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Functional Diversity of Epibenthic Communities on the Chukchi and Beaufort Sea Shelves

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Using Trace Elements in Pacific Walrus Teeth to Track the Impacts of Petroleum Production in the Alaskan Arctic

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Abstract

Functional diversity of epibenthic communities on the Chukchi and Beaufort Sea shelves were compared using biological trait analysis (BTA). BTA functionally characterizes epibenthic organisms based on morphology, life history, and behavior, and it leads to an understanding of ecosystem functioning that is independent of the taxonomic affiliation of the taxa. Understanding the functional diversity of communities is important because it could be disrupted by ecosystem stressors from human resource extraction and climate changes. The objective was to compare the functional diversity of the Chukchi and Beaufort Sea shelf communities using BTA. BTA incorporated natural variability via the plethora of biological traits expressed within communities and large spatial scale this study encompassed. Based on the distinct hydrographic settings of the two seas, the epibenthic communities of the Chukchi and Beaufort Sea shelves were hypothesized to differ in biological trait composition, and thus, ecosystem functional diversity. This study showed epibenthic functional diversity was higher on the Chukchi Sea shelf compared with Beaufort Sea shelf communities. Additionally, this study characterized the Chukchi and Beaufort Sea shelf communities by their dominant biological traits and related these traits to environmental conditions present in each shelf system. Many biological traits were found in both areas in similar proportions; however, the Chukchi Sea communities differed from the Beaufort Sea communities in the biological traits of reproductive strategy, larval development, body form, depth range, and substrate affinity. The Chukchi Sea shelf communities had more planktotrophic larval development, dorso-ventrally compressed organisms, and organisms that prefer soft substrate. The Beaufort Sea shelf communities had more lecithotrophic larval development, organisms with a globulose body form, and organisms that prefer hard substrate. No statistically significant relationships existed between these traits and depth, bottom temperature, or bottom salinity in either Beaufort or Chukchi Sea shelf epibenthic communities.

This work builds on existing epibenthic community information for the Chukchi and Beaufort Seas outer continental shelf to add a new functional perspective to our understanding of epibenthic diversity, which has been based primarily on taxonomic classifications. Improved understanding of ecosystem functioning, defined as the maintenance and regulation of ecosystem processes, may assist in predicting how communities will respond to change or disturbance. Additionally, this project contributed to a pan-Arctic effort to create an online database for biological traits of Arctic benthic organisms.

Introduction

This research improves the understanding of two Alaskan outer continental shelf (OCS) ecosystems that are of public interest due to their oil and energy resources. Functional diversity of epibenthic invertebrate communities on the Beaufort and Chukchi Seas shelves was compared using biological trait analysis (BTA). BTA examines organism characteristics that directly relate to, or describe, the functions these organisms have within overall ecosystem processes. Potential disturbance to these ecosystem processes, from climate stress or oil and gas activities, might then be identified through changes observed in the biological trait composition and functional diversity of a community (Bremner et al. 2006). Benthic communities are especially suitable for this purpose because they integrate processes over timespans of months to years and are, thus, excellent indicators of longer-term impacts instead of short-term, seasonal fluctuations prominent in the Arctic.

Functional diversity describes the range of organismal traits within a community that, combined, influence ecosystem functioning (the maintenance and regulation of ecosystem processes) (Naeem et al. 1999, Tilman 2001, Bremner et al. 2006). Functional diversity explores what communities “do” and complements the “who they are” provided by taxonomic composition (Petchey and Gaston 2002). Functional diversity is increasingly accepted as a key driver of ecosystem processes such as resilience to environmental change and providing ecosystem services (Lailberté and Legendre 2015). BTA is used to assess functional diversity by assigning biological traits to species based on their life history, morphology, and behavior and using this information to identify their function and role in a specific environment (Bremner et al. 2003, 2006). Based on their traits, different taxa can play a similar functional role in a community, and taxonomically similar species can have different functions (Hewitt et al. 2008, Krumhansl et al. 2016). In essence, functional diversity is the balance of the roles of species within a community through different traits and redundancy of shared traits (Díaz and Cabido 2001). High functional redundancy (biological traits shared by several species) and high functional diversity (variety of traits are represented by the species within a community) are presumed to lead to high ecosystem stability and increased resilience to change or disturbance (Hewitt et al. 2008). High redundancy and diversity ensure that a specific function will still occur if one or several species were removed from the system by a disturbance.

The Chukchi and Beaufort OCS benthic communities may be impacted by climate stresses and anthropogenic perturbations such as from oil and gas exploration (Blanchard 2015, Tu et al. 2015). Benthic systems are well poised to study anthropogenic disturbances as well as recovery over time (Krumhansl et al. 2016). Arctic benthic invertebrates are long-lived and respond to disturbances over long time scales but are relatively inert to very short-term climate fluctuations (Grebmeier and Barry 1991). Therefore, functional diversity using BTA of arctic benthic invertebrates has promise in assessing disturbance effects on community function. Taxonomically based analyses have traditionally been used for benthic community-level research on the Chukchi and Beaufort Sea shelves, focusing on species abundance and biomass (e.g., Bluhm et al. 2009; Ravelo et al. 2014, 2015). However, it is difficult to discern community-level function and responses simply based on taxonomy. For example, increasing evidence points to the northward migration and invasion of arctic shelf systems by temperate species due to warming temperatures (Mueter and Litzow 2008, Renaud et al. 2015), but it is currently impossible to predict how these species may complement or disrupt the functional roles within the existing communities. This research builds on the existing taxonomic knowledge in the Chukchi and Beaufort OCS regions to lay the groundwork for monitoring potential changes in functional diversity over time. Globally, studies using BTA to assess functional diversity have increased in the past decade as evidenced by an increase in peer-reviewed articles from just ten in 2000 to over 115 by 2017. While much of this work centered on benthic invertebrates (Beauchard et al. 2017), none addressed benthic invertebrate communities in the Alaskan Arctic.

Benthic communities in the Beaufort and Chukchi Sea OCS support important bottom-up food webs, which can channel anthropogenic or climatic perturbations to upper trophic levels (Iken et al. 2010, Divine et al. 2015a, Tu et al. 2015). High fat content in both upper trophic level consumers and at the base of the Arctic food chain, which includes water respiring benthic invertebrates (Villa et al. 2017), puts ringed seals (*Pusa hispida*) at risk to experience elevated levels of persistent organic pollutants due to biomagnificaiton and bioaccumulation. These upper trophic levels include ecologically, socially, and economically important bearded seals, walruses (Fay 1982, Oliver et al. 1983), demersal fish (Whitehouse et al. 2016), crabs (Divine et al. 2015b), and birds (Lovvorn et al. 2003). These benthic food webs are supported by tight pelagic-benthic coupling to the overlying primary production (Grebmeier and Barry 1991, Carmack and Wassmann 2006, Iken et al. 2010, Divine et al. 2015a). However, the two shelf systems differ distinctly in their oceanographic setting and primary production levels (Sakshaug 2004; Carmack and Wassmann 2006). The Chukchi Sea has a shallow inflow shelf characterized by a high-nutrient influx from upwelling in the Bering Sea, which leads to high primary productivity. In contrast, the Beaufort Sea has a narrow interior shelf with less primary productivity driven by low-nutrient supplies from the oligotrophic Beaufort Gyre and freshwater influx from the Mackenzie and Colville Rivers (Carmack and Wassmann 2006, Grebmeier and Maslowski 2014; Figure 1). Differences in primary productivity are reflected in differences in pelagic-benthic coupling and may subsequently cause differences in biological traits of their epibenthic communities and related ecosystem function. Consequently, the responses and resilience of the benthic communities to perturbations will likely differ between these two shelf systems.

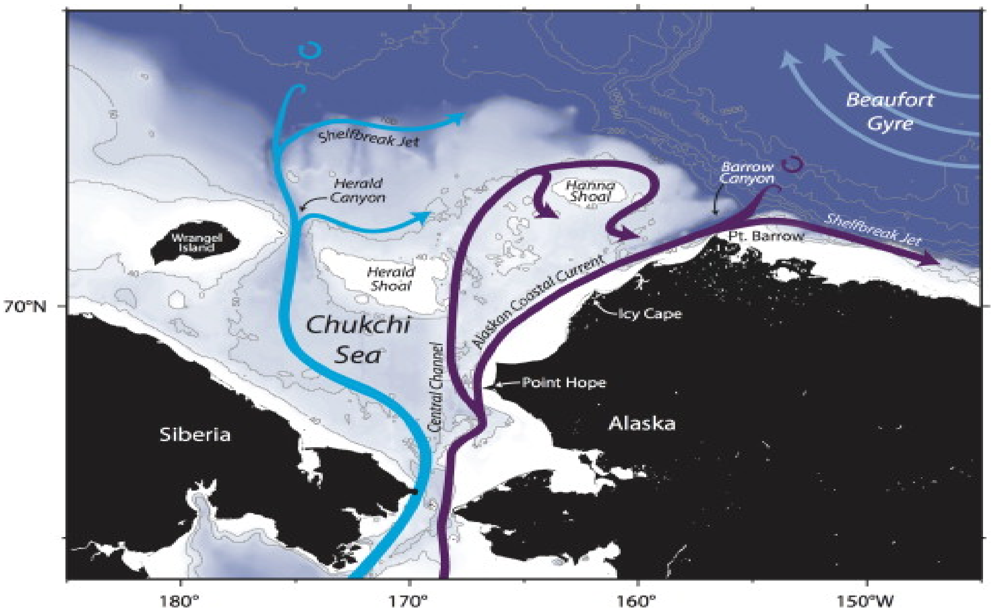


Figure 1. The Chukchi and Beaufort Seas are influenced by different water currents, bathymetry, and primary production regimes (adopted from Gong and Pickart 2015).

Objectives

The objectives of this study were to

1) compare the functional diversity of the Chukchi and Beaufort Sea epibenthic shelf communities using BTA, and

2) develop a benchmark of biological traits for these OCS communities for future comparisons.

The epibenthic communities of the Chukchi and Beaufort Sea shelves were hypothesized to differ in biological trait composition, and thus, epibenthic functional diversity and ecosystem functioning. In addition to a regional comparison between these communities, this work is part of a pan-Arctic effort to create an open-source database of Arctic benthic biological traits through the Arctic Traits Project (http://www.univie.ac.at/arctictraits). Data collected from this project will contribute to this international effort to further the understanding of benthic functional diversity across pan-Arctic scales.

Methods

Study Site

Epibenthic invertebrates were collected during four cruises funded by the Bureau of Ocean Energy Management (BOEM) in 2014 and 2015 on the Chukchi and eastern Beaufort Sea OCS. Stations were distributed across the dominant hydrographic conditions in each of the shelf systems. Environmental data included bottom salinity, bottom temperature, and depth taken during each of the cruises. Stations from these cruises used for this study ranged in depths of 9 m –64 m to keep sampling depth consistent for both shelf systems (Table 1, Figure 2).

Table 1. Station information from four cruises used for BTA on the Chukchi and Beaufort OCS.

Cruise	Sea	Depth Range (meters)	# of Stations	Latitude (° N)	Longitude (°W)
AMBON 2015	Chukchi	13 - 56	66	67°-73°	158°-168°
ANIMIDA 2014	Beaufort	10 - 64	23	69°-72°	141°-153°
ANIMIDA 2015	Beaufort	9 - 62	10	70°-71°	142°-152°
Transboundary 2014	Beaufort	13 - 60	15	70°-71°	140°-146°

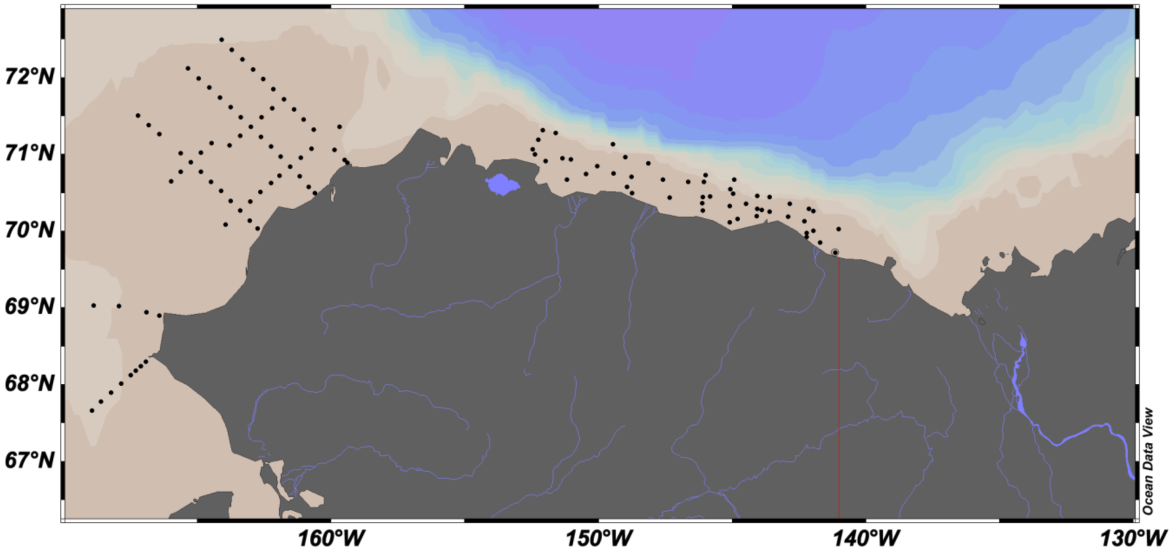


Figure 2. Stations sampled in the Chukchi and Beaufort Seas in 2014 and 2015.

Sample Collection

Epibenthic communities were sampled during all cruises towing a 3.05 m plumb-staff beam trawl with a 7 mm mesh and a 4 mm codend liner with a 1.2 m by 2.6 m mouth opening (modified after Gunderson and Ellis 1986). Average trawl time was 4–5 min, depending on station depth and station bottom conditions. Catch per unit effort was used to calculate abundance and biomass for epibenthic invertebrates at each station based on trawl time by trawled area and normalized to 1000 m^{-2}. Invertebrates were identified on board to the lowest taxonomic level, net wet weight was recorded for biomass using digital hanging scales, and the number of individuals was counted, where possible, for abundance measurements (not possible for colonial taxa). Vouchers for unknown taxa were fixed in either 10% formalin solution or 100% molecular ethanol for later identification with the help of taxonomic experts.

A biological traits database was created, which encompassed measurable properties of organisms, usually at the taxon level that can be used comparatively across different taxa (McGill et al. 2006, Reiss et al. 2009, Violle et al. 2007). The biological traits matrix was assembled through a combination of qualitative traits based on observations (e.g., some morphological traits) and traits derived from extensive literature research (i.e., life history traits, behavioral traits). BTA includes traits related to morphology (body form, fragility, sociability), behavior (living habit, adult movement, feeding habit, depth range, substrate affinity), and life history (reproductive strategy and larval development). Each trait was further separated into modalities. For example, the trait ‘reproductive strategy’ was separated into four modalities: asexual reproduction/budding, broadcast spawning, sexual planktonic reproduction, and sexual brooding (Table 2).

Table 2. Code and description for Biological Traits Analysis (BTA).

