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Prehistoric and Modern Ecological Dynamics in Southern South American Marine Food Webs

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ABSTRACT OF THE DISSERTATION

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Marine food webs in coastal southern South America are thought to have been impacted over time in response to humans in the late Holocene to the Anthropocene. Archaeological sites on the coast of Tierra del Fuego, Argentina, provide a biogeochemical record that can potentially inform us about ecological dynamics over this time period. This record appears as bone collagen from Otariids, southern fur seals (Arctocephalus australis) and sea lions (Otaria flavescens), high trophic level predators. To quantify ecological relationships, we measured bulk and compound specific stable isotope ratios from organic tissues in Otariids and several other associated animals, several of which were potential otariid prey and basal food web resources. Variations in bulk stable isotope ratios of carbon and nitrogen are linked to potential dietary differences and habitat specialization (coastal areas or open ocean) in populations ranging in age from 7000 cal. years BP to the modern day. We observed increases in the variability of these isotopic compositions over time, which suggests a diversity in the diets and habitats of Otariids. Shifts in marine food webs occurred during the transition

from subsistence hunting of Otariids to industrial hunting and expanded human influence. $\delta^{13}C$ ratios of amino acids suggest shifts from coastal to offshore foraging in otariids from past to present, while $\delta^{15}N$ in amino acids showed little change in overall ecological baseline shifts but large variation in trophic level and foraging location in individual otariids. $\delta^{2}H$ dynamics in amino acids largely corroborated results found in $\delta^{13}C$ and $\delta^{15}N$ amino acids. We conclude that direct human influences, such as hunting and habitat alteration, were the major drivers of ecological change in southern South American marine ecosystems rather than climate change.

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Introduction

There are two clear drivers that have shaped the diversity and distribution of organisms in both terrestrial systems in South America and marine systems off the coast of South America: the movement and organization of tectonic plates, allowing or disallowing dispersal, and changes in climate, which acts as an environmental filter. The movement of tectonic plates operates on the scale of millions of years and slowly ease or hinder the transfer of genes geographically. Climate change, on the other hand, can act fast (Zachos et al., 2001). In recent years, climate has been altered at a rate heretofore unseen due to humans (IPCC, 2014) who have also had a hand at filtering out numerous species, and threaten to reduce biodiversity significantly more in the near future (Barnosky et al., 2012).

Elucidating the relationship between humans and other lifeforms, from microbiota to macroecological scales, since *Homo sapiens* emerged has often been troublesome to discern (Lande, 1998). Many approaches in different disciplines have been proposed to consider this relationship since the foundations of science dating back to antiquity, from Plato's philosophical discussion of what distinguishes humans from animals (Kitts, 1987) to statistical models based on the laws of physics and chemistry that precisely define this relationship quantitatively (Muhar et al., 2017). While gross scales of human influence on flora and fauna are well understood in the contemporary paradigm (IPCC, 2013) much uncertainty remains in the finer details.

This uncertainty exists both temporally and spatially: Observations of ecosystems and climate are arguably adequate for understanding the 20th and 19th centuries but begin to falter as we move back in time (Barnosky et al., 2017). Before this time the technology for understanding essential components of Earth's life, climate and geography were rudimentary or simply didn't exist; global satellite imagery, thermometers, techniques for surveying landscapes and the like were not invented, much less mature, until relatively recently. Even modern techniques for testing ecological theory are often troublesome (Cadotte et al., 2017). Spatially uncertainty in our understanding of earth processes exists due to sociopolitical inequity geographically. Despite efforts, developed nations associated with access to more research funding and education are still far better studied than the rest of the world (PISA, 2016). This necessarily results in a problem: How do we understand Earth's past throughout different regions?

Proxies exist in the form of physical and chemical remnants of past events and can be used to reconstruct snapshots of species interactions between humans and animals (Davis and Pineda Munoz, 2016). One particularly strong tool for this is the measurement of stable isotopic ratios (West et al., 2006). Isotopic tools are useful not only for understanding species interactions but for climate (Hamilton, 2009), as well as informing our understanding of many different processes where kinetic or equilibrium isotopic effects occur and are recorded (Lehmann, 2016).

Such techniques are especially useful to answer questions that span multiple disciplines, including paleoecology, archaeology and anthropology: Did humans alter ecosystems prehistorically as they do today? In this dissertation, I set out to identify potential differences in how human behavior towards may or may not have resulted in ecological changes throughout time in marine southern South America.

I hypothesize that human impacts overtake climatic impacts as the primary cause of changes in the food web as time progresses. Human impacts manifested themselves both directly through hunting and indirectly by alteration of habitat for prey and seals, which has cascading effects on the rest of the food web (Rick and Erlandson, 2008; Doney et al., 2012). Climate and ecological changes appear unlikely as the Holocene is characterized by a relatively stable climate and there is little reason to suspect non-human ecological shifts (Kilian and Lamy, 2012). To explore the nature of human interactions with the marine community of Tierra del Fuego, I seek to quantitatively define the relative influence of humans, climate and ecological interactions on marine food web structure and ecological dynamics through several biogeochemical analyses to distinguish whether these variables impacted animal diets, food chain length or habitat or prey specialization spatially or temporally.

Coastal and offshore diets are recorded in the stable isotopic signature of marine animals. Using these measurements, I expect to see a distinction between animals that primarily rely on coastal kelp forest ecosystems or feed in benthic or pelagic settings, where phytoplankton comprise the dominant primary producers (Larsen et al., 2013).

Beyond this spatial distinction, I expect to see shifts in diet preference between these two end members temporally. Three distinct time periods distinguish different paradigms of human interactions with the marine community of Tierra del Fuego.

The prehistoric time, lasting from 7000 years until 150 years ago, is known from archaeological sites and shows gradual change in human activity but is evidenced by marine resource procurement for subsistence purposes. Previous research (Zangrando, 2009b) shows that among vertebrate remains in archaeological shell middens excavated in Tierra del Fuego, Otariids are the most abundant species and decline through time while other terrestrial and marine resources, such as fishes, birds and guanacos, become more abundant. These findings raise questions about dietary preference and prey availability to humans, whether they were ecologically or technologically limited, or simply had cultural preferences for certain prey items over others. Regardless, overhunting or habitat degradation should result in effects to populations of Otariids, reflected by gradual shifts their diets over time, contemporaneous with changes observed between archeological sites.

The second time period is characterized by rapid European colonization, the arrival of industrial hunting of pinnipeds, and decline of indigenous resource procurement. This period of time, lasting between 200 years ago until the 1950s, was characterized by massive declines in pinniped populations in in the southern hemisphere (Nye, in press). Commercial fishing of species important for marine food webs of southern South America also grew during this time. In contrast to the gradual

shifts that are expected to be observed in the previous time period, rapid changes in Otariid diets are expected. However, these shifts may result in a counterintuitive benefit to diet quality in the surviving Otariids: I predict a general shift in stable isotopic values from a lower trophic level to a higher trophic level as a smaller Otariid population results in less interspecific and intraspecific competition (Vales et al., 2016). It also appears likely that Otariid populations would have retreated to more remote areas and would have a stronger preference for feeding in benthic or pelagic ecosystems rather than become easy targets in easily accessible kelp forests.

The last, shortest and most recent time period lasts from the 1950s and continues to this day. It is distinguished by the recovery of marine mammals due to bans on hunting them at the beginning of this period. Despite the stoppage of hunting of marine mammals, which has resulted in population recovery of Otariids, commercial fishing continues has increased globally (Rick and Erlandson, 2008). As the Otariid populations reach their carrying capacities and prey items are exhausted, whether from Otariids or fisherman, trophic levels will likely decrease. However, due to lack of hunting pressure and habitat restoration, Otariids may well have a stronger coastal signal in their diets as they recolonize areas abandoned earlier.

Biogeographic and geologic context

South America has traditionally been viewed as an isolated continent, leading to the perception of "splendid isolation" of floras and faunas (Wilf et al., 2013). South America, as part of Gondwanaland, diverged from Laurasia (the former being the

southern supercontinent and the latter the northern supercontinent derived from Pangea) in the mid to late Mesozoic. Full isolation of South America occurred in the Eocene, when Gondwanaland split into Australia, Antarctica and South America (Prevosti and Forasiepi, 2018d).

Though most South American organisms are derived from Gondwanaland, many Laurasian relatives traveled the shallow early Atlantic Ocean as the land masses were diverging. Notable Laurasian taxa that dispersed after isolation of South America include therian mammals from the late Cretaceous (Prevosti and Forasiepi, 2018c). Organisms also dispersed over the open ocean long after the South American continent drifted from Africa and the rest of Gondwanaland, evidenced by similar flora observed in western Africa and eastern South America (Wilf et al., 2013). Laurasian organisms did not migrate en masse again to South America until the relatively recent "great American interchange" of the Pliocene, the connection of North and South America (O'Dea et al., 2016a).

During the period after therians immigrated to the continent (~115 Ma), much of the flora and fauna developed into recognizable South American organisms, such as those found in the clade Xenartha, including *Megatherium* (Prevosti and Forasiepi, 2018d). The relatively recent formation of the Panama isthmus (~15-3 Ma) resulted in a major transformation of the ecology of South America as many North American organisms traveled and colonized the continent (O'Dea et al., 2016). Many of the

animals currently populating South America, including the iconic llamas, were introduced during this time (Domingo et al., 2012)

The distribution of fauna found during the late Pleistocene and the Holocene, including llamas, have largely been found to be shaped by dispersal paths through the South American continent: A western path (the Andean Corridor) and an eastern path. Isotopic evidence points to these different pathways led to the speciation of different floras and ecological patterns even before immigrants from North America came south, resulting in diversification of immigrant faunas (Domingo et al., 2012). Climate changes during the late Quaternary acted as a strong environmental filter limiting the types of flora throughout South America which in turn altered diversity of the more recent immigrants (Prevosti and Forasiepi, 2018a).

The coasts of South America have similarly been subject to significant climatic events, which in turn resulted in biogeographical changes over time (Kilian and Lamy, 2012). The Pinnipeds (seals and sea lions) underwent significant dynamism in the Cenozoic (Berta et al., 2018). In the Plio-Pleistocene, a major transition from Phocidae (true seals) to Otariidae (sea lions and fur seals) occurred in South America, as phocids were largely extirpated from South American coasts (Churchill et al., 2014). This transition is similarly found throughout the Southern Hemisphere, with contemporaneous phocid to otariid dominance in South Africa and New Zealand (Valenzuela-toro et al., 2013).

Biotic factors related to both climate and plate tectonics, such as evolutionary history, can explain anomalies in biogeographic patterns that cannot be explained by climate or the movement of tectonic plates alone (Segovia et al., 2013). For example the niche variation hypothesis, that populations with more broad niches should have more diet specialization within individuals, plays an important role in passerine bird populations found in central Chile (Karin et al., 2017). Variation in nitrogen isotope ratios in populations with smaller niches had fewer specialists, related to their phylogeny. Likewise, marine systems offshore of South America also show evidence of niche variation and specialization as populations of fur seals and sea lions colonized the South American coastline (Drago et al., 2017). These patterns suggest that evolutionary history is an important link between climate and tectonics in shaping diversity and distribution of species, contributing to the very biologically diverse marine ecosystem around southern South America today (Friedlander et al., 2018).

Past and current climate

Recognizable landforms and bodies of water that affected both the biogeography of marine and terrestrial organisms, as well as human colonization later, formed in the Cenozoic. The coalescence of Antarctica around the south pole and the emergence of the circumpolar current not only induced glacial-interglacial cycles globally but resulted in the major ocean currents that regulate climate in South America today (Prevosti and Forasiepi, 2018d). The Andean Orogeny produced massive mountains that restricted dispersal of organisms from the Pacific and Atlantic oceans

and have also impacted climate in South America. The high elevation of Andes are responsible for a significant rain shadow, creating the dry Pampas of southeastern South America and large ice sheets, such as the Darwin Cordillera (Heusser, 1998; Rabassa et al., 2000). The large glaciers of the Pliocene and Pleistocene scoured great valleys throughout southern South America, creating the fjords along the western and southern coasts of the continent and leaving behind glacial sedimentary features, such as drumlins. Marine transgression and regression created various terrace features well into the Holocene.

Southernmost South America is characterized by a high latitude sub-antarctic climate, including Patagonia and the Patagonian archipelago, the straits of Magellan, the island of Tierra del Fuego, and the Beagle Channel. Today this region contains diverse terrestrial climates, including the southern Andes, ice sheets (the Darwin Cordillera), wetlands and bogs, deciduous forest and grasslands. The Southern Ocean dominates the direction of surface water flow with the eastward flowing Antarctic circumpolar current. Cold nutrient rich waters traveling with this current in the Pacific Ocean hit the continent's sharp western margin and break into the Humboldt and Cape Horn currents, which travel north and south parallel to the coastline, respectively. As the Cape Horn and Antarctic circumpolar currents bend east around Drake Passage and the southern tip of continent, the water flows over the broad and shallow Atlantic continental margin northward as the Falklands/Malvinas Current, which extends out to the Falklands/Malvinas Islands. The Cape Horn and Malvinas currents meet in the channels

that divide the Patagonian archipelago, creating a blend of cold nutrient rich Southern Ocean water, relatively nutrient starved Atlantic water, and terrestrial freshwater (Acha et al., 2004).

The biogeographic patterns of marine vertebrates vary between the coasts of Pacific and Atlantic Oceans near South America. Off the Pacific coast, vertebrates show a rise and fall in diversity during the Neogene (Villafaña and Rivadeneira, 2014). An exceptional increase in diversity along the temperate Pacific coast of South America, unlike the global trend, is tied to the formation of the Humboldt Current System. This current originated during the Miocene climatic optimum and is associated with coastal upwelling. These conditions led to warm, productive waters that favored diversification. A major, non-random turnover in vertebrates occurred later during the Plio-Pleistocene transition when the loss of productive coastal environments due to the formation of the Atacama Desert and marine transgression (sea level rise and tectonic subsidence). The resulting trophic cascades propagated by these climate shifts proved to be the death knell for many organisms.

Some of the marine vertebrate taxa affected by past climate change include phocids. Pinniped turnover from phocids to otariids has been ascribed to climate forcings that limited habitat for phocid haul out sites and rookeries. Marine transgression would have decreased the number of sandy beaches, preferred haul out sites for phocids, and increased rocky shorelines. Alternatively, oceanographic characteristics that changed productivity (as described above) may have limited

resources. These hypotheses are not mutually exclusive, so a combination of these factors may have led to the loss of phocids and created opportunity for otariids to colonize empty niches left behind (Valenzuela-toro et al., 2013).

Tierra del Fuego experienced deglaciation during the Plestiocene-Holocene, with definitive ice retreat occurring around 10 ka (Heusser, 1998; Borrero, 1999). During this time the terrestrial environment transitioned from tundra and cold steppe into subantarctic forest, characterized by *Nothofagus* trees seen today (Heusser, 1989; Rabassa et al., 2000; Markgraf and Huber, 2010). Paleoenvironmental records such as oxygen isotopes from bivalve shells (an SST proxy; Gordillo et al., 2015), pollen analyses of plants associated with different terrestrial climates (Ponce et al., 2017) and sediment records from lakes (Waldmann et al., 2010) suggest that a climatic optimum was achieved in the middle Holocene (3500 and 1000 BP), followed by cooling in the late Holocene associated with the little ice age ca. 500 BP with the advance of glaciers and drop in sea level during this time (Zangrando et al., 2016).

Deglaciation results in increased sediment input from the land to the sea, bringing a rich supply of nutrients that boosts oceanic productivity (Acha et al., 2004). Melting of glaciers and high precipitation in the Fuegian archipelago results in cooler and fresher water than the exposed shelf (Rabassa et al., 2000). Nitrogen isotopic measurements of coastal and Beagle Channel waters suggests macronutrient inputs from runoff from the continents, however micronutrients are introduced from the Antarctic circumpolar current (Garzón et al., 2016). Otariids likely benefitted from these

productive waters as humans began to exploit them in the middle Holocene (Saporiti et al., 2014b).

Climate records from sediments have shown some dynamism in climate from the Holocene to the present. The dominant climate forcing in southern South America is related to westerlies, which influence both temperature and precipitation. Pollen and spore analysis from Tierra del Fuego indicates periods of relative humidity and warming alternating with cool dry periods (Mansilla et al., 2018). Coincident with the first human occupations of Tierra del Fuego is a humid period lasting until 11 ka, followed by dry periods at 10.5-10 ka and 8.5-6.5 ka, with more moist conditions thereafter (Mansilla et al., 2018). Records of plant material in peat bogs agree, indicating changes in increases in precipitation and warming in the late Holocene, associated with changes in the southern westerly wind belt (van Bellen et al., 2016). On decadal scales, these westerlies show cyclical variations in strength, likely due to Antarctic Oscillation or El Niño Southern Oscillation.

Ecology and food webs

Ecologists have used several theoretical frameworks to explain the relationship and assembly of ecosystems in time and space, often using either environmental explanations (Jackson and Blois, 2015), biological interactions (Leibold et al., 2004; Lessard et al., 2012) or neutral-stochastic processes (McGill et al., 2006) to explain community assembly. The identification of niche partitioning, and evidence of biological interactions via dietary reconstruction is useful for understanding community assembly

in southern South America. Ecologists often use the niche as a conceptual tool to help elucidate which of these agents or interactions may be responsible for assemblages of species within an ecosystem. The niche concept itself mirrors the divisions in theory of species assembly, with the Eltonian and Grinnellian niches and these niches are often usefully considered as scenopoetic (environmental) and bionomic axes, as described in Hutchinson's n-dimensional hypervolume (Hutchinson, 1978; Chase and Leibold, 2003). Analysis of food webs and the associated trophic structures is one way to clearly define the bionomic axis and indirectly the scenopoetic axis of an organism's niche, as the base of a food web is directly dependent on abiotic factors (Newsome et al., 2007).

To fully characterize an ecosystem, it is useful to understand the niches of species that specify different trophic levels (Cabral and Kreft, 2012; Lessard et al., 2012). High trophic level organisms with varied diets present an optimal study organism, as they inherently reflect their diets and ultimately the ecosystem as a whole (Newsome et al., 2007). Understanding how species found in zooarchaeological settings were affected by the introduction of humans can elucidate how ecological communities react to human disturbances (Zangrando et al., 2014a).

One of the most powerful proxies to distinguish niches between different organisms is by studying diets. Diets can largely be studied three ways: direct observation, analysis of stomach contents or fecal matter, or indirectly through the analysis of stable isotopic ratios of elements incorporated into the tissues of organisms

(Franco-Trecu et al., 2013). While each of these have their own limitations, only analysis of the remains of tissues is generally viable for archeological specimens.

Stable isotope analysis (SIA) has long been used to identify ecological and environmental characteristics on many different scales. In biological and ecological applications, stable isotope studies have been used to great effect in analyses of tissues in individuals within a species to large scales of meta-communities (Michener and Lajtha, 2008; Ben-David and Flaherty, 2012). SIA is used to approximate theoretical variables of the niche, with certain elements representing scenopoetic and bionomic axes (Newsome et al., 2007; Rossman et al., 2016). Though the isotopic niche may not perfectly conform to the ecological niche, the isotopic niche remains useful as other methods to identify an organism's niche are often not feasible for many studies, such as paleoecological cases where often only proxies can be used.

Stable isotope geochemistry has been well established as a method for identifying marine trophic relationships as well (Newsome et al., 2010). Stable isotopes fractionate as they are shuffled via chemical reactions, with heavier isotopes becoming more abundant in higher trophic levels (Fry, 2006; Sharp, 2017). SIA can be applied to many different types of tissues, however bone collagen represents an excellent resource for representation of animal diets over several years of the animal's lifespan due to its long turnover rate, while often being the only available tissue in archaeological sites (Koch et al., 2009; Zangrando et al., 2014a). Stable isotopic measurements are reported

in delta notation, which relates the ratio of the heavy to light isotope in the sample to a standard. Values are expressed in parts per mil (Equation 1).

Equation 1: $\delta X_j = \left[\frac{\left(\frac{X_j}{X_i}\right)_{Sample}}{\left(\frac{X_j}{X_i}\right)_{Standard}} \right] - 1$

Where X_j represents the heavy isotope and X_i the lighter isotope of carbon or nitrogen. In organic tissues of consumers, $\delta^{13}C$ values generally reflect habitat preferences and trophic levels, while $\delta^{15}N$ values reveal dynamics in ocean productivity, nutrient sources as well as trophic position (Michener and Lajtha, 2008). $\delta^{2}H$ relates to sources of water consumed by an organism, however, significant metabolic fractionation occurs as well (Newsome et al., 2017).

While isotopic ratios of bulk collagen can provide significant insights about an organism's behavior or environment, analysis of individual amino acids (AAs) can elucidate even more information. In nitrogen, some AAs fractionate with trophic level as they undergo changes in animal's given biochemistry (trophic AAs) while others are hardly altered (source AAs). With compound specific analysis of $\delta^{15}N$ one can differentiate food web dynamics and baseline shifts in productivity as source AAs are largely climate controlled while trophic AAs may indicate ecologically independent differences (Chikaraishi et al. 2014, McMahon 2010). A common metric for measuring food chain length (FCL) is to estimate trophic discrimination factors (TDFs), or fractionation between each trophic level (Bradley et al., 2015; McMahon and McCarthy, 2016; Ishikawa et al., 2017). TDFs are calculated by correcting the difference between a

trophic and source AA, with the most commonly used trophic and source AAs used for this metric being glutamic acid (Glu) and phenylalanine (Phe), respectively. However, problems in assumptions of biochemical pathways and resulting fractionation that lead to the creation of AAs in animal tissues may mean relying these two individual AAs solely is problematic, and recommended standard practice is to view the relationships of other source and trophic AAs to corroborate Glu-Phe results (Bradley et al., 2015; McMahon and McCarthy, 2016). Corrections to estimating trophic position and food chain length vary significantly between different consumers due to differences in diet quality; or whether an organism needs to synthesize its own AAs. In effect, careful consideration of diet quality, availability of AAs, and nitrogen excretory pathways needs to be taken in account when calculating FCL of a consumer (McMahon and McCarthy, 2016). When consideration of different TDFs are applied, measurement of $\delta^{15}N$ in AAs is extremely useful for trophic studies.

While nitrogen is largely useful for identifying trophic position, measurements of $\delta^{13}C$ of AAs is useful for identifying the pathway of primary production that led to the formation of a tissue in an organism (Newsome et al., 2014). For example, whether a sea lion's essential amino acids were formed originally from kelp, phytoplankton or bacteria. Essential amino acids carbon isotope ratios are largely unaltered from The identification of the commonality in essential amino acids due to peculiarities of primary production falls into phylogenetic categories, forming an isotopic "fingerprint" (Larsen et al., 2013). Thus, with a $\delta^{13}C$ measurement of essential amino acids in a consumer, one

could match a fingerprint with a consumer. Non-essential $\delta^{13}C$ of AAs on the other hand reflects biochemical pathways of formation in consumers, similar to $\delta^{15}N$ (Hare et al., 1991).

Hydrogen isotopic ratios of AAs are less well constrained as an indicator of trophic level and food web dynamics, though they this isotopic system has been shown to contribute to these subjects (Newsome et al., 2011). However, with the knowledge that $\delta^{2}H$ values in bulk organic tissues reflects both the water consumed by organisms, as well as metabolic fractionation (Newsome et al., 2017), active investigation into exploration of differences in primary producers similar to the fingerprinting analyses developed for $\delta^{13}C$ measurements is ongoing. Meanwhile, large TDFs are being found in consumers, including marine mammals such as polar bears and sea lions. Proline, for example, is highly enriched in $\delta^{2}H$.

Study organisms

Several distinct marine biomes exist near the coasts of southern South America. Nearshore rocky habitats are common in temperate, sub-polar and polar waters associated with large macrophytes that alter the water column by slowing down the flow of seawater, reducing light penetration, and increasing sedimentation and oxygenation (Bruno et al., 2017). The large macrophytes, brown algae commonly known as kelp, are keystone species that form forests supporting a vast diversity of wildlife. The most well-known of these species is *Macrocystis pyfera*, found in abundance in southern South America. Kelp forests are arguably the most productive of cold water ecotypes

supporting diverse communities of invertebrates, fish and marine mammals (Friedlander et al., 2018).

Beyond rocky shorelines where depth becomes too much for kelp forests to grow, the open ocean provides a less concentrated but still significant source of energy for marine organisms (Friedlander et al., 2018). Food webs less complex than those found in kelp forests are supported by phytoplankton. Higher trophic level organisms often feed either in the pelagic or benthos, though much of energy in higher in the food chain is derived from benthic grazing and scavenging (Riccialdelli et al., 2017).

Isotopic measurements of marine primary producers show significant differences between macroalgae and phytoplankton (Larsen et al., 2013). Macroalgaes such as *Macrocystis* appear enriched in $\delta^{13}C$, likely due to light and CO_2 availability (Drobnitch et al., 2017). Phytoplankton, on the other hand, show more depleted $\delta^{13}C$ values due to higher availability of light and CO_2 in the open ocean. Terrestrial nitrogen inputs to coastal systems result in enriched $\delta^{15}N$ in nearshore areas in the Fuegian archipelago (Garzón et al., 2016). As a result, coastal and pelagic primary consumers reflect these variations in isotopic values, with migratory animals resulting in a mix between these two ecotypes (Hopkins and Ferguson, 2012; Parnell et al., 2013).

Otariidae, the family of fur seals and sea lions, diverged in the Pacific and colonized the southern hemisphere, exploiting optimal climatic conditions, 6 to 7 Ma (Valenzuela-toro et al., 2013; Churchill et al., 2014). Phocids, or true seals, were dominant on South American shorelines prior to this time. However oceanographic

changes, largely repeated transgressions, resulted in the reduction of sand beaches and increased the abundance of deeper coastal waters and rocky shorelines. Sandy beaches being the preferred haul out and breeding sites by phocid were likely reduced enough to extirpate populations from South American shores and led to colonization by Otariids. Otariids are completely dominant among pinnipeds along the South American coastline by the late Pliocene and Pleistocene, with modern forms of *Otaria* and *Arctocephalus* present in the Pleistocene (Valenzuela-toro et al., 2013; Villafaña and Rivadeneira, 2014).

Today, two species of Otariids dominate the South American coast line: *Arctocephalus australis*, the South American fur seal, and *Otaria flavescens* (sometimes referred to as *O. byronia*), the South American sea lion (Cappozzo et al., 2008). *Arctocephalus*, the genus that contains all fur seals (except the Northern Fur Seal, *Callorhinus ursinus*), has been subject to taxonomic shuffling since the first descriptions of Fur seals to the present day with continued disagreements over classification. Prior to genetic classification, *A. australis* was often confused for *A. galopagensis* due to similar appearance (Bonner, 1981). *A. australis* was divided into two subspecies during this era based on skull morphometrics (*A. australis australis* and *A. australis gracilis*), (King, 1954; De Oliveira and Brownell, 2014), however, this distinction was later disregarded. Phylogenetic arguments point to the traditional genus designation *Arctocephalus* being paraphyletic, leading some researchers to insist reclassifying most fur seals into a new genus, *Arctophoca*, and only the Brown fur seal (*A. pusillus*) retaining the genus

Arctocephalus (Berta and Churchill, 2012). However, the marine mammal community have continued the traditional use of *Arctocephalus* as skeletal morphometrics show continuity between members of the genus (De Oliveira and Brownell, 2014; Tarnawski et al., 2015) and further phylogenetic analysis suggesting the differentiation proposed by Berta and Churchill was premature (Nyakatura and Bininda-Emonds, 2012). Within the South American fur seals, *A. australis*, a population off the coast of Peru has been identified as different enough to justify the designation of different subspecies, but is as of yet unnamed pending further investigation to its geographically adjacent relative, the Galapagos fur seal, *A. galopagensis* (De Oliveira and Brownell, 2014). A definitive classification of fur seals in South America remains elusive, though Fuegian fur seals clearly belong to *A. australis*.

As high level predators of the Beagle Channel, modern Otariids apply top down pressures on marine community composition (Franco-Trecu et al., 2014). One way they do this is by reducing populations of lower level marine predators like fish and squid. However, evolution of Otariid influence on food webs in this region over the past 10 Ka are poorly known (Zenteno et al., 2015). Modern populations of fur seals and sea lions in southern Argentina are largely limited to the fringes of Tierra del Fuego (Franco-Trecu et al., 2014; Zangrando et al., 2014b). Top level predators that forage closer to the coast near Tierra del Fuego have been shown to have a higher trophic level than those that forage in deeper oceanic waters, given that a larger food web exists near the coast due to higher nutrient and energy availability (Riccialdelli et al. 2010). Other than humans,

the only predator of Otariids includes Orcas, *Orcinus orca* (Baylis et al. 2015). Most colonies are located within the continental shelf break, with fur seals and sea lions feeding on outer shelf species (Franco-Trecu et al. 2014). The majority of observed dietary components in modern southern fur seals are cephalopods, including the Patagonian squid (*Loligo gahi*) and fish, including the Falkland herring (*Sprattis fugensis*) and various Patagonothen species. Crustaceans make up a small portion of the Southern Fur seal's diet (Baylis et al. 2015, Vales et al. 2015; Casper et al. 2006). *A. australis* and *O. byronia* undergo dietary shifts between different life history stages, much like other marine mammals (Vales et al. 2014, Zenteno et al. 2015). Southern fur seals have a short weaning period within their first year, attaining high quality protein from milk (Cane et al. 2005). Younger, smaller fur seals will hunt for prey closer to shore while older bigger adults have access to prey from deeper and more distant waters, with some hunting beyond the continental shelf break (Franco-Trecu et al. 2014).

Mitochondrial DNA evidence shows that *A. australis* suffered a large bottleneck between 64 and 110 Ka, likely due to intensive glaciation during this time (Tunez et al. 2013). After this event, populations largely remained constant, with little variation in population structure within the past 5 Ka. While population changes during pre-European human contact times are unknown, hunting during the last few centuries of industrial sealing resulted in dramatic declines for *A. australis*. By the mid-20th century, just under 30,000 individuals remained. In the late 20th century, populations rebounded to 280,000 individuals, with around 5,000 located in the Tierra del Fuego/Isla Estados

region (Vales et al., 2013). Today, *A. australis* is split into three different genetic ecological subunits (ESUs) in northern Chile/central Peru, Tierra del Fuego, and Uruguay (Túnez et al., 2013).

Overlap in niche preference has been identified in modern populations of both the South American sea lion (*O. byronia*) and the South American fur seal (*A. australis*). Observations of both stomach contents and stable isotope ratios from tissues of modern *Otaria* show benthic and pelagic dietary preferences, including feeding on various fish and Patagonian squid (Baylis et al., 2014; Zenteno et al., 2014). *Arctocephalus* off the Patagonian coast share many of the same dietary preferences, however their diets vary with ontogeny (Vales et al., 2015). Such overlap is argued to reflect a lack of competition due to the collapse of Otariid populations during industrial hunting in the 19th and 20th centuries, however, niche separation appears to be increasing as populations of both species recover (Drago et al., 2017).

Archaeological context

Coastal sites have been occupied since at least 6 ka, when sub-Antarctic forest was found in the lower elevations (Borrero, 1999; Orquera et al., 2011). The indigenous population that occupied southern South America developed into various groups identified by ethnographers in the late 19th century, including the Yamana (or Yaghan), the Alakuf, and the Selknam which shared many cultural aspects but varied in the language, rituals, and diet preferences (Lothrop, 1928). All of these groups were semi-nomadic hunter gatherers, relying on terrestrial and marine resources to sustain

themselves. The Yamana and Alakuf relied more on marine resources than the Selknam, who largely occupied the interior of the island of Tierra del Fuego.

Archaeological investigations of southern South America began with the first contact with indigenous cultures in the 17th and 18th centuries (Lohrop, 1928; Orquera et al., 2011) however zooarchaelogical focused research did not begin in earnest until the late 1970s and 1980s with excavations of sites southern coast of the island of Tierra del Fuego by Orquera and Piana. With these investigations, archaeologists found substantial evidence of marine mammal exploitation, primarily of pinnipeds. Indigenous peoples exploited cetaceans when available, though lacked the means to actively hunt them and relied on intermittent strandings to obtain cetacean resources.

The first humans arrived in southern South America following the Last Glacial Maximum, during the Pleistocene-Holocene transition. The earliest known sites of human occupation in Patagonia from 14 Ka at the Monte Verde site, on the Pacific side of the Andes (Dillehay et al., 2008). Evidence of human occupation to the east of the Andes in Patagonia are no older than 9.5 Ka, and are more commonly known at 8-7 Ka (Miotti and Salemme, 2003). On what is currently the island of Tierra del Fuego, which was connected to the mainland due to lower sea level, the Tres Arroyos site dates back to 10 Ka with evidence of human exploitation of extinct and extant animal resources (Massone, 2003). Occupation of these early sites are discontinuous, which is attributed to fluctuations in climate during this volatile time (Miotti and Salemme, 2003).

The most significant climate fluctuation of the Holocene was marine transgression, lasting from 8-7 Ka to 4.5 Ka. It was during this time that evidence of marine hunter gatherers appeared (Orquera and Piana, 2009). Sites of human occupation are evidenced be shell mounds and middens that were occupied discontinuously, but often repeatedly. The earliest coastal sites off the Beagle Channel, Tierra del Fuego, are no older than 8 Ka and contain only lithic tool remains without any faunal remains at the Imiwaia I site. The first animal remains appear in the lower components of the Tunel I site, dating from 6.4 Ka to 4.9 Ka. Many other sites along the coast of the Beagle Channel and the eastern part of Tierra del Fuego, Peninsula Mitre, provide a record of faunal remains that continued until European colonization of the region in the 19th century (Orquera et al., 2011).

Several distinct indigenous groups have populated the Tierra del Fuego region, with the Yamana hunter gatherers occupying the area around the Beagle Channel. The archaeological record indicates that the early indigenous people were more likely to migrate farther in the middle Holocene (8-6 ka) relative to the late Holocene (2 ka until recently), but remained semi-nomadic until European contact (Orquera et al., 2011). Ethnographic accounts stipulate that the Yamana would occupy sites along the coast for several weeks, then travel to new sites via canoe. They would most commonly hunt A. australis with harpoons, which the Yamana could easily haul in with their two-meter-long canoes. These canoes generally could hold the smaller *A. australis* individuals, rather than larger and heavier prey items like *O. flavescens* which could potentially

capsize the canoes (Lohrop, 1928). Early Yamana tended to rely almost entirely on *A. australis* for food, but eventually included a more varied diet by the time of European contact, including fish and guanacos (Orquera and Piana, 1999; Zangrando, 2009b). In the 19th and 20th centuries, commercial hunting severely Otariid population. After laws passed in the mid-20th century banning hunting of marine mammals, populations began to rebound (Tunez, Juan ICappozzo and Cassini, 2008; Baylis et al., 2014).

