations sufficient to withstand differential rates of mortality (National Oceanic and Atmospheric Administration 2005). Genetics reflects groupings of individuals over generational time scales, whereas the analysis of the composition of teeth reflects groupings of individuals over the lifetime of individuals (Outridge et al. 2003). Alternative methods for investigating stock structure in the Pacific walrus are of particular interest because of the difficulty in accessing walruses during their breeding period in winter.

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