Biological trait	*Modality*	*Abb.*	*Description*
Body Form	Globulose	BF1	Round
	Vermiform	BF2	Wormlike
	Dorso-ventral compressed	BF3	Dorso-ventral compressed or encrusting
	Laterally compressed	BF4	Thin
	Upright	BF5	Body forms upward from the seafloor
Fragility	Fragile	F1	Likely to break due to physical impact
	Intermediate	F2	Suffer minor damage due to physical impacts
	Robust	F3	Unlikely damaged due to physical impacts
Sociability	Solitary	SO1	Single individual
	Gregarious	SO2	Single individuals found in groups
	Colonial	SO3	Living in permanent colonies
Reproduction	Asexual	R1	Budding
	Broadcast spawner	R2	Eggs/sperm released into the water
	Sexual - planktonic larvae	R3	Larvae hatches; dispersed in the water column
	Sexual - brooder	R4	Eggs are brooded, larvae/mini-adult hatches

Table 2. Continued

Biological trait	*Modality*	*Abb.*	*Description*
Larval Development	Planktotrophic	LD1	Larvae feed and grow in the water column
	Lecithotrophic	LD2	Larvae with yolk sac, pelagic for short periods
	Direct development	LD3	No larval stage (eggs develop into juveniles)
Living Habit	Free-living	LH1	Not limited to any restrictive structure
	Crevice dwelling	LH2	Inhabiting coarse/rock or algal holdfasts
	Tube-dwelling	LH3	Tube lined with sand, mucus or $CaCO_3$
	Burrow dwelling	LH4	Species inhabiting burrows in the sediment
	Epi/endo zoic/phytic	LH5	Biogenic species or algal holdfasts
	Attached	LH6	Adherent to a substratum
Adult Movement	Sessile/none	MV1	No movement as adult
	Burrower	MV2	Movement in the sediment
	Crawler	MV3	On surface via movements of appendages
	Swimmer	MV4	Movement above the sediment
Feeding Habit	Surface deposit feeder	FH1	Removal of detrital material from the surface
	Subsurface deposit feeder	FH2	Removal of detrital material from sediment
	Filter/suspension feeder	FH3	Filter food from the water column
	Opportunist/scavenger	FH4	Scavenges food
	Predator	FH5	Actively hunts prey
	Parasite	FH6	Feeds off other organisms
Depth Range	Shallow	DR1	0–20 m
	Shelf	DR2	20–200 m
	Shelf-slope	DR3	200–1000 m
	Slope-basin	DR4	> 1000 m
	Depth generalists	DR5	Everywhere
Substrate Affinity	Soft	SA1	Prefers soft substrate
	Hard	SA2	Prefers hard substrate

Fuzzy Coding Approach

A fuzzy coding approach was used for BTA created by Chevenet et al. (1994). Fuzzy coding allows species to express multiple modalities within a trait based on their affinity to that modality (Chevenet et al. 1994, Bremner et al. 2006). Using a 0–3 scoring system, where 0 means no affinity and 3 is a high affinity to a modality, each species was assigned a number based on the modality exhibited (Chevenet et al.1994). For example, a species that is strictly a predator received a score of 3 for the modality “predator.” However, if a species has a high affinity to predation but also has a low affinity to scavenging, the species was assigned 2 as a predator and ‘1’ as a scavenger.This approach identifies that both feeding mechanisms are present, but the species is predominantly a predator. Species may also exhibit equally high or low affinity to modalities within a biological trait. For example, the bryozoan *Alcyonidium disciforme* has a high affinity for shallow water and equally low affinities for shelf and slope depths. It was coded as 2 for DR1, 1 for DR2, and 1 for DR3 (Table 2, Figure 4). These modality scores were later weighted so that all modalities within a trait of a species summed to 1 (see Statistical Analyses below). Ten biological traits were used (see Table 2) to create unique biological trait profiles for all epibenthic invertebrate taxa (Figure 4).

Statistical Analyses

Fuzzy coded species biological trait profiles were multiplied by each species' biomass at each station and summed for all species at a given station to produce a quantitative station biological trait profile (SBTP, Chevenet et al.1994, Bremner 2003, Oug et al. 2012). SBTPs were then standardized so fuzzy coded modalities within a biological trait would sum to 1. Standardized SBTPs were compared between the Chukchi and Beaufort Seas using analysis of similarity (ANOSIM) on a Bray-Curtis resemblance matrix and visualized in non-metric multidimensional scaling (nMDS) plots using the PRIMER statistical package (v.7 Clarke and Gorley 2017). A functional diversity index (FDpg) was calculated for each SBTP based on the sum of dendrogram branch lengths of the hierarchical clustering for each SBTP (Figure 3, FD package R studio; Laliberté et al. 2015, Petchey and Gaston 2002). FDpg scores were then compared within and between the Chukchi and Beaufort Seas shelves.

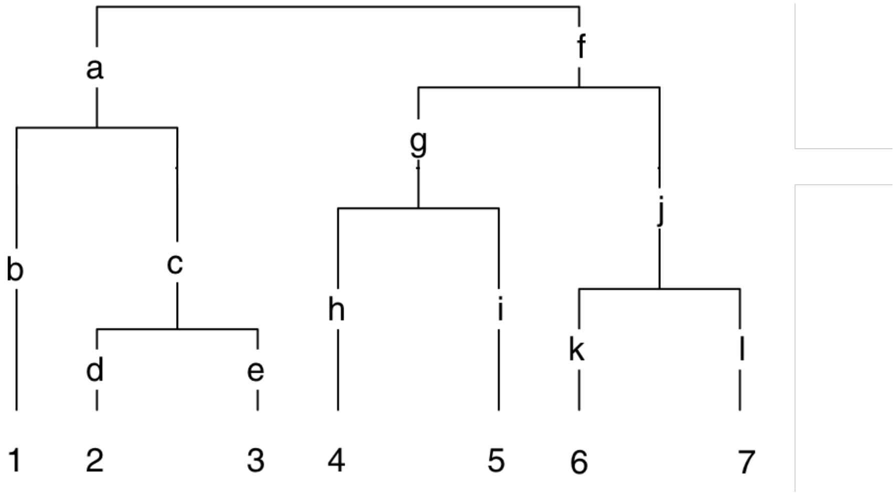
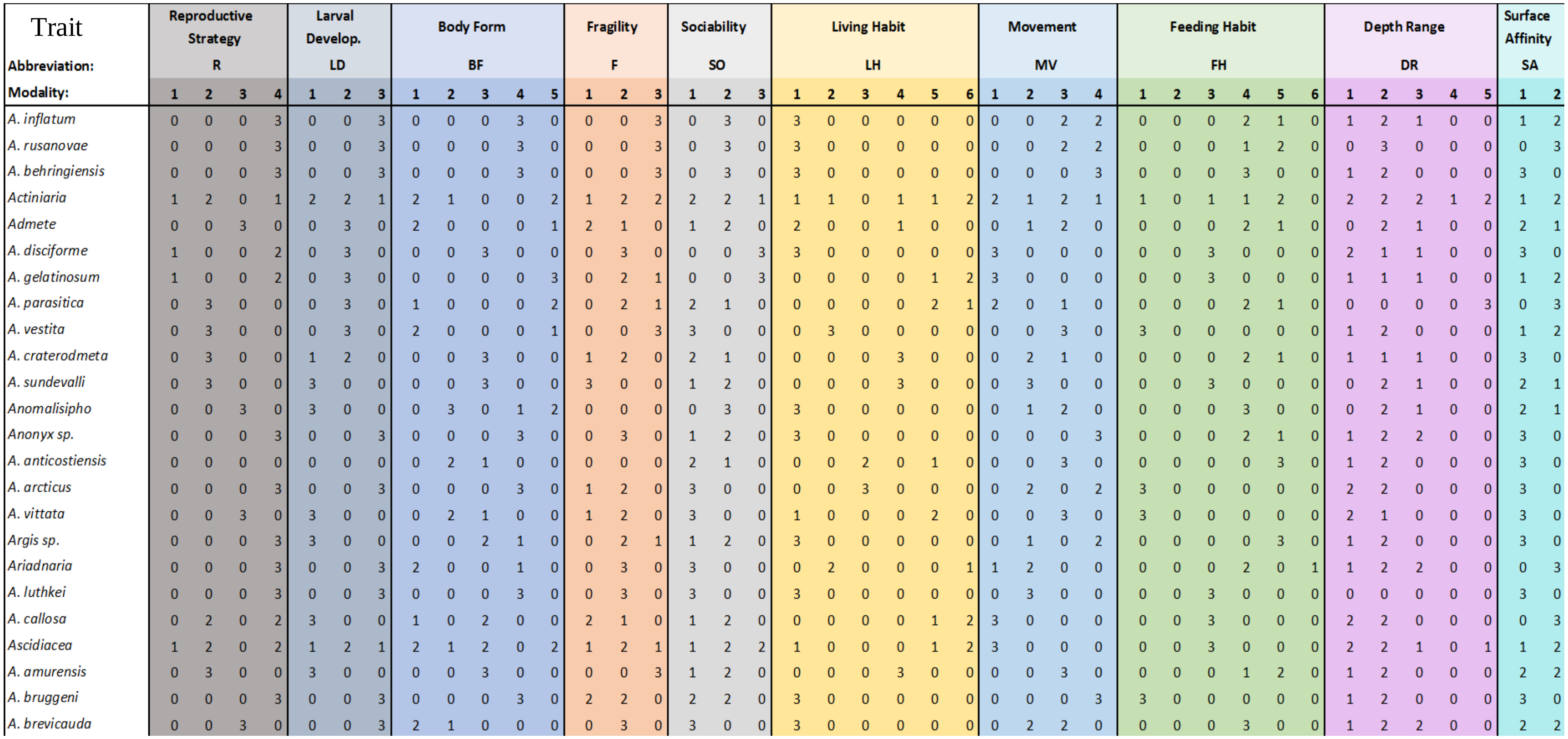


Figure 3. Cluster dendrogram of an example community. Species (1–7) are shown below the branches, and each branch length is labeled (a-l). FDpg sums the total branch lengths for each community (adapted from Petchey and Gaston 2002).



Trait	Reproductive Strategy				Larval Develop.			Body Form					Fragility			Sociability			Living Habit						Movement				Feeding Habit						Depth Range					Surface Affinity	
Abbreviation:	R				LD			BF					F			SO			LH						MV				FH						DR					SA	
Modality:	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**1**	**2**
A. inflatum	0	0	0	3	0	0	3	0	0	0	3	0	0	0	3	0	3	0	3	0	0	0	0	0	0	0	2	2	0	0	0	2	1	0	1	2	1	0	0	1	2
A. rusanovae	0	0	0	3	0	0	3	0	0	0	3	0	0	0	3	0	3	0	3	0	0	0	0	0	0	0	2	2	0	0	0	1	2	0	0	3	0	0	0	0	3
A. behringiensis	0	0	0	3	0	0	3	0	0	0	3	0	0	0	3	0	3	0	3	0	0	0	0	0	0	0	0	3	0	0	0	3	0	0	1	2	0	0	0	3	0
Actiniaria	1	2	0	1	2	2	1	2	1	0	0	2	1	2	2	2	2	1	1	1	0	1	1	2	2	1	2	1	1	0	1	1	2	0	2	2	2	1	2	1	2
Admete	0	0	3	0	0	3	0	2	0	0	0	1	2	1	0	1	2	0	2	0	0	1	0	0	0	1	2	0	0	0	0	2	1	0	0	2	1	0	0	2	1
A. disciforme	1	0	0	2	0	3	0	0	0	3	0	0	0	3	0	0	0	3	3	0	0	0	0	0	3	0	0	0	0	0	3	0	0	0	2	1	1	0	0	3	0
A. gelatinosum	1	0	0	2	0	3	0	0	0	0	0	3	0	2	1	0	0	3	0	0	0	0	1	2	3	0	0	0	0	0	3	0	0	0	1	1	1	0	0	1	2
A. parasitica	0	3	0	0	0	3	0	1	0	0	0	2	0	2	1	2	1	0	0	0	0	0	2	1	2	0	1	0	0	0	0	2	1	0	0	0	0	0	3	0	3
A. vestita	0	3	0	0	0	3	0	2	0	0	0	1	0	0	3	3	0	0	0	3	0	0	0	0	0	0	3	0	3	0	0	0	0	0	1	2	0	0	0	1	2
A. craterodmeta	0	3	0	0	1	2	0	0	0	3	0	0	1	2	0	2	1	0	0	0	0	3	0	0	0	2	1	0	0	0	0	2	1	0	1	1	1	0	0	3	0
A. sundevalli	0	3	0	0	3	0	0	0	0	3	0	0	3	0	0	1	2	0	0	0	0	3	0	0	0	3	0	0	0	0	3	0	0	0	0	2	1	0	0	2	1
Anomalisipho	0	0	3	0	3	0	0	0	3	0	1	2	0	0	0	0	3	0	3	0	0	0	0	0	0	1	2	0	0	0	0	3	0	0	0	2	1	0	0	2	1
Anonyx sp.	0	0	0	3	0	0	3	0	0	0	3	0	0	3	0	1	2	0	3	0	0	0	0	0	0	0	0	3	0	0	0	2	1	0	1	2	2	0	0	3	0
A. anticostiensis	0	0	0	0	0	0	0	0	2	1	0	0	0	0	0	2	1	0	0	0	2	0	1	0	0	0	3	0	0	0	0	0	3	0	1	2	0	0	0	3	0
A. arcticus	0	0	0	3	0	0	3	0	0	0	3	0	1	2	0	3	0	0	0	0	3	0	0	0	0	2	0	2	3	0	0	0	0	0	2	2	0	0	0	3	0
A. vittata	0	0	3	0	3	0	0	0	2	1	0	0	1	2	0	3	0	0	1	0	0	0	2	0	0	0	3	0	3	0	0	0	0	0	2	1	0	0	0	3	0
Argis sp.	0	0	0	3	3	0	0	0	0	2	1	0	0	2	1	1	2	0	3	0	0	0	0	0	0	1	0	2	0	0	0	0	3	0	1	2	0	0	0	3	0
Ariadnaria	0	0	0	3	0	0	3	2	0	0	1	0	0	3	0	3	0	0	0	2	0	0	0	1	1	2	0	0	0	0	0	2	0	1	1	2	2	0	0	0	3
A. luthkei	0	0	0	3	0	0	3	0	0	0	3	0	0	3	0	3	0	0	3	0	0	0	0	0	0	3	0	0	0	0	3	0	0	0	0	0	0	0	0	3	0
A. callosa	0	2	0	2	3	0	0	1	0	2	0	0	2	1	0	1	2	0	0	0	0	0	1	2	3	0	0	0	0	0	3	0	0	0	2	2	0	0	0	0	3
Ascidiacea	1	2	0	2	1	2	1	2	1	2	0	2	1	2	1	1	2	2	1	0	0	0	1	2	3	0	0	0	0	0	3	0	0	0	2	2	1	0	1	1	2
A. amurensis	0	3	0	0	3	0	0	0	0	3	0	0	0	0	3	1	2	0	0	0	0	3	0	0	0	0	3	0	0	0	0	1	2	0	1	2	0	0	0	2	2
A. bruggeni	0	0	0	3	0	0	3	0	0	0	3	0	2	2	0	2	2	0	3	0	0	0	0	0	0	0	0	3	3	0	0	0	0	0	1	2	0	0	0	3	0
A. brevicauda	0	0	3	0	0	0	3	2	1	0	0	0	0	3	0	3	0	0	3	0	0	0	0	0	0	2	2	0	0	0	0	3	0	0	1	2	2	0	0	2	2

Figure 4. Biological trait profiles for a subset of species found in the Chukchi Sea. See Table 2 for modality abbreviations and descriptors. Each species was fuzzy coded (0–3) for each modality within a trait class.

Fuzzy correspondence analysis (FCA) revealed which suite of biological traits accounted for variation across the Chukchi and Beaufort epibenthic shelf communities and which modalities best represented the respective communities. FCA is a parametric linear ordination tool based in eigenanalysis that maximizes correlation ratios and investigates similarities of biological traits among different species present in a community (Chevenet et al. 1994, Bremner et al. 2006, Beauchard et al. 2017). The FCA was based on the standardized SBTP. As opposed to the above ANOSIM, which simply stated a difference in communities between shelves, the FCA focused on which biological traits and which modalities had the most influence on community functional similarity. Correlation ratios tell us how much of the community variance of a specific trait is explained by a given axis (Chevenet et al. 1994). Biological traits with the highest correlation values were represented in the axes of the FCA. Based on previous studies that used FCA, the largest correlation values (values greater than 0.2 for this study) were considered ecologically influential on communities (Degen 2015, Bremner et al. 2006).

Biological traits were compared to environmental variables using RLQ and fourth-corner analyses, which are based in a three-table ordination method (R is the environmental variable by station table, L is the species biomass by station table, and Q is the species by traits table, Figure 5) testing the relationships between traits and abiotic factors (Mouillot et al. 2013; Dray et al. 2014). RLQ provided ordination scores that evaluate the relationship between the above-mentioned three tables. RLQ analysis maximized the covariance between the biomass-weighted traits per species and environmental variables (Dray et al. 2014). Fourth corner analysis evaluated the relationships of each modality with each environmental variable (Dray et al. 2014). These two methods are commonly used in tandem to evaluate trait-environmental relationships holistically. First, a multivariate test evaluated the global significance of the traits-environment relationship based on the total inertia of the RLQ analysis. If a significant result was found, then the fourth corner analysis was employed to show where significant relationships between modalities and environmental variables exist (Dray et al. 2014). The ade4 and FD packages in the software R-studio were used for all analyses.