Isotopic evidence in marine hunter gatherer humans suggest that diets have changed very little during pre-contact times (6400 cal. Bp. to the 19th century, Tafuri et al., 2017). Little variation in both $\delta^{13}C$ and $\delta^{15}N$ in human remains suggests high resilience in human populations, despite changes in the abundance and proportion of different vertebrate fauna found in archaeological sites (Zangrando et al., 2014b).

In archaeological marine mammals, however, significant shifts in diet have been observed. Higher niche partitioning in archaeological Otariids has been observed in the middle Holocene than in recent times (Drago et al., 2017). In previous studies archaeological fur seal values show little variation in $\delta^{13}C$ but decreases in $\delta^{15}N$ within the Beagle Channel, coincident with the reduction in reconstructed average body size of individuals found in archaeological sites (Zangrando et al., 2014b). These results suggest human hunting pressures have been instrumental in dietary changes of Otariids, as they were forced to seek out different and lower quality diets while being hunted by prehistoric hunter gatherers and were free from interspecific competition for resources when their populations were decimated by industrial hunting more recently. Finally,

modern populations are experiencing increased niche partitioning as interspecific competition increases.

Controversy in the reasons behind the increase in niche partitioning among modern Otariids exists however. Vales et al., (2013) argue that fishing intensification since the 20th century ban has not had a significant impact on Otariid populations due to specialization of fishing on prey items not favored by Otariids. This is reflected in isotopic values that reflect feeding on pelagic resources. In contrast, Saporiti et al. (2014a) argue that hunting of mid-trophic level organisms should result in changes in food web dynamics of Otariids, specifically an increase in competition due to less prey availability. However, Vales et al., (2013) does note a change in species hunted by fisherman could result in changes to Otariid populations. Currently, both *A. australis* and *O. byronia* are species of least concern under the IUCN Red List (2017).

Chapter 1 - Temporal and Population Trends in Human Exploited Pinnipeds from Tierra del Fuego

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Abstract

Marine food webs in coastal southern South America are thought to have been impacted over time in response to humans in the late Holocene to the Anthropocene. Archaeological sites on the coast of Tierra del Fuego, Argentina, provide a biogeochemical record that can potentially inform us about ecological dynamics over this time period. This record appears as bone collagen from Otariids, southern fur seals (*Arctocephalus australis*) and sea lions (*Otaria flavescens*), high trophic level predators. To quantify ecological relationships, we measured bulk stable isotope ratios from collagen in Otariids and several other associated animals, several of which were potential otariid prey. Variations in bulk stable isotope ratios of carbon and nitrogen are linked to potential dietary differences and habitat specialization (coastal areas or open ocean) in populations ranging in age from 7000 cal. years BP to modern. We observed increases in the variability of these isotopic compositions over time, which suggests a diversity in the diets and habitats of Otariids. Shifts in marine food webs occurred during the transition from subsistence hunting of Otariids to industrial hunting and expanded human influence. We concluded that direct human influences, such as hunting and habitat alteration, were the major drivers of ecological change in southern South American marine ecosystems rather than climate change.

Introduction

Humans significantly impacted animal populations and food webs worldwide prehistorically as well as historically (Barnosky et al., 2012). However, the extent and cumulative impacts at regional scales are less understood, especially at high latitudes. Southernmost South America is one such region. Researchers studying the Fuegian archipelago have yet to come to a consensus on the relative impacts of human and non-human entities on ecological communities (Saporiti et al., 2014b; Bas et al., 2018a; Fernández et al., 2018). Furthermore, quantifying these relative impacts is compounded by uncertainty of ecological responses to disturbance of communities, whether from humans, abiotic influences, interspecies interactions or the varying contributions of all three of these factors.

One potential way to disentangle the complexity of ecosystem responses to disturbance is by investigating food web dynamics of marine ecosystems. The marine food webs of southern South America are supported by coastal/nearshore kelp forests and open pelagic phytoplankton (Friedlander et al., 2018). The proximity of this region to the nutrient rich Southern Ocean and warm waters of the Atlantic Ocean results in high primary productivity (Garzón et al., 2016). This productivity supports a rich array of different organisms due to high endemism, including resident and migratory fish, birds and marine mammals (Friedlander et al., 2018).

At higher trophic levels, two primary species of marine mammals, pinnipeds, are found in southern South American marine ecosystems: The South American sea lion

(*Otaria flavescens*) and the South American fur seal (*Arctocephalus australis*) (Vaz Ferreria, 1978; Bastida and Rodríguez, 2003; Rossi-santos and Editors, 2018b). Most colonies are located within the continental shelf break, with fur seals and sea lions feeding on outer shelf species (Franco-Trecu et al., 2014).

The majority of observed dietary components in modern Southern Fur seals are cephalopods, including the Patagonian squid (*Loligo gahi*), and fish, including the Falkland herring (*Sprattis fugensis*) and various Patagonothen species. Crustaceans make up a small portion of the Southern Fur seal's diet (Baylis et al., 2018; Casper et al., 2006; Vales et al., 2015). *A. australis* and *O. flavescens* undergo dietary shifts between different life history stages, much like other marine mammals (Vales et al., 2015; Zenteno et al., 2015). Fur seals have a short weaning period within their first year, attaining high quality protein from milk (Cane et al., 2005). Younger, smaller fur seals will hunt for prey closer to shore while older, bigger adults have access to prey from deeper and more distant waters, with some hunting beyond the continental shelf break (Drago et al., 2017).

Since fur seals and sea lions are largely generalists, sea lions have been observed to have a very similar diet to fur seals. However, dietary specialization has been observed in contemporary contexts, including most commonly fish and invertebrate species such as merluccid hakes, octopus, squid and anchovy (Crespo and Pedraza, 2000; Drago et al., 2017). Ontogenetic intraspecies diet specialization follows a similar pattern to fur seal (Drago et al., 2009b). Heightened interspecies dietary variation has been observed in

north-eastern extent of the two species ranges but is less well constrained in southern South America (Drago et al., 2017). However, the amount of overlap in diets of these two otariids and how much feeding habits of these animals has changed over time is still an area of active research.

Human impacts on ecology

Pinnipeds are vulnerable to direct hunting as well as habitat encroachment, especially where rookeries are present. Periods of mass exploitation subject populations to genetic bottlenecks and often result in shifts in community dynamics, such as alterations to interspecific and intraspecific competition, changes in dispersal and colonization as well as the long term survival of a species (Romiguier et al., 2014).

Hunting of pinnipeds ensured the survival of marine hunter gatherers of southern South America for thousands of years, between 7500 cal. BP and the 19th century (Orquera and Piana, 2009). Pinnipeds, bearing a high energetic and nutritional density, were valued for subsistence of human populations and valued for their material resources (Zangrando, 2009a). Pinniped remains are abundant throughout the archaeological record of coastal marine hunter gatherers and provide a valuable resource for studying the ecology of this region and its dynamics through several thousand years (Tivoli and Zangrando, 2011).

Coastal sites have been occupied since at least 8500 cal. BP, when open sub-Antarctic forests colonized at lower elevations (Borrero, 1999; Orquera et al., 2011). Several distinct indigenous groups have populated the Tierra del Fuego region, with the

marine hunter gatherers occupying the area around the Beagle Channel (Orquera et al., 2011). The archaeological record indicates that the early hunter-gatherer populations maintained long-distance interaction networks along coastal fjords of the Fuegian archipelago in the Holocene and remained nomadic until European contact (Orquera and Piana, 2009; Orquera et al., 2011). Ethnographic accounts stipulate that the Yamana would occupy sites along the coast for several weeks, then travel to new sites via canoe (Lothrop, 1928). They would most commonly hunt *A. australis* with harpoons, which the Yamana could easily haul in with their canoes (Martinoli and Vázquez, 2017). These canoes generally could hold the smaller *A. australis* individuals, rather than larger and heavier prey items like male *O. flavescens* which could potentially capsize the canoes (Lothrop, 1928). Skeletal remains of vertebrates found in early Yamana sites show these people tended to rely almost entirely on *A. australis* for food (Orquera and Piana, 1999), but eventually included a more varied diet by the time of European contact, including fish and guanacos (Zangrando, 2009).

Pinnipeds were also highly valued in the 18th and 19th centuries by European colonists for their oil (Kovacs et al., 2012). Many of these remains have also been recovered and in this study compared to both more ancient and modern remains, collected after the practice of industrial seal/sea lion exploitation was banned in 1949 (Grandi et al., 2015b; Romero et al., 2017). Both species face threats today from competition with fisheries and population depression from recent hunting, as well as vulnerability to climate change (Kovacs et al., 2012). Trophic interactions of the current

and past populations of these species remain understudied relative to pinniped species in other parts of the world due to unevenness in resource allocation for research in southern South America (Jarić et al., 2015).

Isotope ecology

A variety of investigations into the diets and associated food webs of South American fur seals and sea lions have shown the importance of these species in their ecosystems (Rossi-santos and Editors, 2018b). Studies of modern Pinnipeds from gut content, fecal matter and stable isotopic studies show a variety of potential prey for these predators. However, each of these types of observations include potential biases (Nielsen et al., 2018). Stable isotope analyses (SIA) provide an optimal method for dietary analysis for long time scales and where traditional visual inspection of dietary items is either difficult or impossible, as in the species and time scales studied here.

In archaeological specimens, SIA has been applied to both species of otariids from different populations from different time periods in southern South America, however significant gaps in knowledge remain, specifically due to limited sample sizes and gaps in time. Zooarchaeological analysis suggests increases in hunting intensity may have led to decreases in body size of hunted animals and an increase in the hunting of younger animals, though SIA indicates that $\delta^{13}C$ and $\delta^{15}N$ did not vary significantly over time (Zangrando et al., 2014b). Further SIA suggests slight changes in the diets near the end of the archaeological sequence (2600 BP) with fur seals feeding on more benthic based

organisms coincident with the reduction observed in body size, which may have been associated with reduced primary productivity (Saporiti et al., 2014b; Vales et al., 2016).

Historical specimens

In modern settings, these organisms represent apex predators in overlapping food webs (Drago et al., 2017). *Otaria* is known to feed more prevalently in offshore/pelagic contexts while *Arctocephalus* typically is observed feeding nearshore ecosystems, though commonly feeds pelagically as well (Casper et al., 2006; Drago et al., 2017).

Questions, objectives, and hypotheses

The objective of this study is to elucidate dynamics of the prehistoric marine ecological record, define potential variation within a population to characterize potential ontogenetic dietary variation, compare the transition between prehistoric and historic human actions on marine ecology, and compare these human mediated prehistoric and historic ecological paradigms with the post-industrial recovery of this ecosystem. While there exists some isotopic measurements of archaeological fur seals and sea lions, individuals from more recently excavated and neglected sites were measured to examine temporal gaps in the ecological record. Furthermore, a comprehensive measurement of historical specimens was conducted. We expected to see (1) an increased dependence on offshore predation by fur seals and sea lions coincident with lower abundance of these species found in archaeological sites, and a similar response in historic industrial hunted populations, due to coastal pinniped populations sinks resulting from human hunting practices and habitat encroachment.

We also expect to document (2) increases in isotopic niche size associated with decreases in both interspecific and intraspecific competition due to decreasing populations over time.

Methods

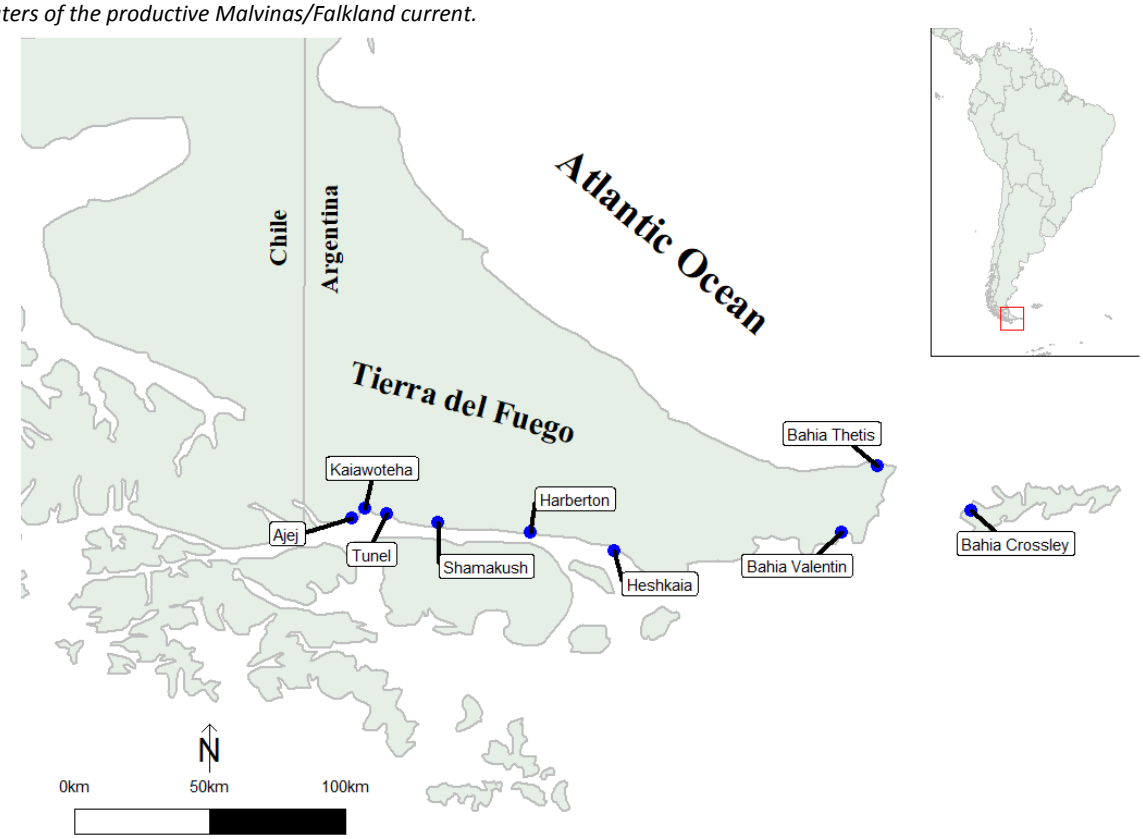
Collection

Samples were of Otariids were collected via excavation of shell middens and from collections at CADIC-CONICET. Bones were selected based on associated zooarchaeological information, such as species, sex and ages of individuals. Anatomical factors, as bone type and laterality, were also considered in the identification of sampled individuals. A variety of sites were chosen, with the Túnel locality containing the highest number of individuals (Table 1). Sites selected vary temporally and longitudinally (Fig. 1, Table 1), including Beagle Channel sites such as Túnel I (7500-4900 BP and 2200-1600 BP), Bahia Valentin (5700-4800 BP), Ajej I (1400-800 BP), Shamakush (1100-1000 BP), Kaiyawoteha II (690-560 BP), and Heshkaia 28 (660-560 BP). Atlantic adjacent sites include Bahia Crossley (3000-1300 BP), Tres Amigos (prehistoric), Bahia Thetis (historic). Modern samples opportunistically collected along the Peninsula Mitre coastline were measured to represent the post-industrial population.

Table 1-1 - Average stable isotope ratios of nitrogen and carbon of otariid populations organized by archeological site.

Site	Cal.years BP (approximate ranges)	$\delta^{15}N$ (‰)			$\delta^{13}C$ (‰)			C:N ratio		n
		Mean	SD	Range	Mean	SD	Range		SD	
Tunel I 2nd Component	7500-4900	18.2	0.8	5.0	-12.6	1.1	5.1	3.3	0.1	176
Bahia Valentin	5700-4800	20.9	0.9	1.9	-12.9	0.9	2.0	3.7	0.1	4
Bahia Crossley	3000-1300	18.5	2.1	5.6	-11.7	1.0	2.3	3.6	0.1	6
Tunel I C1	2200-1600	17.4	1.0	4.0	-12.0	0.6	2.2	3.3	0.2	19
Ajej	1400-800	16.3	0.7	2.2	-12.5	0.5	1.4	3.5	0.2	7
Shamakush	1100-1000	17.7	1.6	3.6	-13.4	0.8	1.4	3.3	0.1	4
Kaiawoteha	690-560	16.6	0.7	2.5	-12.0	0.7	2.5	3.5	0.2	10
Heshkaia	660-550	17.8	1.3	2.7	-14.3	0.5	0.9	3.3	0.0	4
Tres Amigos	Prehistoric (not dated)	14.8	6.2	15.4	-16.1	3.6	8.2	3.2	0.2	6
Thetis Bluff	Historic (not dated)	19.3	0.9	1.7	-12.5	0.5	0.8	3.2	0.1	3
Bahia Thetis	120-70	17.7	1.6	8.7	-13.7	1.2	6.2	3.5	0.3	91
Peninsula Mitre	Post-industrial (not dated)	17.8	3.2	13.1	-13.9	2.5	10.5	3.3	0.2	20

Figure 1-1 - Distribution of archaeological sites sampled for SIA. Beagle Channel sites are defined by their proximity to the terrestrially influenced waters of the Fuegian Archipelago while Atlantic Coast sites are associated with more open waters of the productive Malvinas/Falkland current.



Potential prey and comparative species were also collected from archaeological sites or modern contexts where available. Prey species (summarized in Table 2) include fish such as the Patagonian grenadier (*Macruronus magellanicus* -Merluccid hakes-) and sardines (*Sprattus fuegensis*, Clupidae), derived from the archaeological sites Tunel I and Imiwaia (7800-5700 BP), and Shamakush I (1100-1000 BP). A prey species observed in modern fur seal and sea lion diets, Patagonian squid (*Loligo gahi*), do not occur in archaeological sites but were collected from a local fish market in Ushuaia for comparison. Representing a coastal endmember species, Imperial shag (*Phalacrocorax atriceps*) were collected from Túnel I, Imiwaia I, Bahía Crossley I, Shamakush I, and

Kaiyawoteha II. Finally, four blades of giant kelp (*Macrocystis pyrifera*) were collected from Harberton, Bahia Thetis, and Tres Amigos locality.

Preparation

Bone samples were demineralized and purified into collagen by suspending samples in 5ml 0.5 M HCl which was refreshed every 24 hours until completely demineralized or up to a week. Samples were then suspended in 5ml 0.1 M NaOH to remove humic acid contaminants and refreshed daily until the solution was transparent. Samples were then combusted on a Costech EA fed into a Thermo Delta V IRMS at the UC Merced Stable Isotope Laboratory and UC Riverside EDGE Laboratory for bulk carbon and nitrogen stable isotope analysis. If samples returned C:N ratios higher than expected for collagen (>4) then samples were treated for lipid extraction using a 2:1 chloroform methanol solution and ran again. Kelp blades and squid muscle were washed, dried and weighed into aluminum boats in a similar manner to collagen samples. All samples are summarized in Table 2.

Table 1-2 - Average nitrogen and carbon stable isotope ratios with corresponding carbon to nitrogen ratios, an indicator of preservation quality, in animals analyzed for stable isotope ratios

			$\delta^{15}N$ (‰)			$\delta^{13}C$ (‰)			C:N Ratio		
	Species	Common name	Mean	SD	Range	Mean	SD	Range		SD	n
Otariids	*Arctocephalus australis*	South American fur seal	18.1	1.1	10.9	-12.6	1.2	9.1	3.3	0.2	201
	Otaria Flavescens	South American sea lion	17.8	2.0	13.3	-13.5	1.6	11.7	3.5	0.3	126
	Unidentified	-	16.7	2.8	13.3	-13.2	2.0	8.6	3.4	0.2	23
Fish	*Macruronus magellanicus*	Patagonian grenadier	17.2	0.6	2.1	-13.6	0.6	2.3	3.5	0.2	13
	Clupidae	Sardine	12.5	1.0	3.7	-13.6	0.6	2.4	3.5	0.1	19
Birds	*Phalacrocorax atriceps*	Imperial shag	17.5	2.0	8.7	-12.1	0.8	3.5	3.5	0.2	17
Invertebrates	*Loligo gahi*	Patagonian squid	11.3	0.9	2.2	-17.6	1.0	2.5	4.3	0.6	5

Stable isotope geochemistry has been well established as a method for identifying marine trophic relationships (Zangrando et al. 2014; Newsome et al. 2010; Boecklen 2011). Stable isotopes fractionate as they are shuffled via chemical reactions, with heavier isotopes becoming more abundant in higher trophic levels. SIA can be applied to many different types of tissues, however bone collagen represents an excellent resource for representation of animal diets over several years of the animal's lifespan due to its long turnover rate, while often being the only available tissue in archaeological sites (Ambrose 1990). Stable isotopic measurements are reported in delta notation, which relates the ratio of the heavy to light isotope in the sample to a standard. Values are expressed in parts per mil (Equation 1).

Equation 1: $$\delta X_j = \left[\frac{\left(\frac{X_j}{X_i}\right)_{Sample}}{\left(\frac{X_j}{X_i}\right)_{Standard}} \right] - 1$$

X_j represents the heavy isotope and X_i the lighter isotope of carbon or nitrogen. $\delta^{13}C$ values show habitat preferences and trophic levels, while $\delta^{15}N$ values show changes in ocean productivity over time as well as trophic position. Isotope values were corrected to international standards, Vienna-Pee Belemnite Limestone for carbon and atmospheric N_2. Internal standards used to correct for drift and linearity were acetanilide, glycine, and USGS 42. Standard deviations for internal standard replicates for $\delta^{15}N$ of acetanilide at both laboratories was 0.61‰, 0.81‰ for glycine and 0.51‰ for USGS 42. For $\delta^{13}C$, replicates of acetanilide had a standard deviation of 0.93‰, glycine 1.16‰, and for USGS 42, 0.24‰.

Data were then compared in R to identify potential within population sex or age differences before using Bayesian statistical tools SIBER (Jackson et al., 2011) and FRUITS (Fernandes et al., 2014) to model potential food web dynamics and trophic positions. Linear regression models and Kendall rank correlation tests were performed to identify any potential relationships between variables. Pairwise Wilcoxon and Kruskal-Wallis similarity indices were applied to identify differences between means for

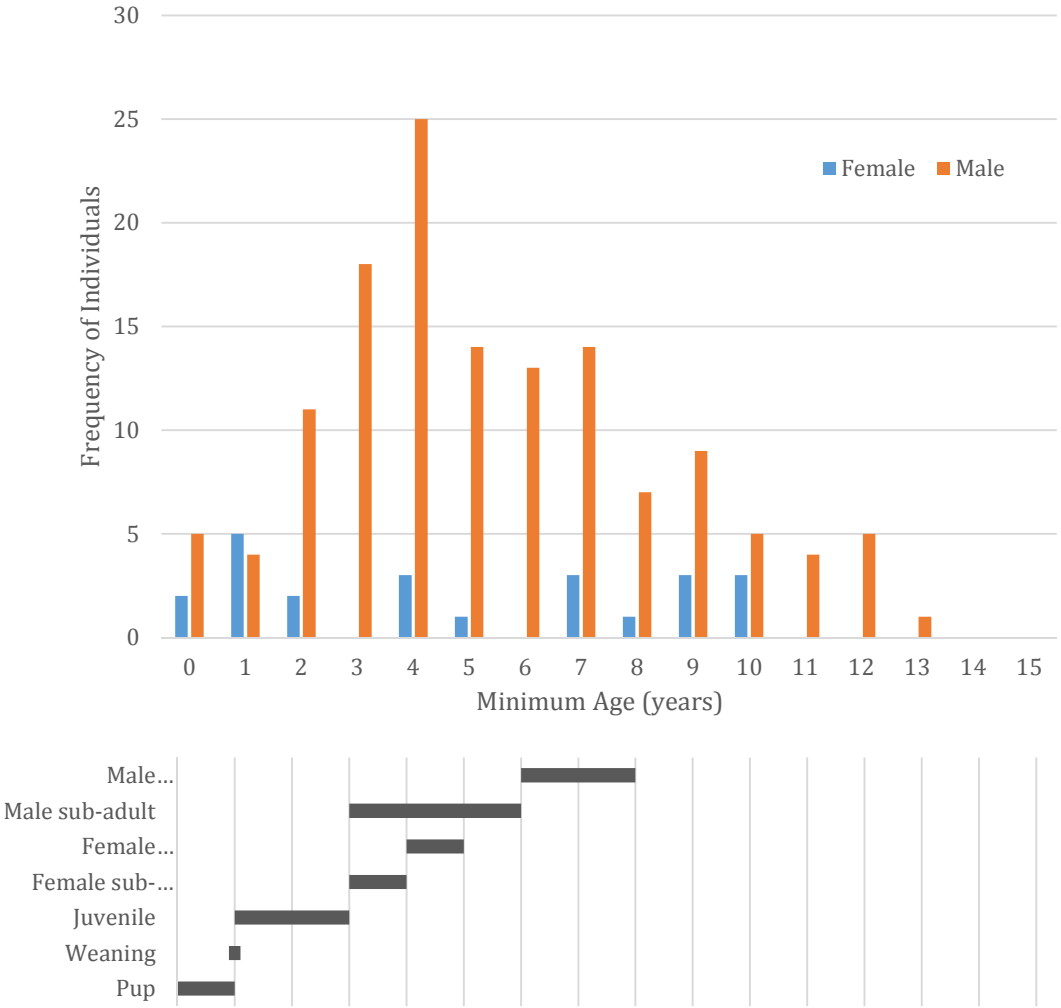


Figure 1-2 – (a) The distribution of A. australis individuals divided by sex found at the site Tunel I that are ideal for stable isotope analysis. (b) The life history development stage age ranges in age for A. australis from Lima and Paez (1997).

groups rather than t-tests and ANOVA since Shapiro tests showed sample groups were not normally distributed (Fig. 2).

Results

Interspecific carbon and nitrogen isotopic compositions and differences

Isotopic values for all of the samples of most of species we measured showed significant overlap in both carbon and nitrogen isotopic space (Table 2 and Fig. 3). Kendall Rank correlations were unable to resolve differences between fur seals, sea lions, imperial shag, and Merluccid fish (Table 2). Only Patagonian squid had $\delta^{15}N$ 11.3±0.9) and $\delta^{13}C$ (-17.6±1) that were more negative than the organisms above. Sardines also had $\delta^{15}N$ values (12.5±1) that were different but similar in terms of $\delta^{13}C$. Kelp showed a very large range in $\delta^{13}C$, primarily due to a single outlier, with the other three kelp samples clustering around -12.5‰. $\delta^{15}N$ values for kelp were the lowest measured and did not vary between individuals at around ~6‰.

Spatial and temporal site differences in otariids

Although there were few significant differences in mean values among species, significant variations in $\delta^{15}N$ and $\delta^{13}C$ in fur seals and sea lions were observed between different time periods and longitude. Kruskal-Wallis measures and Kendall rank correlations comparing $\delta^{13}C$ with mean age and longitude of archaeological sites were both significant, suggesting carbon isotopic values change both over time and space (Table 3, p-values <0.05). Kendall rank correlation τ coefficients are non-zero indicating dependence between archaeological sites and $\delta^{13}C$. A positive τ coefficient of 0.29

shows $\delta^{13}C$ values decrease in otariids as time progresses toward the present (Table 3, figure 7a). Similarly, $\delta^{15}N$ expresses significant variation over time as confirmed by these two statistical tests (Table 3). The Kendall rank correlation τ coefficient is similarly positive suggesting $\delta^{15}N$ values decrease as time progresses. A τ coefficient of 0.13, however, indicates that the strength of this correlation is less than that for carbon. Spatially, a τ coefficient of -0.33 shows a strong correlation of decreasing $\delta^{13}C$ from samples collected at sites from east from the Beagle Channel out to the Atlantic Ocean. The $\delta^{15}N$, on the other hand, shows disagreement between the two statistical measures

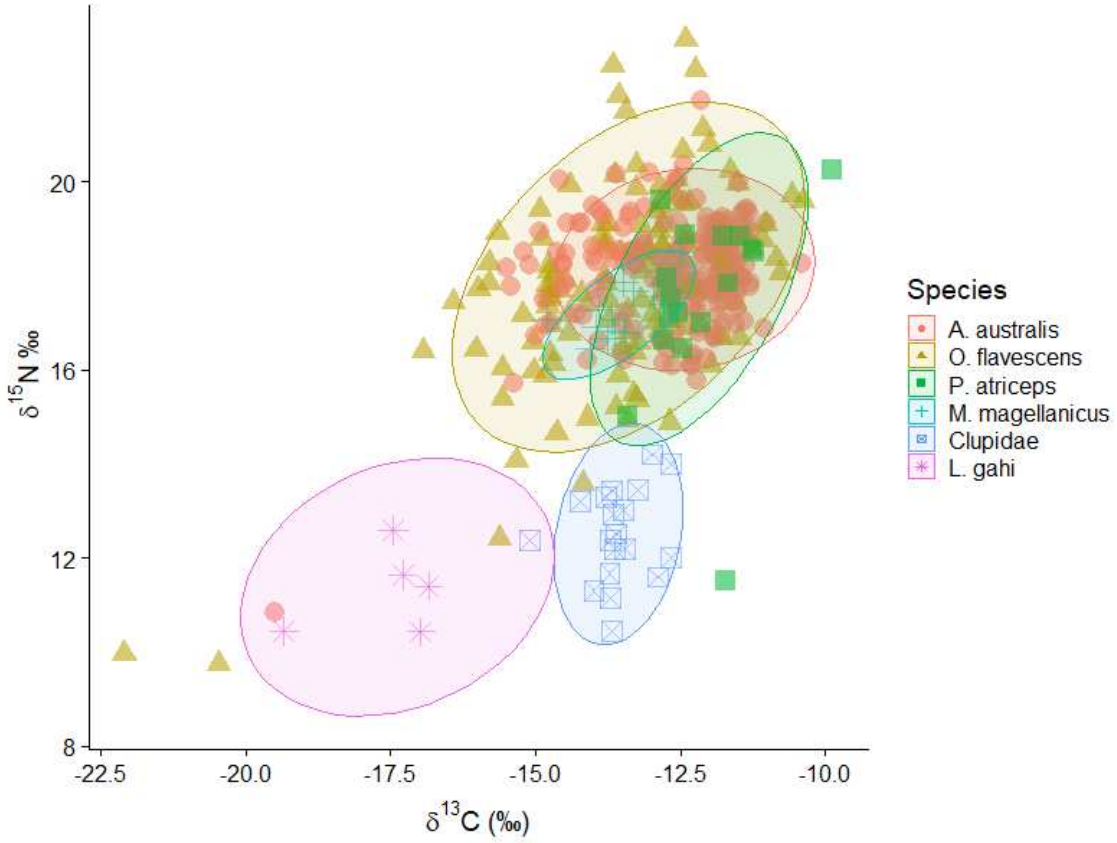
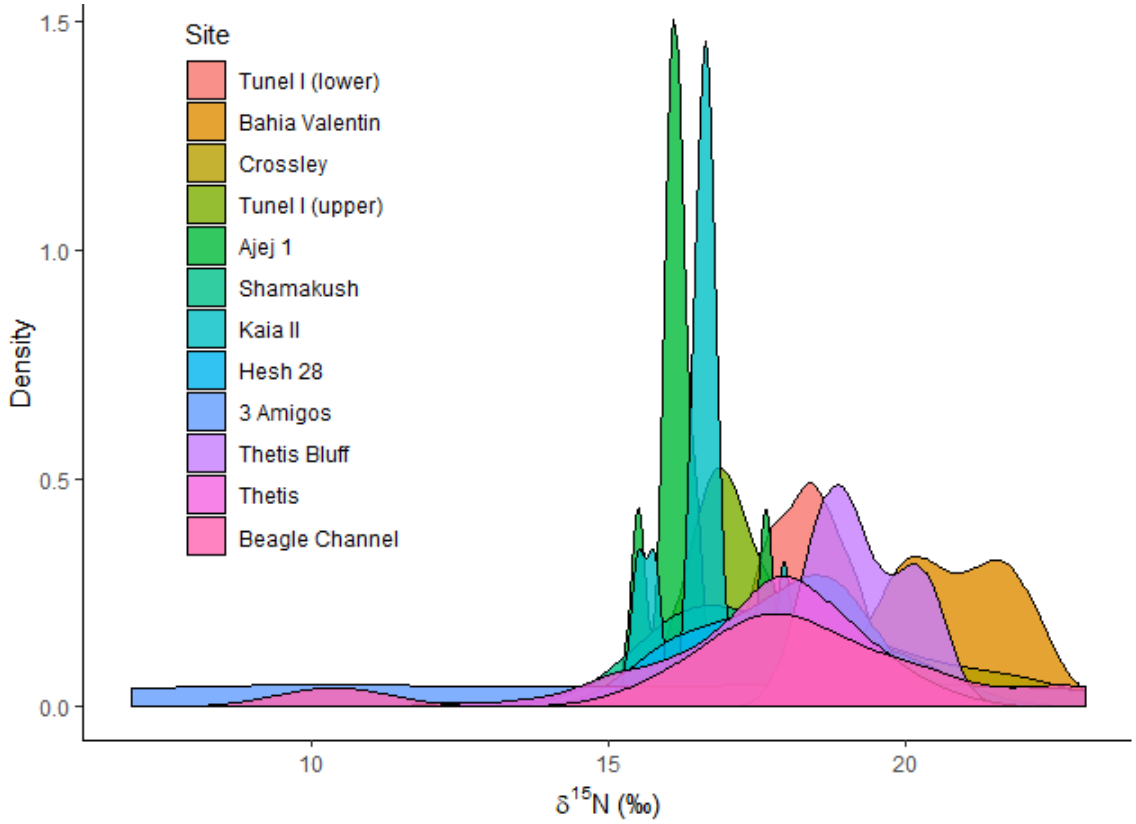


Figure 1-3 – Carbon and nitrogen isotopic space of all organisms (see table 1) organized by species. Ellipses represent 95% confidence intervals. More negative $\delta^{13}C$ values indicate offshore dominated influence while more positive indicate nearshore influence. $\delta^{15}N$ largely indicates trophic level for these organisms. Overlapping ellipses in both carbon and nitrogen space indicate little separation between species.

Figure 1-4 – Density plot of nitrogen isotopic values in otariids divided by archaeological site. Sites are ordered by age. While some sites show dense clustering of sampled individuals, this may be an artifact of small sample sizes for these sites. Overall, there is significant overlap in $\delta^{15}N$ between sites.



employed here. While longitude of archaeological site significantly varies with $\delta^{15}N$ values, no significant correlation is observed (Table 3).