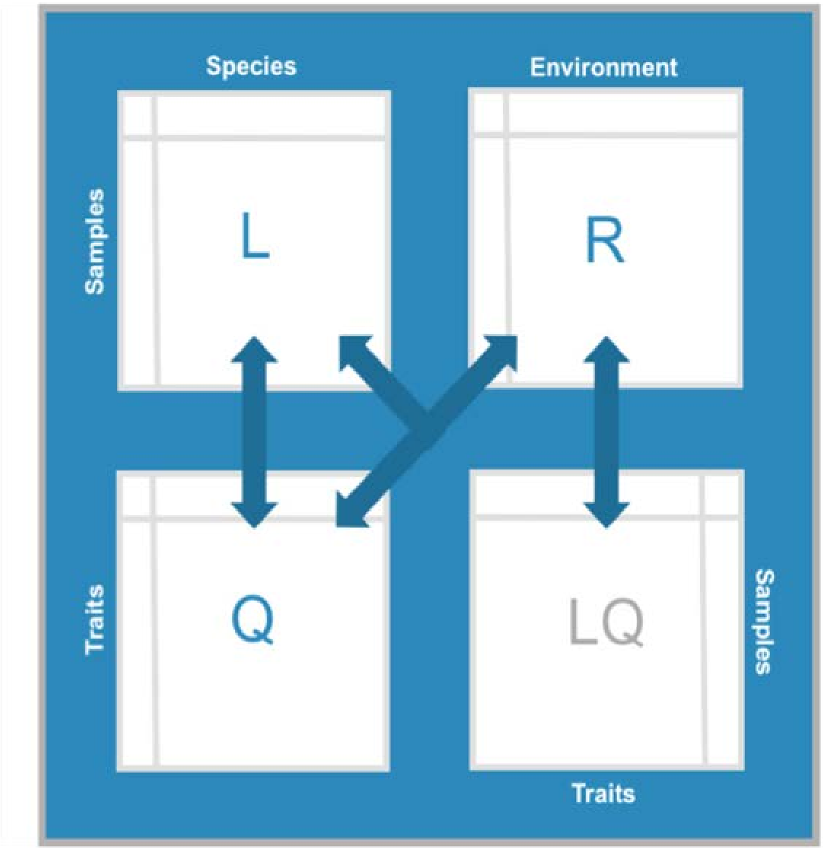


Figure 5. Relationships of the RLQ tables for environment, samples, and traits shown in white boxes while arrows show the possible interaction between these matrices (adopted from Degen et al. 2018).

Results

Functional Diversity Shelf Comparison

The epibenthic communities of the Chukchi Sea and the Beaufort Sea were moderately but significantly functionally distinct (ANOSIM, R=0.15, p<0.001) although many SBTPs overlapped from both seas (Figure 6). Functional diversity (FDpg) values of the Chukchi Sea communities were significantly higher than those of the Beaufort Sea (t-test, p<0.001). The Beaufort FDpg values had a much larger range than those of the Chukchi Sea (Figure 7). The mean and median FDpg values for the Beaufort were 31.99 and 33.67, respectively, while the mean and median FDpg values for the Chukchi were 36.36 and 37.30, respectively (Figure 7).

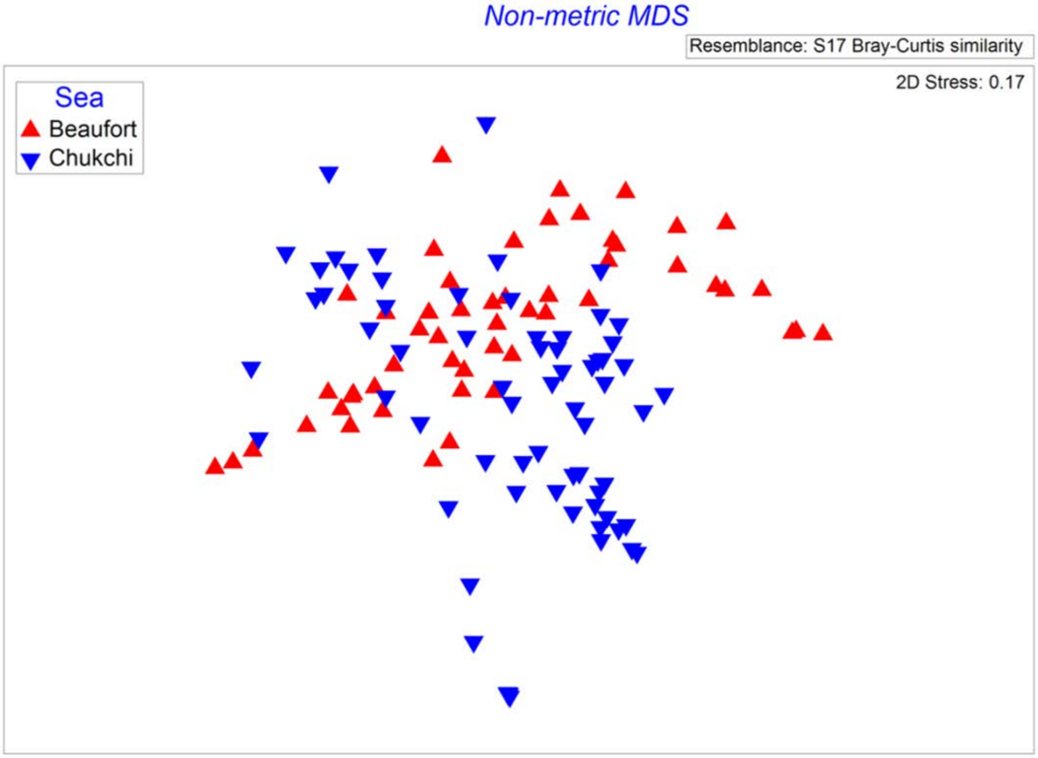


Figure 6. Chukchi and Beaufort Sea station biological trait profiles (SBTP) were moderately distinct but statistically significant from each other (R=0.15, p<0.01).

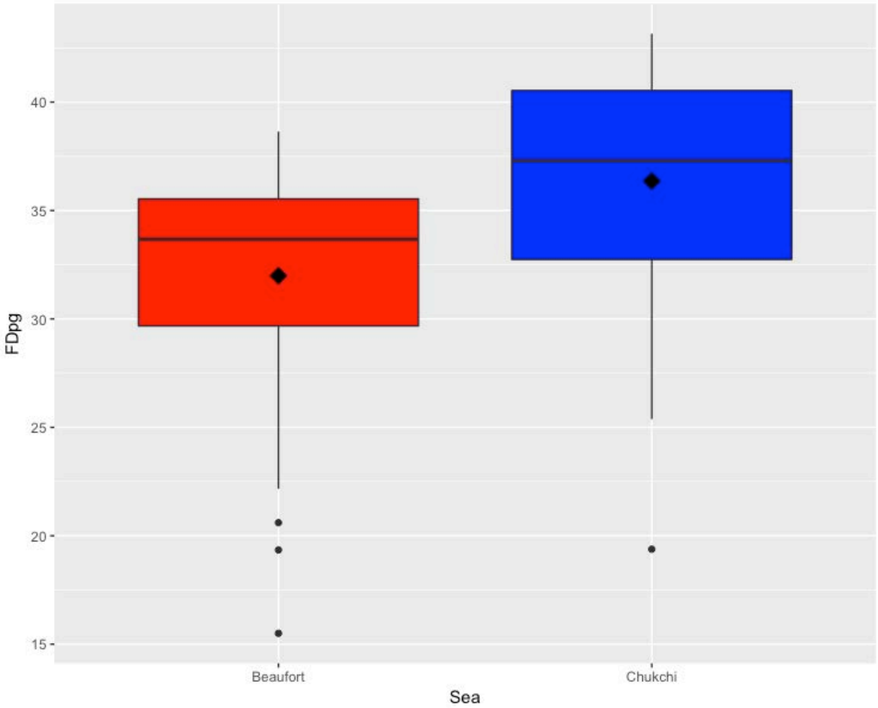


Figure 7. FDpg values of the Beaufort (red) and Chukchi (blue) communities. Black diamonds represent the FDpg shelf mean, and solid black horizontal lines represent the median FDpg values. The lower and upper lines of the boxes represent the first and third quantiles of FDpg values, respectively. Vertical lines represent the range of the first and third quantiles to the smallest and largest FDpg values. Black dots represent outliers within each shelf.

FDpg increased from south to north in the Chukchi Sea epibenthic communities. The highest FDpg values were found at communities in the nearshore and in the northwestern Chukchi Sea (Figure 8). FDpg values on the Beaufort Sea shelf were less spatially distinct. Beaufort Sea FDpg values were lower offshore and at the central nearshore communities than at many nearshore communities, although one nearshore Beaufort Sea station had a particularly low FDpg value (FDpg=15). FDpg values were highest in the far western and far eastern communities in the Beaufort Sea study area (Figure 8).

Fuzzy Correspondence Analysis

The FCA was performed on all stations (SBTP) sampled in the Beaufort and Chukchi seas. The first two FCA axes accounted for 57.79% of the variability in biological trait composition among SBTP. Axis 1 accounted for 35.41% while axis 2 accounted for 22.38% of this variability (relative inertia, Table 3). Biological traits with the greatest influence on differences in communities within the Beaufort and Chukchi seas, as evidenced by their large correlation ratios, were body form, reproductive strategy, larval development, depth range, and substrate affinity (Table 3).

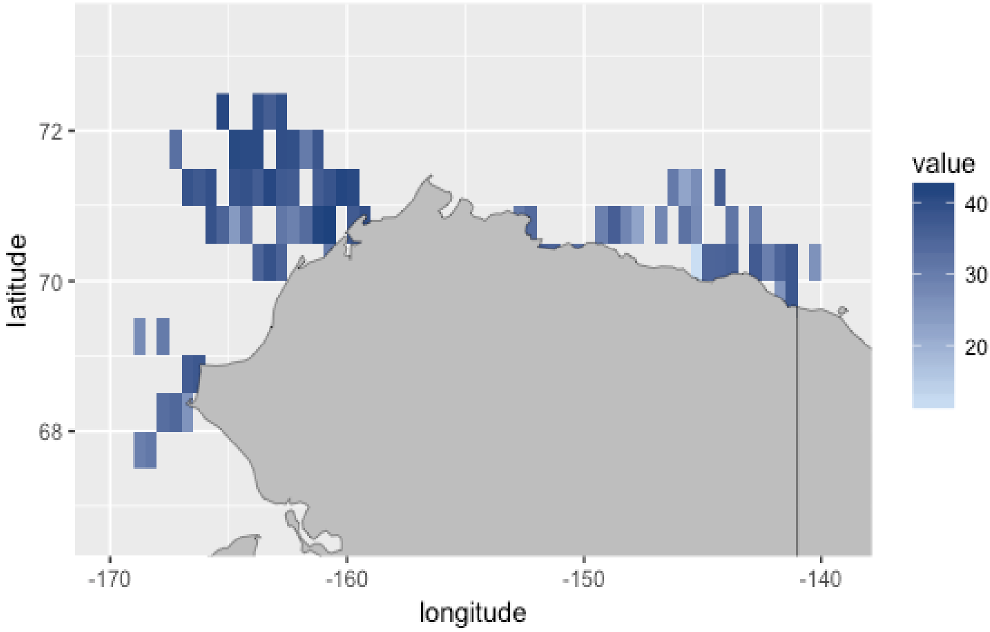


Figure 8. Functional diversity (FDpg) values across the Beaufort and Chukchi shelf communities. Darker blue colors indicate a higher FDpg value while lighter colors indicate a lower FDpg value.

Table 3. The relative inertia values show how much variation was accounted for by the FCA. Biological traits with the highest influence on functional diversity among communities, indicated by correlation values greater than 0.2 on either axis, are shown in bold.

	Axis 1	Axis 2
Relative Inertia (%)	35.41	22.38
Correlation ratio (r)		
Body Form	**0.224**	0.166
Fragility	0.007	0.103
Sociability	0.024	0.074
Reproductive Strategy	0.141	**0.222**
Larval Development	**0.366**	0.166
Living Habit	0.067	0.040
Adult Movement	0.187	0.109
Feeding Habit	0.194	0.051
Depth Range	0.049	**0.240**
Substrate Affinity	**0.251**	0.017

Proportion of Modalities on the Shelf Level

The proportions of modalities in many biological traits were similar between the Beaufort and Chukchi Sea epibenthic shelf communities (Figure 9). The two shelf communities had similar proportions of modalities within the biological traits of fragility, sociability, and feeding habit. There were some differences in the proportion of the biological traits of living habit and adult movement between the two shelf systems (Figure 9). In contrast, there were large differences in body form, reproductive strategy, larval development, depth range, and substrate affinity that influenced the functional diversity of communities within the two shelf systems (Figure 9). The Chukchi Sea shelf communities had proportionally more dorso-ventrally compressed organisms, planktotrophic larval development, more eurybathic, and more organisms that prefer soft substrate. Conversely, the Beaufort Sea shelf communities had proportionally more organisms with a globulose body form, included a sexual planktonic reproductive strategy that was missing in the Chukchi Sea, more lecithotrophic larval development, a greater proportion of organisms with a 20–200 m depth affinity, and more organisms that prefer hard substrate (Figure 9).

Spatial Comparisons of FCA and FDpg

The Beaufort and Chukchi Seas shelf communities were functionally different; however, many modalities were also shared between the shelves (Figure 9). Visual inspection of the ordination in the FCA revealed four SBTP groupings that coincided with the geographic placement of the SBTP (Figures 10, 11): Northern Chukchi Sea (n=29), Nearshore Beaufort Sea (n=6), Nearshore Chukchi/Offshore Beaufort (n=35), and one group (Mixed) with all other stations (n=44) (Figures 10, 11). Northern Chukchi Sea SBTPs were characterized by broadcast spawners, planktotrophic larval development, dorso-ventrally compressed body forms, eurybathic affinities, and preferences for soft substrate (red shade, Figures 10, 11). Nearshore Beaufort Sea SBTPs were characterized by communities with direct larval development and sexual brooding (green shade, Figures 10, 11). Nearshore Chukchi/ Offshore Beaufort SBTPs were characterized by high affinities for hard substrate, lecithotrophic larval development, and globulose body forms (teal shade, Figures 10, 11). The Mixed group had less distinct characteristic biological traits, shared many modalities with the surrounding SBTPs, and did not show any geographical pattern (purple shade, Figures 10, 11). There was a significant difference in FDpg among these geographic-based groups (ANOVA, $p<0.01$, Figure 12), with significantly lower FDpg in the Nearshore Beaufort Sea group (Tukey HSD, $p<0.01$, Figure 12). There were no significant differences between the Mixed, Nearshore Chukchi/Offshore Beaufort, and Northern Chukchi Sea SBTP groupings (Tukey HSD, $p>0.05$, Figure 12).