To visualize differences in isotopic ratios of otariids between individual archeological sites, that vary both in their location and in age of deposition (Table 1), we made density plots to compare probability distributions of nitrogen isotopic ratios (Fig. 4) and carbon isotopic ratios (Fig. 5). $\delta^{15}N$ of the different sites tend to have higher probabilities of falling within a smaller range of values (Fig.4) than for $\delta^{13}C$ (Fig. 5) despite an apparent larger absolute range of values for $\delta^{15}N$ (Table 1). On the other hand, the probability of an otariid's $\delta^{13}C$ falling within a more constrained range is

lower. Ultimately the range of the probability distribution in ‰ increases with the number of samples per site, so extremely high density probabilities for sites with fewer individuals may be biased by a small sample size (Table 1).

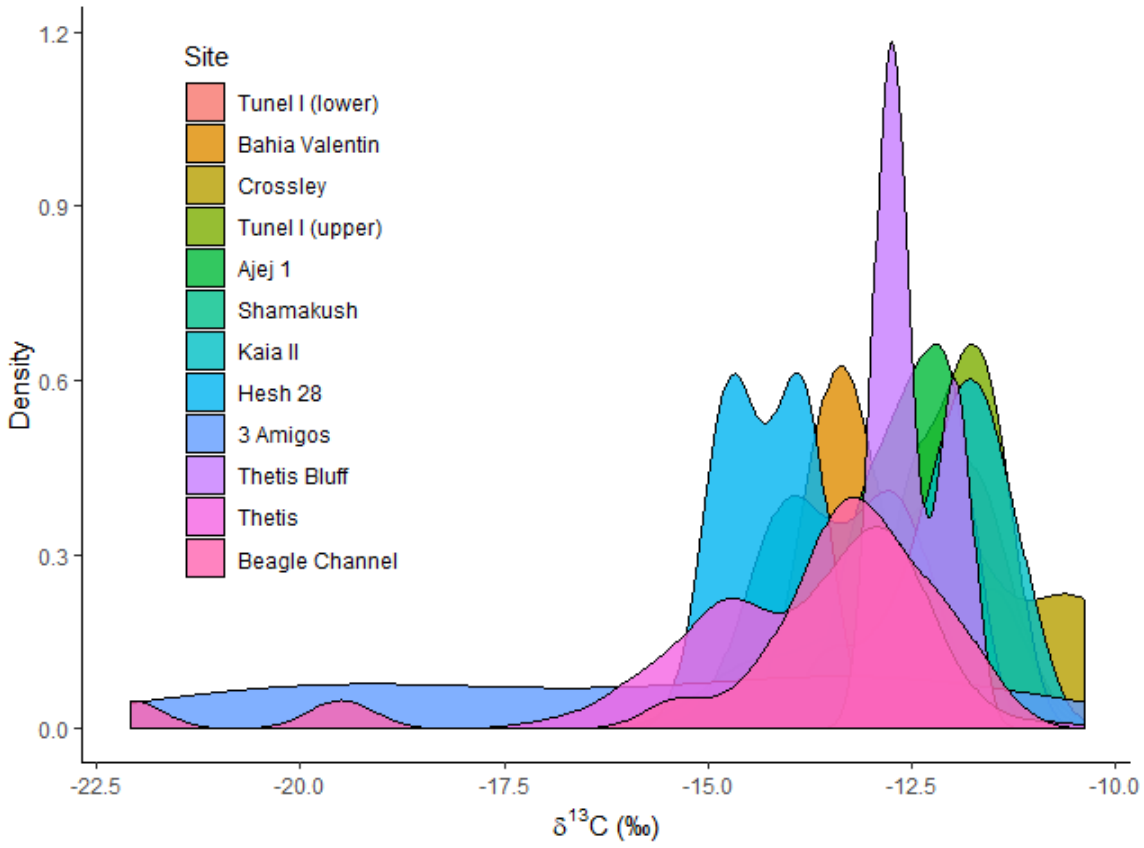


Figure 1-5 – Density plot of carbon isotopic values in otariids. Sites are ordered by mean radiocarbon age with more recent sites in the foreground. More recent sites show large ranges in $\delta^{13}C$ values and significant overlap is observed in all sites over time.

Table 1-3- Summary of statistical tests to determine significance of different variables on carbon and nitrogen isotopic ratios of otariids. Carbon isotopic ratios significantly varied or correlated between species and between mean age and longitude of archaeological sites while nitrogen isotopic values significantly varied or correlated between different sexes and mean age of archeological sites.

	$\delta^{13}C$					
	Kruskal-Wallis test		**Kendall Rank Correlation**			
	p-value	**Significance (<0.05)**	**p-value**	**Significance (<0.05)**	**τ**	**Correlation strength**
Age of individual	0.07	not significant	0.03	*	0.12	*
Sex	0.95	not significant	0.95	not significant	0.00	not correlated
Species (*O. flavescens* vs. *A. australis*)	<0.001	*	<0.001	*	0.29	**
Mean age of archaeological site	<0.001	*	<0.001	*	0.29	**
Longitude of archaeological site	<0.001	*	<0.001	*	-0.33	***
	$\delta^{15}N$					
	Kruskal-Wallis test		**Kendall Rank Correlation**			
	p-value	**Significance (<0.05)**	**p-value**	**Significance (<0.05)**	**τ**	**Correlation strength**
Age of individual	0.12	not significant	1.00	not significant	<0.001	not correlated
Sex	0.05	*	0.05	*	0.12	*
Species (*O. flavescens* vs. *A. australis*)	0.25	not significant	0.25	not significant	0.05	not correlated
Mean age of archaeological site	<0.001	*	0.00	*	0.13	*
Longitude of archaeological site	<0.001	*	0.46	not significant	0.03	not correlated

Population dynamics

We found significant differences and correlation between the two otariid species in their $\delta^{13}C$ values, but not for $\delta^{15}N$ values. Carbon isotopic measurements were significantly more negative in sea lions (*O. flavescens*) than in fur seals (*A. australis*) as indicated by a Kendall rank correlation of 0.29 (Tables 1 & 3). Conversely, no significant differences between species were indicated by a Kruskal-Wallis test and Kendall rank correlation in $\delta^{15}N$ (p-values >0.05, Tables 1 & 3). We also found the age class of individuals are not significantly related or correlated with nitrogen isotopic values (Table 3). Age class of individuals may be weakly correlated with their $\delta^{13}C$ values ($\tau = 0.12$), however a Kruskal-Wallis test did not show significant variation between the two variables. No significant variation or correlation was found in $\delta^{13}C$ and sexes of individuals, however a weak correlation ($\tau = 0.12$) and significant variation was observed in $\delta^{15}N$ and sexes of individuals (Table 3).

To assess potential isotopic variability without the complications of location, we measured 164 individuals from the archaeological site with the most abundant fur seal remains (Tunel I 2nd component, Figure 2, Tables 1 & 4). Mean $\delta^{15}N$ values showed no significant differences for any age class. Variations in $\delta^{15}N$ values, however, ranged by 4.3‰ (16.1-20.3‰) and were observed in all age categories from pups to adults. A Kruskal-Wallis comparison of mean $\delta^{15}N$ values suggests no significant differences between age categories (p=0.57), and a Kendall rank correlation likewise suggests no

significant differences (p=0.99). Male adults had the highest maximum $\delta^{15}N$ values and pairwise Wilcoxon tests suggest significant variation between mean values of male and female adult fur seals (p=0.032). Variation in $\delta^{13}C$ was significant as well, with a range of 4.5‰ and varied similarly between different age categories. We observed significant variation between means of age using Kruskal-Wallis tests (p=0.0051). The two age groups with the most variable means included juveniles, who had more negative $\delta^{13}C$ values, and adults, who were most positive in $\delta^{13}C$ (p=0.0076). Like nitrogen, we observed no significant differences between mean values of $\delta^{13}C$ between males or females using a Kruskall-Wallis comparison (p=0.91) and Kendall rank correlation highlighted no differences (p=0.95).

Table 1-4– Average stable isotope ratios by ontogenetic age class of fur seals (Arctocephalus australis) from Tunel 1 2nd component. Few differences were observed between individuals of different age classes. The only statistically significant difference was observed between juveniles and adult fur seals in $\delta^{13}C$ values (Wilcoxon, p=0.0076).

		$\delta^{15}N$ (‰)			$\delta^{13}C$ (‰)			C:N Ratio		
Sex	Age class	Mean	SD	Range	Mean	SD	Range		SD	n
NA	pup	18.2	1.0	2.4	-12.5	1.4	3.4	3.3	0.1	5
NA	juvenile	18.1	0.8	4.1	-13.1	1.2	4.1	3.2	0.1	38
F	sub-adult	17.5	1.1	2.3	-11.8	0.3	0.6	3.5	0.3	3
M	sub-adult	18.4	0.9	3.4	-12.7	1.0	3.8	3.3	0.1	38
F	adult	17.9	0.3	1.0	-12.5	0.8	2.3	3.2	0.1	10
M	adult	18.4	0.8	4.1	-12.5	1.0	4.2	3.3	0.2	54

SIBER, FRUITS – Food web/trophic dynamics

Using the SIBER Bayesian analysis package in R, we calculated the ellipse areas and convex hull areas from several different time periods of communities reconstructed from different species (Table 2) based on the areas calculated from nitrogen and carbon isotope values (Fig. 8). Different time periods show significant differences in their convex hull areas. The community from the earliest time period had the highest convex hull area while the modern period had the most potential variation in area. Uncertainty in convex hull area shows significant overlap between these two time periods. The community between 3000-500 BP has fewer unique organisms present, and as a result

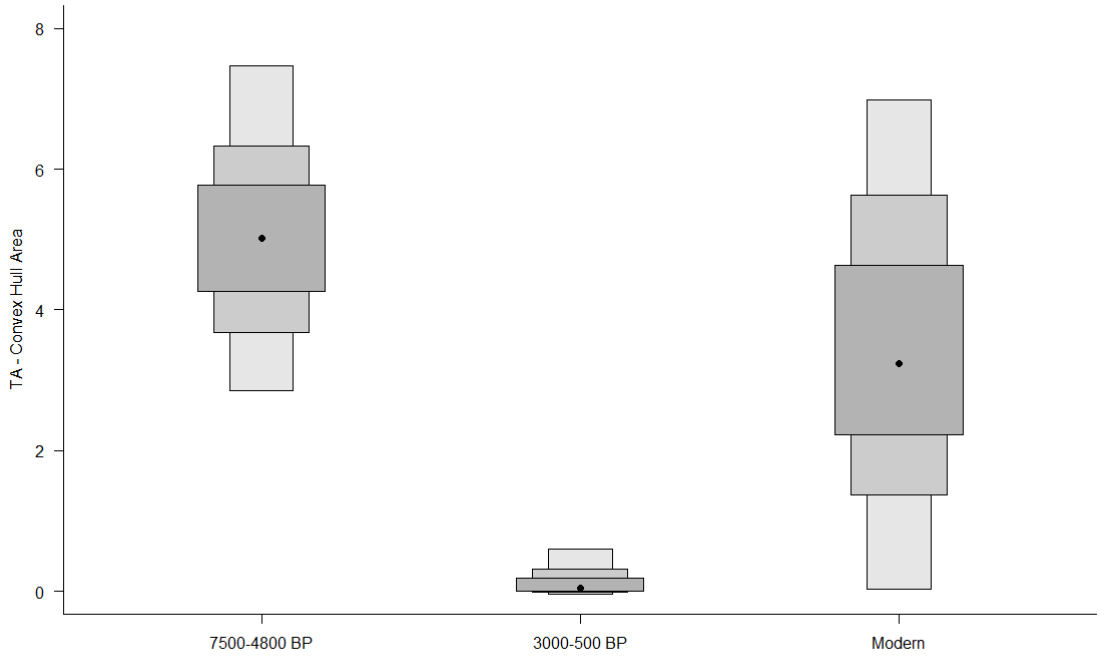


Figure 1-6 – Convex hull areas of all archaeological sites (7500-4800 BP, 3000-500 BP) compared to all modern sites (120-70 BP, historic and post-industrial). While average convex hull area is less in the modern community, there is much higher variation in modern carbon-nitrogen isotopic space. Hull area is also much smaller in the intermediate time span, suggesting less dynamic isotopic niches during this time.

shows a very low convex hull area (Fig. 8). The calculated means and ranges of ellipse areas for otariids increase as time progresses toward the present, from 12.5 ‰^{2} from 7500-4800 BP to 43.6 ‰^{2} in the modern community. The uncertainties between the middle and late Holocene do not overlap, while the late Holocene and modern time period show overlap in their uncertainties.

Discussion

Both species of otariids as well as many other organisms found in coastal Tierra del Fuego have similar isotopic niche spaces (Table 2 & Fig. 3). Squid (*L. gahi*) and sardines (Clupidae) are the only groups of organisms that essentially do not overlap with each other in isotopic niche space and are largely separate from other species in nitrogen isotopic space while being roughly similar to each other. We interpret the wide range in δ^{13}C values as a continuous range between offshore (phytoplankton, -21‰) and nearshore (kelp forest, -11.7‰) ecosystems (Riccialdelli et al., 2017). Sardines and squid represent lower trophic level organisms that reflect reliance on carbon routed through different primary producer biochemical pathways.

Otariids are the most diverse in their ranges for δ^{13}C. Sea lions, *O. flavescens*, clearly show reliance on both offshore and nearshore primary producers, while fur seals, *A. australis,* have δ^{13}C more biased toward nearshore ecosystems, though still derive carbon from a mix of offshore and nearshore sources (Table 2). Patagonian grenadier (*M. magellanicus*) also have intermediate δ^{13}C values indicative of their being reliant on a mix of nearshore and offshore carbon. On the other hand, Imperial Shag (*P. atriceps*)

has $\delta^{13}C$ values that indicate a firmer reliance on nearshore carbon. Despite differences in carbon sources, representing different primary producers, all four of these species have a similar trophic level, as indicated by their overlapping nitrogen isotopic values (Table 2 & Fig. 3). These findings are consistent with modern day observations of these species (Bas et al., 2018b).

The abundance of fur seal remains at the oldest site, Túnel I Second Component (Table 2 & Fig. 2), provides enough samples to characterize the population and ontogeny in a middle Holocene population of fur seals. The presence of more males than females suggests that this locality was not directly adjacent to a breeding colony (Schiavini, 1993), as females are more often associated with rookeries while males are observed to travel more broadly (Carrara, 1952; Siielefeld et al., 1978; de Lima et al., 2019). This observation is supported by the abundance of older individuals as well. Fewer pups and yearlings are observed in the data set (Schiavini, 1993). While some might argue that these individuals may have been selected preferentially by humans, it should be noted that hunter gatherers were only limited by their ability to hunt the largest of animals due to weight capacity of their boats. Animals hunted would only have excluded sea lion males at 300 kg or more (Cárdenas-Alayza et al., 2017; Martinoli and Vázquez, 2017).

Isotopically, we observe several differences between age class and sex (Fig. 3). Pups reflect the diets of their mothers, while yearlings and juveniles, restricted in hunting ability and prey sources, show lower $\delta^{15}N$ values than adults that can forage for

larger, more difficult to hunt prey from a broader array of sources. $\delta^{13}C$ values are represented in a continuous range between offshore (phytoplankton, -21‰) and nearshore (kelp forest, -11.7‰) sources in adults, where they are unrestricted in hunting habitat (Riccialdelli et al., 2017). Yearling and juvenile $\delta^{13}C$ values tend to cluster in groups associated with either nearshore or offshore diets, indicating that these individuals foraged in one or the other habitat rather than in both habitats as adults. Ultimately, we observe ontogenetic variation within a single community several thousand years ago that is not unlike modern communities.

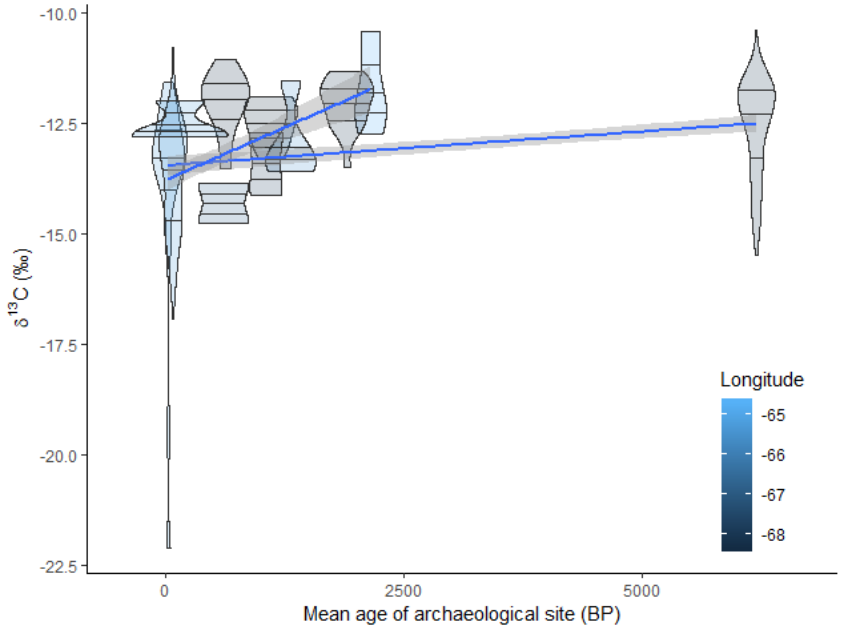
However, drivers of changes in whole populations and communities have a number of different possible causes. Environmental change has long been regarded as a driver of ecological change (Prevosti and Forasiepi, 2018b). Climate change events have had notable effects on food webs throughout the Holocene in various parts of the world, and their effects are noted in the ecosystems where marine hunter gatherer societies existed, including the California Channel Islands (Jazwa et al., 2015), the fjords of modern day Norway (Pääkkönen et al., 2018), and many others. In the southern hemisphere notable changes are seen in the Ross Sea and the Antarctic Peninsula (Koch et al., 2019). The unique nature of the Antarctic circumpolar current led to a relatively stable climate in southern South America. That is not to say climate did not change in the Fuegian archipelago, however, it was relatively attenuated relative to other parts of the world (Caniupán et al., 2014).

Primary producers, including the two most significant contributors in the Fuegian marine ecosystem, phytoplankton and kelp, will vary in biomass production from nutrient and energy supplies. Changes in climate affects the base of the food web, which can result in a potential baseline shift, in which isotopic values at the base of the food web are offset and are reflected in higher trophic levels. The results from our study indicate that changes in isotopic values can be explained by 1) a shift in baseline, 2) a change in food chain length or 3) fundamental change in the food web, the latter of which could be explained by ecological factors, such as shifts in competition between populations or species.

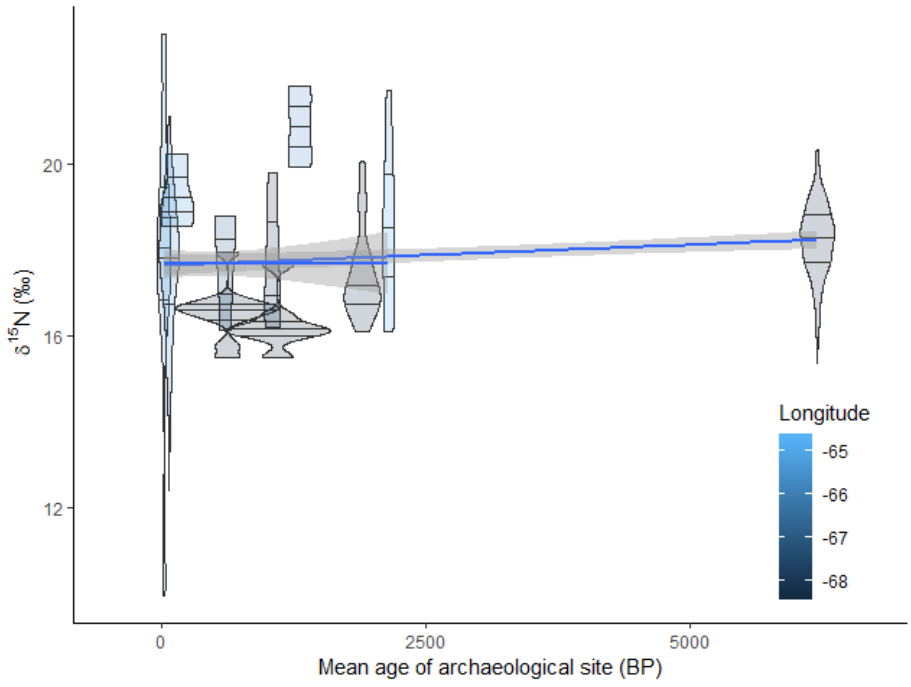
Several papers have advanced the idea that environmental baseline shifts are the proximate cause for variation in carbon and nitrogen isotopic values in higher trophic levels in the prehistoric time period (7500-250 BP). Saporiti et al. (2014) found a steady depletion in $\delta^{18}O$ values from Beagle Channel limpets until the Little Ice Age (500 BP), a period associated with higher primary productivity. Isotopic values returned to those previously observed shortly thereafter owing to lower productivity with the termination of this climatic event, from ~0.1‰ to 0.25‰. These observations are consistent with $\delta^{15}N$ obtained from pinnipeds in our study which shifted from 18.2‰ to 17.8‰ (Table 1 & Fig. 4). While one could speculate about pinnipeds in a similar fashion, the $\delta^{15}N$ values measured in pinnipeds are complicated by the more varied prey sources, including prey from both the coast and open ocean. Changes in productivity could be attenuated or routed directly through the length of the food web and reflected

in high trophic level organisms. The former case cannot be discounted as spikes in productivity could be mediated in intermediate trophic levels through resource partitioning via shifts in species interactions, for example. This results in a common dilemma in archaeological stable isotope studies, without measuring all prey items at all time periods and all members of the food web directly we cannot know with a high degree of confidence, short of using more advanced types of analyses like compound specific stable isotope analysis (Nielsen et al., 2018).

Figure 1-7– Overlain violin plots of carbon and nitrogen isotopic ratios of otariids organized by mean year of site age. Longitude is represented by color with lighter blue farther east and black/grey farther west. 25th, 50th and 75th percentile lines are represented on each violin. Linear regression model of means between each site indicate a weak correlation between site age and $\delta^{13}C$ values ($r^2 = 0.11$ for all sites and $r^2 = 0.2$ for sites less than 2500 BP). There was no correlation between site age and $\delta^{15}N$ values ($r^2 = 0.03$ for all sites and $r^2 = -0.006$ for sites less than 2500 BP)



A



B

In southern South America marine ecosystems, it has been suggested that changes in sea level and sea surface temperature have been linked to changes in productivity (Saporiti et al., 2013; Caniupán et al., 2014). However, our results suggest that these changes have not appeared to affect trophic level, with apparent little change over time in $\delta^{15}N$ values (0.4‰, Table 2, Figs. 4 & 7). The more apparent and significant changes occur in carbon isotopic niche space, which is more closely associated with habitat regime. Significant changes in productivity could appear as a change in trophic level, as a more complex food web would offer more opportunity for a longer food chain length. Higher productivity may very well shorten food chain length as predators take advantage of a more readily available basal resource (Doi and Hillebrand, 2019). In any case a lack of change in either direction of trophic level indicates potential productivity changes were not significant for otariids. A more likely explanation for dynamic changes in isotopic niches of otariids is direct human activities forcing changes in otariid habitat. The observation of decreasing $\delta^{13}C$ values in the prehistoric time period agrees with our hypothesis that seals are foraging more offshore, even when considering potential climatic effects. It should be noted however that this decreasing trend of mean values of archaeological is most notable in the latest 2500 years (Fig. 7a).

Variation in both carbon and nitrogen isotopic values in the historic and modern time periods likely reflect ecological trends influenced by human activities, e.g. hunting, rather than climate or changes in productivity. Due to industrial hunting and habitat encroachment, a competitive release where niches that were human-impacted

previously were liberated and available for otariid foraging. A significant population bottleneck introduced by industrial sealers in the 19th and 20th centuries may have allowed for subsequent increased dispersal after hunting was banned. A lack of interspecific and intraspecific competition likely allowed otariids to forage in previously crowded environments, despite less coastal habitat. With less intraspecific and interspecific competition between pinnipeds, individuals could forage in larger niche spaces. This interpretation appears to confirm our second hypothesis that niche size increased over time, though we were not expecting such large variations within the modern population. Regardless, the historic and modern populations represent the most dynamic in isotopic niche space values, coincident with greater human influence on the Fuegian marine ecosystem.

Conclusion

In contrast with some previous findings from archaeological, historic, and modern ecological analyses, we find little evidence of climatic variation affecting higher trophic level organisms from the middle Holocene to the near present. In this region and during the last 7,500 years, climatic variation pales in comparison to direct human influences on the marine ecosystems in the Fuegian archipelago. In contrast to our expectation that otariids would increase their trophic level over time, we see little evidence of that change. However, the range of dietary sources and habitats in which otariids forage increases dramatically as time progresses toward the present. This observation is congruent with our hypothesis that human impacts, in both hunter-

gatherer and industrial societies, have altered species interactions in coastal Tierra del Fuego. The processes of human hunting of pinnipeds, through prehistory to historical time periods, and habitat encroachment, which continues from prehistory to the present day, have resulted in significant variations in the diets of pinnipeds, most significantly within the past several hundred years.

Chapter 2 - Shifting basal resource use in southern South American fur seals and sea lions

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Abstract

The contribution of apex predators to community dynamics and marine ecosystems is well studied; however, the relationship of high trophic level predators to basal resource use over millennial time scales is less understood. Using archaeological remains found on coastal Tierra del Fuego, Argentina, we measured the $\delta^{13}C$ of individual amino acids in South American fur seal (*Arctocephalus australis*), South American sea lions (*Otaria flavescens*), Imperial shag (*Phalacrocorax atriceps*), Merluccid fish, giant kelp (*Macrocystis pyfera*) and Patagonian squid (*Logligo gahi*). We used principle components analyses, linear discriminant analyses and Bayesian statistical analyses to identify patterns between essential and non-essential amino acids among basal resources and consumers. Using this approach, an amino acid $\delta^{13}C$ fingerprint was developed. We found that the apex predators (fur seals and sea lions) significantly and dynamically shifted their basal resource use between coastal and offshore habitats over the time period between 7,000 years before present and today. Fur seals appear to be acquiring more offshore resources in younger samples, while sea lions shift their diet to a more even mix of coastal-offshore basal resources. These dietary dynamics are likely related to, if not caused by, prehistoric and historic hunting practices and habitat destruction by humans. This study demonstrates the long-term impacts of human alterations to marine ecosystems, the responses of apex predators to disturbance and the adaptive capacity of generalist marine predators.

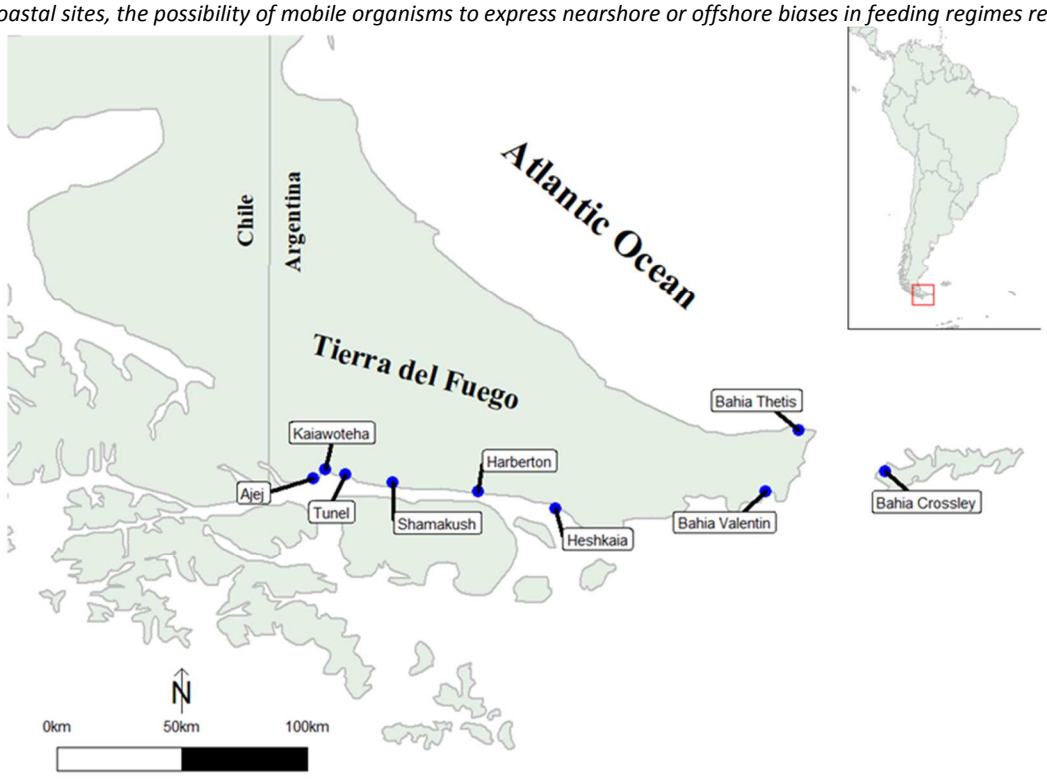
Introduction

The ecological niche of pinnipeds of southern South America has changed over time in response to changes in climate and human interactions through the Holocene and into the Anthropocene (Nye et al., 2019a; Drago et al., 2017; Rossi-santos and Editors, 2018; Zangrando et al., 2014). Responses of top predators to perturbations in diet, habitat and interspecies interactions are complicated by species interactions and population dynamics at lower trophic levels (Sugihara et al., 2012; Carozza et al., 2018). These responses can manifest themselves in different ways, for example changes in food chain length or shifting feeding regimes (Ferrier-Pagès and Leal, 2018; Stoner et al., 2018).

Pinnipeds, as apex predators, hold a large influence over other species in their respective food webs through predation and competition (Vales et al., 2016; Drago et al., 2017). Two of these Pinnipeds species (family Otariidae), the South American fur seal (*Arctocephalus australis*) and the South American sea lion (*Otaria flavescens*), are abundant in the region, though their populations have fluctuated significantly in the recent past (Romero et al., 2017; Rossi-santos and Editors, 2018a). Their historic abundance proved to be useful for human populations as pinnipeds were exploited from the middle Holocene to the middle of the 20th century. The reasons for and levels of exploitation of these species changed over time. Two significant proximal human activities have influenced these particular Otariid population dynamics: destruction of

habitat and hunting. The potential responses of *A. australis* and *O. flavescens* to these perturbations are numerous and range from relocation of rookeries to alterations in diet (Zenteno et al., 2015). These responses may not only affect the species in question, in fact, changes in Otariids can reverberate further and affect interspecies relationships of marine ecosystems around the Fuegian Archipelago (Fig. 1).

Figure 2-1 - Distribution of archaeological sites sampled for stable isotope analyses. While all individuals are collected in coastal sites, the possibility of mobile organisms to express nearshore or offshore biases in feeding regimes remains.



Modern observations of both species show they primarily eat fish and squid, though stomach content analyses on modern *O. flavescens* have revealed lesser amounts of crustaceans (Drago et al., 2017). Otariids have been observed to forage offshore and coastally, though how much time they spend feeding in these habitats

through time is less well known. Bones of these animals appear in archaeological sites in a near continuous record over several thousand years, from roughly 7000 years before present (BP) to the 20th century (Table 1, Nye et al., 2019a). Remains of Otariids and several of their prey or otherwise coexisting organisms provide a valuable resource for investigating food web dynamics and niche preferences using stable isotope techniques (Zangrando et al., 2014a). Though not all potential prey items are found in archaeological sites, examples of organisms associated with distinct marine ecosystems have been found, such as Imperial Shag (*Phalacrocorax atriceps*), a coastal shorebird (Tivoli and Zangrando, 2011). Other organisms found in sites, such as Patagonian grenadier (*Macruronus magellanicus*) and sardines (family Clupidae), have been observed to migrate between open ocean and coastal areas (Orquera and Piana, 2009). Though not found in archaeological sites, Patagonian squid (*Loligo gahi)*, is restricted to a pelagic habitat (Drago et al., 2009a; Muñoz et al., 2013). Coastal, intermediary, and open ocean species acquire carbon from different sources and this is reflected in their tissues.

Collagen, the organic tissue present in bones of these organisms, acts as a long term dietary proxy due to the long turnover rate of protein that is incorporated into the tissue relative to other tissues, like blood, muscle, skin or keratin (Vander Zanden et al., 2015). The stable isotopic ratios of carbon largely reflect the biochemical pathways of the primary producers that were consumed by prey, which predators then subsequently consume. Using compound specific amino acid (CS-AA) analysis of carbon from purified

bone collagen, the source of essential amino acids to the pinnipeds diet can be linked to the source of carbon at the base of the food chain. Essential amino acids, which often exhibit very little isotopic fractionation as they are transferred along a trophic pathway, can be matched with biochemical synthesis pathways as they are made by primary producers (Hare et al., 1991; Larsen et al., 2012). Differences in carbon uptake and metabolism is apparent between broad taxonomic groups, such as kelp and phytoplankton, resulting in unique fingerprints for these groups (Larsen et al., 2013). The nonessential amino acids can be routed directly from an organism's diet or synthesized *de novo* using all of the available dietary macromolecules ((McMahon et al., 2010; Newsome et al., 2014).

Although many types of primary producers exist worldwide, functionally two are important for distinguishing the niches of marine predators in Tierra del Fuego: kelp, a brown algal, dominant species, in particular the giant kelp (*Macrocystis pyfera)* that only grows close to the coastline versus various phytoplankton species which occupy the photic zones of the open ocean, offshore. Brown algae in particular have more positive carbon isotopic ratios relative to phytoplankton. Diffusion of CO_2 into kelp blades is limited seasonally and bicarbonate uptake proceeds in an energy intensive series of reactions in order for the organism to keep photosynthesizing, which concentrate greater proportions of ^{13}C in the tissue (Foley and Koch, 2010).

Bulk $\delta^{13}C$ and $\delta^{15}N$ isotopes has shown significant dynamism in Fuegian marine ecosystems (Nye et al. 2019a, Zangrando et al., 2014b; Vales et al., 2016). While

significant decreases in average $\delta^{13}C$ are observed over several thousand years which likely indicate changes in habitat and resource use from coastal to pelagic ecosystems, it is unclear if these dynamics are resulting in changes in behavior of organisms at higher trophic levels or if there are shifts at the base of the food web. Ranges in bulk isotopic compositions are also highly variable at different time periods. Without being able to measure all components of the food web, including all likely prey items of pinnipeds at all time periods, there is potential that the food chain length and shifts in ecological baseline are not well represented by bulk isotopic data. To fully characterize the food web, measurements from the $\delta^{13}C$ of essential amino acids, which represent the ecological baseline, are needed.