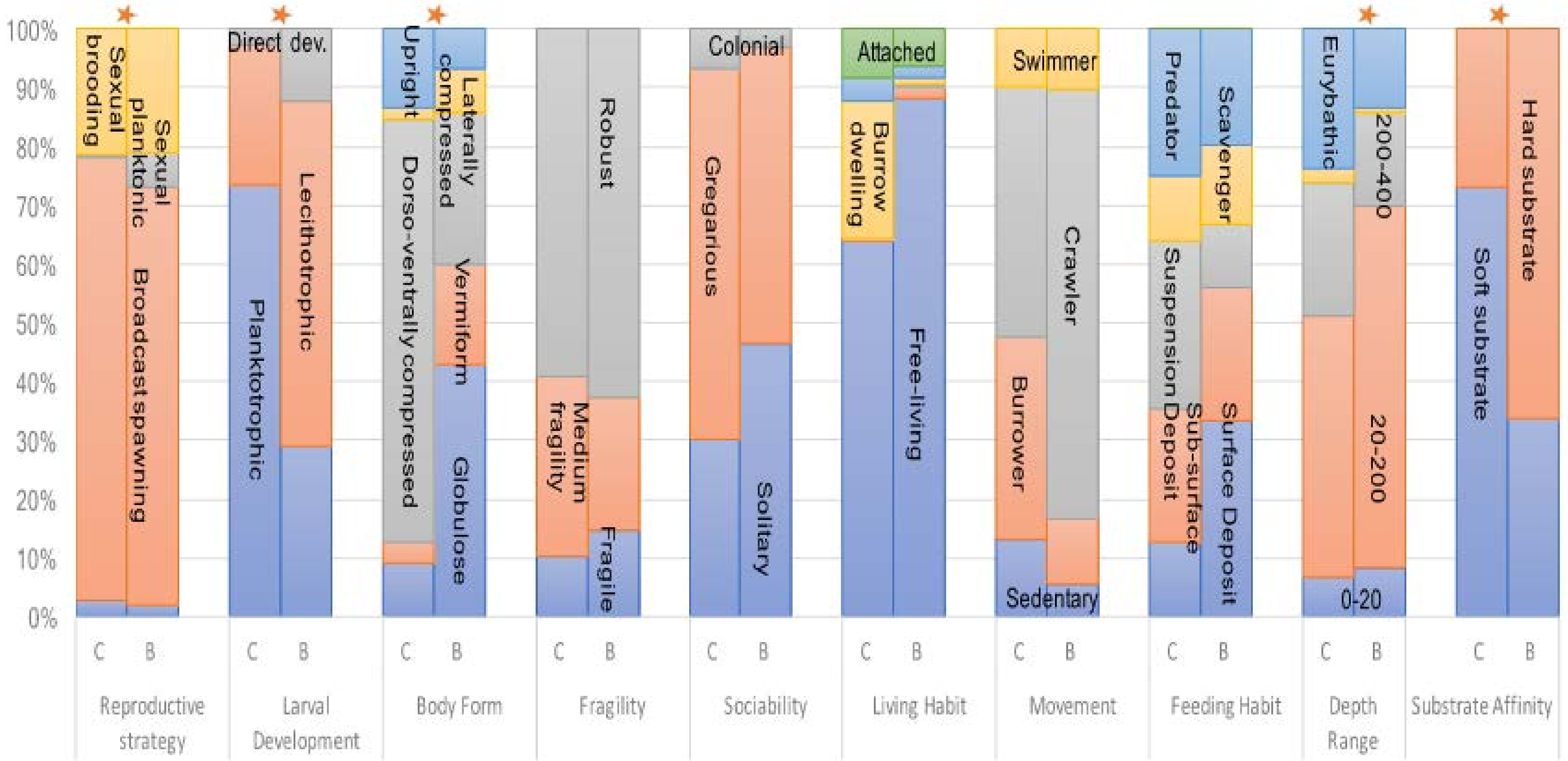


Figure 9. The proportions of modalities within traits for the Chukchi Sea shelf and Beaufort Sea shelf epibenthic communities. The biological traits with a star were considered most influential on differences in functional trait profiles for communities across the two seas.

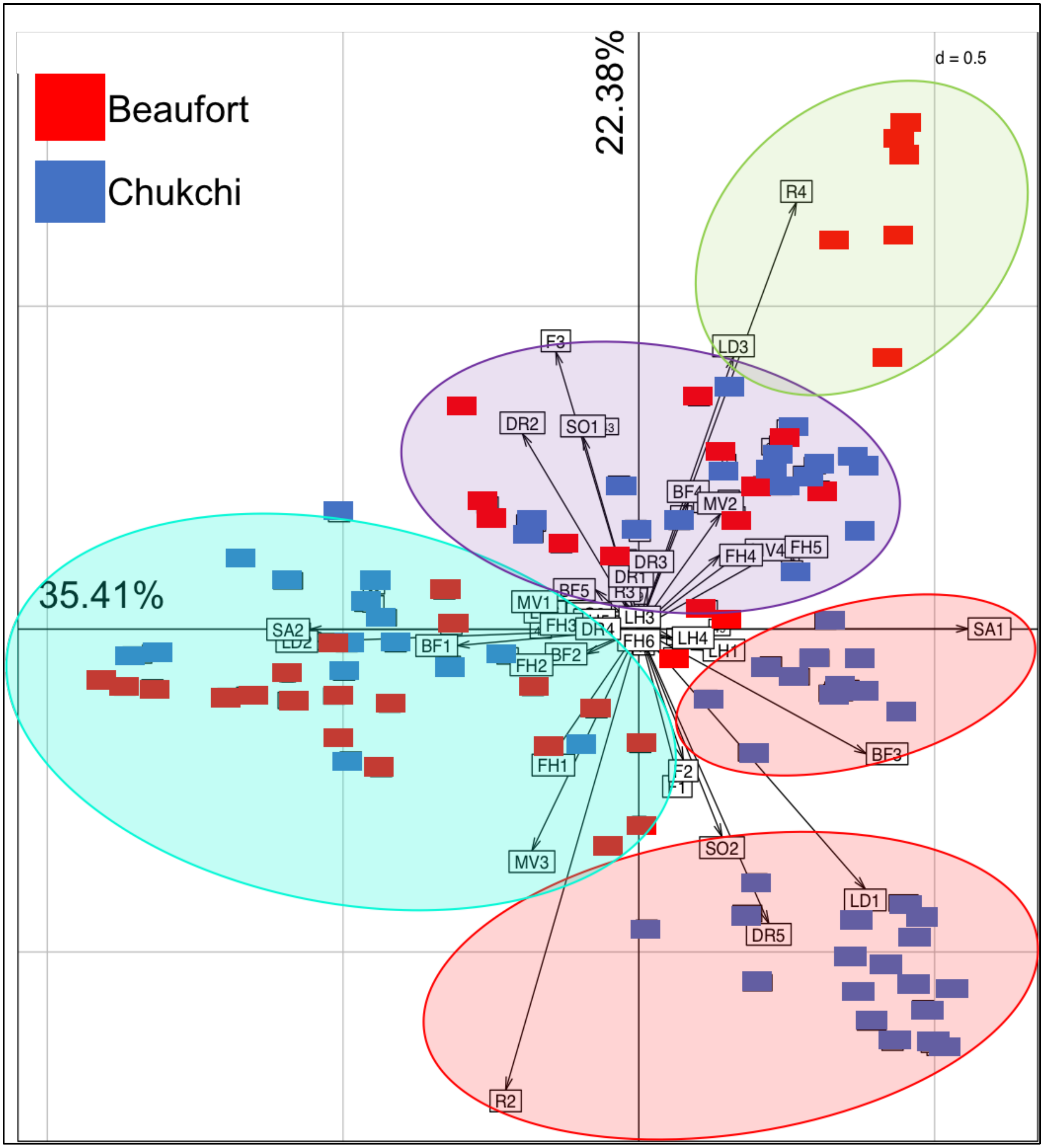


Figure 10. FCA of Beaufort Sea SBTPs (red squares) and Chukchi Sea SBTPs (blue squares). Modalities within biological traits are shown as open boxes, abbreviated as in Table 2. SBTPs that are closer together are more similar than SBTPs that are farther apart. SBTP are grouped by characteristic modalities in colored circles: red circles represent northern Chukchi Sea SBTPs, green circles represent nearshore Beaufort Sea SBTPs, teal circles represent nearshore Chukchi/Offshore Beaufort Sea SBTPs, and purple circles represent Mixed SBTPs.

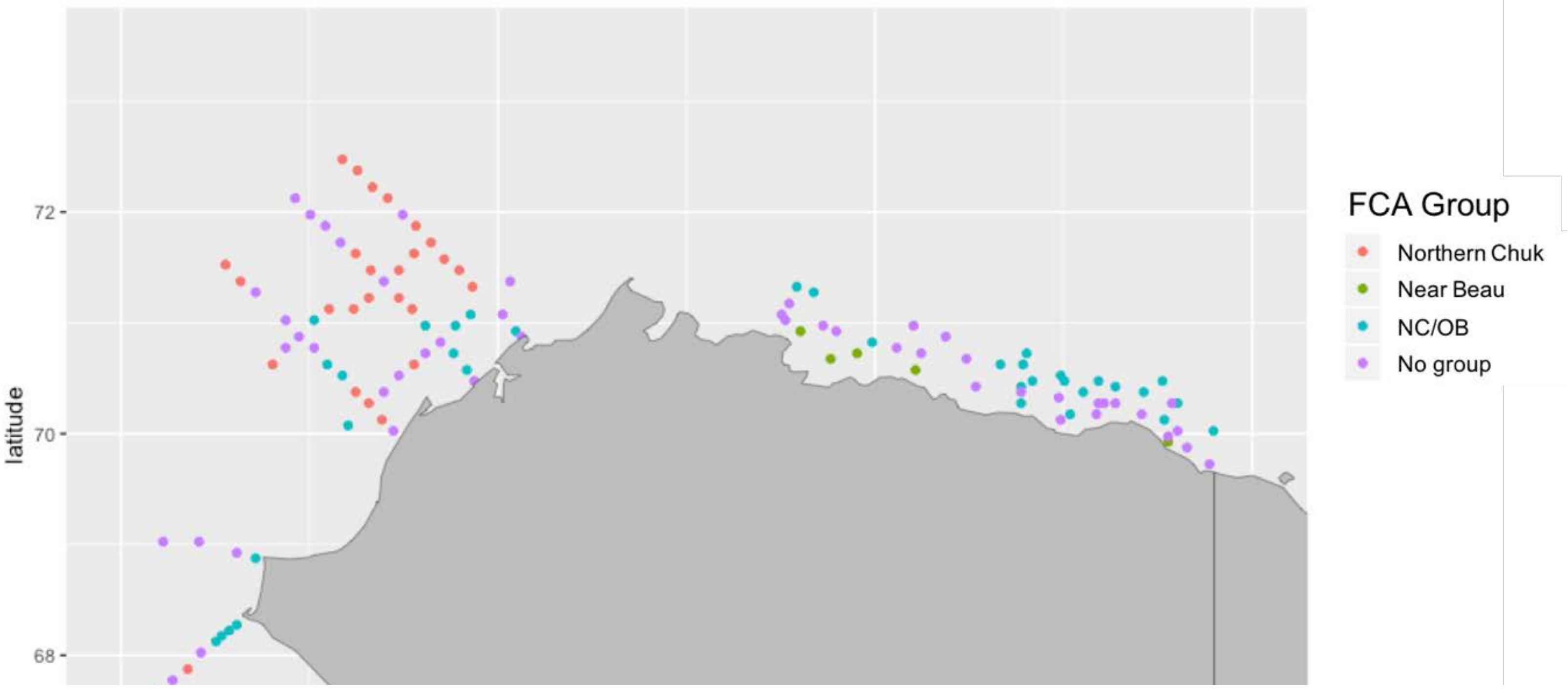


Figure 11. Spatial distribution of the SBTP groupings according to the FCA ordination (Figure 10).

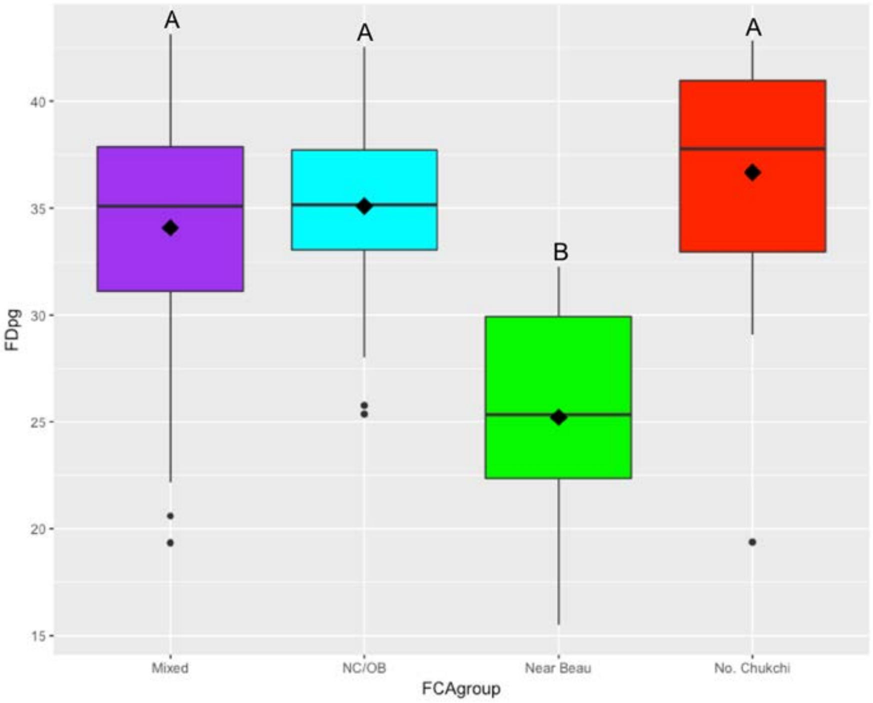


Figure 12. FDpg of the four groups distinguished in the FCA ordination. Groups with significant differences in FDpg are delineated by different letters. Black diamonds represent the mean FDpg values, and black horizontal lines represent the median FDpg value for each group. The lower and upper lines of the boxes represent the first and third quantiles of FDpg values, respectively. Vertical lines represent the range of the first and third quantiles to the smallest and largest FDpg values. Black dots represent outliers within each shelf.

Influence of Environmental Variables

There were no significant relationships between biological traits and environmental variables in the Chukchi Sea epibenthic communities (RLQ analysis, p=0.517) or the Beaufort Sea epibenthic communities (RLQ analysis, p=0.618). There was a significant relationship between environmental variables and SBTPs in the Chukchi Sea. However, due to a non-significant interaction between environmental variables and traits, fourth-corner analyses were not further considered.

Contribution to the Arctic Traits Project

Starting with an inaugural meeting in November 2016 in Vienna, Austria, the researcher has been part of the international Arctic Traits Project, an initiative led by Dr. Renate Degen (University of Vienna, Germany) to create a pan-arctic database of biological traits of arctic benthic species. The effort is described in Degen et al. 2018 a recent publication co-authored by the researcher.

Information on fourteen biological traits was assembled for this research. This includes the ten biological traits used for this analysis (Table 2), as well as information for size, lifespan, motility, and trophic level, which are currently not included in these analyses. Size ranges based on current research data are being determined, but this has not yet been completed for all taxa. The biological trait lifespan was only coded for half of the taxa due to lack of information. The biological traits motility and trophic level were highly correlated with the biological traits movement and feeding habit, respectively, and were excluded from this analysis. All traits will be included in the supplementary material for the researcher's thesis as well as published in the Arctic Traits Database.

Discussion

Functional diversity differed between the Chukchi and Beaufort Sea shelves and was, on average, higher on the Chukchi shelf. In the Chukchi Sea, more species and more functionally unique species contribute to the Chukchi functional traits profiles than observed in the Beaufort Sea. Therefore, Chukchi Sea communities may be more likely to conserve function on a shelf-wide scale than communities in the Beaufort Sea. Using BTA, communities are increasingly seen as assemblages of a multitude of functions rather than an assemblage of species (Cadotte et al. 2011). Therefore, when biological traits drive diversity, patterns in ecosystem function become more apparent than in traditional taxonomic diversity measures based on species diversity. In fact, when species diversity was compared to functional diversity for the Chukchi Sea, function was conserved across large spatial scales and at a high similarity level across the shelf even when species diversity changed (Figure 13, unpublished data presented at the World Conference on Marine Biodiversity, May 2018; Katrin Iken and Lauren Sutton). Regional as well as localized measures of functional diversity could inform future decisions in such things as biological conservation or management decisions (Carmona et al. 2016).

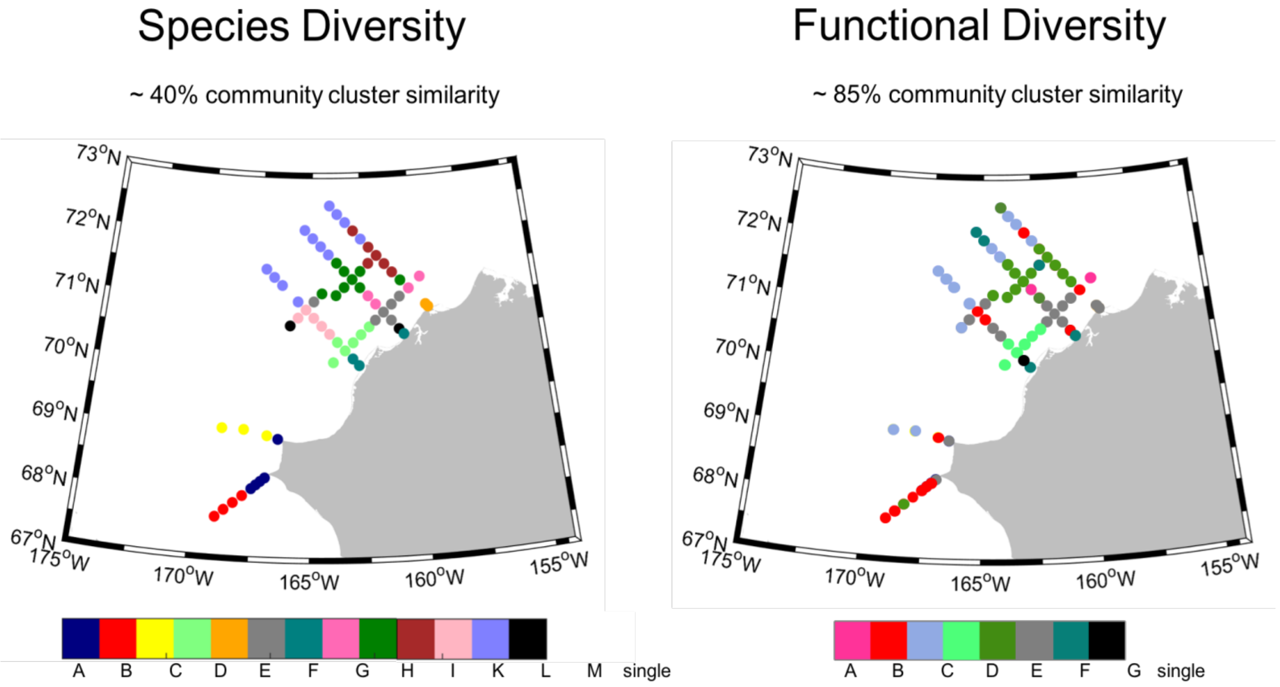


Figure 13. Species diversity and functional diversity grouped according to Similarity Percentages (SIMPER) analysis (40% and 85% for species and functional diversity, respectively). Greater similarity in community clusters for functional diversity implies function was conserved, despite differences in species composition.

The Chukchi and Beaufort Sea communities shared many biological traits and, thus, expressed many similar functions in similar proportions, but the two shelf systems also differed in several traits. The most influential biological traits that drove functional differences were body form, reproductive strategy, larval development, depth range, and substrate affinity. Individual modalities within these biological traits can inform likely community responses to change, disturbance, and energy flow and resource partitioning within a given system (Rand et al. 2017). For example, characteristic modalities in the Chukchi Sea communities included typical reproductive strategies of broadcast spawning and planktotrophic larval development, which directly affects these communities' ability to spread fast and far (Węsławski et al. 2011). On the one hand, this may allow Chukchi Sea communities to respond and recover quickly following a local disturbance, such as intense walrus feeding. On the other hand, the same communities may be negatively affected if environmental conditions for these reproductive strategy modalities (e.g., productive water column for planktotrophic feeding; large dispersal corridors for broadcast spawning) are disrupted. In contrast, a higher proportion of k-selected lecithotrophic larval development on the Beaufort shelf may make this system less susceptible to changes in water column productivity. The Beaufort and Chukchi shelf communities had similar distribution of modalities within several of the biological traits and would likely react similarly to disturbances involving those traits. For example, trawling has negative effects on benthic species with low motility (i.e., sedentary movement / attached living habit) and high vulnerability to impact (i.e., fragile) that are resident on soft substrate and filter feed (Juan et al. 2007). Most of these modalities, apart from an affinity for soft substrate, were similar in proportion for the Chukchi and Beaufort Sea epibenthic communities. These examples show that the vulnerability and resilience of these two shelf systems will likely depend on the specific disturbance and how it may interact with the specific set of modalities expressed on each shelf system.

Results from this project supported the hypothesis that the Chukchi Sea and Beaufort Sea epibenthic shelf communities differed in functional diversity and at least in some biological traits. A closer analysis also showed more detailed spatial patterns in epibenthic functional community structure. While some of these small-scale spatial patterns were within each shelf system (i.e., Nearshore Beaufort Sea SBTPs), others were distributed across shelf systems (i.e., Nearshore Chukchi/Offshore Beaufort Sea SBTPs). This demonstrates that functional diversity approaches can be used to test *a priori* hypotheses such as differences between the two shelf systems based on primary productivity and oceanographic features. It also shows that this approach can be useful to determine *a posteriori* groupings that may be ecologically relevant. These *a posteriori* groupings may be driven by small-scale environmental conditions. Based on the cross-shelf similarities, environmental variables did not show a significant relationship with trait-based patterns. However, model 2 for the Chukchi Sea, which tested the relationship of the environmental factors and a station-by-species matrix based in biomass, showed a significant relationship. It could be that environmental variables influence functional diversity patterns but that these drivers act on smaller spatial scales than the shelf-wide scale tested here. Assessing how environmental drivers shape the relative proportions of functions within and between communities is an ongoing field of research (Pacheco et al. 2011). Relationships between the environment and taxonomically-based community patterns have been well established and may act similarly for functional community patterns. For example, the epibenthic community functional diversity pattern in the Beaufort Sea (low diversity nearshore to higher diversity offshore) closely follows patterns of species diversity (Ravelo et al. 2015). Ravelo et al. (2015) found that depth was an important environmental driver for species diversity. In this study, depth-range was limited to 9–64 meters (just the shelf) and no depth-effect was identified for functional diversity. But different environmental variables for this analysis may elucidate correlations. Studies in both shelf areas have shown that sediment properties in addition to bottom water temperature, salinity, and depth may play important roles in driving epibenthic community patterns. Sediment grain size, sediment pigments, and sediment organic matter tend to have relationships with taxonomic species diversity (Bluhm et al. 2009; Ravelo et al. 2015). Sediment properties, for example, also are related to the flow regime, with faster flow resulting in coarser sediment structure, which influences functional properties of benthic feeding types (Pisareva et al. 2015). In addition, food supply as measured by integrated water column chlorophyll or sediment chlorophyll may influence epibenthic functional patterns. The Chukchi Sea shelf, which is a more productive system than the Beaufort Sea shelf (Feder et al. 1989; Carmack and Wassmann 2006; Grebmeier and Maslowski 2014) and with longer seasonally ice-free periods (Feder et al. 1989), may support more functionally diverse communities than the Beaufort Sea shelf communities. The latter communities may be more specialized due to the oligotrophic influences from the high Arctic and low-nutrient riverine input from the Mackenzie and Colville rivers (Bell et al. 2016).

Lack of information available for determining modalities in biological traits of Arctic invertebrates was the most significant challenge in this analysis and is a well-known problem in trait-based studies of functional diversity (Bremner 2006; Beauchard et al. 2017; Zhu et al. 2017; Degen et al. 2018). Many biological traits in this study were coded according to either southern congeners or closely related species. Queiros et al. (2013) argued that “in the absence of specific information, we consider it likely that genetically and physically similar taxa are likely to be functionally similar.” This general trend that similar species share similar functional roles was an important baseline for most of this research. However, species, and especially invertebrates, are extremely plastic in their morphology, life history, and behavior (Degen et al. 2018). The fuzzy coding approach, which allows species to exhibit multiple modalities, is a robust analytical tool and may account somewhat for partial miscoding (e.g., an organism was coded as more predator than scavenger but is actually more scavenger). In these results, the Beaufort Sea communities were less functionally diverse than the Chukchi Sea communities. This may be related to the fact that the Beaufort Sea communities were composed of a greater number of lesser-known species than the Chukchi Sea communities, meaning that more taxa had to be coded based on other, surrogate species. A possible effect of this on the outcome of functional diversity trends is unknown. This emphasizes that there is a pressing need for more basic studies on species life history, morphology, and behavior, especially for Arctic invertebrates. In addition, gathering information from taxonomic experts in this region who are currently or may soon be retiring is a tangible objective for data archives such as the Arctic Traits Database. Much biologically relevant information such as species behavior observed in the field needs to be documented to further our knowledge of biological traits.

Outreach

Outreach for this project included a hands-on activity with first and second-grade classes at Anne Wien Elementary School in Fairbanks (Figure 14). A touch-tank of live invertebrates including sea stars, crabs, and nudibranchs were used to explore biological traits and learn about functional diversity. Students were given stickers with pictures of invertebrates and instructed to think about the size, body form, and movement of the depicted organisms and characterize the invertebrates by trait. Students handled the invertebrates and talked to each other about their scientific observations and reconsidered their characterizations. For example, some invertebrates that were initially characterized as "no movement" changed to "crawler" at the end of the observation time when students realized that sea stars and nudibranchs do in fact move, albeit slowly.

Figure 14. Photo of Anne Wien Elementary outreach project. First and second graders sort stickers of organisms into different modalities of biological traits based their personal observations.

Graduate student support from this project also facilitated bringing the touch tank experience to the Fairbanks Children's Museum and Joy Elementary School and participation as a mentor in the Girls on Ice program through UAF.

Acknowledgments

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Using Trace Elements in Pacific Walrus Teeth to Track the Impacts of Petroleum Production in the Alaskan Arctic

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Abstract

Offshore oil and gas exploration and extraction have the potential to introduce harmful materials into marine food webs. These pollutants are typically associated with sediments; thus, benthic communities are most likely to be impacted. Trace element analysis is an effective tool for determining the presence of these materials in the marine environment. Pacific walruses (*Odobenus rosmarus divergens*) are benthic foragers, consuming a wide variety of prey items across broad areas of the seafloor. For this reason, walruses are considered a sentinel species for benthic communities. Walrus teeth grow continually and incorporate trace elements in concentrations proportional to those in the body of the walrus. These teeth can thus act as archives of element exposure, recording trace element concentrations experienced by walruses as they forage in benthic systems across the Alaskan Arctic. This study measured concentrations of 15 trace elements in the teeth of 99 female Pacific walruses collected between 1933 and 2016. Teeth were sectioned, and laser ablation inductively coupled plasma mass spectrometry (ICP-MS) was used to measure element concentrations in a transect across the tooth cementum, thereby reconstructing a lifetime history of element concentrations experienced by each walrus. The objectives of this study were (1) to quantify the variability of 14 trace elements (V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Sr, Mo, Ag, Cd, Ba, Pb) in teeth of female walruses, (2) to assess changes in 11 elements commonly associated with oil and gas activities (V, Cr, Mn, Fe, Ni, Cu, Zn, As, Cd, Ba, Pb) during different phases of petroleum exploration and extraction in the Alaskan Arctic, and (3) to analyze longitudinal changes in trace element concentrations in the teeth of individual female walruses to identify lifetime trends in trace element exposure.

Overall, most trace elements typically associated with oil and gas exploration and extraction did not change during the study period. In some cases, concentrations of these elements were lowest during the decades coinciding with the most intense petroleum-related activities (1970s–1980s, 1990s). The element concentrations presented here can, therefore, be treated as baseline values for future studies, with the exception of vanadium, arsenic, and iron, which changed substantially within the study period. Some elements showed consistent patterns within the teeth of individual walruses. Manganese, zinc, and lead tended to increase across a walrus' life, whereas copper, strontium, and barium typically declined. Zinc and lead were strongly correlated with one another and with the microstructure of the tooth, exhibiting peak concentrations in the dark layers of the cementum and minima in the light layers. These elements also tended to reach an inflection midway through the life of the animal, after which their rates of accumulation increased substantially. Given the importance of zinc to reproduction and the timing of these inflection points (median = 5 years), we developed a working hypothesis that this change in uptake represents attainment of reproductive maturity. Examinations of changes in this parameter across the study period matched well with known fluctuations in the size of the walrus population, lending support to this hypothesis. This technique has the potential to become a valuable tool for managers to track the status of the Pacific walrus population.

Introduction

Since the 1940s, the Alaskan Arctic has been the focus of widespread exploration for oil and gas reserves (Naidu et al. 2012). After the discovery of oil near Prudhoe Bay in 1968, these activities were greatly intensified, reaching a maximum in the 1980s. To date, more than six thousand wells have been drilled on the North Slope, the majority of them on land or on manmade islands in shallow, coastal waters (Alaska Department of Natural Resources 2014). In recent years, however, the focus has shifted farther offshore, where the majority of undiscovered reserves are believed to be (Gautier et al. 2009). This push to expand offshore petroleum exploration and production has raised concerns about the potential impacts these activities might have on marine ecosystems in the region, which are already experiencing rapid biological and physical shifts driven by the changing Arctic climate.

The impacts of offshore petroleum exploration and extraction on the marine environment have been the subject of considerable research. The two primary concerns associated with these activities are the discharge of potentially toxic drilling mud into marine systems and the possibility of contamination of pelagic and benthic environments by oil during extraction production (Boothe and Presley 1987; Marcaccio et al. 2003; Breuer et al. 2004, 2008; Lacerda et al. 2013). Barium has been identified as the most effective way to trace contamination by drilling muds, which typically contain large quantities of barite ($BaSO_4$), whereas vanadium is a tracer of crude oil in marine sediments (Khalaf et al. 1982; Trefry et al. 1985). Here, we assess the concentration of 11 petroleum-related elements as well as cobalt (Co), strontium (Sr), molybdenum (Mo), and silver (Ag). Some of these elements may occur at high enough concentrations to detrimentally impact marine ecosystems (Holdway 2002), while others may serve as a means of tracking environmental contamination associated with petroleum production (Trocine and Trefry 1983).

The biological effects of contaminants related to oil and gas production are expected to be most prominent in benthic communities, as sediments are their primary vector of dispersal into the environment (Brakstad et al. 1994). The size of the discharge footprint varies with the depth of the seafloor at the drilling site, the depth at which the material is discharged, and the strength and direction of ocean currents. It is often restricted to within 250 m of the drilling platform but has been documented to spread across distances as great as 3 km (Engelhardt et al. 1989; Kingston 1992). Depending on the rate of sedimentation, benthic organisms living near the platform may be smothered by sediment discharge; however, even when sedimentation rates are lower, community composition is often impacted (Kingston 1992). Despite the high rate of sedimentation and toxicity of sediments, areas close to production sites may exhibit an increased abundance of benthic organisms, as oil in the sediments is biodegraded and organic carbon is used (directly or indirectly) by macrofauna (Kingston 1987). Benthic communities in the Alaskan Arctic tend to be abundant and diverse, with a large biomass of both suspension and deposit feeders (Grebmeier et al. 1989a; b), thus the impacts to these communities and to benthic-foraging predators may be large.

The Pacific walrus (*Odobenus rosmarus divergens*, hereafter walrus) is a sentinel species for benthic systems in the Alaskan Arctic. Walruses are generalist benthic foragers and consume organisms from over 100 taxa (Sheffield and Grebmeier 2009). As a result, walruses effectively "sample" the seafloor throughout their lives. Female walruses spend the summer foraging in the Chukchi Sea and, to a lesser extent, the western Beaufort Sea (Jay et al. 2012). This summer foraging habitat includes regions of current and proposed offshore oil and natural gas production, overlapping substantially with the Bureau of Ocean Energy Management (BOEM) oil and gas lease areas in the Chukchi Sea (Figure 1). As a result, these animals may serve as a valuable means of monitoring the impacts of petroleum extraction on benthic communities as well as other predators foraging in this area. (e.g., bearded seals (*Erignathus barbatus*), gray whales (*Eschrichtius robustus*), and bowhead whales (*Balaena mysticetus*). Walruses are also an important subsistence resource for Alaska (and Russian) Native communities (Garlich-Miller et al. 2006), thus monitoring the potential exposure and impacts of petroleum production on this species is paramount to the sustained health and culture of communities in this region.

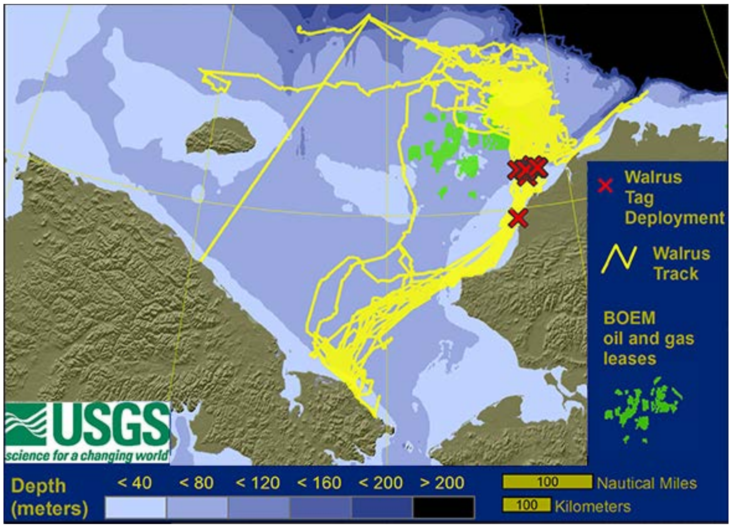

Figure 1. Movements of satellite-tagged walruses (yellow) in relation to BOEM oil and gas lease areas (green) in the Chukchi Sea (courtesy of the USGS, 2014).