This study aims to investigate whether pinnipeds shifted their ecological niche preference in response to human exploitation, and if so, how significant of shift this was. We also seek to find the relationship of basal resource use of Otariids to coexisting species, such as the end-member coastal Imperial shag and open ocean Patagonian squid, as well as intermediary species like Patagonian grenadier. We predict that Otariids shift their resource use and feeding habitat in response to human activities. We aim to answer these questions using analysis of $\delta^{13}C$ of amino acids in tissues of different organisms.

Methods

Sample selection

Otariid samples were selected from sites along the south and southeastern coastlines of Tierra del Fuego (Fig. 1). Samples were separated into different time bins, based on radiocarbon dates, for each species to identify temporal trends in $\delta^{13}C$ of essential amino acids. Modern kelp and archaeological shag from the region were also selected to represent coastal end members, whereas squid were collected from a local supermarket in Ushuaia to characterize the pelagic amino acid carbon isotopic signature. Archaeological grenadier was selected as both a potential prey source for Otariids and as a representative of an intermediary migratory species.

Preparation

All samples were measured for their bulk $\delta^{13}C$ values before hydrolysis and derivatization of amino acids (Nye et al., 2019a). Ten milligrams of organic tissue from samples were hydrolyzed for 20 hours at 110° C in 1 milliliter of 6N hydrochloric acid in vials flushed with N_2. The resulting hydrolysate of the samples and a mixture of amino acid standards were dried down under N_2 and reacted with a 4:1 Isopropanol-Acetyl Chloride solution for 1 hour at 110° C. Samples and standards were then dried under N_2 and washed twice with 200 microliters of dichloromethane (DCM). We then derivatized the samples and standards using 500 microliters of triflouroacetic acid anhydride (TFAA) in solution with 500 microliters of DCM for 10 minutes at 110° C. Samples and standards were then cooled to room temperature and dried under N_2, washed twice with 200

microliters of DCM and resuspended in 500 ml of DCM before injection into the GC-IRMS system (Silfer et al., 1991 I think).

Samples were analyzed using a suite of Thermo Scientific instruments at the UCR Edge Laboratory consisting of a Trace 1310 Gas Chromatograph with a GC-Isolink II routed through a Conflo IV interface into a Delta V Plus isotope ratio mass spectrometer (Thermo Fisher Scientific). Injections into the Trace 1310 are performed via a Triplus RSH autosampler. The autosampler syringe is washed x times in DCM before and after every 1 microliter injection. Typical samples were injected at a concentration of 1mg/200uL in triplicate with an amino acid standard injection between sample injections. The Trace 1310 GC is equipped with a 60-meter DB-5 column (1 micron film thickness) that ramps from 50° C to 320° C over 25 minutes to ensure adequate separation between amino acid peaks. Regular maintenance includes periodic baking at 320° C to remove potential contaminants in the column and replacement of the injection liner upon deviations in GC performance. The combustion reactor in the GC-Isolink II is heated to 1000 ° C to ensure complete combustion of amino acids into CO_2 which is then routed into the Conflo IV interface. After an initial longer oxidation of immediately after installation of a new reactor, we maintain sufficient oxygen levels in the combustion reactor by performing short 1 minute seed oxidations every injection to ensure complete combustion of samples. The combustion reactor is monitored and maintained at regular intervals by inspecting peak shape, retention times, values of standards and number of injections. The combustion reactor is typically replaced after oxidation of the reactor

becomes ineffective, we observe poor peak shapes and retention times, or identify poor reproducibility of standards which usually occurs after 500 injections.

All samples are corrected to an in-house standard mixture of 13 amino acids (alanine, glycine, threonine, serine, valine, leucine, isoleucine, proline, aspartic acid, glutamic acid, phenylalanine, tyrosine, and lysine) after measurement on the Delta V Plus IRMS. The amino acids in the standard mixture have been corrected to the international standard for carbon, Vienna Pee Dee Belemnite, via independent measurements using a continuous flow EA-IRMS in the same laboratory (see Nye 2019a). We corrected the samples by calculating the $\delta^{13}C$ value of carbon introduced by TFAA and isopropanol from derivatization in the standard mixture (O'Brien et al.), identifying the proportion of carbon added to each individual amino acid, and then subtracting the average difference of the TFAA's carbon isotopic value of three standard injections with the average of three sample injections. Reproducibility of standards were within 1.1‰ for all amino acids, with an average of 0.8‰.

Analyses

It is assumed that essential amino acids display minimal isotopic fractionation between trophic levels. Therefore, essential amino acid $\delta^{13}C$ values should not be significantly different in higher level organisms as these amino acids are directly routed into tissues. We applied a mixing model approach to identify which primary producers might be contributing the most to animal diets. Essential amino acids (EAAs) were compared in R (ver. 3.5.2) where Principal Components Analyses (PCA) and Linear

Discriminant Analyses (LDA) were performed. PCAs were run with all organisms to identify which EAAs were responsible for the most variation among species, while LDAs were trained using the end-member and intermediary species. Thus, primary producer biochemical pathways should be mirrored despite trophic distinctions. Comparisons of Otariid carbon CS-AA fingerprints to end member and intermediary species were constructed from different archaeological age-based bins. Bayesian mixing model software FRUITS was also used and an independent method to estimate relationships of species to one another by comparing essential amino acids (Fernandes et al., 2014). We chose to use a non-weighted model with no concentration dependence and 2‰ offsets between food sources and consumers to account for potential fractionation.

Results

We observed significant variation in the isotopic values of carbon in both essential and non-essential amino acids in different organisms (Table 1, Fig. 2). $\delta^{13}C$ values of individual amino acids varied from -32.4‰ found in fish leucine to 13.5‰ in imperial shag glycine, a range of 45.9‰. Relative differences between essential amino acids appear similar between basal food web resources and consumers, except for threonine in imperial shag. Non-essential amino acids appear very different between basal food web resources and consumers. These amino acids showed larger variation and had higher standard deviations than essential amino acids in both basal food web resources and consumers, and there was much more variation in non-essential amino acids between the two groups. Within basal resources, the most positive essential

amino acids were generally found in kelp, and they were similar in fish and squid, except for leucine. In non-essentials, kelp and fish had similar values while squid were significantly different in their serine, aspartic acid and glutamic acid $\delta^{13}C$ values. We also observed significant variation in the $\delta^{13}C$ of amino acids in the giant kelp samples (Table 2). Non-essential amino acids showed the highest variation within kelp samples, most notable serine, glycine and alanine.

Figure 2-2 - $\delta^{13}C$ of amino acids in (a.) basal food web resources and (b.) consumers. Patterns in essential amino acids are conserved in consumers while non-essential amino acids can differ significantly due to de novo synthesis in consumers.

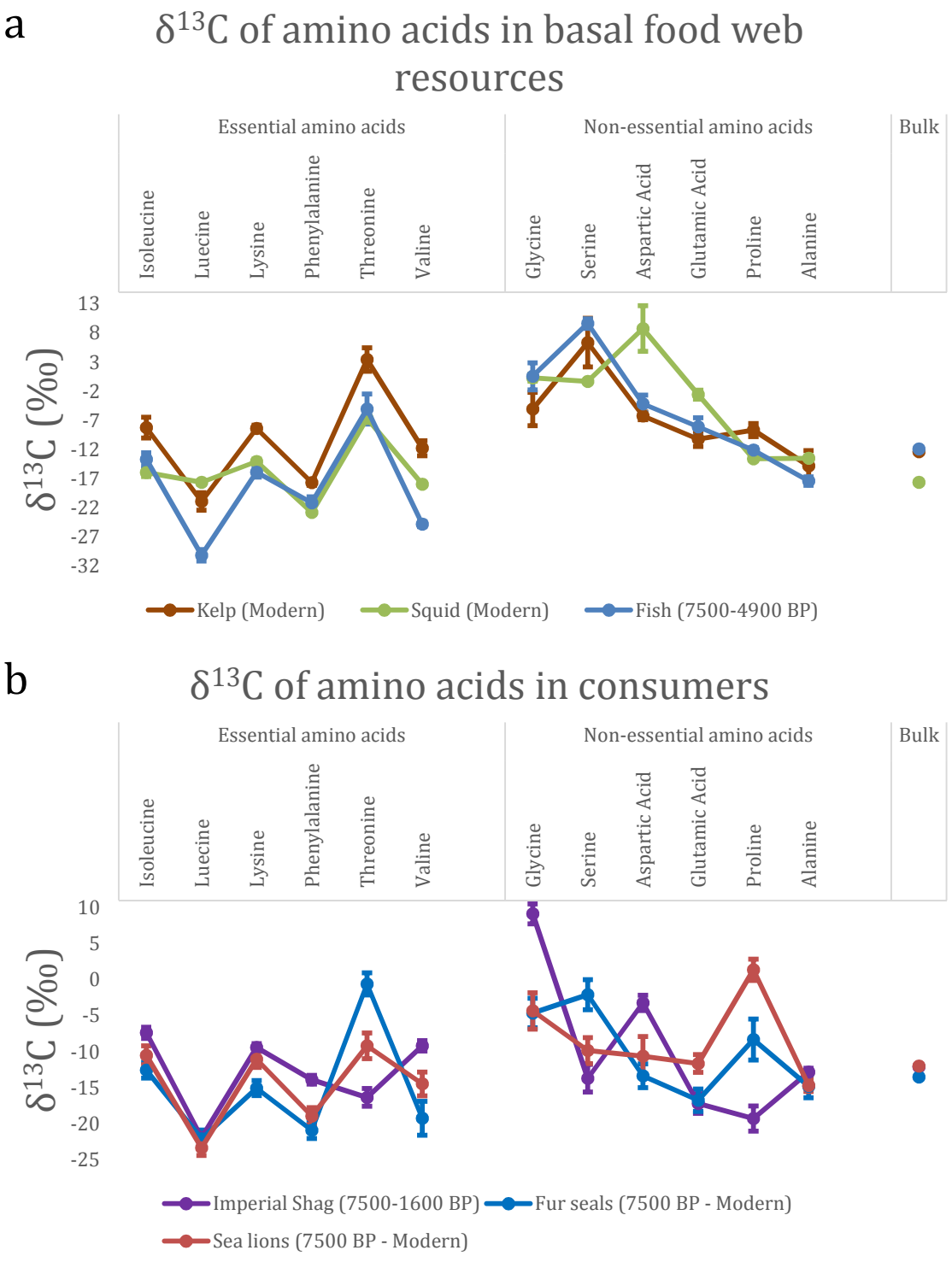


Table 2-1 - $\delta^{13}C$ values of AAs in fur seals and primary producers/consumer nearshore and offshore end members.

	$\delta^{13}C$ values of essential amino acids (‰)						$\delta^{13}C$ values of non-essential amino acids (‰)							
	Isoleucine	Leucine	Lysine	Phenylalanine	Threonine	Valine	Glycine	Serine	Aspartic acid	Glutamic acid	Proline	Alanine	Bulk $\delta^{13}C$ (‰)	n
Fur seals (7500-4900 BP)	-10.5±1.0	-20.4±1.4	-15.2±1.2	-16.9±1.1	3.4±1.7	-14.5±1.1	-4.9±1.5	4.8±2.0	-11.6±1.2	-12.9±0.7	-14.6±0.9	-12.2±1.4	-13.5	9
Fur seals (2200-1600 BP)	-11.3±3.7	-20.0±1.6	-9.6±1.5	-21.7±0.3	1.2±6.4	-15.6±2.2	-3.7±2.2	-0.3±4.4	-17.3±3.1	-16.3±1.8	-14.0±1.7	-12.1±3.9	-11.8	7
Fur seals (Modern)	-15.8±2.1	-25.9±4.3	-20.4±3.6	-24.2±5.3	-6.4±1.1	-27.6±10.9	-5.3±8.6	-10.8±6.1	-11.1±5.6	-21.0±6.8	3.7±14.5	-20.0±4.3	-15.3	4
Sea lions (7500-4900 BP)	-11.7±2.7	-24.5±2.0	-11.7±2.9	-18.6±2.3	-12.2±5.3	-13.1±2.2	-7.4±2.4	-16.5±5.1	-11.9±5.0	-11.6±2.9	-0.1±1.9	-14.3±1.6	-11.2	7
Sea Lions (2200-1600 BP)	-5.9±4.3	-22.3±3.6	-11.0±2.1	-16.0±4.1	-10.4±3.5	-12.5±7.1	0.1±5.5	-8.8±4.4	-8.4±10.1	-11.7±4.0	-2.8±6.2	-14.1±2.8	-11.8	7
Sea Lions (Modern)	-13.9±0.9	-23.3±0.6	-10.4±1.7	-22.1±0.6	-4.9±1.8	-17.8±0.6	-5.7±7.3	-4.2±1.2	-11.6±1.3	-11.5±1.3	7.0±0.8	-15.7±0.6	-12.8	5
Kelp (Modern)	-8.2±3.5	-20.9±3.0	-8.4±1.1	-17.7±0.9	3.5±4	-11.8±2.6	-5.0±5.7	6.4±8.4	-6.2±1.3	-10.2±2.5	-8.7±2.2	-14.8±5.3	-12.3	4
Squid (Modern)	-15.9±1.4	-17.6±0.6	-14.1±0.9	-22.8±0.5	-6.7±0.6	-18±0.4	0.3±1.2	-0.3±0.3	8.8±7.8	-2.6±1.6	-13.6±0.5	-13.5±0.7	-17.6	4
Fish (7500-4900 BP)	-13.7±2.2	-30.1±2.0	-15.9±1.6	-21.1±2.1	-5.0±5.2	-24.8±0.8	0.6±4.6	9.6±1.7	-4.2±3.0	-8.1±3.1	-12.1±0.8	-17.4±1.4	-11.9	8
Imperial Shag (7500-1600 BP)	-7.4±1.5	-21.9±1.2	-9.4±1.0	-13.9±1.2	-16.3±2.4	-9.2±1.4	9.1±2.8	-13.7±3.8	-3.2±2.1	-17.2±2.7	-19.3±3.5	-12.8±1.0	-12.1	8

Table 2-2 – Variation in δ13C values of amino acids in giant kelp (‰)

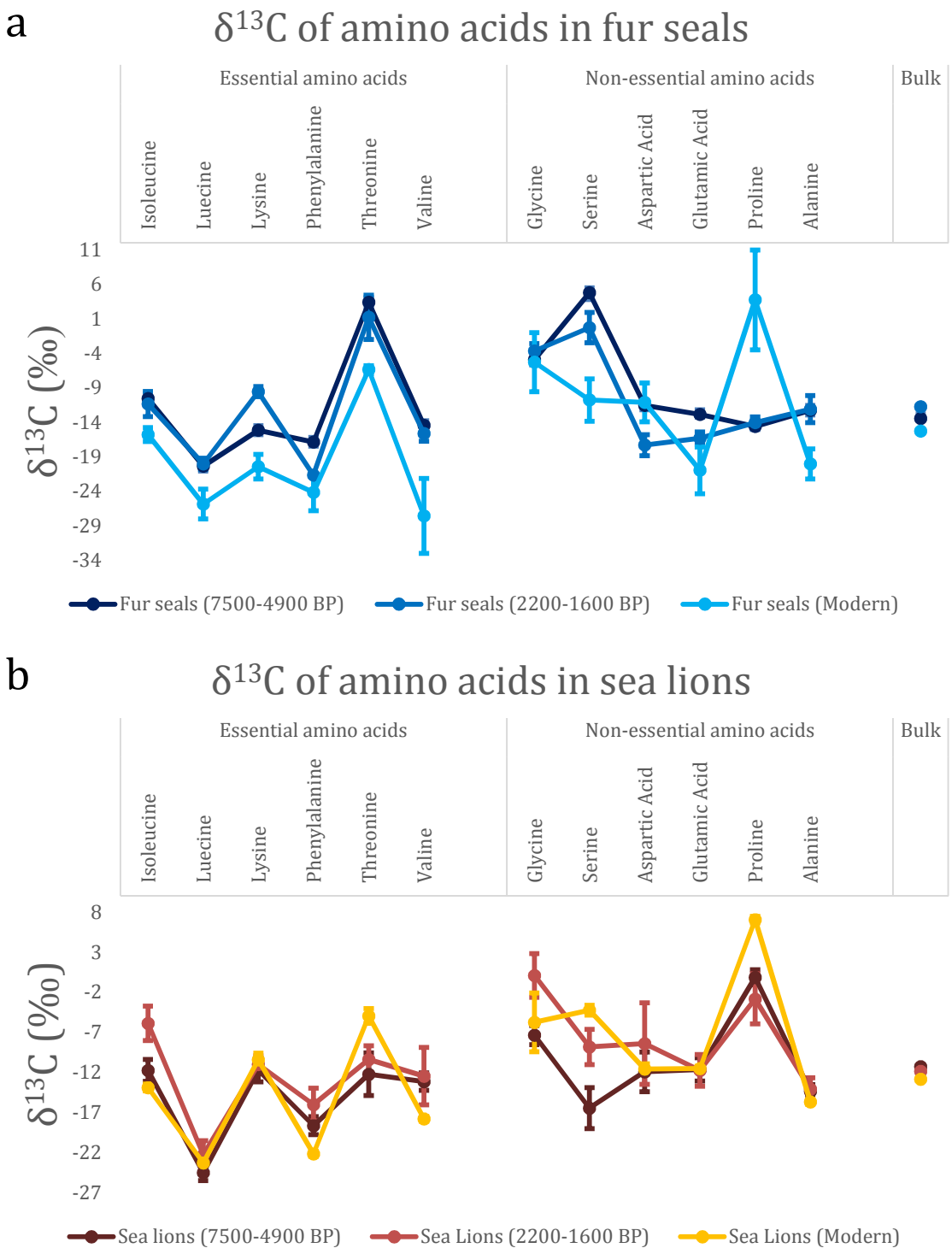
$\delta^{13}C$ values of amino acids in giant kelp (‰)

	3Amigos	TH-Kelp1	Kelp1	Kelp2	Average	Standard Deviation
Non-essential						
Glycine	-1.0	-0.6	-5.5	-13.0	-5.0	5.7
Serine	17.1	8.4	2.4	-2.4	6.4	8.4
Aspartic acid	-5.3	-8.2	-5.4	-6.0	-6.2	1.3
Glutamic acid	-8.5	-9.2	-9.0	-14.0	-10.2	2.5
Proline	-7.3	-8.7	-6.9	-11.8	-8.7	2.2
Alanine	-7.4	-19.5	-14.9	-17.4	-14.8	5.3
Essential						
Threonine	7.1	6.1	2.5	-1.8	3.5	4.0
Leucine	-17.5	-20.1	-21.2	-24.7	-20.9	3.0
Isoleucine	-7.5	-4.3	-8.2	-12.9	-8.2	3.5
Valine	-9.1	-12.2	-10.6	-15.1	-11.7	2.6
Phenylalanine	-17.3	-17.4	-16.9	-19.0	-17.7	0.9
Lysine	-7.9	-7.3	-8.4	-9.8	-8.3	1.1
Bulk	-10.9	-11.3	-11.6	-15.6	-12.3	2.2

There were significant differences between species of pinnipeds in both essential and non-essential amino acids (Table 1, Figs. 2 & 3). In essential amino acids, lysine, threonine, and valine significantly differed while in non-essential amino acids serine, glutamic acid and proline significantly differed, with proline and threonine being the most different between the two. We observed significant differences between pinniped species in different time periods (Table 1, Fig. 3). In fur seals, modern $\delta^{13}C$ values were more negative by about 5‰ when compared with $\delta^{13}C$ from earlier time periods. Non-essential amino acids were similar between different time periods with several notable

exceptions, serine being more negative and proline significantly more positive than earlier time periods. In sea lions differences between different time periods were lesser in magnitude than in fur seals. Essential amino acids with significantly different values between different time periods were apparent in isoleucine, phenylalanine and valine (both increasingly negative with time), and threonine (more positive with time). In non-essential amino acids serine values progressively decreased while proline progressively increased over time.

Figure 2-3 - $\delta^{13}C$ of amino acids in (a.) fur seals and (b.) sea lions of different time periods



Several amino acids were similar to bulk diet when applying a diet to collagen correction, collagen being 5‰ more positive than diet (Ambrose and Norr, 1993). In essential amino acids, lysine, phenylalanine and valine were more likely to be representative of diets in consumers, while glutamic acid in the non-essential amino acid group was more likely to represent routing from dietary sources due its similarity in isotopic ratio with essential amino acids.

PCA of the $\delta^{13}C$ of essential amino acids showed separation among kelp and basal food web resources (Fig. 4). The most variation is seen within Giant Kelp, where 95% confidence intervals overlap with other basal food web resources. Principal component 1 accounts for 66.1% of the variation in $\delta^{13}C$ of essential amino acids. Lysine appears to be the greatest contributor to PC1's variation, though all contribute some amount. PC2, accounting for 23% of the variation, is largely driven by the $\delta^{13}C$ of leucine and a combination of the other AAs except for lysine. PCA of consumer essential amino acid $\delta^{13}C$ (Fig. 5) showed significant overlap for species, except between fur seal and imperial shag. The majority of variation was driven by phenylalanine, lysine, valine and isoleucine while nearly a quarter of the variation was being driven by threonine and leucine.

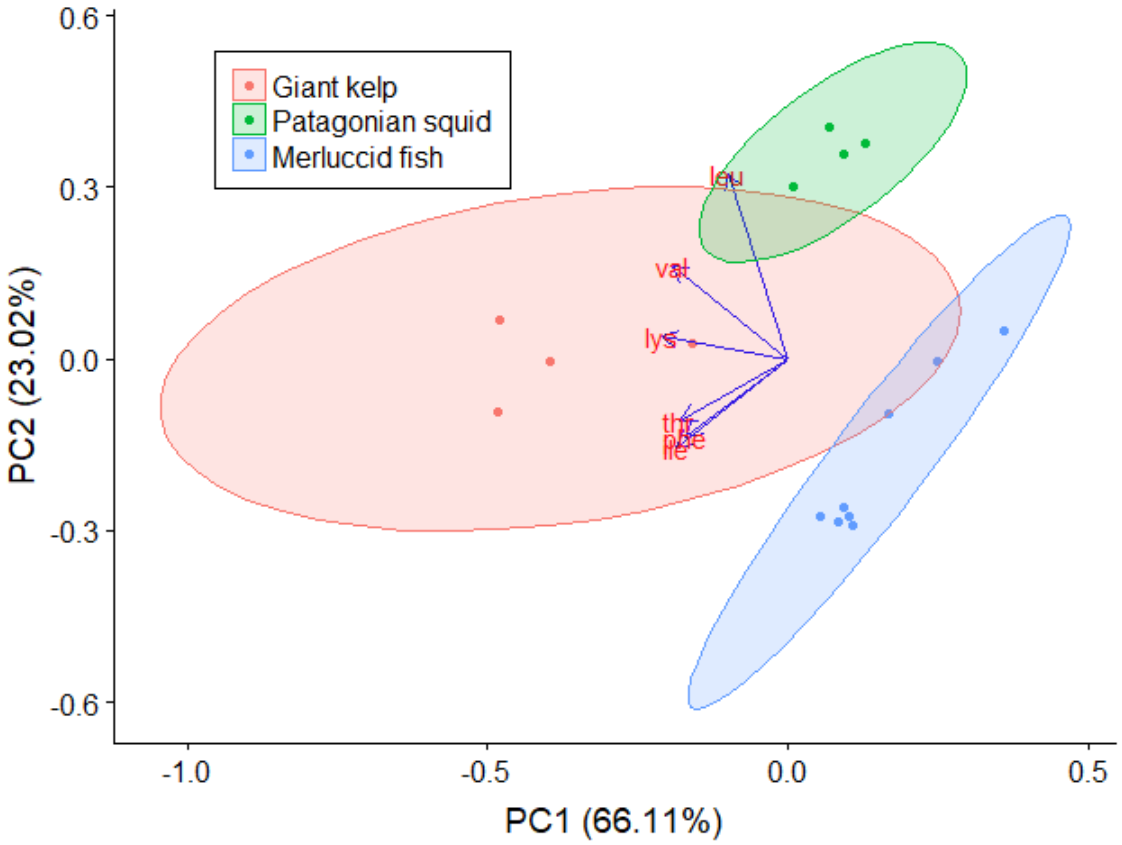


Figure 2-4- PCA of different potential primary producer/primary producer proxies using essential amino acids. The majority of variation (PC1=Principal component 1) is expressed through all of the essential amino acids and biased by more extreme values found in kelp with significant overlap in fish and Patagonian squid. A significant minority of variation (PC2=Principal component 2) is expressed through leucine, showing separation between fish and squid, but more overlap between kelp and fish. (leu=leucine, val=valine, lys=lysine, thr=threonine, phe=phenylalanine, ile=isoleucine).

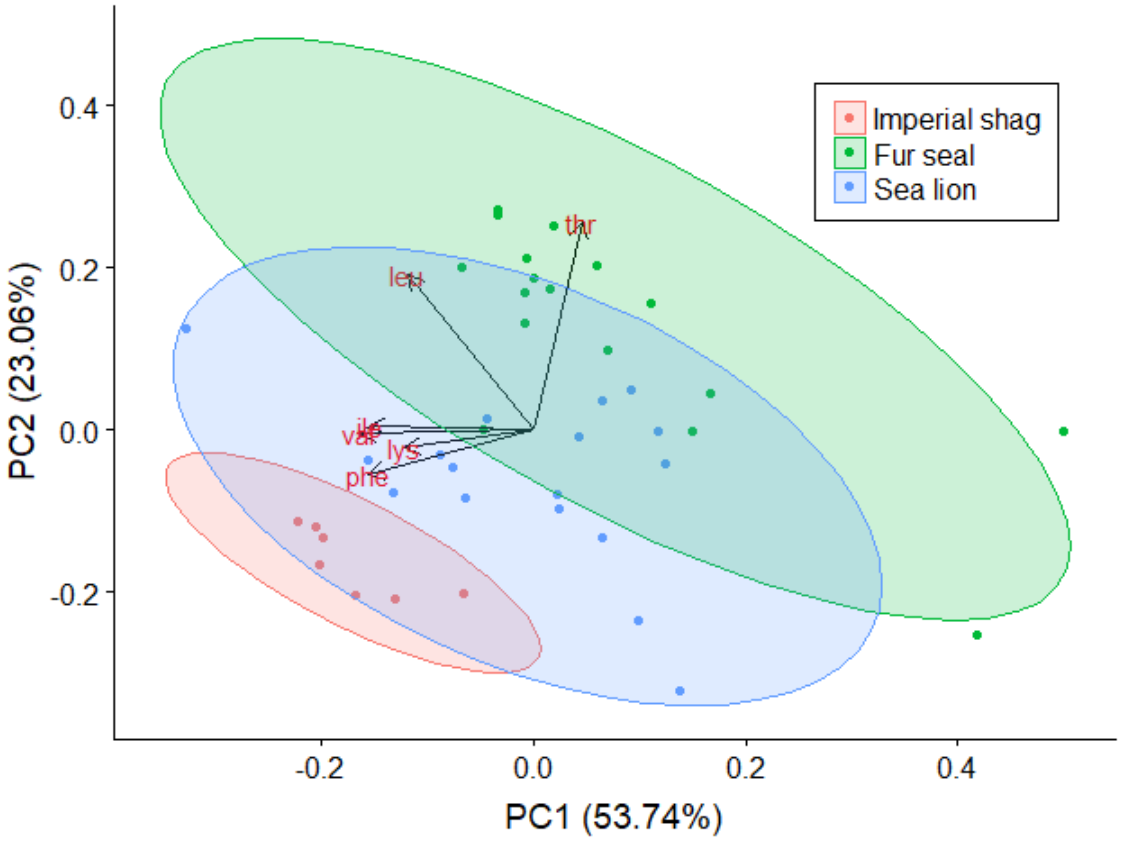
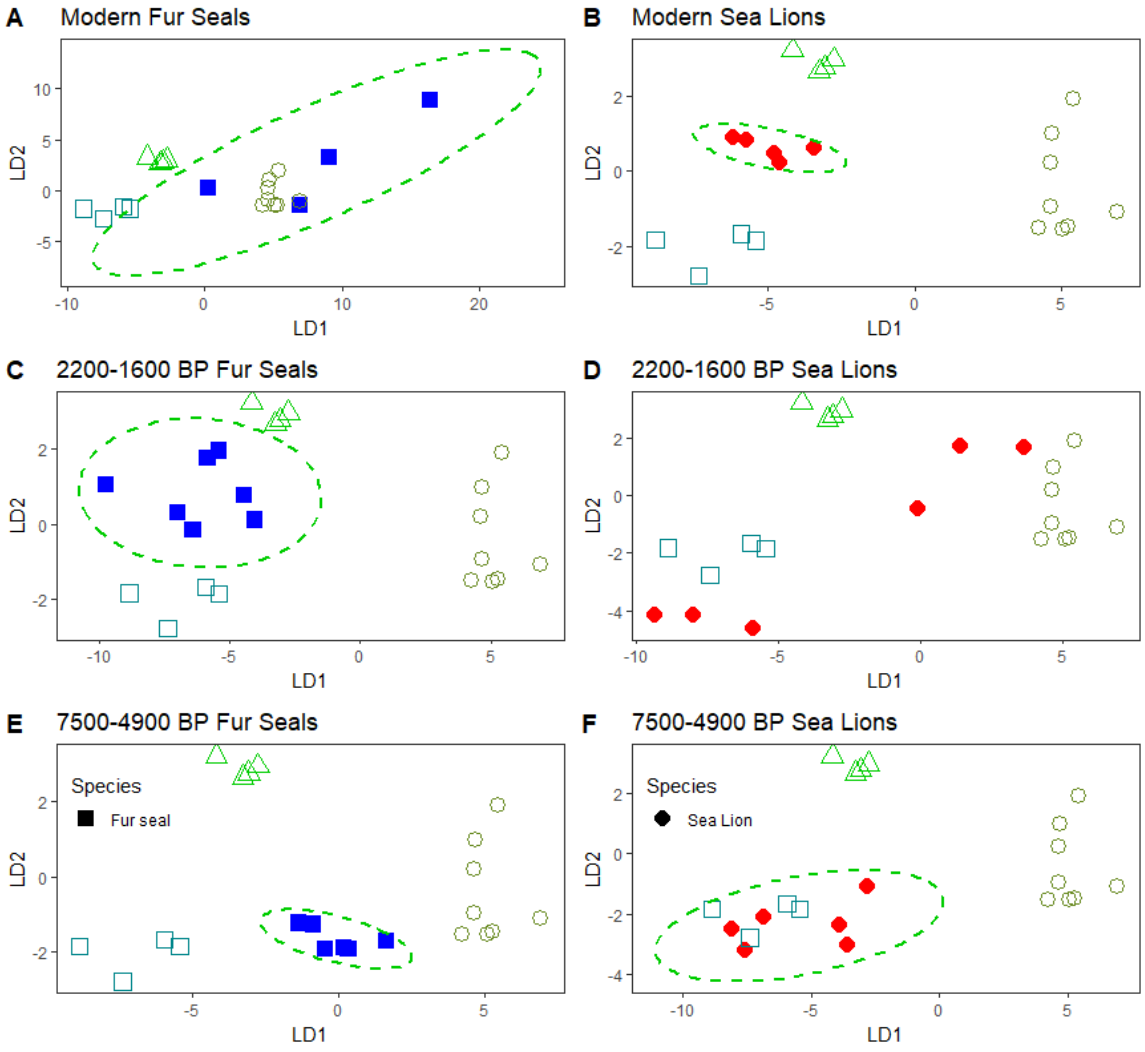


Figure 2-5 – PCA of essential amino acid $\delta^{13}C$ in consumers. The majority of variation is being driven by several amino acids (PC1=Principal component 1, ile=isoleucine, val=valine, lys=lysine, phe=phenylalanine) while a significant minority is bet two essential AAs (PC2=Principal component 2, leu=leucine and thr=threonine). PC1 shows significant overlap in essential amino acids between all three consumer groups, while PC2 shows separation between imperial shag and fur seals, indicating potential differences in basal resource utilization.

Following results of the primary producer proxy PCA, an LDA model was trained using $\delta^{13}C$ values derived from essential amino acids in giant kelp, Patagonian squid and grenadier. $\delta^{13}C$ values of Imperial Shag, fur seals and sea lions from different time periods were then analyzed relative to the primary producer proxies. Imperial Shag appear closest to giant kelp in linear discriminant space (Fig. 7). Both fur seals and sea lions significantly differ in linear discriminant space in time relative to intraspecies populations (Fig. 6). In fur seals, the 7500-4900 BP population is tightly packed in mixed

carbon space between kelp and fish (Fig. 6e), the 2200-1600 BP population is loosely clustered but mixed between kelp and squid (Fig. 6c), while the modern population is scattered closest to fish (Fig. 6a), but with outliers unlike any of the three basal food web resources. In sea lions, the 7500-4900 BP population is closely aligned with kelp (Fig. 6f) and the modern population is tightly grouped between kelp and squid (Fig. 6b). Sea lions from 2200-1600 BP are separated into two distinct groups, half are closely associated with kelp while the other half appears mixed between fish and squid (Fig. 6d).