Walrus teeth contain an unusually large cementum layer, which is laid down seasonally in dark and light bands (Fay 1982). Trace elements are believed to be included in the matrix of the tooth cementum in concentrations that generally reflect those of the environment in which walruses live and feed (Evans et al. 1995). By measuring these trace element concentrations, the lifetime history of an animal's exposure to specific trace elements may be reconstructed. This method has the potential to capture changes in element exposure within an individual's lifetime. It also allows for comparisons of trace element exposure across longer timeframes. Walrus teeth are relatively abundant in museum collections, allowing a time series of element concentrations to be generated that spans many decades. Natural variation in trace element concentrations can be established by measuring element concentrations in walrus teeth from historic and modern walruses. These data can be used to examine variability in the concentrations of trace elements typically associated with oil and gas exploration and extraction, and to investigate whether these concentrations have changed since the beginning of intensive petroleum-related activities in the Alaskan Arctic. If no changes are detected, trace element concentrations in present-day walrus teeth can be treated as baseline values for future monitoring of the impacts of petroleum production on benthic systems and benthic predators in Alaska.

Objectives

1) Quantify the variability of 14 trace elements (V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Sr, Mo, Ag, Cd, Ba, Pb) in teeth of female walruses and establish baseline values for these elements.
2) Assess changes in 11 elements commonly associated with oil and gas activities (V, Cr, Mn, Fe, Ni, Cu, and Zn, As, Cd, Ba, and Pb) during different phases of petroleum exploration and extraction in the Alaskan Arctic.
3) Analyze changes in trace element concentrations across the tooth cementum of individual female walruses to identify lifetime trends in trace element exposure.

Methods

Sample Preparation

Teeth of 99 female Pacific walruses were borrowed from the University of Alaska Museum of the North in Fairbanks, Alaska, and the National Museum of Natural History, in Washington, D.C. Collection dates for these specimens ranged from 1932 to 2016, with consistent sample coverage (≥10 individuals/decade) beginning in the 1960s and continuing through the 2010s. Older samples were collected during scientific expeditions, whereas the majority of recent samples were taken as part of the Alaska Native subsistence harvests in the communities of Gambell and Savoonga on Saint Lawrence Island, Alaska. Each tooth was cut longitudinally with a low speed, water-cooled saw using a diamond blade to create a 1.5 mm-thick cross-section of the center of the tooth. This cross section was then affixed to a microscope slide and polished using a 3000 grit diamond smoothing disc on a rotary polishing wheel. Samples were rinsed thoroughly with ultra-pure water then allowed to dry after polishing and again immediately prior to analysis.

Laboratory Analysis

Trace element analyses were conducted in the Advanced Instrumentation Lab at UAF, in Fairbanks, Alaska. Concentrations of 15 elements (Table 1) were measured using an Agilent 7500ce inductively coupled plasma mass spectrometer (ICP-MS) coupled with a New Wave UP213 laser. Element concentrations were compared with a United States Geological Survey micro-analytical phosphate standard (MAPS-4), as well as a National Institute of Standards and Technology Standard Reference Material (NIST610). ^{43}Ca was used as an internal standard for these analyses. Laser transects were ablated with a beam width of 25 µm, at 55% power, with a pulse frequency of 10 Hz, and a transect speed of 5 µm/s.

Table 1. Trace elements measured with typical (median) limits of detection. All limits of detection are expressed in parts per million (ppm).

Element	Symbol	Limit of detection (ppm)	Element	Symbol	Limit of detection (ppm)
vanadium	^{51}V	0.026	arsenic	^{75}As	0.141
Chromium	^{53}Cr	0.234	strontium	^{88}Sr	0.177
magnesium	^{55}Mn	0.116	molybdenum	^{95}Mo	0.026
iron	^{57}Fe	2.877	silver	^{107}Ag	0.019
cobalt	^{59}Co	0.018	cadmium	^{111}Cd	0.134
nickel	^{60}Ni	0.098	barium	^{137}Ba	0.347
copper	^{63}Cu	0.095	lead	^{208}Pb	0.047
zinc	^{66}Zn	0.515			

Each tooth was sampled at a location chosen to maximize distance from the root, where cementum growth layer groups converge and become distorted, while avoiding areas of tooth wear near the crown where not all cementum layers were present for sampling. Each transect was ablated from the cementum-dentin interface (beginning of the first year of life) to the outer edge of the tooth (end of the final year of life), thereby measuring lifetime changes in element concentrations for each animal.

Data Processing and Statistical Analysis

Trace element data were extracted and processed in Igor Pro version 6.37 using the Iolite software package version 3.0. All statistical analyses were conducted using R version 3.4.1 (R Core Team 2014) with Studio version 1.0.153 (RStudio Team 2015). Limits of detection were calculated separately for each sample run using the standard method applied by the Iolite software (limit of detection = three times the standard deviation of each element's measured background concentrations; Table 1). Measured element concentrations that fell below the limit of detection were replaced with a value of one half the limit of detection, the accepted method in the published literature (U.S. Environmental Protection Agency 2000). Data points more than four standard deviations from the mean were considered outlier and removed from the analysis.

Testing for Differences Among Time Periods

One-way analyses of variance (ANOVAs) were used to test whether trace element concentrations in walrus teeth differed among four periods: 1930s–1960s ($n = 22$), 1970s–1980s ($n = 23$), 1990s–2000s ($n = 38$), and 2010s ($n =16$). These periods were delineated based on the timeline of petroleum exploration and extraction activities in the Alaskan Arctic (Naidu et al. 2012). Trace element data tended to have strongly right-skewed distributions, so the data were natural log-transformed prior to analysis. In cases where ANOVAs indicated significant differences among periods ($p < 0.05$), Tukey's Honest Significant Difference *post hoc* tests were used to examine which periods differed from one another. Median element concentrations were calculated for each animal and used to test for differences among animals collected during each of the four periods. Vanadium, chromium, and arsenic concentrations were not measured in one animal, so sample sizes for these elements were smaller by one ($n = 98$) than those of the remaining elements ($n = 99$).

Examining Lifetime Patterns

After analysis on the ICP-MS, teeth were photographed under an optical microscope using reflected light. Walrus tooth cementum accrues one light and one dark growth layer each year (Kryukova 2014). Growth layer groups in the tooth cementum were counted by three observers and used to estimate the age of each animal. Counts of growth layer groups were conducted on at least two separate days for each tooth to ensure consistency of age estimates. Photographs were centered on laser ablation scars so that changes in element concentrations could be directly compared to tooth structure (dark/light growth layers). In this way, changes in trace element concentrations within a transect could be associated with specific years of an animal's life.

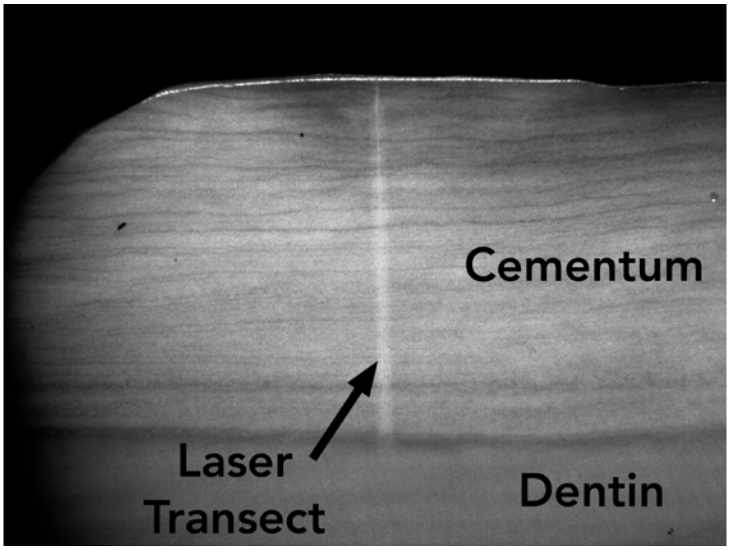

Figure 2. Image of a walrus tooth after analysis on the ICP-MS. Dentin (darker material at the bottom) and cementum (lighter material with visible dark/light growth layers) can be seen clearly, as well as the ablation scar left by the laser transect (vertical, light-colored line).

Patterns of trace element accumulation within the lives of individual walruses were assessed through visual examinations of the data. A centered moving average with a width of 35 data points was calculated for all elements from each walrus. The smoothed data from all walruses were then plotted together to determine whether consistent patterns (*e.g.*, lifetime increase/decrease in element concentrations) existed for any elements. Data were overlaid on photographs of teeth so changes in element concentrations could be directly related to growth layer groups.

Results

Summary Statistics and Differences Among Time Periods

Detectable concentrations of all 15 trace elements were measured in the teeth of female walruses. Strontium, zinc, and iron were present in the highest concentrations, whereas silver, cobalt, and molybdenum were present in the lowest concentrations (Table 2). ANOVAs indicated significant differences among the 1930s–1960s, 1970s–1980s, 1990s–2000s, and 2010s for nine of the elements measured and no differences among these periods for six elements (Table 3, Figures 3 and 4). Vanadium concentrations were greater in the teeth of walruses collected during the 1930s–1960s and 1970s–1980s than those that were killed in the 1990s–2000s and the 2010s ($F_{3,94} = 12.21$, $p < 0.001$). Chromium concentrations were slightly higher in the 2010s than in the other periods; however, this difference was not significant ($F_{3,94} = 2.41$, $p = 0.072$). Walruses killed during the 1930s–1960s had manganese concentrations that were significantly greater than those of walruses collected during the 1990s–2000s ($F_{3, 95} = 3.41$, $p = 0.021$). Average iron values increased from the 1930s–1960s to the 2010s, with walruses from the 1990s–2000s and 2010s both having significantly greater iron concentrations than those collected in the 1930s–1960s ($F_{3, 95} = 3.51$, $p = 0.018$). There were no significant differences among any of the periods for cobalt ($F_{3,95} = 1.732$, $p = 0.166$). Nickel concentrations were greatest in the 2010s, and were significantly different from the 1970s–1980s and 1990s–2000s ($F_{3,95} = 5.26$, $p = 0.002$). There were no other differences among time periods for nickel. Copper concentrations were greatest in walruses killed in the 1930s–1960s, which had significantly higher values than walruses collected in the 1970s–1980s, and in the 2010s, which were significantly greater than in animals from the 1990s–2000s ($F_{3,95} = 4.89$, $p = 0.003$). Zinc did not differ among time periods ($F_{3,95} = 0.18$, $p = 0.913$). Arsenic concentrations were generally low, but were much greater in animals from the 1990s–2000s than in walruses from any other time period ($F_{3,94} = 9.22$, $p < 0.001$). Concentrations of strontium were significantly lower in the 2010s than in any of the other time periods, which were similar to one another ($F_{3,95} = 4.72$, $p = 0.004$). Molybdenum concentrations were similar among all four time periods ($F_{3,95} = 1.24$, $p = 0.300$). Silver was significantly higher in the 1990s–2000s than in the 1930s–1960s ($F_{3,95} = 4.38$, $p = 0.006$), but no other differences existed among periods for this element. Walruses collected in the 1990s–2000s had significantly lower cadmium concentrations in their teeth than animals killed in the 1930s–1960s and the 1970s–1980s but did not differ significantly from those collected in the 2010s ($F_{3,95} = 4.34$, $p = 0.007$). No other differences existed for cadmium. Concentrations of barium were lower in the 1970s–1980s than in the 1930s–1960s, though all other periods were similar to one another ($F_{3,95} = 2.81$, $p = 0.044$). Finally, lead concentrations were similar among all four time periods ($F_{3,95} = 2.39$, $p = 0.074$).

Table 2. Summary statistics (sample size, mean ± 1 standard deviation, median, minimum, maximum) for the concentrations of trace elements in the teeth of female walruses. All element concentrations are expressed in parts per million (ppm).

Element	**n =**	**Mean (± 1 SD) (ppm)**	**Median (ppm)**	**Min (ppm)**	**Max (ppm)**
V	98	1.316 ± 0.658	1.154	0.529	4.699
Cr	98	0.199 ± 0.151	0.149	0.068	1.24
Mn	99	0.909 ± 0.486	0.859	0.439	5.057
Fe	99	50.636 ± 47.658	33.444	17.893	281.612
Co	99	0.024 ± 0.020	0.018	0.003	0.124
Ni	99	0.205 ± 0.167	0.172	0.047	0.918
Cu	99	1.453 ± 3.024	0.829	0.319	25.588
Zn	99	170.758 ± 39.579	164.351	91.982	262.661
As	98	0.127 ± 0.172	0.076	0.029	1.215
Sr	99	272.337 ± 54.461	262.535	188.87	472.441
Mo	99	0.033 ± 0.014	0.031	0.01	0.072
Ag	99	0.012 ± 0.008	0.01	0.003	0.063
Cd	99	0.226 ± 0.146	0.199	0.049	0.615
Ba	99	4.772 ± 1.224	4.427	2.98	9.812
Pb	99	1.690 ± 0.594	1.586	0.734	3.732

Table 3. Mean (± 1 standard deviation) concentrations of trace elements in the teeth of female walruses collected during the 1930s–1960s (n = 22), 1970s–1980s (n = 23), 1990s–2000s (n = 38), and 2010s (n = 16). Periods that do not share a superscript (A, B, C) differ significantly ($p < 0.05$).

	1930s–1960s (ppm)	**1970s–1980s (ppm)**	**1990s–2000s (ppm)**	**2010s (ppm)**
V	1.495 ± 0.727^{A}	1.741 ± 0.742^{A}	1.111 ± 0.524^{B}	0.955 ± 0.224^{B}
Cr	0.210 ± 0.163^{A}	0.174 ± 0.081^{A}	0.171 ± 0.090^{A}	0.286 ± 0.268^{A}
Mn	1.131 ± 0.910^{A}	$0.953 \pm 0.309^{A,B}$	0.794 ± 0.180^{B}	$0.812 \pm 0.206^{A,B}$
Fe	31.532 ± 20.633^{A}	$43.663 \pm 22.151^{A,B}$	53.394 ± 36.404^{B}	80.377 ± 93.177^{B}
Co	0.020 ± 0.014^{A}	0.023 ± 0.011^{A}	0.021 ± 0.014^{A}	0.037 ± 0.037^{A}
Ni	$0.230 \pm 0.183^{A,B}$	0.176 ± 0.087^{B}	0.160 ± 0.139^{B}	0.319 ± 0.234^{A}
Cu	$1.527 \pm 1.305^{A,B}$	$1.144 \pm 1.344^{C,D}$	$0.783 \pm 0.290^{A,D}$	$3.390 \pm 7.014^{B,C}$
Zn	170.345 ± 50.602^{A}	173.735 ± 32.073^{A}	169.944 ± 36.423^{A}	168.980 ± 43.009^{A}
As	0.064 ± 0.031^{A}	0.073 ± 0.021^{A}	0.214 ± 0.251^{B}	0.082 ± 0.056^{A}
Sr	$275.769 \pm 70.057^{A,B}$	$260.464 \pm 32.037^{A,B}$	291.192 ± 53.916^{A}	239.906 ± 39.680^{B}
Mo	0.037 ± 0.012^{A}	0.032 ± 0.014^{A}	0.032 ± 0.015^{A}	0.034 ± 0.016^{A}
Ag	0.009 ± 0.007^{A}	$0.012 \pm 0.006^{A,B}$	0.013 ± 0.005^{B}	$0.012 \pm 0.014^{A,B}$
Cd	0.299 ± 0.160^{A}	0.273 ± 0.151^{A}	0.174 ± 0.129^{B}	$0.192 \pm 0.106^{A,B}$
Ba	5.283 ± 1.593^{A}	4.225 ± 0.638^{B}	$4.801 \pm 1.241^{A,B}$	$4.786 \pm 1.000^{A,B}$
Pb	1.933 ± 0.776^{A}	1.807 ± 0.642^{A}	1.518 ± 0.381^{A}	1.597 ± 0.558^{A}