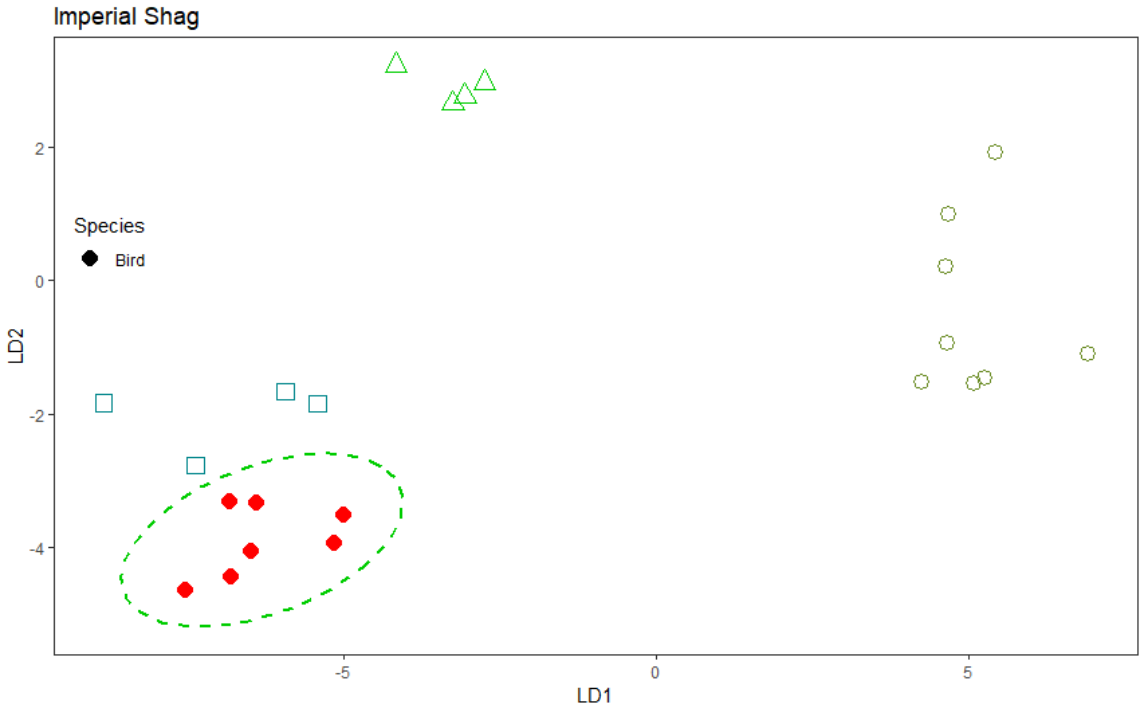
Figure 2-6 – Linear Discriminant Analysis (LDA) of fur seals and sea lions trained with kelp, squid and fish essential amino acid $\delta^{13}C$ values. Open shapes represent basal food web resources, turquoise squares=Giant kelp, green triangles=Patagonian squid, yellow circles=Merluccid fish. Filled shapes represent consumers, and dashed ellipses represent 95% confidence intervals. Blue filled squares represent fur seals while red circles represent sea lions. Both species of otariids dynamically shift in LDA space and ultimately move away from coastal resources (kelp) over time.



Populations of the two pinniped species differ in linear discriminant space at similar time periods as well. At the earliest time period (7500-4900 BP) fur seals are mixed between kelp and fish while sea lions are closely aligned with kelp (Fig. 6e & 6f). At 2200-1600 BP, some sea lions are still closely associated with kelp while others are

mixed between fish and squid (Fig. 6d). In contrast, fur seals are mixed between kelp and squid (Fig. 6c). Modern fur seals and sea lions also show no overlap, sea lions are densely packed between kelp and squid while fur seals scattered (Fig. 6a & b). One individual fur seal appears mixed between fish and squid, another is aligned with fish, and the remaining two have unique combinations of linear discriminants entirely (Fig. 6a).

Figure 2-7 - LDA analysis with Imperial Shag (Bird) trained with kelp, squid and fish essential amino acid $\delta^{13}C$ values. . Open shapes represent basal food web resources, turquoise squares=Giant kelp, green triangles=Patagonian squid, yellow circles=Merluccid fish. Filled red circles represent the consumer, imperial shag, and dashed ellipses represent 95% confidence intervals. These shorebirds are most closely associated with kelp, a coastal resource.



To further quantify potential carbon sources that may contribute to these animals, Bayesian analysis using FRUITS was applied (Fernandes et al., 2014). Imperial shag appears to have a diet the most closely associated with giant kelp with 72.7% match

(Figs. 5 & 8). Both fur seals and sea lions have their highest proportion of kelp-based carbon at the earliest time period (7700—4900 BP), which decreases as time progresses toward the present (Figs. 5, 6 & 7). Proportional similarity to other carbon sources (like fish and squid) are more complicated between time periods in both pinniped species. However, modern pinnipeds are more similar to fish and squid than kelp.

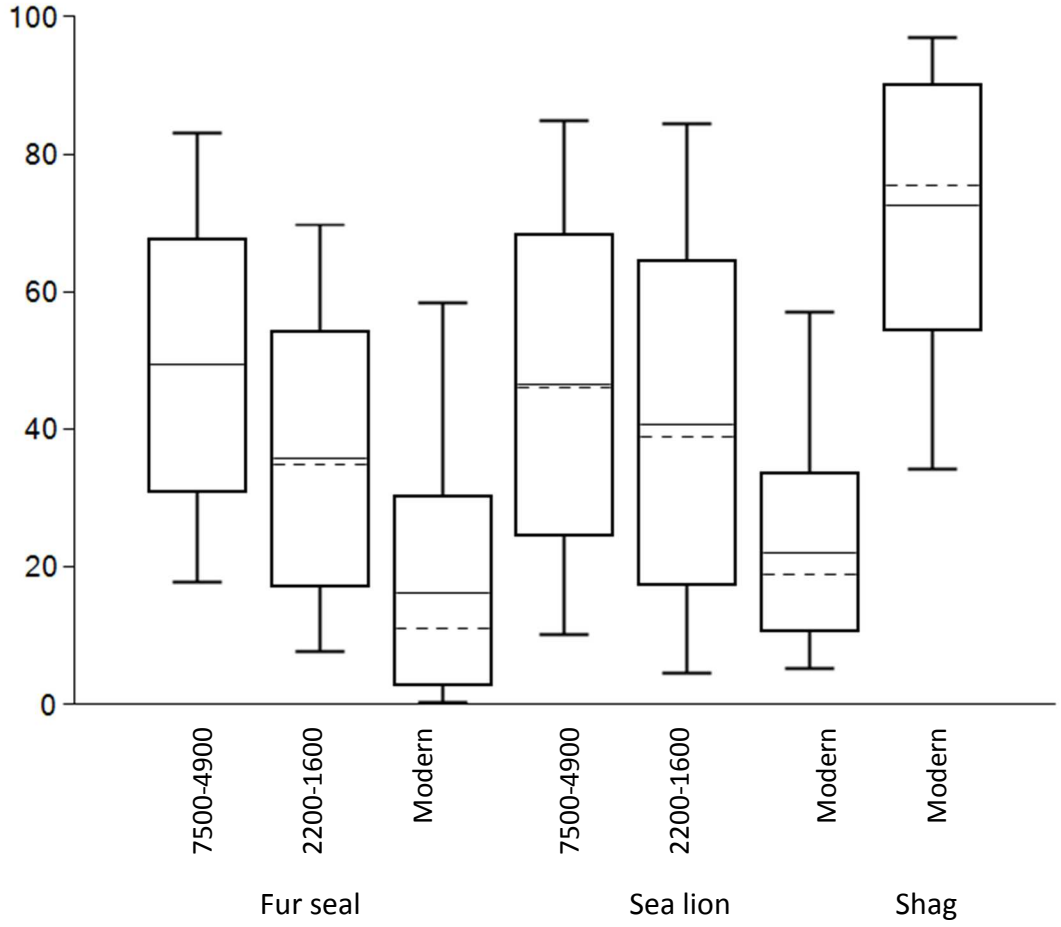


Figure 2-8 - Likelihood of kelp reliance over time for fur seals (A. australis), sea lions (O. flavescens) and imperial shag (P. atriceps) using Bayesian isotope mixing model software FRUITS. The mixing model was fed data from $\delta^{13}C$ measurements of the essential amino acids isoleucine, leucine, lysine, phenylalanine, threonine, and valine. Reliance on kelp decreases as time progresses to the present for both pinniped species. Cormorant carbon values largely match kelp values as expected for a shorebird.

Nonessential amino acid similarity varied considerably (Fig. 9). Similarity to different basal food resources generally matched fish and kelp in fur seals, and mostly kelp in imperial shag and sea lions. Variability in matching nonessential amino acid $\delta^{13}C$ values shows a low degree of confidence for matching fur seals with potential dietary sources relative to essential AAs. Sea lion and shag confidence was higher, but still highly variable, especially for kelp.

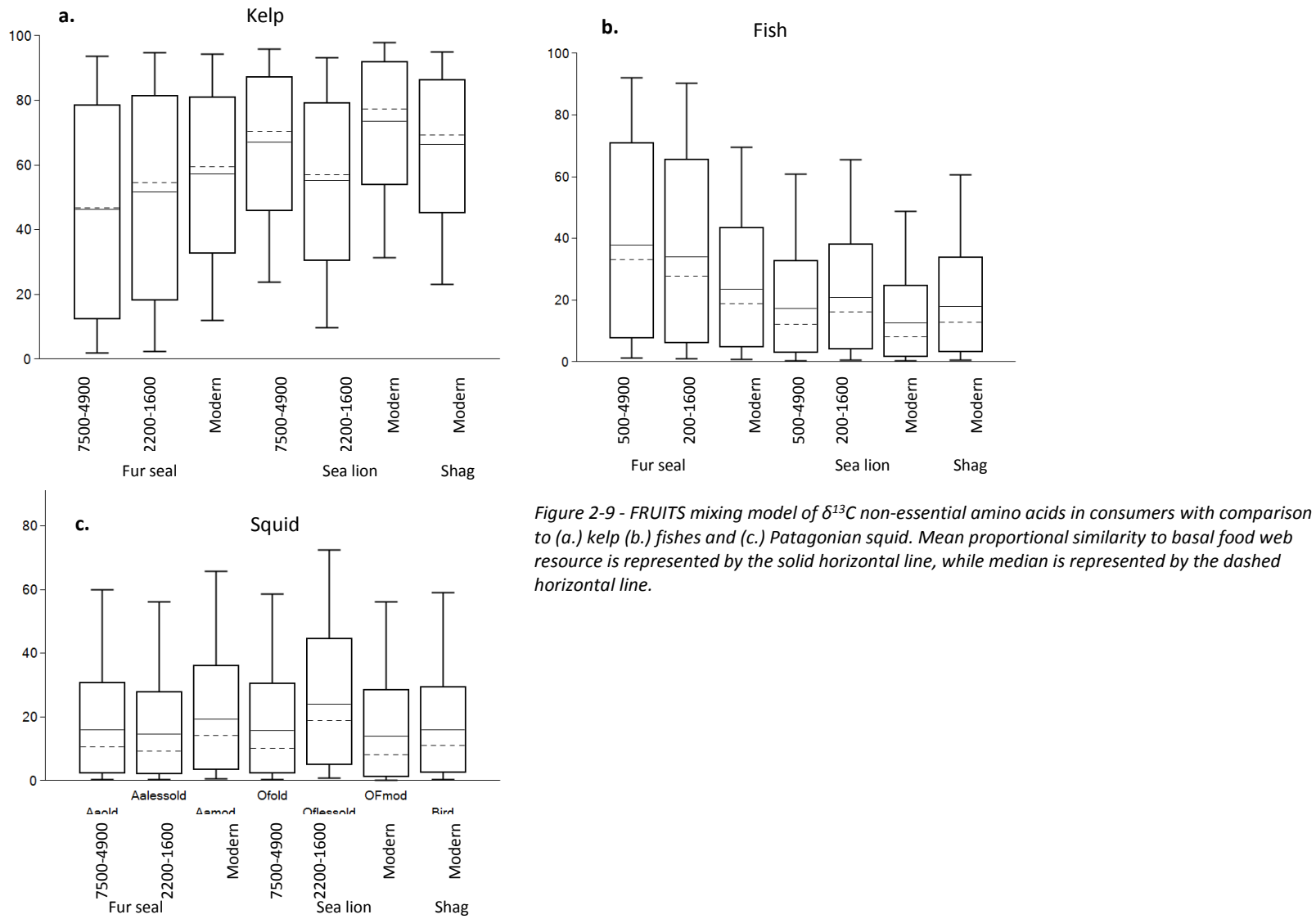


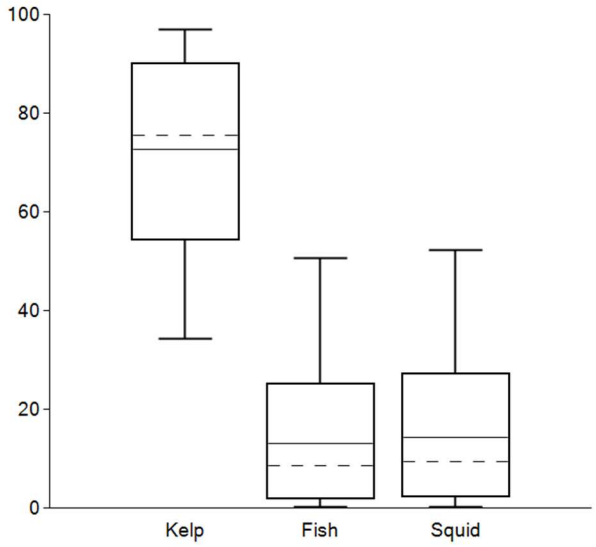
Figure 2-9 - FRUITS mixing model of $\delta^{13}C$ non-essential amino acids in consumers with comparison to (a.) kelp (b.) fishes and (c.) Patagonian squid. Mean proportional similarity to basal food web resource is represented by the solid horizontal line, while median is represented by the dashed horizontal line.

Discussion

The distinction between essential amino acids in primary producer proxies agrees with what one would expect given different photosynthetic pathways that lead to their synthesis (Abelson and Hoering, 1961; Hare et al., 1991). The isotopic patterns can be conserved in consumers, such as squid and fish (Larsen et al., 2013). Patagonian squid, relying on pelagic protein originating from phytoplankton, are distinguishable from proteins in giant kelp (Fig. 4). Despite many possible species that may contribute to the carbon in a squid's essential amino acids, they are distinctly different from those in macroalgae like giant kelp. What is notable, however, is the large range of variation between different individuals of giant kelp. Despite similar timing of collection, local effects may be resulting in this variation, from relative influences of terrestrial carbon to differences in sunlight availability modifying photosynthetic rate on individual kelp blades at different depths (Foley and Koch, 2010; Elliott Smith et al., 2018).

Patagonian grenadier presents an interesting combination of basal carbon resources. The species is known to migrate between nearshore and offshore areas (Lloris and Matallanas, 2005). It is likely to incorporate a variety of essential amino acids derived from different photosynthetic strategies, including those from phytoplankton in the open ocean and coastal macroalgae. Thus the $\delta^{13}C$ of their collagen AAs are a combination of their broad geographic distribution. Their carbon isotopic fingerprints are distinct from the other two dietary components.

Figure 2-10 - Primary producer dependence in Imperial shag (P. atriceps) using Bayesian isotope mixing model software FRUITS. The mixing model was fed data from $\delta^{13}C$ measurements of the essential amino acids isoleucine, leucine, lysine, phenylalanine, threonine, and valine. Most of the carbon that is incorporated into these shorebirds matches with carbon from kelp.



The comparison of essential amino acid patterning between the two pinniped species and the Imperial shag to primary producer proxies through linear discriminant analyses and mixing model results yielded results predicted from a highly dynamic and changing ecosystem. First, the imperial shag (Fig. 7 & 9), as a test of this analysis, confirms that this coastal seabird does in fact rely on coastal resources with its essential amino acid patterning closely matching that of giant kelp (Harris et al., 2016). Likewise, at a broad scale, both generalist pinniped species appear to incorporate resources in varying habitats, hinting at their dynamic life histories and in agreement with other observations of these species. In agreement with broader macroecological and food web theory, the two species show niche partitioning at any given time, never actually overlapping with each other (Drago et al., 2017). This is a finding that we were unable to detect using only bulk isotopic values (Nye 2019a).

Arctocephalus australis shows high adaptive capacity in that they accessed different basal resources at each time period (Figs. 3, 6 & 10). At 7500-4900 BP, fur seals

we analyzed are split between the migratory grenadier and kelp, indicating that they are biased towards coastal resources. At 2200-1600 BP, fur seal diets were split between coastal and pelagically derived carbon, showing that *Arctocephalus australis* are beginning to feed more offshore. In the modern era fur seals show an extremely dynamic pattern, largely aligned with migratory fish, but not kelp or squid exclusively. This dietary shift indicates two things: first, that fur seals are getting their essential amino acids from a mix of offshore and nearshore resources similar to grenadier, and secondly, the variation suggests these individual fur seals may not be a part of the same population. Instead, these individuals may be foraging in non-traditional habitats, such as the Southern Ocean.

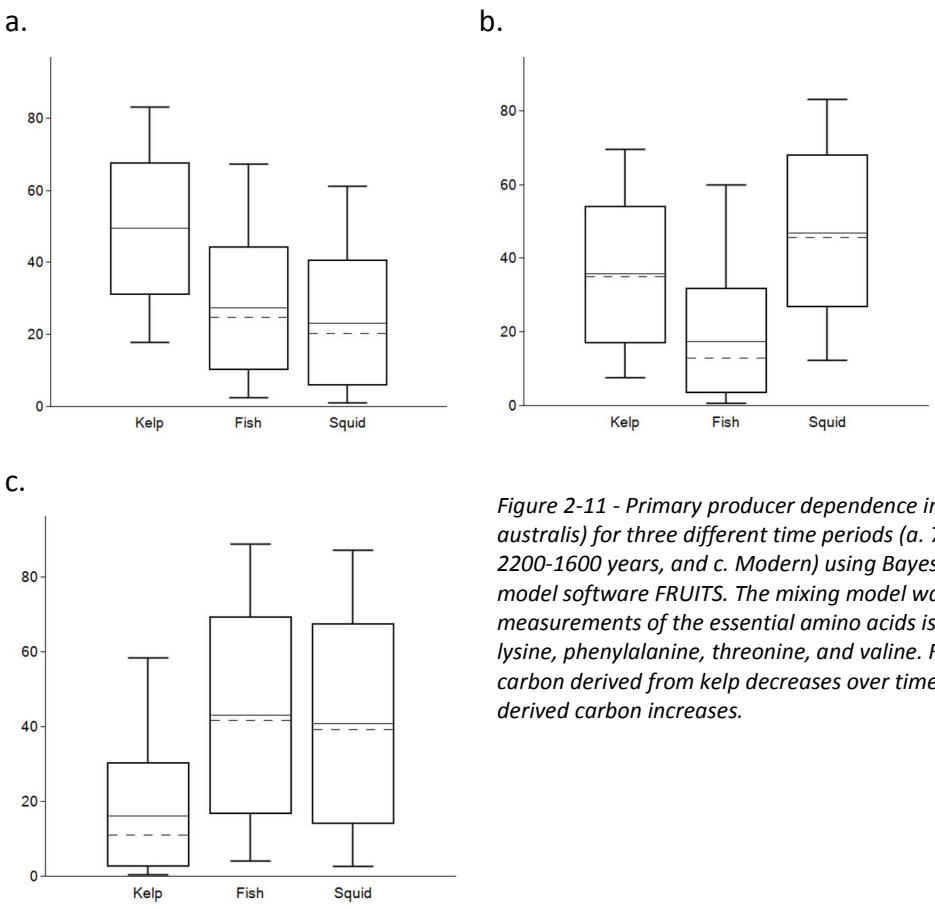


Figure 2-11 - Primary producer dependence in fur seals (A. australis) for three different time periods (a. 7500-4900 years, b. 2200-1600 years, and c. Modern) using Bayesian isotope mixing model software FRUITS. The mixing model was fed data from $\delta^{13}C$ measurements of the essential amino acids isoleucine, leucine, lysine, phenylalanine, threonine, and valine. Fur seal reliance on carbon derived from kelp decreases over time, while open ocean derived carbon increases.

Sea lions at 7500-4900 BP, unlike fur seals, overlap with giant kelp almost entirely. *Otaria flavescens* currently are deriving their protein carbon from coastal resources. At 2200-1600 BP sea lions appear to have split into two recognizable populations, one that is using coastal resources like their predecessors from several thousand years earlier and another that has shifted to mixed-offshore carbon sources like that of the migratory fish and squid. In modern sea lions, unlike fur seals, these individuals are tightly grouped, reflecting a niche that indicates a mixed coastal-offshore

diet between kelp and squid that was abandoned by fur seals over a thousand years earlier.

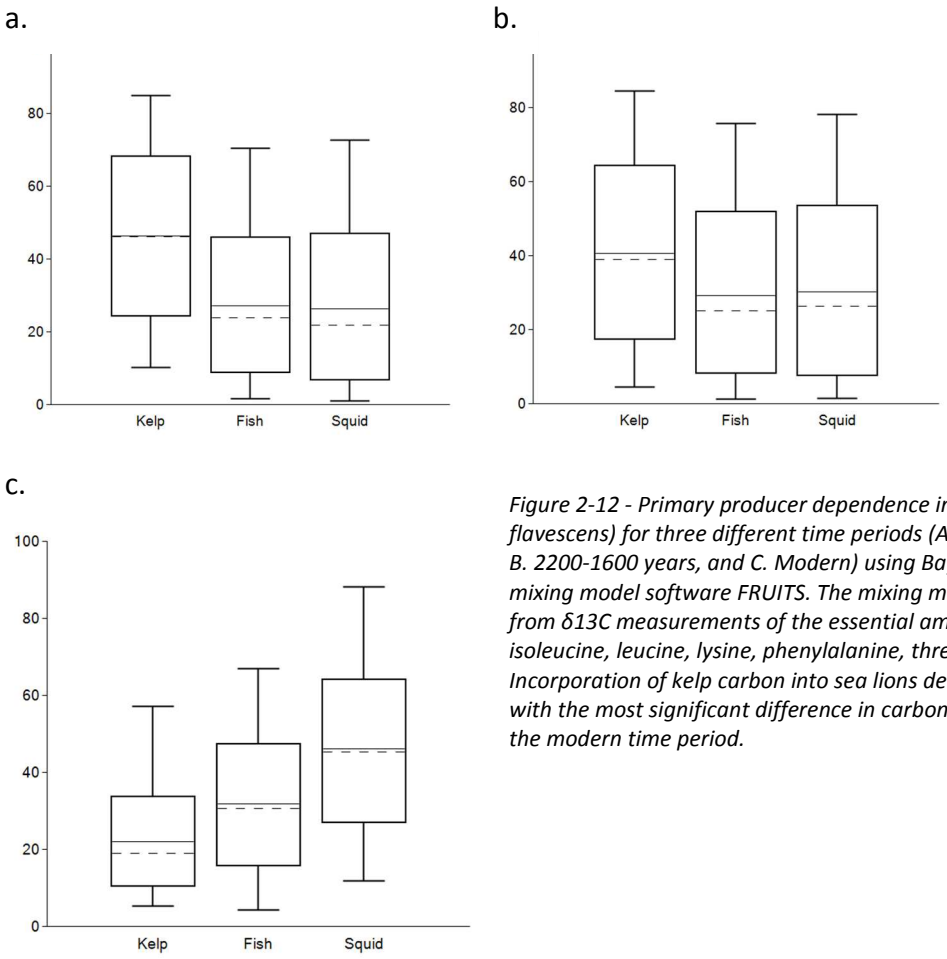


Figure 2-12 - Primary producer dependence in sea lions (O. flavescens) for three different time periods (A. 7500-4900 years, B. 2200-1600 years, and C. Modern) using Bayesian isotope mixing model software FRUITS. The mixing model was fed data from δ13C measurements of the essential amino acids isoleucine, leucine, lysine, phenylalanine, threonine, and valine. Incorporation of kelp carbon into sea lions decreases over time, with the most significant difference in carbon source present in the modern time period.

These findings agree with the independent Bayesian mixing model analyses using FRUITS (Fig. 8). Both LDA and Bayesian statistics show that imperial shag is relying on coastal resources. Also in agreement pinniped $\delta^{13}C$ values of essential amino acids deviate in their proximity to kelp $\delta^{13}C$ values over time. In 2200-1600 BP, the mixing model approach shows the higher proportion of squid in fur seal diet relative to fish, though for sea lions the diet is averaged and does not highlight the separate populations

like LDA does. However, the decrease in similarity of essential amino acids in pinnipeds to kelp is more apparent using this mixing model.

There are multiple potential explanations for the trends observed. The proxy approach for primary producers in this study could potentially be flawed due to the age of the specimens and the Suess effect, which has decreased $\delta^{13}C$ values in the atmosphere over time (Eide et al., 2017). Although this could potentially bias the $\delta^{13}C$ values in the kelp, squid and modern sea lions and fur seals, in marine environments, the dissolved inorganic carbon is likely being assimilated from pre-industrial sources due to the ocean's reservoir effect (Albero et al., 1986). The results of the mixing model also contradict this supposition as the most likely organism to be biased by the Suess effect, giant kelp, becomes the least matched carbon source by modern organisms. Conversely, the imperial shag, which was analyzed using pre-industrial remains, matches with the contemporary kelp despite the potential Suess effect bias.

The LDA approach circumvents this problem altogether as this type of analysis identifies patterns by classifying data rather than comparing raw values, as in the mixing model approach (Larsen et al., 2013). The fact that LDA agrees with mixing model data suggests that the Suess effect is not a concern for these data. A second potential concern with the age of the primary producer proxies is that these basal sources of carbon may have changed over time. However, the patterns of photosynthetic pathways that lead to the creation essential amino acids are unlikely to have changed significantly enough in the past 7500 years to alter the validity of these results.

A potential explanation for the trends observed in temporal pinniped basal resource dynamics is a bottom-up transformation of the trophic structure due to factors such as changes in primary productivity, which can be influenced from changes in climate. It is unclear what would cause a decline in coastal resources over time due changes in productivity from coastal macroalgae. Though we do not have an estimate of coastal macroalgae abundance in past contexts, there is still an abundance of kelp in the Fuegian archipelago today.

The most likely source of niche shifts in pinnipeds is from direct human activities, such as hunting and habitat occupation. In response to these human activities, it is likely that both pinniped species adapted their diets as they were restricted to more remote areas to house rookeries. In turn, both species began relying more on offshore resources. Archaeological evidence shows between 7500 BP and the 20th century, fur seals were the primary species hunted by humans, while sea lions were less preferred due their larger mass (Zangrando, 2009a). During this time, populations of sea lions are more closely aligned to coastal dietary niches while fur seals are not (Figs. 6 & 8).

With industrial sealing in the 19th and 20th centuries, sea lions became the preferred species to hunt with the increased ability to carry larger animals, and thus nearshore sea lions were nearly eliminated (Nye et al., 2018). Modern post-industrial sea lions had not refilled the coastal niche decimated by this activity, which is reflected by the lesser coastal resource utilization. Likewise fur seals, also affected by industrial sealing and thousands of years of direct hunting pressures, rely on more offshore

resources in post-industrial times. At least one of the fur seal individuals appears to be deriving carbon from a completely different resource than its predecessors, which could mark an expansion into new foraging territory with different photosynthetic pathways not measured here. These individuals may be feeding in spatially unique areas, such as outer areas like the Falkland Islands/Las Malvinas (Rodrigues et al., 2018).

Conclusion

The identification of resource use of top-level predators through primary producer proxies is a valuable way to characterize ecological processes and food web dynamics. Quantifying patterns of essential amino acids through methods such as PCA, LDA and mixing models allows us to decipher the isotopic and foraging niches over long time scales. Pinnipeds appear to have shifted their dietary niche preferences in response to human impacts, away from coastal resource use to offshore resources. As fur seals (*Arctocephalus australis*) and sea lions (*Otaria flavescens*) are now protected from direct hunting, we may expect to see prior niches re-occupied. However, with climate change becoming an increasing threat to wild organisms, especially in high-latitude regions, fur seals and sea lions are likely to shift their diets more as bottom-up trophic dynamics play an increasing role in food web structure.

Chapter 3 - Stability and variation in trophic position of southern South American pinnipeds over time

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Abstract

Our understanding of the variability in pinniped trophic positions in marine food webs of southern South America is limited by a lack of sampling availability of prey species and basal food web resources. Compound specific amino acid analyses of $\delta^{15}N$ in South American fur seals (*Arctocephalus australis*), South American sea lions (*Otaria flavescens*) and Merluccid Hakes allows us to independently estimate trophic position of pinnipeds. To accomplish this, we measured and compared the $\delta^{15}N$ of phenylalanine, a source amino acid, with the $\delta^{15}N$ of glutamate, a trophic amino acid, to estimate trophic position. The difference between the $\delta^{15}N$ of glutamic acid and phenylalanine is used in a calculation based on a trophic discrimination factor common to marine predators. We found that both pinnipeds had a trophic position of 3. Populations of pinnipeds from different time periods were also feeding at the same trophic level. However, we observed significant variation in basal resource procurement among individuals within each group, but not at population or species level scales. Modern pinnipeds showed the largest amount of variation in trophic position while hake occupied a high trophic level.

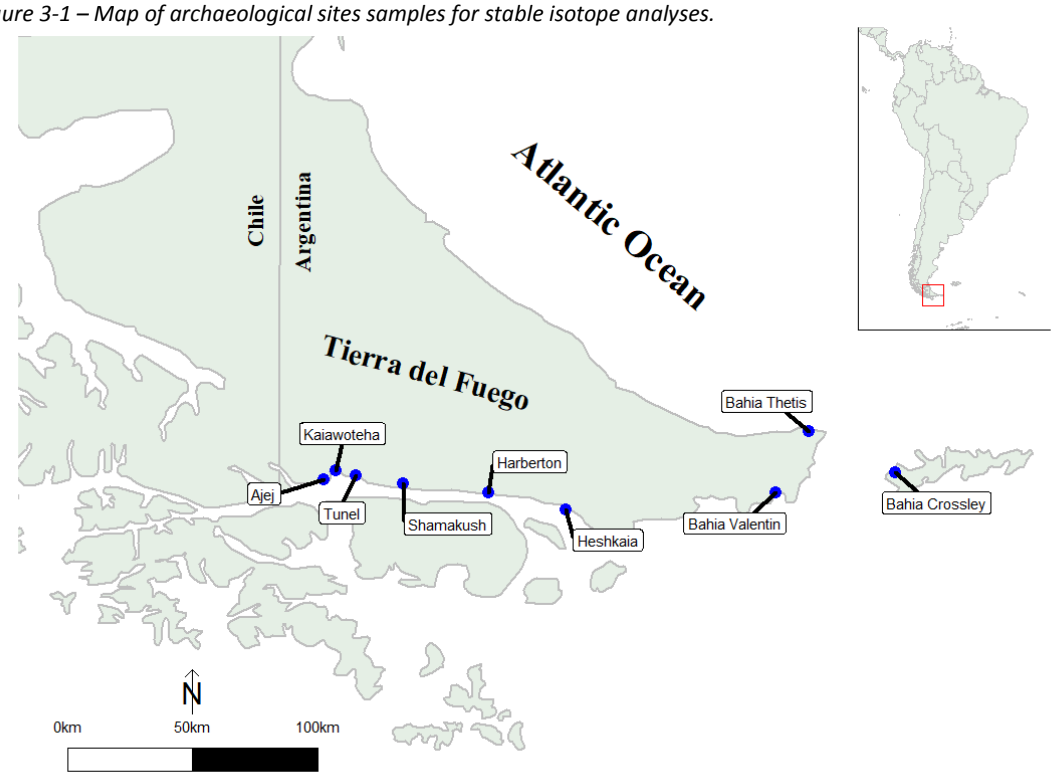
Introduction

Predator-prey dynamics are hotly studied and regarded as an important component of community assembly (Drago et al., 2017; Polito et al., 2019). Changes in populations and shifting species interactions are tightly integrated with trophic structures (Boecklen et al., 2011; Riccialdelli et al., 2017). Trophic structural changes often arise from ecological disturbance and population shifts (Kiszka et al., 2015; Barak et al., 2016). This phenomenon has been observed in terrestrial ecosystems but is less clear in marine ecosystems (Pirotta et al., 2017). Changes in community assembly and species interactions may not be so obvious in marine ecosystems due to the ability of generalist high trophic level species to shift their diets between multiple potential habitats and resources (Baylis et al., 2015).

Pinnipeds, as apex predators, exert a large influence over other species in their respective food webs through predation and competition (Vales et al., 2016; Drago et al., 2017). Two of these Pinnipeds species (family Otariidae), the South American fur seal (*Arctocephalus australis*) and the South American sea lion (*Otaria flavescens*), are abundant in coastal South America from Peru, south to Cape Horn, and north to Uruguay as well as the Falkland Islands/Las Malvinas. Their populations have fluctuated significantly in the recent past (Romero et al., 2017; Rossi-santos and Editors, 2018a). Their historic abundance proved to be useful for human populations as pinnipeds were exploited from the middle Holocene (7500 BP) to the middle of the 20th century. The reasons for and levels of exploitation of these species changed over this time period.

Two significant proximal human activities have influenced these particular Otariid population dynamics: destruction of habitat and hunting. The potential responses of *A. australis* and *O. flavescens* to these activities are numerous and range from relocation of rookeries to alterations in diet (Zenteno et al., 2015). These responses may not only affect the species in question, in fact, changes in Otariids can reverberate further and affect interspecies relationships of marine ecosystems around the Fuegian Archipelago (Fig. 1).

Figure 3-1 – Map of archaeological sites samples for stable isotope analyses.



Modern observations of both species show they primarily eat fish and squid, though stomach content analyses on modern *O. flavescens* have revealed lesser amounts of crustaceans (Drago et al., 2017). Otariids have been observed to forage

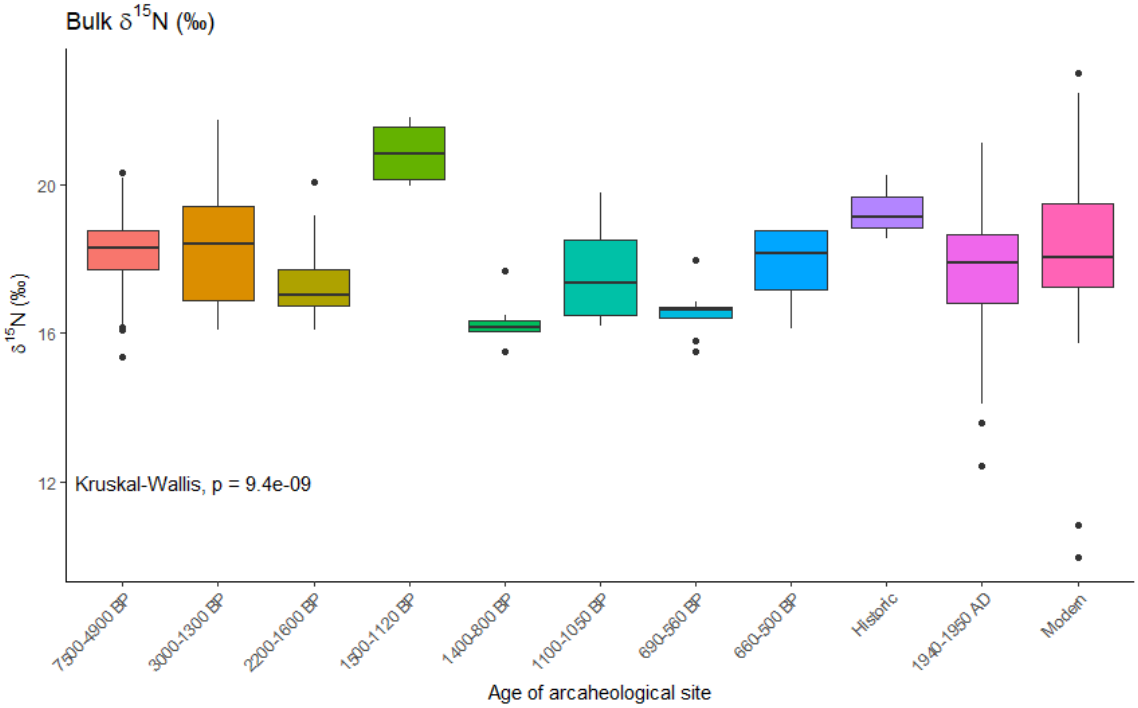
offshore and coastally, though how much time they spend feeding in these habitats through time is less well known. Bones of these animals appear in archaeological sites in a near continuous record over several thousand years, from roughly 7000 years before present (BP) to the 20th century (Table 1, Nye et al., 2019a). Remains of Otariids and several of their prey or other coexisting organisms provide a valuable resource for investigating food web dynamics and niche preferences using stable isotope techniques (Zangrando et al., 2014a).

Traditionally measurements of trophic structure are performed by direct observations of the species in question or through indirect methods, such as observations of stomach contents or bulk stable isotope analyses. Bulk stable isotope analysis has been employed on a large scale in studies of ecosystems both modern and archaeological. In the context of the Fuegian archipelago, many studies of pinniped trophic position have been performed (Nye 2019a). While these studies are valuable, especially in modern contexts, to be fully informative it is essential to have measurements of all potential prey items to characterize the trophic position of predators.

In complex and ancient food webs this is a difficult proposition due to lack of ability to sample all potential prey items (Bradley et al., 2015). Differences in primary productivity can complicate direct comparisons between food webs from different temporal or spatial contexts as shifts at the base of the food web offset the rest of the food web (Casey and Post, 2011; Lorrain et al., 2015). Ancient coastal Tierra del Fuego is

one example of such a food web (Saporiti et al., 2014a). Nye et al. (2019a, Fig. 2) showed that there was significant variation in $\delta^{15}N$ values at various points between 7500 BP and the 20th century in pinnipeds, however, we did not observe overall trends between time periods. What variation we do see between populations of different time periods can be explained by variation in trophic level (potentially 2-3 trophic levels) between individuals or differences in the baseline $\delta^{15}N$ values of primary producers offsetting values at higher trophic levels. Prey items of pinnipeds are limited to zooarchaeological remains that do not include several noted dietary components of pinnipeds, like squid, crustaceans and certain fish species (Zangrando, 2009a).

Figure 3-2 – Bulk nitrogen isotopic values found in pinniped archaeological sites. Despite high variation in isotopic values over time, no clear trend was observed over time, which may be obscured by differences in the ecological baseline over time rather than true tropic differences.



Compound specific amino acid analyses of $\delta^{15}N$ allow for an independent measurement of trophic position by comparison of source and trophic amino acids, with trophic amino acids fractionating with each trophic step (glutamic acid, aspartic acid, alanine, isoleucine, leucine, proline and valine) and source amino acids (phenylalanine and lysine) fractionating very little (McMahon and McCarthy, 2016). The difference in $\delta^{15}N$ between glutamic acid and phenylamine ($\Delta^{15}N$ Glu-Phe) has commonly been used to quantify food chain length (FCL). However, due to differences in biochemical pathways in different primary producers, source amino acids from different primary producers need to be accounted for in calculations of trophic level. These differences in the $\delta^{15}N$ of source amino acids are represented by the β-factor which can vary between different ecosystems. A second complication relates to trophic amino acids. It has been observed that fractionation of trophic amino acids decreases with subsequent trophic steps (McMahon et al., 2015; Bradley et al., 2015). Therefore $\Delta^{15}N$ Glu-Phe calculations and similar metrics may not represent "true" FCL. To ascertain a more realistic FCL, multiple attempts have been made to correctly account for fractionation at each trophic level. For example experiments reveal there is decreased fractionation at higher trophic levels (Germain et al., 2012; Bradley et al., 2015). Similarly, the trophic discrimination factor (TDF) has been observed to vary based on the type of diet to consumer pairing (McMahon and McCarthy, 2016).

Due to uncertainties in the ability to identify the proximal causes of variability of variation in $\delta^{15}N$ of pinnipeds over time, we designed a study in which we aim to

reconstruct the trophic position of pinnipeds of the beagle channel through compound specific stable isotope analysis of archaeological remains. We expect to find evidence for one of the following hypotheses: (1) that differences in foraging between individuals is supported by variation in food chain length indicating trophic shifts; or, (2) a lack of difference in food chain lengths between individuals, coupled with variation in source amino acid $\delta^{15}N$, would indicate differences in basal food web resource consumption (see Nye 2019b).

Methods

Sample selection

Otariid samples were selected from sites along the south and southeastern coastlines of Tierra del Fuego (Fig. 1). Samples were separated into different time bins for each species to identify temporal trends in nitrogen isotopic composition of essential amino acids.

Preparation

All samples were measured for their bulk nitrogen isotope values before hydrolysis and derivatization of amino acids (Nye et al., 2019a). 10 milligrams of organic tissues from samples were hydrolyzed for 20 hours at 110° C in 1 milliliter of 6N hydrochloric acid in vials flushed with N_2. The resulting hydrolysate of the samples, as well as a separate mixture of amino acid standards were dried down under N_2 and reacted with a 4:1 Isopropanol-Acetyl Chloride solution for 1 hour at 110° C. Samples and standards were then dried under N_2 and washed twice with 200 microliters of

dichloromethane (DCM). We then derivatized the samples and standards using 500 microliters of trifluoracetic acid anhydride (TFAA) in solution with 500 microliters of DCM for 10 minutes at 110° C. Samples and standards were then cooled to room temperature and dried under N_2, washed twice with 200 microliters of DCM and resuspended in 500 ml of DCM before injection into the GC-IRMS system.

Samples were analyzed using a suite of Thermo Scientific instruments at the UCR Edge Laboratory consisting of a Trace 1310 Gas Chromatograph with a GC-Isolink II routed through a Conflo IV interface into a Delta V Plus isotope ratio mass spectrometer. Injections into the Trace 1310 are performed via a Triplus RSH autosampler. The autosampler syringe is washed five times in DCM before and after every injection. Typical samples were injected at a concentration of 1mg/200uL in triplicate with an amino acid standard injection between different sample injections. The Trace 1310 GC is equipped with a 60-meter DB-5 column with a 1 micron film thickness that ramps from 50° C to 320° C over 25 minutes to ensure adequate separation between amino acid peaks. Regular maintenance includes periodic baking at 320 ° C to remove potential column bleed. The combustion reactor in the GC-Isolink II is heated to 1000 ° C to ensure complete combustion of amino acids into CO_2 and N_2. Carbon dioxide was trapped out with liquid nitrogen prior to entering the Conflo IV interface.

After an initial longer oxidation of 4 hours immediately after installation of a new reactor, we maintain sufficient oxygen levels in the combustion reactor by performing

short 1 minute seed oxidations every injection to ensure complete combustion of samples. The combustion reactor is monitored and maintained at regular intervals by inspecting peak shape, retention times, $\delta^{15}N$ values of standards and number of injections. The combustion reactor is typically replaced after oxidation of the reactor becomes ineffective, we observe poor peak shapes and retention times, or identify poor reproducibility of standards, which usually occurs after about 500 injections.

All sample $\delta^{15}N$ are corrected to an in-house standard mixture of 13 amino acids with known $\delta^{15}N$ values (alanine, glycine, threonine, serine, valine, leucine, isoleucine, proline, aspartic acid, glutamic acid, phenylalanine, tyrosine, and lysine) after measurement on the Delta V Plus IRMS. The amino acids in the standard mixture have been corrected to the international standard for nitrogen, atmospheric N_2, via independent measurements using a continuous flow EA-IRMS in the same laboratory (see Nye 2019a). Between standards, the average stand deviation was 1.95‰ with the highest deviation observed in alanine at 2.4‰.

For calculations of trophic level (FCL), we used a generalized equation (Equation 1) for calculating trophic position from compound specific amino acid $\delta^{15}N$ (TP_{CSIA}, McMahon and McCarthy, 2016):

$$(1.) \quad \mathrm{TP}_{\beta} = 1 + \left(\frac{\delta^{15}\mathrm{N}_{\mathrm{Glu}} - \delta^{15}\mathrm{N}_{\mathrm{Phe}} - \beta}{\mathrm{TDF}_{\mathrm{Glu-Phe}}}\right)$$

Given the marine ecosystem context and diet of pinnipeds consisting of teleost fish and crustaceans, we assumed a β-factor of -3.4‰ and a trophic discrimination factor ($TDF_{Glu\text{-}Phe}$) of 7.6‰.

We also used an equation (Equation 2) from Germain et al. (2013) derived from an controlled feeding study in harbor seals (*Phoca vitulina*), the closest relatives to *A. australis* and *O. flavescens* to date.

$$(2.) \quad TP_{MultiTDF} = 2 + \left(\frac{\delta^{15}N_{Glu} - \delta^{15}N_{Phe} - \beta - TDF_{Seal}}{TDF_{Plankton}}\right)$$

Results

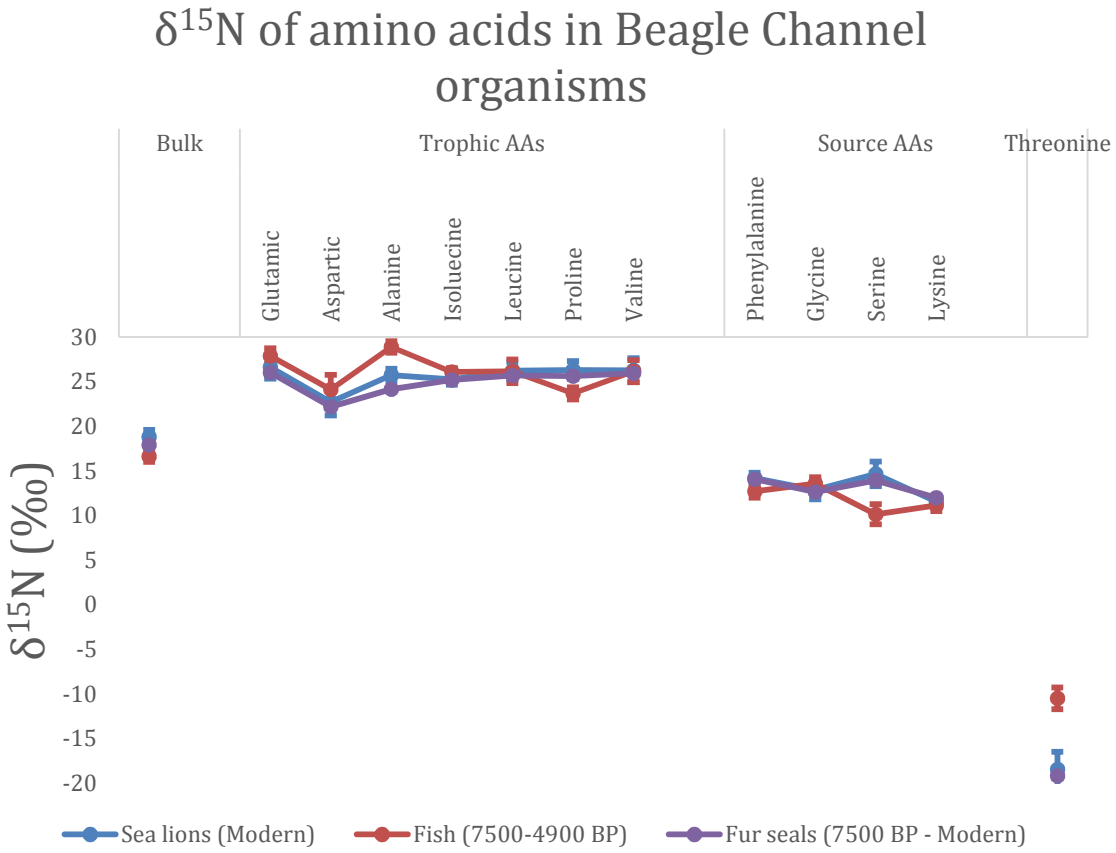
We observed differences in the patterns of the $\delta^{15}N$ values of amino acids between different species (Table 1, Fig. 2). The most positive $\delta^{15}N$ value was found in glutamic acid of a sea lion at 30.5‰ while the most negative value was observed in threonine of a fur seal at -25.9‰, a range of 56.4‰. Among source amino acids, the most negative $\delta^{15}N$ value was observed in fur seal glycine at 8.6‰. Threonine was most different from bulk $\delta^{15}N$ values, while aspartic acid was the most similar. Between different species, values for $\delta^{15}N$ of amino acids were similar. The exceptions to this were between alanine, proline, serine and threonine in fishes and pinnipeds (Table 1).

We observed variation in phenylalanine between different individual fur seals throughout the 7500 BP-Modern time periods (Table 1). However, we did not observe an obvious trend or pattern in the overall patterns of amino acids between different time periods (Fig. 4).

Table 3-1 – Nitrogen isotopic values of amino acids in consumer species of different time periods.

	Bulk ($\delta^{15}N$ ‰)	$\delta^{15}N$ values of Trophic AAs (‰)							$\delta^{15}N$ values of Source AAs (‰)				
		Glutamic	Aspartic	Alanine	Isoluecine	Leucine	Proline	Valine	Phenylalanine	Glycine	Serine	Lysine	Threonine
Fur seals (7500-4900 BP)	17.8±1.6	26.4±2.1	22.7±1.8	23.9±2.9	24.5±1.3	26.2±1.3	25.7±1.6	25.9±2.2	14.3±1.7	15.0±2.2	14.3±1.5	11.3±1.2	-19.5±3.3
Fur seals (2200-1600 BP)	18.0±1.3	25.7±1.0	21.8±1.0	23.7±1.7	24.7±1.4	25.0±1.5	26.0±1.3	25.5±1.7	13.4±1.3	11.2±2.2	13.2±1.6	12.6±1.8	-19.8±3.7
Fur seals (Modern)	17.8±1.9	25.9±2.8	22.1±2.5	24.9±2.1	26.4±0.4	25.8±2.2	25.1±1.9	26.3±2.2	14.5±1.5	11.7±2.2	14.4±0.3	11.4±2.3	-18.2±1.8
Sea lions (Modern)	18.8±1.5	26.6±2.6	22.7±3.1	25.7±1.4	25.3±1.2	26.2±2.0	26.3±2.0	26.3±2.7	14.2±1.2	12.8±2.1	14.7±2.8	11.6±1.2	-18.5±4.0
Fish (7500-4900 BP)	16.6±1.2	27.9±1.7	24.1±3.3	28.9±1.3	26.1±0.9	26.2±2.7	23.7±1.4	26.2±2.5	12.7±1.4	13.6±1.5	10.1±2.3	11.1±1.3	-10.5±2.4
Fur seals (7500 BP - Modern)	17.9±0.3	26.0±0.9	22.2±0.7	24.2±0.6	25.2±0.6	25.7±0.5	25.6±0.3	25.9±0.3	14.1±0.2	12.6±0.03	13.9±0.7	12.0±0.4	-19.2±1.0

Figure 3-3 - $\delta^{15}N$ of amino acids in Beagle Channel consumers. Trophic amino acids are significantly more positive than both source amino acids and bulk tissue, source amino acids are more negative than bulk tissue and trophic amino acids, and threonine is far more negative than bulk and all amino acids, and atmospheric nitrogen.



Estimates of trophic position using both equations showed little variation between otariids of different time periods (Table 2, Fig. 5). The individual with the greatest $\Delta^{15}N$ Glu-Phe was a sea lion with 15.9‰, whereas the smallest $\Delta^{15}N$ was measured in another sea lion at 8.8‰. Their corresponding calculated trophic positions using equation 1 are 3.5 and 2.6, nearly a full trophic step apart from one another. Both individuals are modern sea lions that are more variable than the entirety of fur seals, which had a maximum $\Delta^{15}N_{Glu\text{-}Phe}$ of 14.4‰ observed in an archaeological individual and 9.9‰ in a modern individual, with trophic positions of 3.3 and 2.7 respectively (equation

Table 3-2 – Trophic level metrics in otariids and fish from different time periods. Trophic position (TP) calculations vary with TDF applied.

					Fur seals (7500-4900 BP)					
Sample ID	3632	1930	3635	0169	3533	3786	7288	2609	3719	Average
Phe (δ^{15}N ‰)	15.7	14.0	15.7	17.4	14.5	13.0	12.2	12.5	13.8	14.3±1.7
Glu-Phe (Δ^{15}N ‰)	12.0	12.7	11.6	13.4	11.3	11.0	13.3	13.3	10.2	12.1±1.2
TP (eq. 1, TDF=7.6)	3.0	3.1	3.0	3.2	2.9	2.9	3.2	3.2	2.8	3.0±0.2
TP (eq. 1, TDF=4.3)	4.6	4.7	4.5	4.9	4.4	4.4	4.9	4.9	4.2	4.6±0.3
TP (eq. 2)	3.5	3.5	3.4	3.7	3.4	3.3	3.6	3.6	3.2	3.5±0.2
					Fur seals (2200-1600 BP)					
Sample ID	5880	2527	0194	0195	7702	2421	6957	2349		Average
Phe (δ^{15}N ‰)	13.2	12.4	14.1	12.5	11.7	14.0	15.6	14.1		13.4±1.3
Glu-Phe (Δ^{15}N ‰)	10.6	12.2	12.1	14.2	14.4	11.9	11.1	11.9		12.3±1.3
TP (eq. 1, TDF=7.6)	2.8	3.1	3.0	3.3	3.3	3.0	2.9	3.0		3.1±0.2
TP (eq. 1, TDF=4.3)	4.3	4.6	4.6	5.1	5.1	4.6	4.4	4.6		4.7±0.3
TP (eq. 2)	3.3	3.5	3.5	3.7	3.8	3.4	3.3	3.4		3.5±0.2
					Fur seals (Modern)					
Sample ID	BCAP2	A1315	2858							Average
Phe (δ^{15}N ‰)	14.9	15.8	12.8							14.5±1.5
Glu-Phe (Δ^{15}N ‰)	12.0	12.3	9.9							11.4±1.3
TP (eq. 1, TDF=7.6)	3.0	3.1	2.7							2.9±0.2
TP (eq. 1, TDF=4.3)	4.6	4.7	4.1							4.4±0.3
TP (eq. 2)	3.5	3.5	3.2							3.4±0.2
					Sea lions (Modern)					
Sample ID	OF-FLA1	OF-L2	OF-MLA	OF-L4	OF-R					Average
Phe (δ^{15}N ‰)	14.3	14.6	15.7	13.9	12.4					14.2±1.2
Glu-Phe (Δ^{15}N ‰)	13.8	15.9	8.8	10.9	13.0					12.5±2.7
TP (eq. 1, TDF=7.6)	3.3	3.5	2.6	2.9	3.2					3.1±0.4
TP (eq. 1, TDF=4.3)	5.0	5.5	3.8	4.3	4.8					4.7±0.6
TP (eq. 2)	3.7	4.0	3.0	3.3	3.6					3.5±0.4
					Fish (7500-4900 BP)					
Sample ID	IMI-08	86284	Mac-Sh-03							Average
Phe (δ^{15}N ‰)	13.2	13.8	11.1							12.7±1.4
Glu-Phe (Δ^{15}N ‰)	15.7	14.4	17.4							15.9±1.5
TP (eq. 1, TDF=7.6)	3.5	3.3	3.7							3.5±0.2

1), roughly half a trophic step. Considering averages of Δ^{15}N Glu-Phe values and calculations of trophic position between different species and fur seals of different time periods, there were no significant differences found between any group (Table 2). A Kruskal-Wallis non-parametric statistical test between fur seals of different time periods indicated an insignificant difference between groups.

We observed significant differences when using different equations to calculate trophic level in otariids (Table 2, Figs. 6 & 7). When calculating trophic level of otariids using the more generalized equation 1, we used two TDFs, 7.6, observed from a multitude of marine organisms, including teleost fish (McMahon and McCarthy, 2016) and 4.3, a value experimentally derived from harbor seals (*Phoca vitulina*). With a TDF of 7.6 applied to both otariids and fish, otariids are nearly half a trophic level below hake. When a TDF of 4.3 is applied to otariids (keeping fish at a TDF of 7.6), otariids are half a trophic level higher than fish. Finally, using a multi-TDF approach (equation 2) we find that otariids and hake effectively are at the same trophic position.

Discussion

The slight differences in δ^{15}N of amino acids between pinnipeds and fish are expected given differences in biochemical pathways of nitrogen between these two taxonomically diverse groups (Germain et al., 2012). Nitrogen excretion in fish is in the form of ammonia, whereas mammals excrete urea. The fundamental metabolic difference between these groups of animals may be responsible for different δ^{15}N values in alanine, proline, serine and threonine. Dietary differences between these

species may also potentially explain these differences, as these species have been observed to occupy similar trophic positions, with some of the fish (Merluccid hakes) potentially occupying higher trophic level niches through cannibalism (Ocampo Reinaldo et al., 2011).

Unknown TDFs for otariids due to a lack of experimental data leads to some uncertainty in estimated their trophic position. Using the more generalized single TDF approach (equation 1) to estimate trophic position of otariids resulted in either apparent underestimation (Table 2, Fig. 6a) or overestimation (Fig. 6b). A more realistic approach is to use a multi-TDF approach (equation 2), accounting for changes in fractionations from one trophic level to the next, as fractionation has been demonstrated to decrease at higher trophic levels (McMahon et al., 2015). Using equation 2, we found a more plausible trophic position for otariids, with an average trophic position of 3 for otariids.

The consistency in $\delta^{15}N$ of amino acids between otariid populations of different times is striking, however, as the $\delta^{15}N$ values do not seem to vary significantly in time or by species (Table 1, Figs. 2 & 3). What variation we do observe is most prevalent in modern pinnipeds, with most of the $\delta^{15}N$ variations occurring in modern sea lions. This finding is consistent with bulk $\delta^{15}N$ values observed in Nye 2019a, where we also observed the highest variation in values in the modern time period. Despite this observation, variations in amino acid $\delta^{15}N$ and trophic level are expressed at the individual scale rather than at the population level. Within population specialization has

been observed in modern sea lions through direct observation as well (Baylis et al., 2015).

Measurements of food chain length and calculations of trophic position also conform to a pattern of stability with respect to trophic position of pinniped populations over time (Table 2, Fig. 5). All trophic position estimates and Δ^{15}N Glu-Phe measurements at the population scale are within each other's standard deviation. Once again, variation in the Δ^{15}N Glu-Phe we observe in the modern samples at the individual scale (Fig. 4). These results suggest that our second hypothesis, that individuals may be foraging on different basal food web resources at any given time signified by the large range in δ^{15}N variation of phenylalanine. Furthermore, the variation in these basal food resources do not appear to increase or decrease between time period or species of pinnipeds or show any trend (Fig. 4). Nye 2019a and 2019b show that there is significant variation in basal food web resource consumption, consistent with this study.

Figure 3-4 – Density plot of the nitrogen isotopic values of phenylalanine (a.) and the difference between canonical trophic and source amino acids. Large ranges in phenylalanine indicate significant variation in foraging areas of pinnipeds. Similarly, large variation in Glu-Phe values indicate large individual variation in trophic level. Significant overlap in both metrics indicate little change over time in overall population trends.

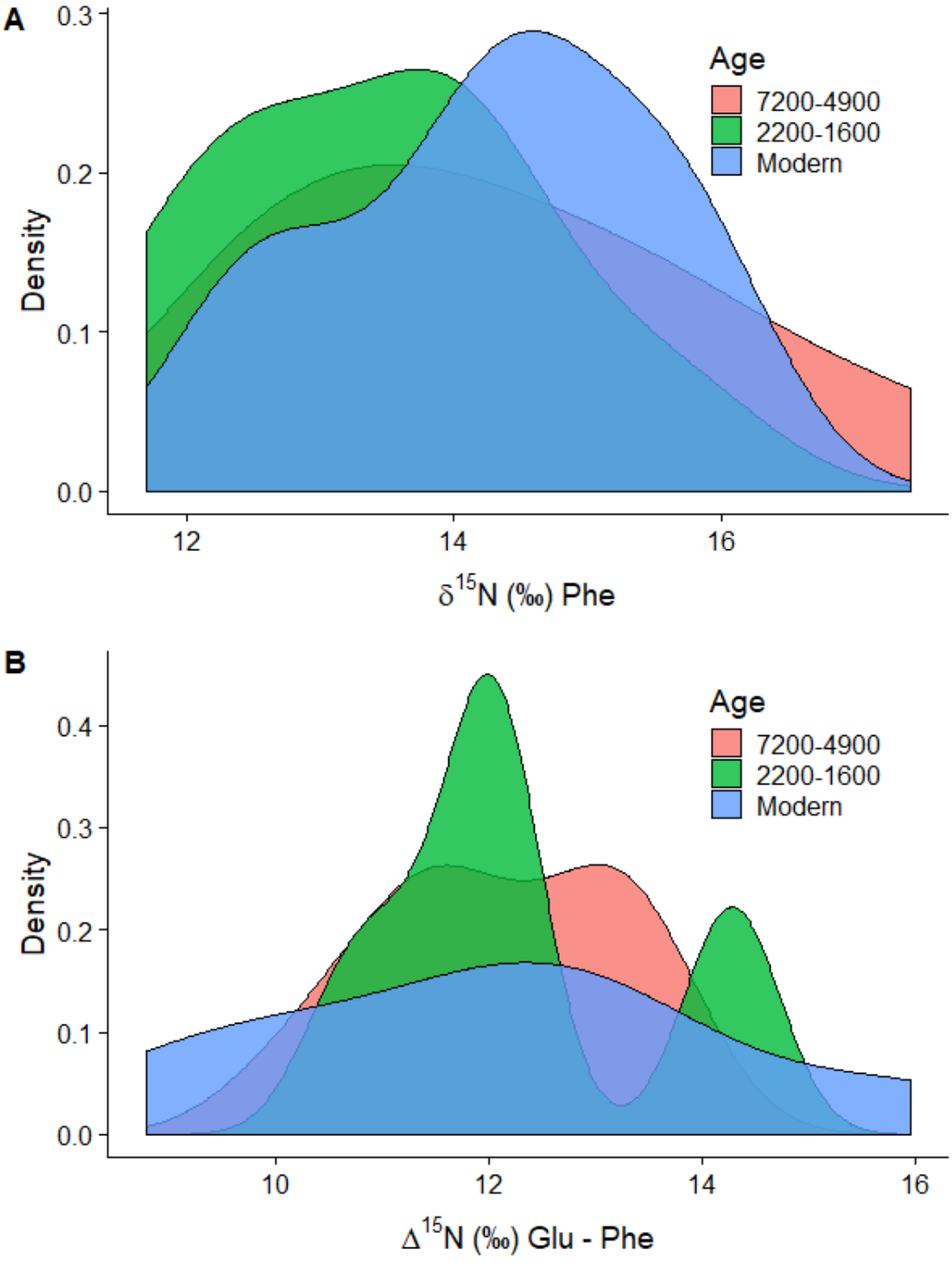
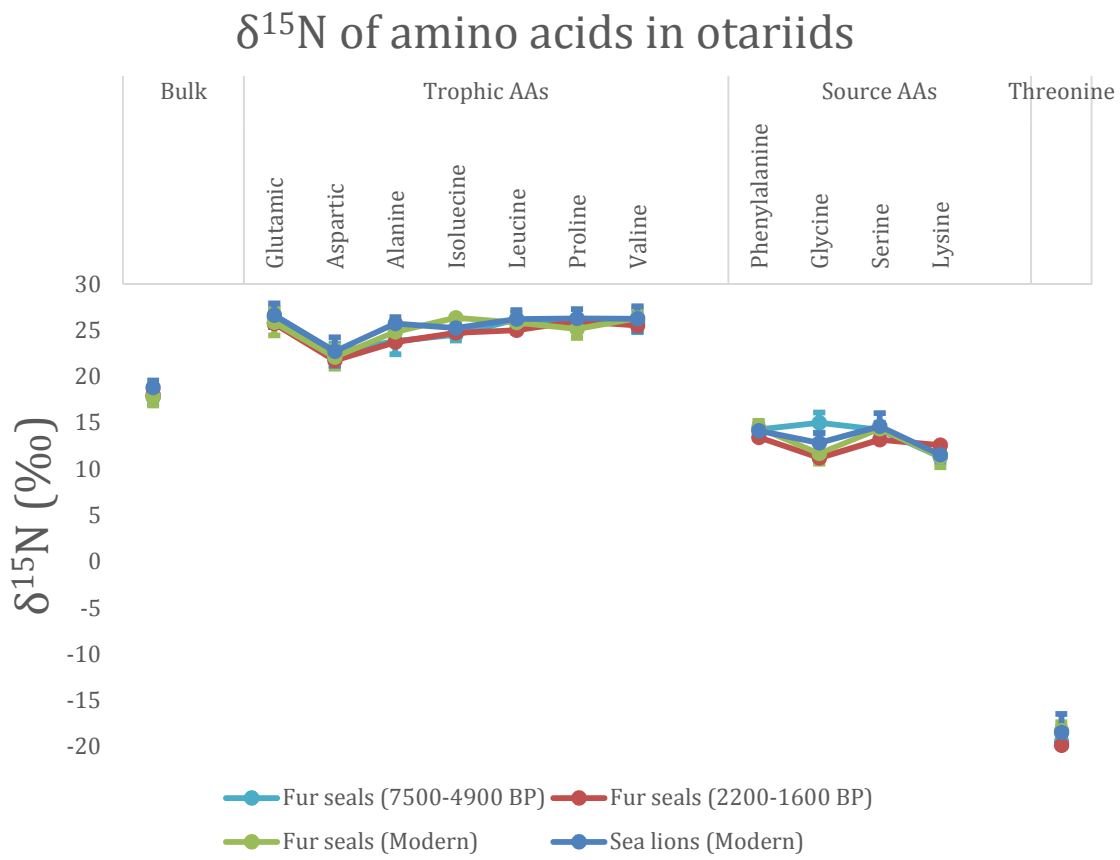


Figure 3-5 - $\delta^{15}N$ of amino acids in otariids



One indication of the significant basal food web variation is the unique results for the canonical source amino acid, phenylalanine, which differ from results of many other marine ecosystems. While average phenylalanine $\delta^{15}N$ values do not appear vary appreciably between different groups of pinnipeds over different time periods, individual variation is significant (Tables 1,2 and Fig. 4a) with the most negative $\delta^{15}N$ value being 12.2‰ and the most positive 15.8‰. The most negative value observed in our study is more positive than most other marine consumers. Morra et al., (2019) found range of $\delta^{15}N_{Phe}$ between -2.2‰ and 11.1‰ in Pacific ocean seabirds, Hetherington et al. -0.7‰ and 10.5‰ in the eastern tropical Pacific marine organisms

(2017), and Ruiz-Cooley et al. found a range between 7‰ and 15‰ in sperm whales (*Physeter macrocephalus,* 2014). In captive harbor seals, $\delta^{15}N_{Phe}$ values range between 9-12‰ (Germain et al., 2013). More positive phenylalanine values are observed in higher trophic level organisms. Despite the characterization of phenylalanine as a source amino acid, there does appear to be trophic fractionation resulting in higher $\delta^{15}N$ values, as observed in other marine mammals, but especially so in pinnipeds measured here.

Nye 2019a & 2019b suggest that there may be shifts between coastal and offshore basal food web resources over time, but these two ecosystems are not easily distinguishable with $\delta^{15}N$ measurements, because $\delta^{15}N$ of basal resources do not vary in the context of primary production between kelp and offshore phytoplankton. There may be exceptions to this at more localized scales, for instance, individual modern pinnipeds may be incorporating nitrogen from anthropogenic sources nearby human populated areas (Diodato et al., 2012). Ultimately, sampling modern pinnipeds of known local origin can elucidate modern impacts of anthropogenic nitrogen on pinniped populations of Tierra del Fuego.

Figure 3-6 – Estimated Trophic level positions of pinnipeds and fishes using single TDF approaches adapted from McMahon et al. (Eq. 1). Neither TDF seems a realistic result, with a TDF of 7.6 (a) underestimating trophic position in otariids and a TDF of 4.3 (b) overestimating trophic position.