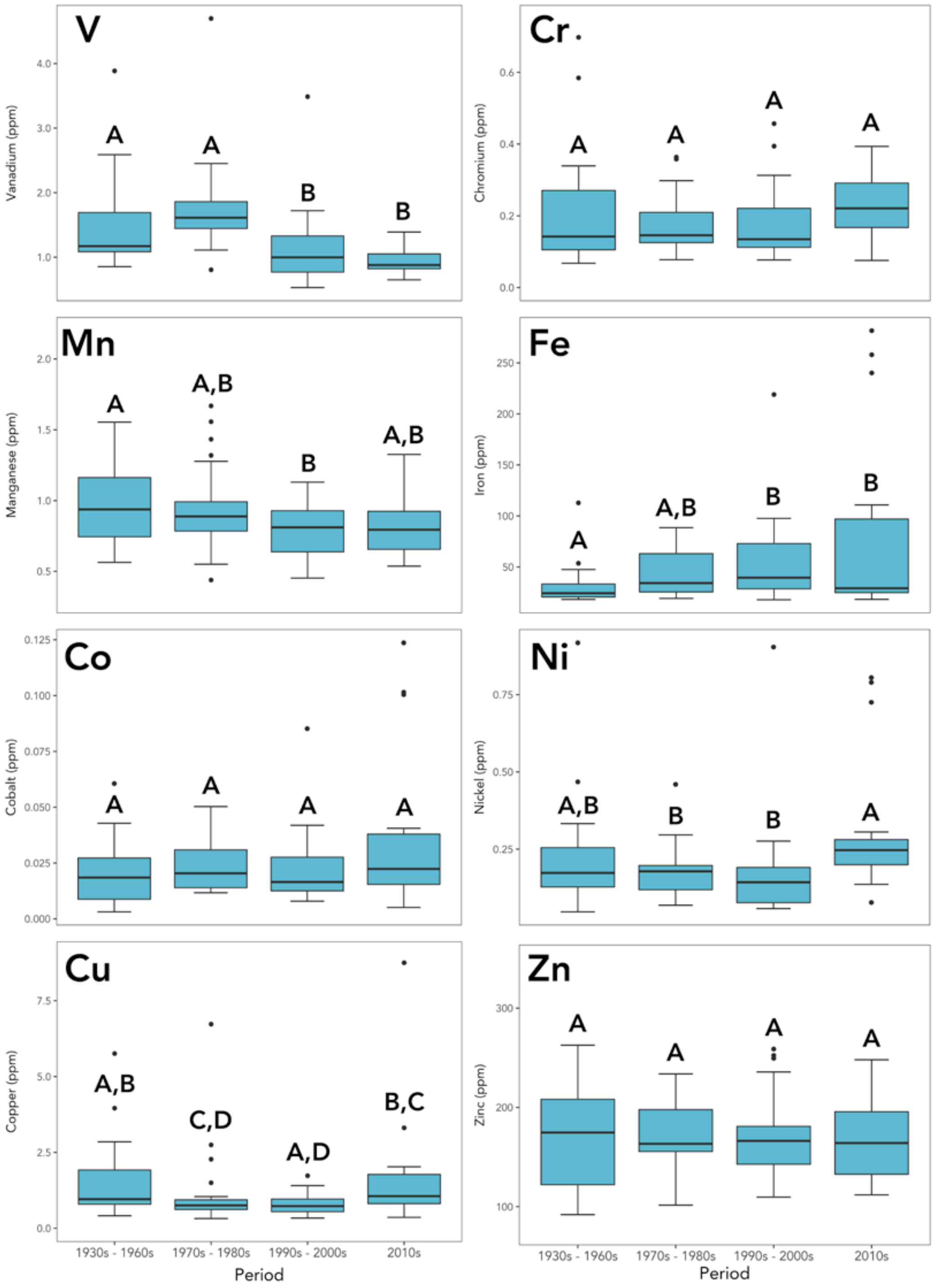

Figure 3. Box plots showing changes in median concentrations (ppm) of eight trace elements in walrus teeth from the 1930s–1960s, 1970s–1980s, 1990s–2000s, and 2010s. Periods that do not share a letter (A, B, C) differ significantly ($p < 0.05$). Medians are indicated by horizontal lines in the middle of each box plot. Bottom and top of each box represent the first and third quartiles of the data, respectively. Whiskers represent the range (minimum and maximum) of the data. Outliers are represented by individual points.

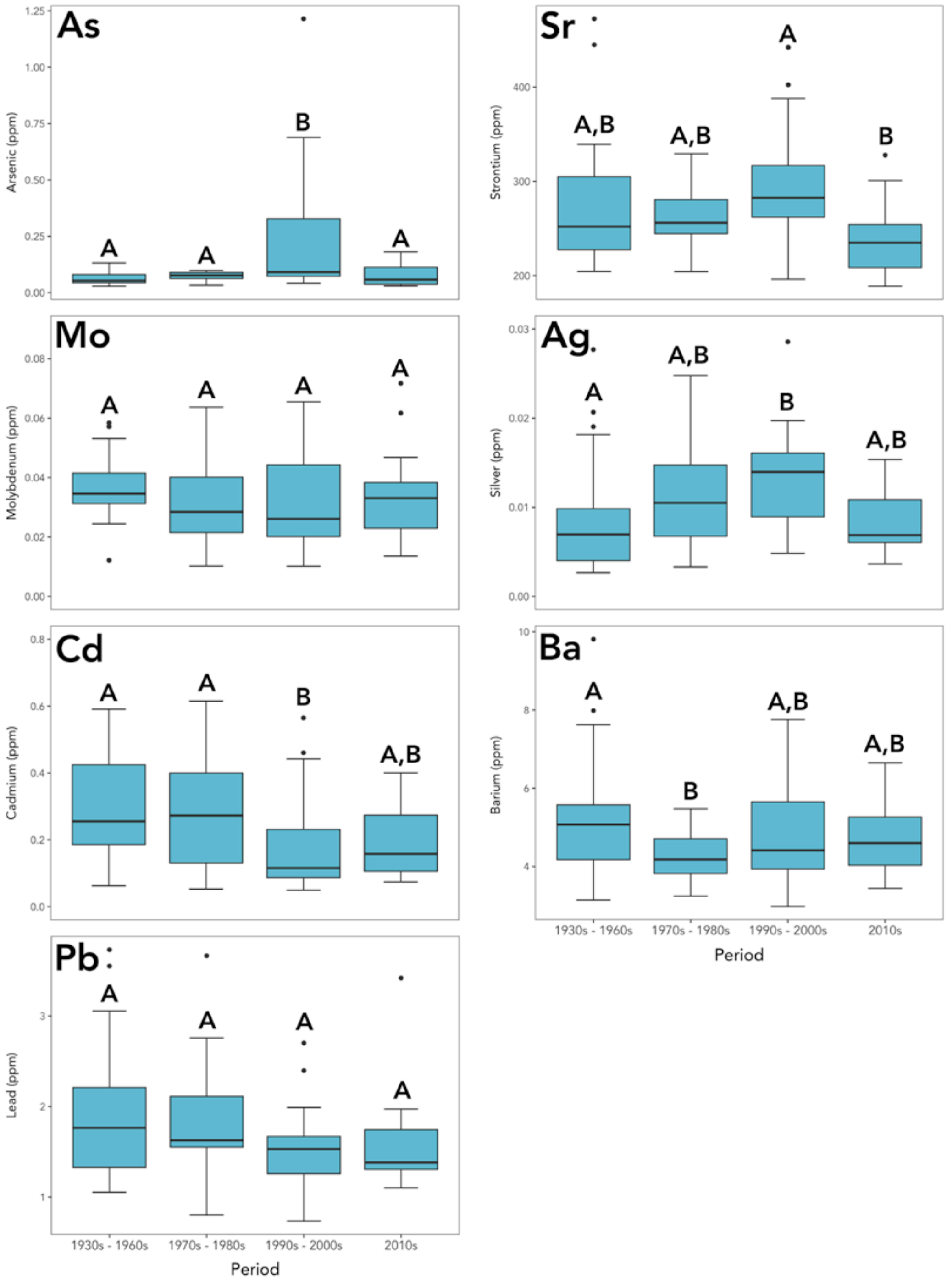

Figure 4. Box plots showing changes in median concentrations (ppm) of seven trace elements in walrus teeth from the 1930s–1960s, 1970s–1980s, 1990s–2000s, and 2010s. Periods that do not share a letter (A, B, C) differ significantly ($p < 0.05$). Medians are indicated by horizontal lines in the middle of each box plot. Bottom and top of each box represent the first and third quartiles of the data, respectively. Whiskers represent the range (minimum and maximum) of the data. Outliers are represented by individual points.

Patterns Within the Lives of Individual Walruses

Six of the 15 trace elements exhibited patterns that were relatively consistent across female walruses examined in this study. Manganese, zinc, and lead tended to increase over the life of a walrus, whereas copper, strontium, and barium tended to decline (Figure 5). The remaining trace elements either showed no trend through time or inconsistent patterns among individual walruses. Zinc and lead were strongly correlated with one another, exhibiting closely matched peaks and troughs in concentrations in addition to their similar overall trends of increase over the lifetime of individual walruses.

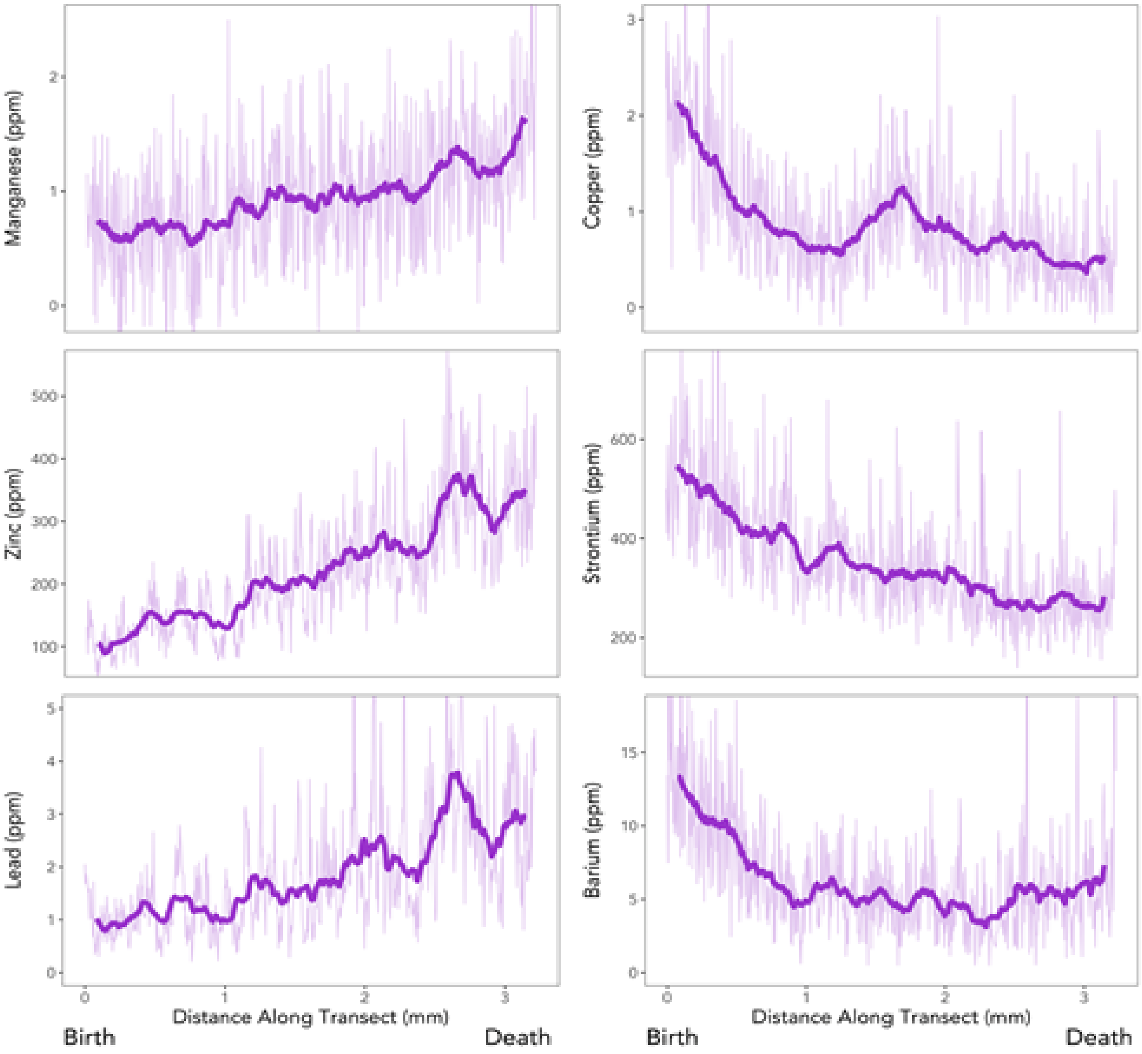

Figure 5. Examples of typical lifetime trends of trace element concentrations (ppm) within the tooth of an individual female walrus. Concentrations of manganese (Mn), zinc (Zn), and lead (Pb) all tended to increase across the life of the animal, whereas copper (Cu), strontium (Sr), and barium (Ba) concentrations typically declined. The thin, light purple line represents the raw data. The dark purple line is a centered moving average with a width of 35 data points.

Zinc and Lead as Potential Indicators of Age at Reproductive Maturity

Overlaying trace element data on tooth photographs revealed further patterns in zinc and lead concentrations that were not apparent in earlier examinations of the data. Both elements exhibited cyclical peaks and troughs that aligned with the dark and light growth layers of the cementum, respectively (Figure 6). Additionally, the rates of increase of both zinc and lead concentrations across the lifetimes of individual walruses tended to change partway through the animal's life. Concentrations of these elements were typically stable or slowly increasing for a number of years before reaching an inflection point, after which the rate of accumulation (slope of the line) increased sharply. This unexpected pattern prompted further investigation as to the nature of these inflection points. During uptake from the gut, zinc and lead bind to the same sites on metallothionein proteins responsible for transporting metals within the body (Ahamed et al. 2007). Zinc is an essential element that is critically important for growth and reproduction (Apgar 1985). Thus, it is likely that the patterns in zinc and lead concentrations observed in this study were driven by physiological changes related to zinc uptake, and that changes in lead concentrations were simply mirroring those of zinc due to the similarity of their uptake pathways. Given this, we focused primarily on zinc while developing hypotheses for the changing rate of Zn/Pb uptake and the inflection points observed in the data for both elements.

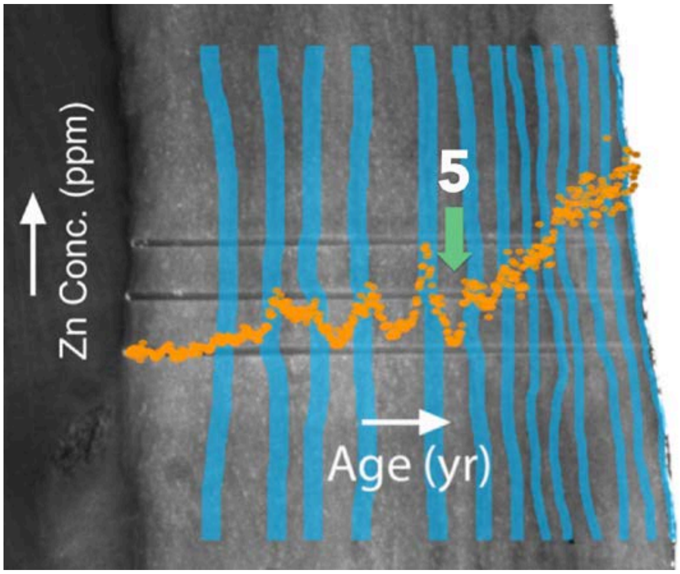

Figure 6. Example of a 13-year-old female walrus tooth with dark growth layer groups highlighted in blue and zinc concentration data overlaid in orange. The green arrow points to an inflection point at 5 years. Pb concentrations exhibited a similar pattern and are not shown.

Given zinc's importance to growth and reproduction and its critical role in pregnancy (Apgar 1985), we explored the hypothesis that these inflection points represented attainment of reproductive maturity for female walruses. To test this theory, the age at which the inflections in zinc and lead occurred was estimated for each animal. The resulting values were compared with literature estimates of age at sexual maturity and first reproduction for female walruses (Fay

1982). The median estimated age at which the inflection point occurred was 5 years, which matched estimates of the average age of attainment of sexual maturity from the literature (5 years; Fay 1982). Furthermore, the range of estimated ages at which the inflection point occurred (2–11 years) was plausible for female walruses (Fay 1982). To further test this hypothesis, the ages at which walruses reached the inflection point in zinc and lead values were plotted against the calendar year in which that inflection point was estimated to have occurred (estimated death year–years of life after estimated inflection point). In this way, we were able to create a timeline of how this parameter changed across the last ~100 years (Figure 7). Changes in the estimated age at which the inflection point occurred closely matched known fluctuations in the size of the Pacific walrus population (Fay et al. 1997), lending support for the hypothesis that the inflection point in zinc indicates attainment of reproductive maturity.