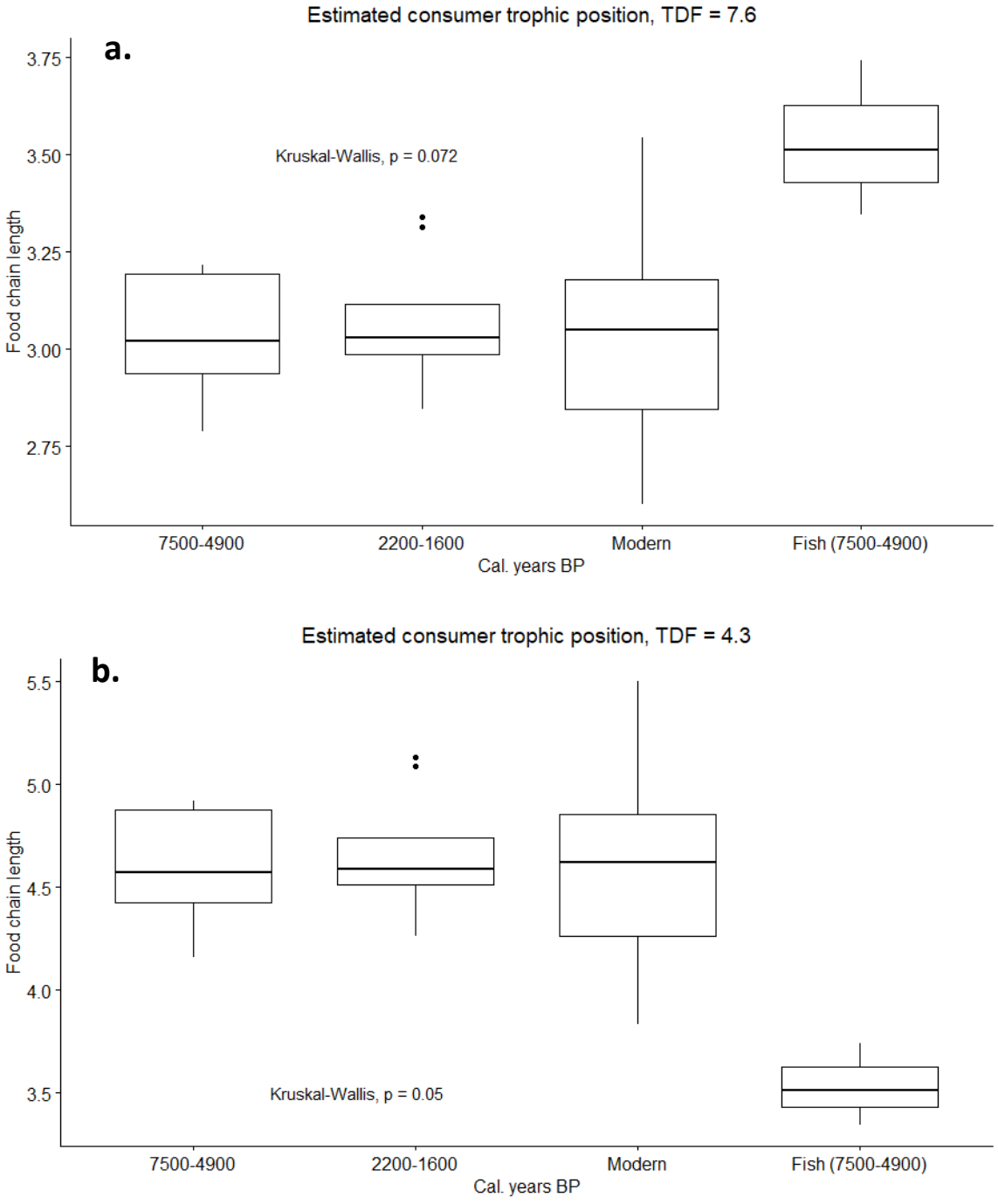
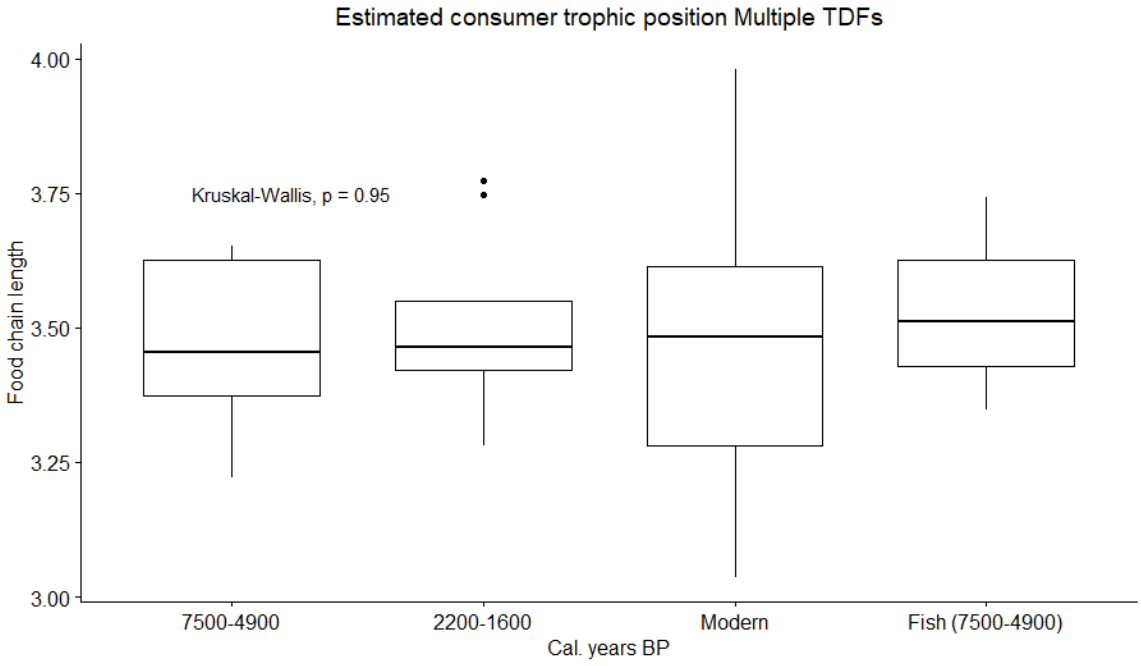


Figure 3-7 - Estimated Trophic level positions of pinnipeds and fishes using Multi-TDF approach adapted from Koch et al. Plausibly realistic.



Conclusion

The stability of trophic positions and food chain length speaks to the resiliency of these two pinniped species. While basal resources and physical niche space may be altered by human presence of the past several millennia (Nye 2019a & 2019b), pinnipeds are still able to maintain their place in the food webs of the Fuegian Archipelago as high trophic level organisms. They also show significant adaptive capacity within species and populations as different individuals can survive at similar trophic positions despite shifting basal resources. What may be concerning is the increased spread in variation between individuals in the modern time period. This may signify that species are responding to the devastating impacts of 20th century industrial hunting by occupying abandoned niches with abundant prey, that certain individuals may be struggling to maintain their trophic position with competition from fisheries, or changes in productivity due to climate change.

Chapter 4 : Hydrogen isotopes in archaeological samples from southern South American marine ecosystems

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Abstract

The analyses of hydrogen isotopes in animal tissues are commonly applied to studies of mobility and migration, but the application to food web ecology in archaeological and marine investigations is uncommon. We used bulk and compound specific amino acid (AA) δ^2H measurements to identify trophic relationships and basal food web resource use in both archaeological and modern populations of two species of pinnipeds, South American fur seals, *Arctocephalus australis* and South American sea lions, *Otaria flavescens.* We compared this data to modern Patagonian squid, *Loligo gahi*, and giant kelp, *Macrocystis pyrifera* from Tierra del Fuego. For bulk hydrogen δ^2H, diagenesis resulted in more variable values within a population. Lipids in squid muscle influenced the δ^2H resulting in more negative δ^2H relative to lipid-extracted collagen from bones. The δ^2H of collagen increased by markedly, as a function of trophic level. For compound specific AAs, we found significant differences between the δ^2H of essential and non-essential amino acids. In fur seals, the δ^2H values of certain amino acids varied as a function of time period, however it is unclear what this means in terms of trophic ecology. Sea lions appear to have a higher trophic position based on the δ^2H of proline, a potential marker for trophic level, than fur seals. A similar trend with respect to the δ^2H of proline was measured between fur seals and their squid prey. Hydrogen isotopes have the potential to provide a new, valuable, and independent way to measure marine food webs from carbon and nitrogen, both in the present and in archaeological contexts

Introduction

Spatiotemporal dynamics in food webs, trophic level, and ecological niche space is commonly measured via stable isotopic proxies including carbon and nitrogen, but can also be measured through hydrogen. Bulk carbon and nitrogen analysis of organic tissues from different organisms provides information not only about niche breadth in different species, but also primary producers that are incorporated into animal diets from the base of the food web. These can be applied in both modern ecological studies, but also to ancient food webs as well (Nye et al., 2019a).

Hydrogen isotopes in animals reflect both ecological niche and geography of animal species. Much like carbon and nitrogen isotopes, hydrogen isotopes in animals are first sourced from their dietary sources and second, fractionated with each trophic step (Estep and Dabrowski, 1980; Macko et al., 1983; Solomon et al., 2009). This relationship allows hydrogen to act as a proxy for food web structure. Relatively uniform metabolic processes in animals result in fractionations that range from 0-60‰ in per trophic step (Estep and Dabrowski, 1981; Macko et al., 1983; Reynard and Hedges, 2008). Respiration and excretion are two processes that influence the fractionation in which the lighter isotope (H) is preferentially lost and the heaver isotope retained in tissues.

Stable isotopes of hydrogen have been used in ecology to learn about migration across landscapes. Because the hydrogen isotopic composition of precipitation varies owing to physical processes (e.g., evaporation and condensation). δ^2H patterns in

precipitation vary systematically across landscapes (Bowen and West, 2019)**.** Analyses of hydrogen in ancient ecosystems is less common because preservation of the original δ^2H signal is may be compromised by diagenesis and exchange. The majority of hydrogen in proteinaceous tissues, however, is derived from organic hydrogen from dietary sources (e.g., Newsome et al., 2017), making hydrogen isotopic measurements strong contenders for dietary studies.

One potential system where measurements of δ^2H may prove useful is in the investigation of ecological dynamics of Tierra del Fuego, southern South America. Archaeological remains of marine organisms from the middle Holocene to the present day provide ample tissues for hydrogen analyses. Species in sites ranging from ancient to modern in age include two species of pinnipeds, South American fur seals, *Arctocephalus australis* and South American sea lions, *Otaria flavescens.* These pinnipeds are largely generalists (Nye et al., 2019a & 2019c), but show evidence of changes in nearshore to offshore influence in their dietary trends over time (Nye et al. 2019a & 2019b). Modern specimens from the region include Patagonian squid, *Loligo gahi,* and giant kelp, *Macrocystis pyrifera,* which represent offshore and nearshore dietary resources, respectively.

Diet vs. Ambient environment

While the majority of hydrogen incorporated into the non-exchangeable portion of organic tissues is from dietary sources (70-80%, Hobson et al., 1999; Birchall et al., 2005) the remainder (20-30%) is from water sourced from the local environment

(O'Brien et al., 2007; Ehleringer et al., 2008). This added complexity can represent a problem in terrestrial ecosystems where questions of autochthonous vs. allochthonous sources of water may be incorporated into tissues (Hobson and Bairlein, 2003; Doucett et al., 2007). On the other hand, the provenance of these ambient waters as identified through isoscape approaches has proved to be beneficial in identifying migratory patterns and questions of allochthony or autochthony of water in many different ecological studies (Ehleringer et al., 2008; Hobson et al., 2019).

Varying sources of hydrogen can result in difficulties for interpreting individual samples, especially if individuals in a population come from different geographic origins or are highly mobile (Langin et al., 2007). Furthermore, while comparisons to carbon metabolism in animals are often made, hydrogen isotope fractionation is underpinned by different physiological characteristics despite similar biochemical pathways (Wolf et al., 2012). In the context of the marine environment of the Beagle Channel, all organisms in this study are ultimately sourcing environmental water from the ocean and therefore should reflect the δ^2H of Standard Mean Ocean Water (SMOW). When similar sources of environmental water are considered, the remaining differences in hydrogen isotopic ratios in a community can be attributed to trophic dynamics (Birchall et al., 2005).

Hydrogen isotopes in aquatic ecosystems

In aquatic ecosystems hydrogen isotopic fractionation results from several processes. Photosynthesis results in negative hydrogen isotopic values, often -100‰ or

more in primary producers (Estep and Hoering, 1980). Aquatic primary producers tend to have very negative δ^2H values relative to their terrestrial counterparts. In land plants, transpiration results in a isotope fractionation in leaves that reduces the abundance of the protium in terrestrial primary producer tissues (Flanagan and Ehleringer, 2006). Little difference is observed in δ^2H between aquatic primary producers that uptake bicarbonate and those that uptake carbon dioxide as their carbon sources. Within aquatic primary producers, differences have been observed between microalgae and macroalgae. Microalgae tend to be the most depleted in deuterium while macroalgae are more variable (Hondula et al., 2014).

In bulk measurements of δ^2H in aquatic consumers differences can be ascribed to not only food web dynamics and environmental water, but also the particular differences between tissues (Soto et al., 2013). Commonly these tissues include collagen, muscle and liver, which are primarily protein, and lipids. Lipids have been recorded to be 60‰ more negative than proteinaceous tissues (Estep and Hoering, 1980; Sessions et al., 1999; Soto et al., 2011). When considering routing of hydrogen into proteinaceous tissues from diet, proportions of macromolecules from different sources incorporated into consumers differ. In tilapia, one of the few aquatic organisms where these proportions have been calculated, lipids account for less than 1% of macromolecules incorporated into muscle and liver (Newsome et al., 2017).

δ^2H in Compound Specific Amino Acids

Compound specific analyses of amino acids from the same tissues go a step further, providing a glimpse into the individual biochemical pathways of each amino acid that makes proteinaceous tissue (Fogel et al., 2016). The δ^2H of essential amino acids in consumers originates from the δ^2H in a consumer's diet through direct routing, similar to carbon isotopic measurements. The hydrogen in nonessential amino acids comes from cellular water, NADPH, and organic H from dietary macromolecules (Newsome et al., Oecologia, submitted). While compound specific analyses of carbon and nitrogen are routinely used to quantify food web characteristics, a third isotope analysis may reveal further information about the biochemistry of food webs or larger ecosystem dynamics: hydrogen.

Hydrogen in amino acids can be closely bound with carbon, which likely goes through similar biochemical pathways of formation. Categories of essential and non-essential as for carbon likely also applies to hydrogen (Hayes, 2001; Fogel et al., 2016). Sources of hydrogen vary between essential and non-essential amino acids, with non-essential amino acids incorporating significantly more water into their structures than essential amino acids. As such, compound specific analyses hydrogen can be used as both a tracer of both water and diet.

The goal of this study is to identify ecological dynamics in niches and trophic position of Beagle Channel pinnipeds through measurements of δ^2H. Given the sources of water are largely the same given the context of marine mammals, birds and squid,

δ^2H should reflect trophic, metabolic and biosynthetic processes in these animals. Although we do not expect metabolic and biosynthetic processes to differ over millennial timescales, carbon and nitrogen results have shown ecological differences between middle Holocene animals and modern relatives (Nye et al. 2019a, 2019b, 2019c) We hypothesize that hydrogen stable isotopes will support our previous observations, while also potentially giving further insight into ecological dynamics as well as metabolism in in different marine organisms.

Methods

Preparation

All samples were measured for their bulk δ^2H values before hydrolysis and derivatization of amino acids. Bone samples were demineralized and purified into collagen by suspending samples in 5ml 0.5 M HCl which was refreshed every 24 hours until completely demineralized or up to a week. Samples were then suspended in 5ml 0.1 M NaOH to remove humic acid contaminants and refreshed daily until the solution was transparent. Samples were then combusted on a Thermo TCEA fed into a Thermo Delta V IRMS at the UC Riverside EDGE Laboratory for bulk hydrogen stable isotope analysis.

For compound specific measurements of amino acids, 10 milligrams of organic tissues from samples were hydrolyzed for 20 hours at 110° C in 1 milliliter of 6N hydrochloric acid in vials flushed with N_2. The resulting hydrolysate of the samples, as well as a separate mixture of amino acid standards were dried down under N_2 and

reacted with a 4:1 Isopropanol-Acetyl Chloride solution for 1 hour at 110° C. Samples and standards were then dried under N_2 and washed twice with 200 microliters of dichloromethane (DCM). We then derivatized the samples and standards using 500 microliters of trifluoracetic acid anhydride (TFAA) in solution with 500 microliters of DCM for 10 minutes at 110° C. Samples and standards were then cooled to room temperature and dried under N_2, washed twice with 200 microliters of DCM and resuspended in 500 ml of DCM before injection into the GC-IRMS system.

Samples were analyzed using a suite of Thermo Scientific instruments at the UCR Edge Laboratory consisting of a Trace 1310 Gas Chromatograph with a GC-Isolink II routed through a Conflo IV interface into a Delta V Plus isotope ratio mass spectrometer. Injections into the Trace 1310 are performed via a Triplus RSH autosampler. The autosampler syringe is washed five times in DCM before and after every injection. Typical samples were injected at a concentration of 1mg/200uL in triplicate with an amino acid standard injection between different sample injections. The Trace 1310 GC is equipped with a 60-meter DB-5 column with a 1 micron film thickness that ramps from 50° C to 320° C over 25 minutes to ensure adequate separation between amino acid peaks. Regular maintenance includes periodic baking at 320 ° C to remove potential column bleed. The HTC reactor in the GC-Isolink II is heated to 1400 ° C to ensure complete combustion of amino acids into H_2.

All sample δ^2H are corrected to an in-house standard mixture of 13 amino acids with known δ^2H values (alanine, glycine, threonine, serine, valine, leucine, isoleucine,

proline, aspartic acid, glutamic acid, phenylalanine, tyrosine, and lysine) after measurement on the Delta V Plus IRMS. The amino acids in the standard mixture have been corrected to the international standard for hydrogen, SMOW (Standard Mean Ocean Water), via independent measurements using a continuous flow TCEA-IRMS. Average standard deviation of δ^2H between amino acid standards was 11.7‰, with the highest standard deviation observed in aspartic acid at 17.4‰.

Results

Table 4-1 – Bulk hydrogen isotopic results of Beagle Channel organisms.

Species	Average δ^2H (‰)	n
Fur seal (7500-4900 BP)	21.7±25.3	10
Fur seal (2200-1600 BP)	25±33.6	6
Fur seal (Modern)	19.7±7.1	4
Sea lion (7500-4900 BP)	35±20.6	7
Sea lion (2200-1600 BP)	-16.6±41.5	7
Sea lion (Modern)	47.6±4.4	6
Kelp (Modern)	-79.8±6.8	4
Squid (Modern)	-100.7±6.2	5

Kelp, at the base of the food web, was characterized by relatively more negative δ^2H values, with all samples falling within a relatively small standard deviation of 6.8‰ and a relatively low average percent hydrogen value of 3.7 (Appendix, Table 5). Similarly, squid also had a very small standard deviation at 6.2‰ and an average hydrogen concentration of 6.1% (Appendix, Table 5). Fur seals and sea lions from various time periods had positive δ^2H values with hydrogen concentrations averaging 4.8%, with the lowest value at 0.7% and the highest at 9.5%. In an analysis to determine if there was any relationship between %H of a sample and its δ^2H value, we compared the δ^2H of every sample to its corresponding C/H ratio and found no relationship, except for some sea lions samples with anomalously high C/H ratios (>15) which had low δ^2H values (Appendix, Fig. 1).

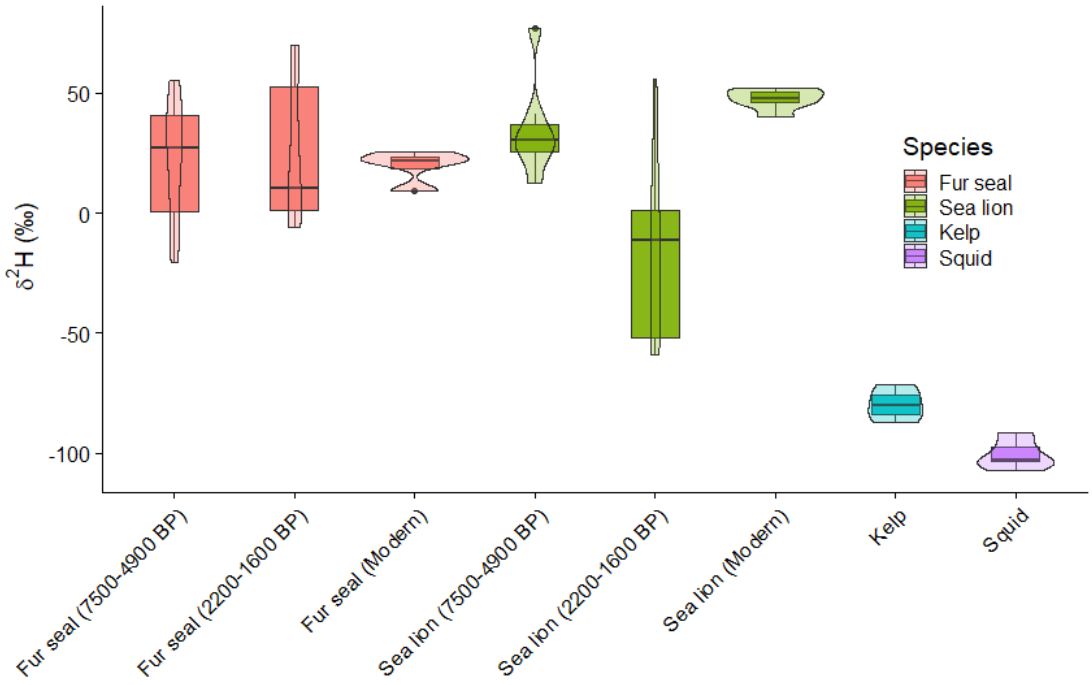
Modern pinnipeds were significantly more positive than kelp and squid (Table 1). Modern sea lions had also the most positive δ^2H values of all organisms in the entire data set, with one individual sea lion at 73.3‰ (Appendix, Table 5). Both modern sea

lions and fur seals had low standard deviations, at 4.4‰ and 7.1‰ respectively. %H for modern pinnipeds was similar for both species at around 5%, however one individual modern fur seal had the largest %H of any sample at 9.5%.

The δ^2H values for sea lions and fur seals were relatively consistent over time, except for some sea lions from 2200-1600 BP, which also had very low %H that averaged at 2.2% for that group (Appendix, Table 1). These samples also had very high C/H ratios, where preservation of non-exchangeable hydrogen, we suspect, may be compromised.

Bulk δ^2H results showed differences between all species, including kelp squid and pinnipeds (Kruskal-Wallis $p=6x10^{-5}$, Table 1, Figure 1). In a pairwise Wilcoxon test between all fur seals and sea lions, we found no significant variation ($p=0.49$). Other species were all found to be significantly different from each other in Wilcoxon tests ($p<0.05$). Means for fur seals did not vary over time (Kruskal-Wallis $p=0.84$). Means between sea lions, on the other hand, did vary over time (Kruskal-Wallis $p=0.01$). δ^2H means sea lion populations from different time periods varied significantly with each other (Wilcoxon $p<0.08$) but did not have a clear trend, likely due to the poor quality of preservation in the sea lion population from 2200-1600 BP. Excluding this population, we found no significant variation between means of modern sea lions and sea lions form 7500-4900 BP. Archaeological samples had the highest variation between individuals, while all modern populations had an order of magnitude lower variation between individuals (Table 1, Figure 1).

Figure 4-1 - Combined box plot and violin plot showing variation and range of bulk hydrogen isotopic values in different organisms.



Compound specific amino acids revealed further differences between different species (Table 2, Figures 2 &3). The only primary producer measured, giant kelp, had an average minimum of -209.9‰ (isoleucine) to a maximum of 65.8‰ (alanine) for a range of 275.7‰. All amino acids, except for three non-essentials (aspartic acid, 51.6‰, and glutamic acid, 0.8‰, and alanine, 65.8‰) had δ^2H values more negative than SMOW (0‰). Primary producers, including kelp, synthesize all these amino acids, forming a baseline for consumers.

Consumers cannot synthesize essential amino acids directly but can synthesize non-essential amino acids. Despite being near the base of the food web, squid have δ^2H values one might expect for a consumer with an average range of 588.1‰ between the most negative amino acid (lysine, -159.7‰) and the most positive (proline, 428.4‰).

While most non-essential amino acids were more negative than sea water, threonine was more positive (127.3‰). Almost all non-essential amino acids were more positive than sea water, except for serine (-3.5‰), which has a δ^2H virtually identical to that of seawater.

Higher trophic level consumers, fur seals and sea lions, showed similar patterns (Table 2). All but one essential amino acid, threonine, have δ^2H values more negative than water in modern fur seals and sea lions. Similar to squid, the only non-essential amino acids more negative than sea water in modern fur seals are aspartic acid (-42.6‰) and glutamic acid (-17.7‰). In modern sea lions, only aspartic acid is more negative than sea water for non-essentials (-24‰). The range between the most positive and most negative δ^2H values is 608.8‰ in modern fur seals and 748.4‰ in sea lions. Fur seals have a range similar to squid but more than twice the range in kelp while sea lions had the largest range.

Ancient fur seals and sea lions showed much more variation, though patterns are still similar to modern consumers. The range in δ^2H of fur seals varies from 601.7‰ in the 7500-4900 BP population, to 592.2‰ in the 2200-1600 BP population, to 608.8‰ in the modern fur seal population, all very similar. In sea lions, the 7500-4900 BP population has a range of 884‰, the largest range of δ^2H values in the data set. Modern sea lions, with significantly smaller range of 748.4‰, still have a very large range of δ^2H values. Some amino acids in archaeological populations are significantly different than modern samples. For example, phenylalanine is more positive than sea water in both

ancient fur seal populations. In non-essential amino acids, serine values are very positive, while aspartic acid and glutamic acid are more negative.

On average essential amino acids have more negative δ^2H values than non-essential amino acids. In our consumers, isoleucine has the most negative average δ^2H value at -156‰ while proline has the most positive at 507‰. The average difference between these two extreme amino acids is 663‰. Kelp, a primary producer, had significantly lower isoleucine and especially low values of proline at -210‰ and -41.5‰. Proline values varied between consumer species (Table 2, Figure 4).

Table 4-2 - Average and standard deviations of δ^2H of essential (a.) and non-essential (b.) amino acids in different organisms of the Beagle Channel.

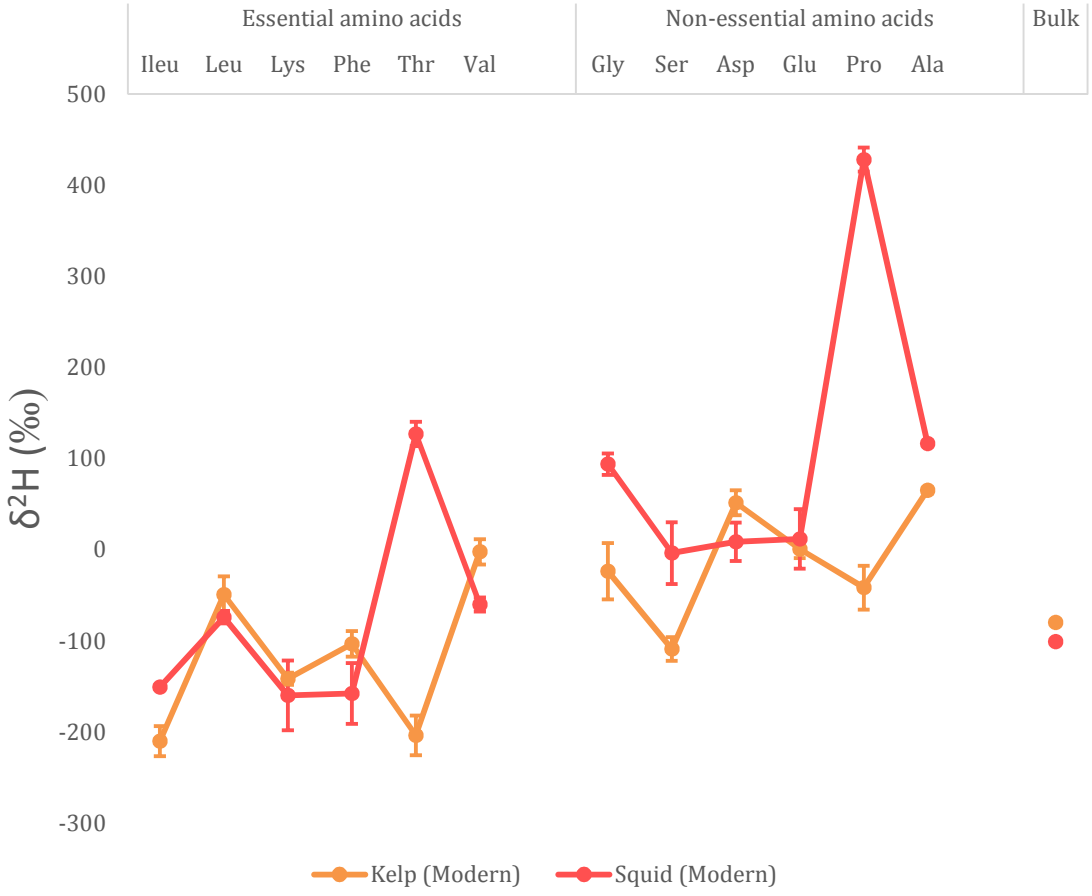
δ^2H values of essential amino acids (‰)

a.	Isoleucine	Leucine	Lysine	Phenylalanine	Threonine	Valine	Bulk δ^2H (‰)	n
Fur seals (7500-4900 BP)	-180.4±10.3	-107.3±6.6	-87.3±9.2	10.0±53.7	-26.2±10.0	-112.3±35.4	36.1±28.7	9
Fur seals (2200-1600 BP)	-141.7±19.8	-132.3±10.5	-92.4±10.1	127.7±63.7		-119.6±12.1	44.9±36.4	8
Fur seals (Modern)	-150.3±21.3	-87.8±3.6	-84.5±2.7	-106.2±12.5	93.7±7.8	-106.8±21.2	38.8±9.3	4
Sea Lions (7500-4900 BP)	-209.0±15.2	-117.3±3.1	-104.8±22.8	-133.6±12.6	44.8±20.6	-201.2±11.0	54.8±22.1	7
Sea Lions (Modern)	-61.4±21.3	-62.8±3.6	-145.6±2.7	-70.8±12.5	193.6±7.8	-36.8±21.2	-5.1±72.6	5
Squid (Modern)	-150.6±0.5	-73.9±6.9	-159.7±38.3	-157.5±33.4	127.3±13.4	-60.0±7.9	-68.0±7.3	3
Kelp (Modern)	-209.9±16.5	-49.2±20.0	-141.5±6.7	-103.2±14.0	-203.6±21.9	-2.0±13.9	-90.6±6.7	4

δ^2H values of non-essential amino acids (‰)

b.	Glycine	Serine	Aspartic acid	Glutamic acid	Proline	Alanine	Bulk δ^2H (‰)	n
Fur seals (7500-4900 BP)	17.2±7.9	224.3±6.5	-123.6±107.7	-132.7±30.5	421.3±37.5	15.4±17.8	36.1±28.7	9
Fur seals (2200-1600 BP)	-33.2±11.4		-7.6±21.3	-35.1±10.8	450.5±65.9	-31.2±16.8	44.9±36.4	8
Fur seals (Modern)	4.2±3.5	55.2±9.6	-42.8±24.6	-17.7±13.7	458.5±15.7	31.9±7.9	38.8±9.3	4
Sea Lions (7500-4900 BP)	26.1±3.2	197.3±8.3	-201.2±11.0	-10.4±4.6	675.0±65.7	-24.8±4.6	54.8±22.1	7
Sea Lions (Modern)	24.9±3.5	28.6±9.6	-24.0±24.6	18.0±13.7	602.8±15.7	47.7±7.9	-5.1±72.6	5
Squid (Modern)	94.0±9.5	-3.5±11.7	8.9±34.0	12.0±21.0	428.4±32.7	116.8±13.3	-68.0±7.3	3
Kelp (Modern)	-23.3±29.0	-108.7±30.8	51.6±13.0	0.8±13.8	-41.5±9.8	65.8±23.9	-90.6±6.7	4

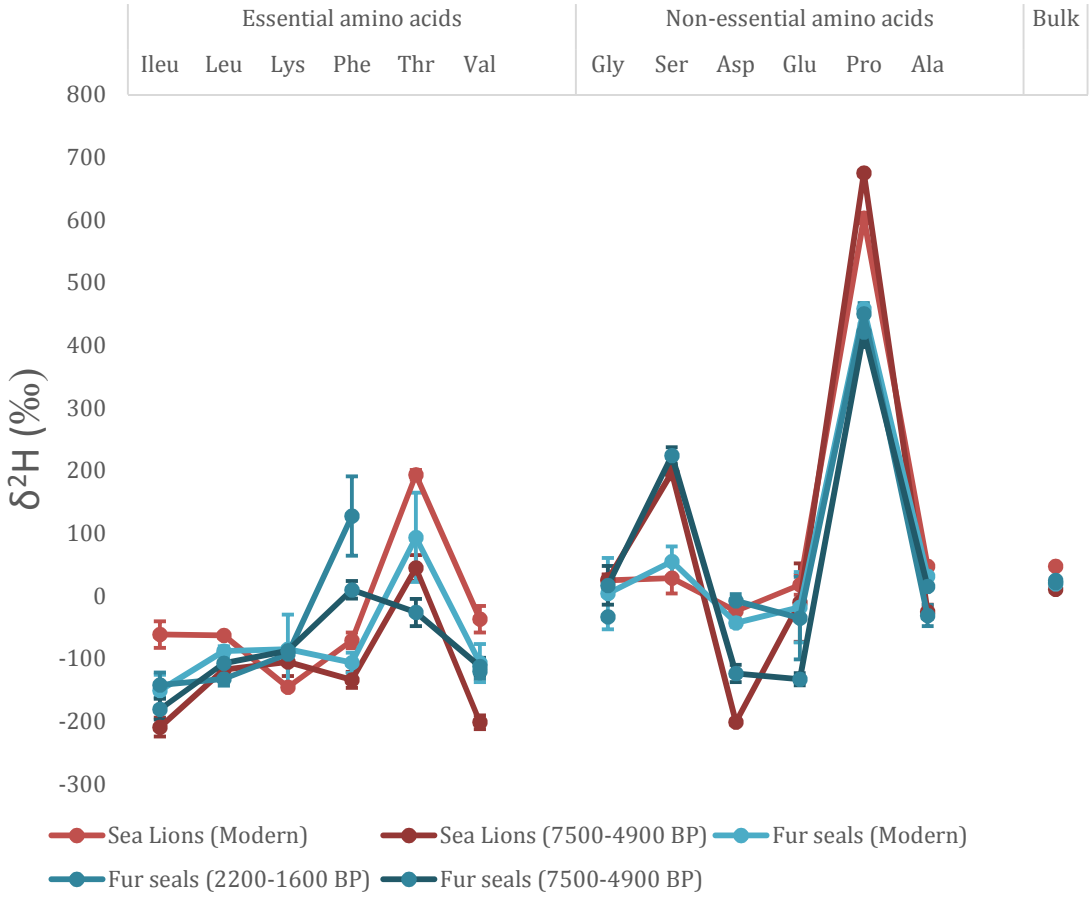
Figure 4-2 - δ^2H of Fuegian organisms at the base of the food web



Principle components analysis revealed significant variation in some amino acids but not others (Figs. 5 & 6). In analysis of all amino acids measured (Fig. 5), the first principal component captured nearly half the variation observed, primarily explained by isoleucine, valine, leucine, alanine threonine, lysine, and serine. The majority of these are essential AAs. Nearly 20% of the remaining variation was explained by the non-essential amino acids. In a PCA focusing solely on essential AAs (Fig. 6), over 60% of the variation was observed in all but one of the essential amino acids. PC2, explain just over

18% of the variation, was primarily driven by phenylalanine, with some contribution from isoleucine and lysine.

Figure 4-3 - Hydrogen isotopic values in pinnipeds of different time periods.