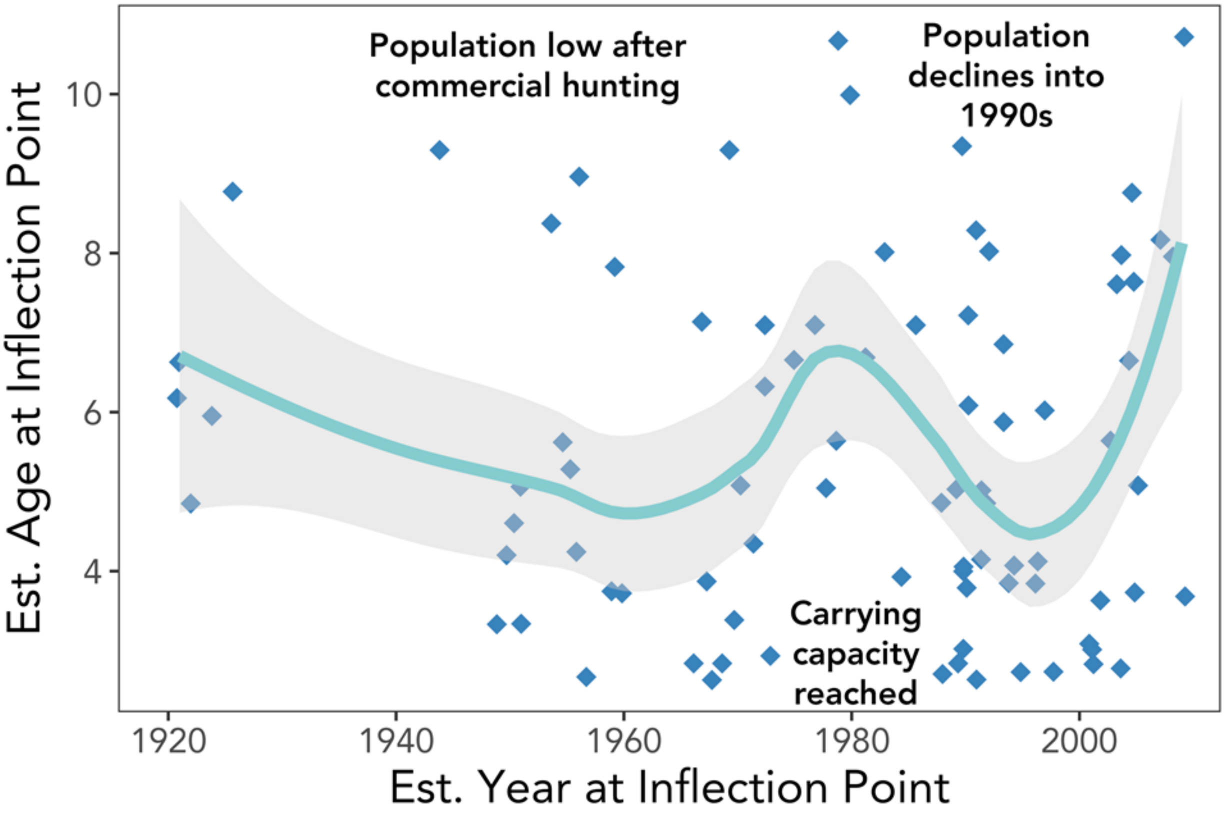

Figure 7. Estimated age at which the inflection point in zinc/lead concentrations was reached (y-axis) plotted against the estimated calendar year in which the inflection point occurred (x-axis; estimated death year–estimated number of years between inflection point and animal's death). The solid teal line is the smoothed conditional mean as estimated by a loess smoother. The gray envelope surrounding the line represents the 95% confidence interval for the smoothed mean. Annotations denote important changes in the size of the walrus population.

Discussion

Baseline Values and Differences Among Time Periods

With the exception of vanadium, iron, and arsenic, the majority of the trace elements measured in the teeth of female Pacific walruses changed very little from the 1930s to the 2010s (Tables 2 & 3, Figures 3 & 4). These data may, therefore, be treated as baseline values against which future changes can be measured. For arsenic, the data from the 1930s–1960s, 1970s–1980s, and 2010s were similar and can likely be treated as baseline values as well. Taken together, the results of this study indicate that petroleum exploration and extraction have not caused substantial changes in the concentrations of trace elements in female walrus teeth. It is possible that trace elements from petroleum-related activities entered the environment, but did so in forms that were not bioavailable. Thus they did not enter benthic food webs. Alternatively, walruses may avoid prey items containing increased concentrations of potentially toxic elements, or some of these elements may experience “biominification” or “biodepletion” as they pass to higher trophic levels (Smith et al. 1990; Outridge et al. 1997). Perhaps a more likely explanation, however, is that contamination from oil and gas-related activities has thus far been minimal and restricted to the areas directly around sites of high-intensity use. This conclusion is supported by examinations of trace element concentrations in Beaufort Sea marine sediment cores, collected in close proximity to drilling sites and at more distant locations (Trefry et al. 2003; 2014). These studies found increased concentrations of some elements (particularly barium) in sediments immediately adjacent to drilling sites and high-use industrial areas, but that sediments a short distance away remained pristine.

In the present study, concentrations of many elements typically associated with drilling activities were often lowest in the teeth of walruses that lived during the period of most intensive petroleum exploration and extraction. This provides further support for the conclusion that contamination from these activities is not affecting walruses, and may not be entering benthic food webs. However, the element vanadium is a possible exception. Concentrations of this element were relatively high in the 1930s–1960s, then peaked in the 1970s–1980s, before diminishing in 1990s–2000s and reaching a minimum in the 2010s (Table 3; Figure 3). Given the correlation between vanadium concentrations in walrus teeth and the intensity of oil and gas exploration in the Alaskan Arctic, it seems feasible that the vanadium in the walrus teeth was, in part, derived from petroleum-related activities. Vanadium is present in high concentrations in Alaska North Slope crude oil and in drilling muds and cuttings on the Alaskan outer continental shelf (Coleman 1978; Naidu et al. 2012). It is possible that the increased vanadium concentrations in the 1970s–1980s resulted from the inadvertent input of crude oil and drilling byproducts into benthic systems. However, analysis of Beaufort Sea sediment cores indicated no significant changes in the composition or concentration of hydrocarbons from 1976 to 1997 (Venkatesan et al. 2013). This suggests another mechanism might be responsible for the observed changes in walrus teeth.

Naidu et al. (2012) detected decreasing vanadium concentrations in marine sediments in Beaufort Lagoon from 1977 to 2003. Near Prudhoe Bay, sediment vanadium concentrations increased from 1977 to 1985 and remained elevated through at least 1997. The authors suggest the trends observed in Beaufort Lagoon and Prudhoe Bay were associated with the closure of a Distant Early Warning Line station and combustion of petroleum products at the Deadhorse gas flaring facility, respectively. These localized sources may have significantly increased vanadium concentrations in local, coastal waters; however, it remains unclear whether such local sources would have released enough vanadium to increase concentrations of this element in walrus teeth. Non-local sources may also be responsible for increased vanadium in the Arctic. The so-called "Arctic Haze" is generated primarily by lower-latitude pollution sources and contains large amounts of vanadium (Rahn and McCaffrey 1980). Changes in the amount or composition of this airborne pollution could be responsible for changes in vanadium concentrations in the walruses observed in this study. Finally, another possible natural source of vanadium for walruses is sessile tunicates, which contain vanadium in high concentrations (Michibata and Sakurai 1990). Pacific walruses are known to consume tunicates, particularly the "sea peach," *Halocynthia aurantium* (Sheffield and Grebmeier 2009). If the abundance of tunicates changed over the study period, it is possible that this could have contributed to the observed differences in vanadium concentrations.

Iron concentrations in walrus teeth increased across the entire study period. Individual walruses did not typically exhibit increases in iron within their lifetimes. Rather, walruses that lived in the 1990s–2000s and 2010s were more likely to have greater concentrations of iron in their teeth throughout their lifetime. Interestingly, not all walruses exhibited these higher iron levels, even in the more recent portions of the study period. Instead, each successive time period contained a greater proportion of walruses with iron concentrations that were substantially elevated above the "baseline" value exhibited by the majority of the animals sampled. The mechanism for this increase remains unknown. When the input of organic matter to the benthos is high, microbial metabolism may release bioavailable iron into the surrounding sediments and overlying water (Van Cappellen and Wang 1996), where it may be taken up by benthic infauna and epifauna. As a result, organisms living in areas with a substantially increased input of nutrients may have higher iron concentrations in their tissues. Walruses eating these organisms might then exhibit greater tooth iron concentrations; however, the viability of this mechanism as a source of iron for walruses remains unknown.

Though generally found in very low concentrations in walrus teeth, arsenic values spiked in walruses harvested in the 1990s–2000s. The exact source of this arsenic remains unknown. Arsenic concentrations in seawater are typically low (Matschullat 2000), but marine sediments may contain this element in higher concentrations as a result of diagenetic processes (Farmer and Lovell 1986; Chaillou et al. 2003). Naturally occurring levels of arsenic in marine sediments are not expected to have changed over the course of the study period. Additionally, these values are typically much lower than those found in locations contaminated by smelting and mining, major sources of anthropogenic arsenic contamination (Matschullat 2000). It is possible that the walruses in this study that exhibited high arsenic concentrations consumed prey that was contaminated by anthropogenic arsenic input into the ocean. Red Dog Mine, one of the world's largest zinc/lead mines, is located in western Alaska. Discharge from the mine drains into the Chukchi Sea near the village of Kivalina. The company that operates the mine has been charged with multiple permit violations related to release or discharge of mining-related contaminants. In 2004 and 2006, wastewater was discharged into Red Dog Creek. Additionally, the mine releases tons of "fugitive dust" each year, which contains high concentrations of metals (Kerin and Lin 2010). It is possible that Red Dog Mine and/or other mining and smelting operations are the source of the increased arsenic concentrations in the teeth of walruses from the 1990s–2000s. Analysis of arsenic in walrus teeth may prove a valuable tool for monitoring the contamination of the marine environment by mining operations. Future work could be conducted on male walruses from Bristol Bay to establish baseline concentrations and monitor for contamination from sources such as Pebble Mine.

Patterns within the Lives of Individual Walruses

Direct absorption from seawater is considered a negligible source of trace elements in marine mammal tissues (Law 1996). Tooth cementum may experience some degree of elemental input from seawater and sediments, as they frequently come into direct contact with the teeth; however, the majority of the tooth is below the gum line, and cementum trace elements are more likely representative of concentrations in the bloodstream and surrounding tissues. The primary routes for trace elements to enter the body are through transplacental transfer prior to birth, through milk when the animal is nursing, and from the diet (Law 1996). Thus, it would be expected that elements for which transfer from mother to offspring is high (either across the placenta or *via* milk) would be found in greater concentrations in younger animals. This is particularly true for elements that are found in low concentrations in the adult diet or are actively excreted from the body. In this scenario, concentrations of such elements in the teeth would be expected to decline from a maximum in the early years of life, as was observed for copper, strontium, and barium in this study. Copper experiences a great degree of transplacental transfer in marine mammals, and the high concentrations in a newborn may be maintained via the milk during the nursing period (Law 1996). These high concentrations typically decline as the animal grows to adulthood, as observed in the teeth of walruses during this study. Information on strontium and barium in marine mammals is sparse, particularly as relates to transplacental transfer and concentrations in milk; however, the results of the present study suggest that walrus calves receive considerable amounts of these elements from their mothers, relative to their dietary intake as adults.

Elements that exhibit increased concentrations over the life of an animal are likely those for which uptake from the diet is greater than transfer from mother to offspring. Increasing concentrations with age can result from the accumulation of certain trace elements within the body or from changes in the rate of element absorption across an animal's life (Honda and Tatsukawa 1983; Tohyama et al. 1986; Law 1996). Little is known about manganese in marine mammals. The increasing concentrations of zinc and lead likely have something to do with the physiological demands of growth and reproduction (discussed in the next section), rather than accumulation in the tissues. Both of these elements are taken up from the gut by metallothionein proteins, which are responsible for the regulation and transport of many metals within the body (Ahamed et al. 2007). These two metals compete for the same binding site on the metallothionein. Thus the proportional uptake of each metal is likely related to its affinity to the binding site. The fact that zinc and lead concentrations in walrus teeth changed concurrently, maintaining the overall proportions of these two metals, suggests changes to their uptake from the gut, rather than their concentrations in the diet. In harbor seals (*Phoca vitulina*), metallothionein levels are positively correlated with age (Tohyama et al. 1986). A similar increase in metallothionein levels with age in walruses would help explain the increase in zinc and lead concentrations across the life of the animal and point to the mechanism responsible for the inflection points observed in the zinc and lead data.

Zinc and Lead as Potential Indicators of Age at Reproductive Maturity

A central concept of population biology asserts that the average age at first reproduction in a population will increase when the population reaches carrying capacity, as increased competition, greater levels of stress and disease, and decreased access to food resources mean that individuals require more time to accrue the energy required to become reproductive (McMahon et al. 2003). Conversely, when the number of animals in a population is well below the capacity of the environment to support that population, resources are relatively plentiful, and competition is low, age at first reproduction is expected to decrease. Though directly linking tooth trace element concentrations to specific life history events is difficult, the evidence compiled in this study suggests that the inflection points observed in the zinc and lead concentrations may be related to attainment of reproductive maturity in female walruses. This hypothesis is supported by the close agreement between the estimated ages at which the inflection point occurred and age at sexual maturity in female walruses (Fay 1982). Examining how these estimates have changed since the early 20th century provided further evidence that the inflection points are somehow related to sexual maturity.

In this study, the estimated ages at which inflection points in zinc and lead occurred closely matched known changes in the size of the walrus population. The Pacific walrus population reached a low in the 1950s as a result of commercial hunting (Fay et al. 1997). The population then recovered and reached or exceeded carrying capacity in the early 1980s, before declining into the 1990s. The average estimated ages at which the inflection points in Zn/Pb were reached exhibited a minimum around 1960, increased to a peak at around 1980, then declined into the 1990s (Figure 7). This correlation between population size and estimated age when the inflection point occurred is the most compelling evidence that this phenomenon is associated with the age at attainment of reproductive maturity for female walruses. The rapid increase in this parameter in the first decade of the 2000s may be an indication that the walrus population is once again approaching carrying capacity, either because walrus numbers are high, or because the capacity of the environment to support the walrus population has changed (or a combination of the two). This technique has the potential to become a valuable tool for monitoring and managing the status of the Pacific walrus population. Additional work should be conducted to develop this method further.

Acknowledgments

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Benthic Habitat Mapping in Eastern Cook Inlet: DISCONTINUED

Principal Investigator: *Amanda Blackburn* Cooperative Agreement Number: M17AC00009

The objectives of this project were to compile and analyze existing data sets from Kachemak Bay, Alaska, to

1) process archived multi-beam backscatter data to a usable form, and

2) construct benthic habitat maps.

The proposed habitat maps were to include classification of substrate, bottom water characteristics, and different habitat types to the maximum possible resolution. The maps were intended to inform management and research related to oil and gas activities by providing information about habitat associations of benthic and demersal species, sediment transport patterns, and potential ties between the pelagic and benthic ecosystems of eastern Cook Inlet.

This project was discontinued due to Ms. Blackburn's departure from the funded graduate studies. A multibeam bathymetric data set was acquired from the NOAA National Centers for Environmental Information. However, the backscatter data was incomplete and not of suitable quality for the intended purpose of the project. Identifying alternate data, adapting objectives, and continuing the project were not possible following Ms. Blackburn's departure.

The Department of the Interior Mission

As the Nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This includes fostering the sound use of our land and water resources, protecting our fish, wildlife and biological diversity; preserving the environmental and cultural values of our national parks and historical places; and providing for the enjoyment of life through outdoor recreation. The Department assesses our energy and mineral resources and works to ensure that their development is in the best interests of all our people by encouraging stewardship and citizen participation in their care. The Department also has a major responsibility for American Indian reservation communities and for people who live in island communities.

The Bureau of Ocean Energy Management

The Bureau of Ocean Energy Management (BOEM) works to manage the exploration and development of the nation's offshore resources in a way that appropriately balances economic development, energy independence, and environmental protection through oil and gas leases, renewable energy development and environmental reviews and studies.