Discussion

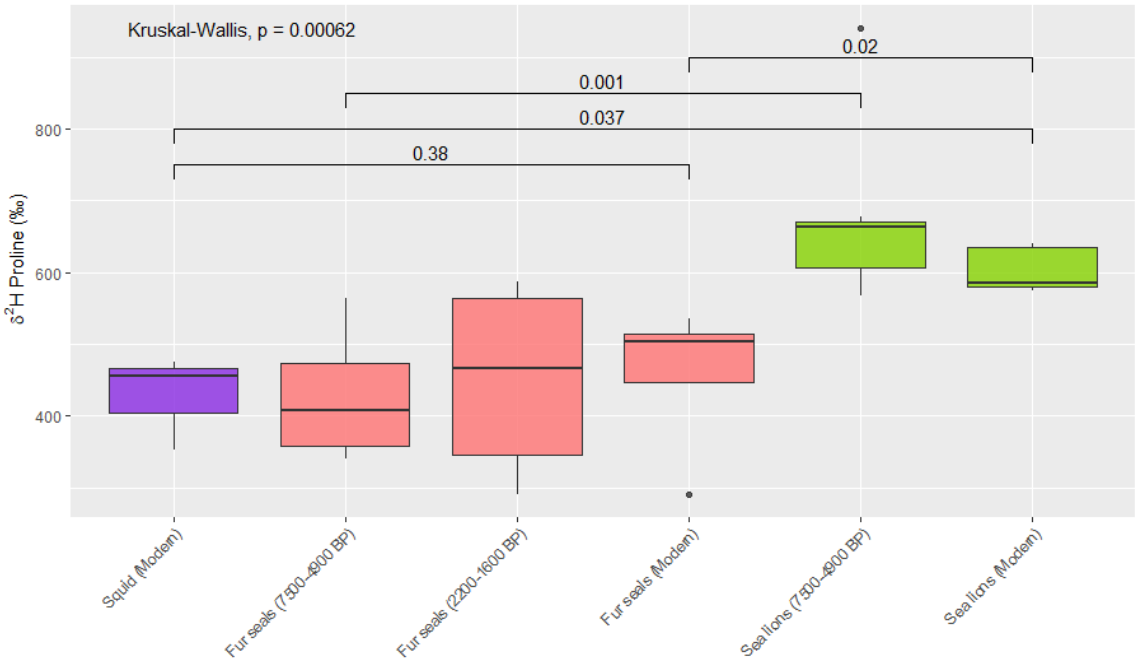
In general bulk δ^2H variation between species points to significant enrichment due to trophic and niche differences. Samples of squid, with the lowest δ^2H values, included both protein and lipids. Hydrogen from lipids likely resulted in lower δ^2H values. Differences between kelp and squid δ^2H values can also be explained by the observation that squid derive their essential amino acids from phytoplankton rather

than kelp as squid are pelagic organisms. A calculation of the average of all amino acids for squid, which excludes the lipid contribution to bulk δ^2H values, puts the squid more in line with bulk values for the pinnipeds at 19.9‰. This is nearly identical to the mean bulk value for modern fur seals (19.7‰, Table 1) but significantly more negative than modern sea lions (47.6‰, Table 1).

One noticeable finding was the large variation observed in prehistoric samples in bulk δ^2H relative to modern samples. This is somewhat contradictory to our results in variation found in $\delta^{13}C$ and in $\delta^{15}N$ (Nye et al., 2019a). Several possibilities could explain this variation. First, there exists variability in population sampling within sites. It is possible that the individual fur seals and sea lions, while found in the same stratigraphic section, are not necessarily from populations as well defined as those collected in modern time periods. For example, within each defined archaeological time period the exists several hundred years of time while modern individuals are known to live within several decades of each other. Second, the possibility of diagenetic effects on the samples themselves could explain this variability. An analysis of C/H ratios (Appendix, Fig. 1) revealed no significant differences in collagen between modern and ancient pinnipeds, suggesting diagenesis may not be a factor, with the exception of sea lions with very high C/H ratios (>15). A third possibility is that exchangeable hydrogen in pinniped tissues may account for this variability, however, all samples have been stored in the same environment for over a year before analysis and likely would have equilibrated with the atmosphere in Riverside, CA. Finally, this variation could represent

a truly higher variability in feeding regimes in ancient populations of these organisms relative to modern populations. However, this interpretation would contradict what we observed in $\delta^{13}C$ and in $\delta^{15}N$ measurements (Nye et al. 2019a & 2019b).

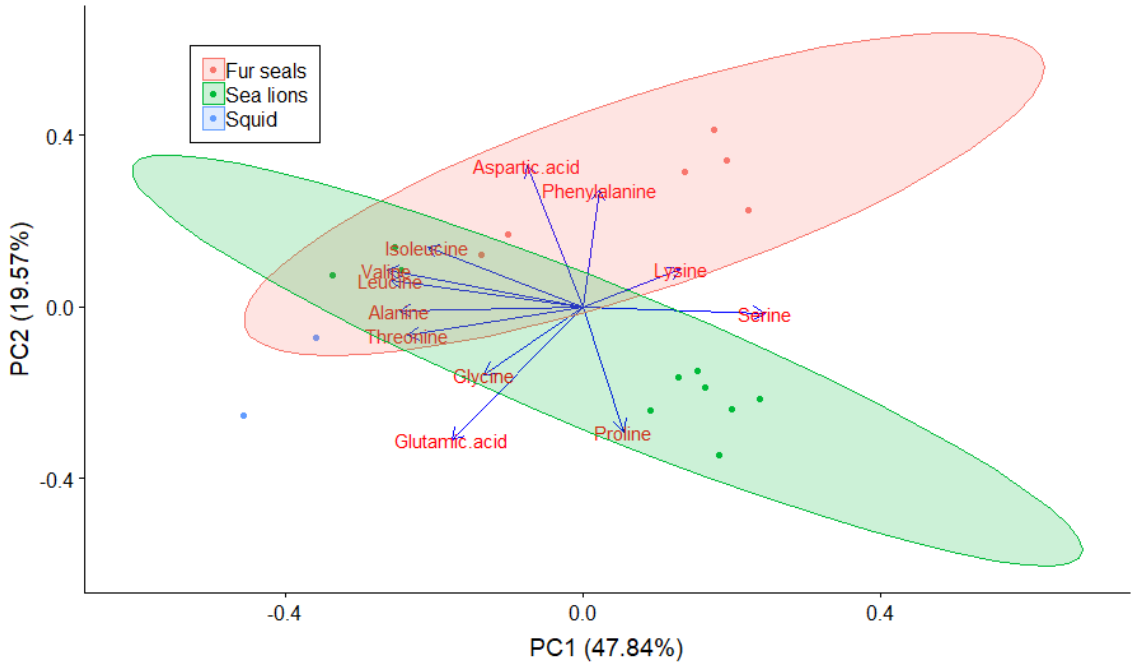
Figure 4-4 - Proline isotopic values over time.



Measurements of $\delta^{2}H$ in amino acids revealed further insights into the relationships between these species and time. Unlike our fingerprinting approach of $\delta^{13}C$ essential amino acids (Nye et al. 2019b), using low trophic level consumers as a primary producer proxy appeared inadequate due to the common patterns observed in consumers. The very positive threonine values observed in squid matched very closely with pinnipeds (Table 2, Figures 2 & 3). Likewise, in non-essential AAs, proline was very positive in squid as one might expect for an animal (Newsome et al., Oecologia submitted). In any case, consumer species showed significant variation in their essential amino acids (Fig. 5), which suggests high individual variability in primary producer

pathways. When populations are separated (Appendix, Fig. 2), principal component axes are largely the same as the variation observed in Fig. 6 while populations overlap with each other. This supports our findings from $\delta^{15}N$ amino acid phenylalanine (Nye et al., 2019c) that suggest high variability in foraging of individuals but little differences between populations of different time periods as a whole.

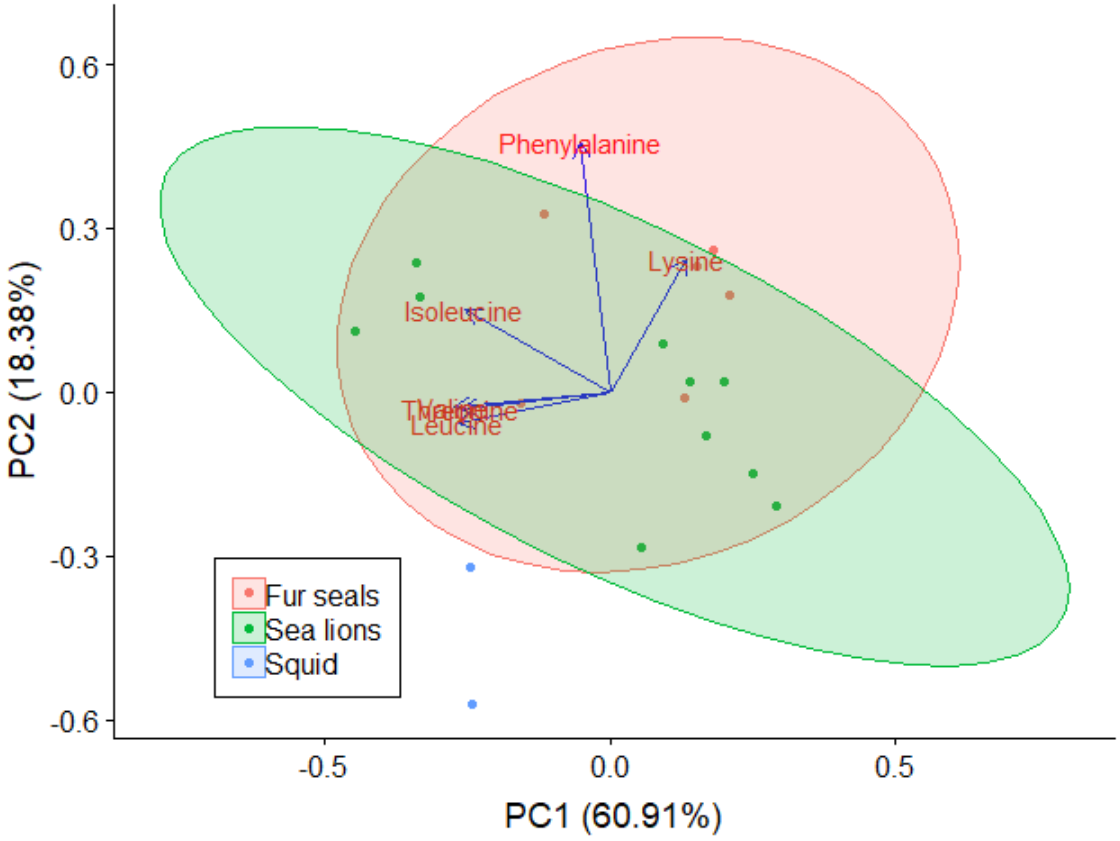
Figure 4-5 – Principal components analysis of all amino acids using measurements of δ^2H in Beagle Channel consumers. Significant overlap between consumers is observed on the first principal component, while divergence in PC2 increases as PC1 reaches more positive values.



We also wanted to test our interpretations of trophic level of organisms as observed in Nye et al., 2019c. Proline, as a non-essential AA and by far the most positive in δ^2H values in consumers, may possibly be used as a trophic level indicator. Despite a lack of understanding of trophic discrimination factors for proline in δ^2H, a statistical approach to absolute values may quantify some differences. As shown in Fig. 4, proline varies between species. However, within fur seals and within sea lions, we see no

significant differences in proline δ^2H values. This observation is supported by $\delta^{15}N$ measurements of glu-phe, a trophic proxy, in the same populations (Nye et al., 2019c).

Figure 4-6 - PCA of essential amino acids of hydrogen isotopes. The majority of variation in essential amino acids is borne by a combination of leucine, isoleucine, threonine, and valine. However, phenylamine, lysine, and to a smaller degree, isoleucine, is explained by a minority grouping.



Conclusion

Ultimately, δ^2H measurements in both bulk tissues and amino acids support our observations from measurements $\delta^{13}C$ and $\delta^{15}N$. In bulk measurements, we observed significant differences between high trophic level and low trophic level organisms, while δ^2H of amino acids indicate significant differences in trophic level between fur seals and sea lions. However, significant overlap in essential amino acids suggests an overall similar basal resource regime despite large individual variation in pinniped populations.

Chapter 5 Conclusion

The goals of this dissertation were to answer complex questions about the influence of humans, climate, and other ecological controls that might be influencing organisms that make up the food webs of marine southern South America. As the last habitable region on earth to be occupied by humans, with a biologically rich fauna, I found that there are significant changes at the highest trophic levels that largely appear to be influenced by human activities--from subsistence hunting and habitat occupation to industrial scale exploitation.

Ultimately, I observed several significant shifts in marine food webs from analyses in $\delta^{13}C$ (Nye et al. 2019a & 2019b). Pinnipeds that once hugged the coastline to avoid the dominant top predators, orcas (*Orcinus orca*), slowly shifted from nearshore to more offshore based diets as the most impactful predator became *Homo sapiens.* The ecological niches of these pinnipeds dynamically shifted over time, perhaps as a plastic response to the new predatory threat of humans. This change in niche accelerated dramatically when human impacts changed from subsistence exploitation from hunter-gatherer cultures to the industrial exploitation practiced by industrial sealers in the 19th and 20th centuries. Impacts on populations became so extreme that intrapopulation competitive exclusion became reduced, resulting in extremely variable diets between modern individuals, perhaps with some individual pinnipeds showing novel foraging regimes.

I expected general trends to show decreases in trophic level of pinnipeds as human exploitation became more pervasive. However, $\delta^{15}N$ analyses showed that overall populations did not show significant shifts in their trophic levels (Nye et al. 2019a & 2019c). Nitrogen analyses also revealed high individual variation in pinniped species, with some individuals occupying more than a full trophic level of difference between each other within a population. This finding supports the idea that these species have a high adaptive capacity with a generalist foraging strategy. Despite intense human predation on pinnipeds, pinnipeds can forage in a variety of habitats to prolong the survival of their species.

These findings are supported by analyses of $\delta^{2}H$ in tissues, where shifts in pinniped diets changed over time (Nye et al., 2019d). Furthermore, I was able to distinguish trophic levels differences between fur seals and sea lions, with sea lions showing substantially larger differences between essential and non-essential amino acids in their $\delta^{2}H$ values than fur seals. This observation could point to the competitive exclusion between species that persists from past to present.

Over the archaeological time period, little variation in climate suggests more influence on food webs was derived from direct human impacts. However, with marine mammal exploitation banned since the mid-20th century, direct human impacts from hunting have fallen off. Indirect human activities, however, remain a concern. Fishing intensification and larger human populations in Tierra del Fuego are impacting prey items, as well as habitat alteration. While these impacts pose a threat to pinnipeds, the

greater concern now is the rapid changes in global climate and the unknown effects of anthropomorphic climate change on the marine food webs of Tierra del Fuego. With rapid warming predicted to impact the oceanographic patterns of major oceans, both predator and prey alike will be forced to adapt quickly to rapidly changing environmental conditions. At least for now, pinniped populations are recovering from human exploitation and the food webs remain strong and resilient.

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Chapter 6 Appendix

Table 6-1 – All bulk $\delta^{15}N$ and $\delta^{13}C$ measurements of otariids.

Sample ID	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	C:N Ratio	Spp	Sex	Age Class	Site	Lat	Long	Site age
MD-AA-LJ	10.8	-19.5	3.1	Aa	NA	juvenile	Beagle Channel	-54.64	-65.25	Modern
MD-Aa-bCap-2	19.4	-13.4	3.1	Aa	NA	NA	Beagle Channel	-54.64	-65.25	Modern
Mod-A1315-cran	18.4	-12.9	3.1	Aa	NA	NA	Beagle Channel	-54.64	-65.25	Modern
Md-A4-RNP-2858	15.7	-15.4	3.3	Aa	NA	NA	Beagle Channel	-54.64	-65.25	Modern
MD-OF-FLA2	16.4	-13.3	3.1	Of	F	adult	Beagle Channel	-54.64	-65.25	Modern
MD-OF-L2	20.8	-12.0	3.2	Of	NA	sub-adult	Beagle Channel	-54.64	-65.25	Modern
MD-OF-L1	16.9	-12.4	3.4	Of	NA	sub-adult	Beagle Channel	-54.64	-65.25	Modern
MD-OF-R	18.6	-12.0	3.4	Of	NA	sub-adult	Beagle Channel	-54.64	-65.25	Modern
MD-OF-L3	17.5	-13.2	3.7	Of	NA	sub-adult	Beagle Channel	-54.64	-65.25	Modern
MD-OF-L4	17.4	-14.1	3.7	Of	NA	sub-adult	Beagle Channel	-54.64	-65.25	Modern
Md-OF-FLA1	19.9	-14.4	3.1	Of	F	adult	Beagle Channel	-54.64	-65.25	Modern
Md-Of-MLA	17.4	-11.6	3.3	Of	M	adult	Beagle Channel	-54.64	-65.25	Modern
MD-OF-MRA	18.5	-13.2	3.4	Of	M	adult	Beagle Channel	-54.64	-65.25	Modern
MD-OF-FRA	17.7	-12.8	3.5	Of	F	adult	Beagle Channel	-54.64	-65.25	Modern
MD-OF-LJ	20.1	-13.6	3.4	Of	NA	juvenile	Beagle Channel	-54.64	-65.25	Modern
Md-OF-LSA	17.6	-12.8	3.1	Of	NA	sub-adult	Beagle Channel	-54.64	-65.25	Modern
Md-Of-Cran	10.0	-22.1	3.3	Of	NA	NA	Beagle Channel	-54.64	-65.25	Modern
MD-Of-scap-M7	22.5	-13.7	3.3	Of	NA	NA	Beagle Channel	-54.64	-65.25	Modern
Md-Of-Seap-1	18.5	-13.5	3.3	Of	NA	NA	Beagle Channel	-54.64	-65.25	Modern
Md-Of-Pelvis-9anos	23.0	-12.4	3.5	Of	NA	adult	Beagle Channel	-54.64	-65.25	Modern
T-27	18.0	-12.7	3.1	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-27	18.4	-12.7	3.1	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-6	18.2	-13.1	3.1	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-6	18.6	-13.1	3.1	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-69	18.9	-12.4	3.1	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-74	17.9	-14.5	3.1	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-78	18.9	-11.9	3.2	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-18	19.6	-12.6	3.2	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-18	20.0	-12.6	3.2	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-24	19.5	-12.9	3.2	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-24	19.9	-12.9	3.2	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-79	18.0	-12.6	3.2	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-87	18.1	-10.8	3.2	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-81	18.9	-12.9	3.2	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD

T-86	19.5	-12.4	3.2	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-29	18.6	-12.8	3.2	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-29	19.1	-12.8	3.2	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-82	17.9	-12.8	3.3	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-26	17.1	-13.7	3.3	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-26	17.5	-13.7	3.3	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-102	16.1	-12.0	3.3	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-41	17.5	-12.7	3.3	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-83	18.7	-12.4	3.3	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-47	16.8	-12.9	3.3	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-7	16.8	-13.4	3.4	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-7	17.3	-13.4	3.4	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-fem-9	17.2	-13.0	3.4	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-43	18.3	-12.4	3.4	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-fem-14	15.5	-13.3	3.4	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-fem-8	17.7	-12.6	3.4	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-15	19.9	-13.3	3.4	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-15	20.3	-13.3	3.4	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-fem-7	19.6	-12.3	3.4	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-fem-21	18.6	-12.8	3.4	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-fem-15	16.6	-13.2	3.4	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-fem-20	15.0	-14.1	3.4	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-fem-19	18.5	-13.1	3.4	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-12	18.7	-13.7	3.4	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-12	19.1	-13.7	3.4	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-63	21.1	-12.1	3.4	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-fem-12	20.2	-11.6	3.4	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-97	17.9	-12.8	3.4	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-94	17.0	-12.9	3.5	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-48	18.1	-13.2	3.5	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-fem-13	17.6	-14.2	3.5	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-54	17.2	-15.2	3.5	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-fem-17	19.1	-13.2	3.5	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-fem-5	19.4	-12.9	3.5	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-84	14.9	-12.7	3.5	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-fem-2	17.9	-12.7	3.5	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-fem-16	20.6	-12.5	3.5	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD

T-25	18.6	-13.8	3.5	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-25	19.1	-13.8	3.5	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-90	14.9	-13.4	3.5	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-fem-6	15.4	-13.2	3.5	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-98	13.6	-14.2	3.5	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-71	19.4	-14.9	3.6	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-103-01	15.2	-13.6	3.6	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-17	17.7	-14.8	3.6	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-17	18.2	-14.8	3.6	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-fem-18	15.9	-13.6	3.7	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-95	18.0	-13.8	3.7	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-49	17.2	-14.4	3.7	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-75	16.8	-14.4	3.7	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-67	18.9	-15.7	3.8	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-99	14.7	-14.6	3.8	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-96	17.8	-13.8	3.8	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-13	17.5	-14.8	3.8	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-13	18.1	-14.8	3.8	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-51	18.5	-13.2	3.8	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-11	17.3	-14.8	3.8	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-11	17.8	-14.8	3.8	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-16	17.8	-15.8	3.9	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-16	18.3	-15.8	3.9	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-52	17.4	-16.4	3.9	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-101-Real	18.0	-14.7	3.9	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-22	12.4	-15.6	3.9	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-37	17.6	-14.8	3.9	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-10	16.0	-15.0	3.9	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-10	16.6	-15.0	3.9	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-4	15.4	-15.6	3.9	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-4	16.0	-15.6	3.9	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-2	16.4	-16.9	3.9	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-93	18.8	-14.9	3.9	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-77	17.7	-16.0	3.9	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-91	14.1	-15.3	3.9	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-46	17.7	-14.6	3.9	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-66	15.9	-14.8	3.9	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD

T-50	16.4	-16.0	4.0	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-23	16.3	-14.7	4.0	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
T-23	16.9	-14.7	4.0	Of	NA	NA	Thetis	-54.64	-65.25	1940-1950 AD
TH-3	19.1	-12.8	3.3	NA	NA	adult	Thetis Bluff	-54.64	-65.25	Historic
TB-2	20.2	-12.0	3.1	NA	NA	juvenile	Thetis Bluff	-54.64	-65.25	Historic
TB-1	18.6	-12.7	3.2	NA	NA	juvenile	Thetis Bluff	-54.64	-65.25	Historic
SH 1765	16.6	-13.9	3.2	Aa	NA	sub-adult	Shamakush	-54.85	-67.85	1100-1000 BP
SH 1655	19.8	-12.7	3.3	Aa	NA	sub-adult	Shamakush	-54.85	-67.85	1100-1000 BP
SH 1751	16.2	-14.1	3.4	Aa	NA	sub-adult	Shamakush	-54.85	-67.85	1100-1000 BP
SH 1511	18.1	-12.7	3.5	Aa	NA	sub-adult	Shamakush	-54.85	-67.85	1100-1000 BP
2705	16.5	-11.9	3.4	NA	M	juvenile	Ajej 1	-54.84	-68.36	1400-800 BP
2020	16.1	-12.6	3.9	NA	M	juvenile	Ajej 1	-54.84	-68.36	1400-800 BP
1031	17.7	-13.3	3.5	NA	M	sub-adult	Ajej 1	-54.84	-68.36	1400-800 BP
2174	16.2	-12.0	3.4	NA	M	NA	Ajej 1	-54.84	-68.36	1400-800 BP
3084	15.5	-12.9	3.5	NA	M	NA	Ajej 1	-54.84	-68.36	1400-800 BP
1405	16.0	-12.4	3.5	NA	NA	NA	Ajej 1	-54.84	-68.36	1400-800 BP
2629	16.2	-12.2	3.5	NA	M	NA	Ajej 1	-54.84	-68.36	1400-800 BP
13225	20.2	-13.0	3.5	Aa	NA	pup	Bahia Valentin	-54.89	-65.46	1500-1120 BP
11275	20.0	-11.5	3.9	Of	F	juvenile	Bahia Valentin	-54.89	-65.46	1500-1120 BP
10246	21.8	-13.6	3.6	Of	NA	pup	Bahia Valentin	-54.89	-65.46	1500-1120 BP
10246	21.5	-13.4	3.7	Of	NA	pup	Bahia Valentin	-54.89	-65.46	1500-1120 BP
35880	17.0	-11.8	3.2	Aa	M	adult	Tunel I (upper)	-54.82	-68.15	2200-1600 BP
42527	16.8	-12.6	3.2	Aa	F	adult	Tunel I (upper)	-54.82	-68.15	2200-1600 BP
40194	19.0	-11.4	3.3	Aa	F	adult	Tunel I (upper)	-54.82	-68.15	2200-1600 BP
35229	17.2	-11.8	3.2	Aa	NA	juvenile	Tunel I (upper)	-54.82	-68.15	2200-1600 BP
42460	16.1	-12.3	3.2	Aa	NA	pup	Tunel I (upper)	-54.82	-68.15	2200-1600 BP
36597	20.1	-12.5	3.2	Aa	NA	pup	Tunel I (upper)	-54.82	-68.15	2200-1600 BP
42742	17.7	-11.7	3.2	Aa	NA	pup	Tunel I (upper)	-54.82	-68.15	2200-1600 BP
37702	19.2	-12.7	3.2	Aa	NA	pup	Tunel I (upper)	-54.82	-68.15	2200-1600 BP
37371	16.7	-11.9	3.1	Aa	NA	sub-adult	Tunel I (upper)	-54.82	-68.15	2200-1600 BP
42614	17.7	-11.5	3.2	Aa	NA	sub-adult	Tunel I (upper)	-54.82	-68.15	2200-1600 BP
136325	16.3	-12.3	3.2	Aa	NA	sub-adult	Tunel I (upper)	-54.82	-68.15	2200-1600 BP
42439	17.8	-11.3	3.2	Aa	NA	sub-adult	Tunel I (upper)	-54.82	-68.15	2200-1600 BP
42416	17.0	-11.6	3.2	Aa	M	sub-adult	Tunel I (upper)	-54.82	-68.15	2200-1600 BP
40878	18.1	-11.8	3.2	Aa	NA	sub-adult	Tunel I (upper)	-54.82	-68.15	2200-1600 BP
35210	17.2	-12.5	3.4	Aa	NA	sub-adult	Tunel I (upper)	-54.82	-68.15	2200-1600 BP
42421	17.0	-11.4	3.4	Aa	NA	sub-adult	Tunel I (upper)	-54.82	-68.15	2200-1600 BP

37401	16.7	-11.9	3.4	Aa	NA	sub-adult	Tunel I (upper)	-54.82	-68.15	2200-1600 BP
37605	16.8	-12.2	3.5	Aa	NA	sub-adult	Tunel I (upper)	-54.82	-68.15	2200-1600 BP
32730	16.5	-13.5	3.9	Of	NA	adult	Tunel I (upper)	-54.82	-68.15	2200-1600 BP
BCI VI 80	18.3	-10.4	3.4	Aa	M	adult	Crossley	-54.81	-64.70	3000-1300 BP
BCI 458	16.1	-12.3	3.7	Aa	F	adult	Crossley	-54.81	-64.70	3000-1300 BP
BCI VI 1045	21.7	-12.1	3.8	Aa	NA	pup	Crossley	-54.81	-64.70	3000-1300 BP
II 879	16.4	-12.7	3.4	Aa	NA	sub-adult	Crossley	-54.81	-64.70	3000-1300 BP
BCI 968	19.7	-10.6	3.5	Of	M	adult	Crossley	-54.81	-64.70	3000-1300 BP
11870	18.5	-11.9	3.6	Of	M	sub-adult	Crossley	-54.81	-64.70	3000-1300 BP
H28-25B1	17.5	-14.8	3.3	NA	NA	NA	Hesh 28	-54.96	-66.81	660-550 BP
H28-9908-F40	18.8	-13.9	3.3	NA	NA	NA	Hesh 28	-54.96	-66.81	660-550 BP
7571	16.1	-14.7	3.3	Of	M	adult	Hesh 28	-54.96	-66.81	660-550 BP
9908	18.8	-13.9	3.3	Of	M	adult	Hesh 28	-54.96	-66.81	660-550 BP
K 9729	18.0	-11.4	3.2	Aa	M	adult	Kaia II	-54.80	-68.28	690-560 BP
K 8470	16.6	-12.8	3.3	Aa	M	adult	Kaia II	-54.80	-68.28	690-560 BP
K 8676	16.4	-13.5	3.5	Aa	F	adult	Kaia II	-54.80	-68.28	690-560 BP
K 8725	15.8	-12.2	3.7	Aa	M	adult	Kaia II	-54.80	-68.28	690-560 BP
K 7756	16.7	-11.8	3.6	Aa	M	sub-adult	Kaia II	-54.80	-68.28	690-560 BP
K 7756	16.9	-11.1	3.8	Aa	M	sub-adult	Kaia II	-54.80	-68.28	690-560 BP
K 8388	16.5	-11.9	3.6	NA	NA	juvenile	Kaia II	-54.80	-68.28	690-560 BP
K 8598	15.5	-12.3	3.8	NA	NA	juvenile	Kaia II	-54.80	-68.28	690-560 BP
K 9037	16.7	-11.8	3.4	Of	F	NA	Kaia II	-54.80	-68.28	690-560 BP
K 8872	16.7	-11.5	3.5	Of	F	NA	Kaia II	-54.80	-68.28	690-560 BP
3 Amigos 1	11.6	-17.4	3.2	NA	NA	adult	3 Amigos	-54.64	-65.25	7500-200 BP
3 Amigos 4	19.2	-13.7	3.1	NA	NA	pup	3 Amigos	-54.64	-65.25	7500-200 BP
3 Amigos 2	18.8	-13.0	3.1	NA	NA	juvenile	3 Amigos	-54.64	-65.25	7500-200 BP
3 Amigos 3	7.0	-19.9	3.5	NA	NA	juvenile	3 Amigos	-54.64	-65.25	7500-200 BP
3 Amigos 6	22.4	-12.2	3.2	Of	NA	juvenile	3 Amigos	-54.64	-65.25	7500-200 BP
3 Amigos 5	9.7	-20.5	3.1	Of	NA	pup	3 Amigos	-54.64	-65.25	7500-200 BP
34326	18.2	-13.2	3.1	Aa	F	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
34466	19.3	-13.4	3.1	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
34248	17.9	-13.8	3.1	Aa	F	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33630	17.8	-14.6	3.1	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
32090	20.0	-14.6	3.1	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
34537	18.5	-12.6	3.1	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33854	18.8	-12.5	3.2	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
34222	17.9	-12.3	3.2	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP

34138	17.9	-12.3	3.2	Aa	F	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
31109	18.3	-13.5	3.2	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
30510	18.5	-14.5	3.2	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
34111	17.8	-14.5	3.2	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33100	18.6	-13.1	3.2	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
31178	18.2	-13.4	3.2	Aa	F	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
34467	18.2	-11.6	3.2	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
34212	18.3	-14.0	3.2	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
31454	20.3	-12.4	3.2	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
31794	18.3	-14.5	3.2	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
32102	17.9	-12.8	3.2	Aa	F	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
31289	17.6	-12.2	3.2	Aa	F	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33454	19.4	-13.9	3.2	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
30486	18.7	-12.0	3.2	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33045	17.7	-13.9	3.2	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
30441	17.4	-11.6	3.2	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
30439	18.3	-11.8	3.2	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
32428	18.4	-12.1	3.2	Aa	F	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
31236	20.0	-12.6	3.2	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33781	18.8	-11.9	3.2	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
31820	17.9	-11.7	3.2	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
34152	17.7	-12.2	3.3	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
30166	19.2	-11.7	3.3	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
32308	18.1	-11.9	3.3	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33624	19.0	-11.9	3.3	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
30634	18.9	-11.7	3.3	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33775	16.2	-13.4	3.3	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
32124	18.1	-12.7	3.3	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33835	19.1	-11.9	3.3	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
30697	19.4	-11.4	3.3	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
32461	19.1	-11.5	3.3	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
31837	18.8	-11.4	3.3	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33490	18.3	-12.4	3.3	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33777	16.3	-12.3	3.3	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
31923	17.5	-11.8	3.3	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
31792	19.2	-12.0	3.3	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
32245	18.9	-11.4	3.3	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP

32351	18.0	-11.5	3.3	Aa	F	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
31872	19.2	-11.8	3.3	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33050	18.3	-11.7	3.3	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
31107	17.4	-11.8	3.3	Aa	F	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
30472	17.6	-11.5	3.4	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
30139	18.7	-11.7	3.4	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
30169	18.7	-11.3	3.4	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33422	17.8	-11.9	3.4	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
34220	19.0	-11.9	3.4	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
32813	18.0	-11.5	3.4	Aa	F	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
32946	18.7	-11.9	3.4	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
32495	18.5	-11.7	3.4	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
31578	18.1	-12.4	3.5	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
32385	18.3	-11.7	3.5	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
32288	18.2	-15.5	3.6	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
32288	17.7	-12.5	3.6	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33936	18.3	-12.5	3.6	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33045	17.0	-13.5	3.8	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33936	17.3	-12.3	4.0	Aa	M	adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
34495	19.1	-14.4	3.1	Aa	M	juvenile	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
34614	17.6	-12.5	3.1	Aa	M	juvenile	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
32040	17.5	-14.8	3.1	Aa	M	juvenile	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33786	16.7	-15.0	3.1	Aa	M	juvenile	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
34434	18.7	-13.1	3.1	Aa	M	juvenile	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
32846	18.5	-13.7	3.1	Aa	F	juvenile	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
32075	17.2	-14.2	3.1	Aa	F	juvenile	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
30765	20.2	-13.6	3.1	Aa	M	juvenile	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
34509	19.4	-13.1	3.1	Aa	M	juvenile	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
34392	17.5	-13.9	3.1	Aa	M	juvenile	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33795	18.3	-13.6	3.2	Aa	M	juvenile	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33745	17.2	-13.5	3.2	Aa	M	juvenile	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
34041	18.5	-13.3	3.2	Aa	M	juvenile	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
34080	18.2	-14.5	3.2	Aa	M	juvenile	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33629	19.1	-14.2	3.2	Aa	M	juvenile	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33518	18.4	-14.0	3.2	Aa	M	juvenile	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33565	18.2	-15.1	3.2	Aa	M	juvenile	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
34038	18.5	-15.2	3.2	Aa	F	juvenile	Tunel I (lower)	-54.82	-68.15	7500-4900 BP

31379	17.9	-11.8	3.2	Aa	M	juvenile	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33155	18.7	-12.0	3.2	Aa	F	juvenile	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
32359	17.2	-14.3	3.2	Aa	M	juvenile	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33785	16.1	-12.6	3.2	Aa	M	juvenile	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
30794	17.7	-11.8	3.2	Aa	M	juvenile	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33160	16.9	-12.2	3.2	Aa	M	juvenile	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
34747	18.4	-11.9	3.2	Aa	M	juvenile	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33522	18.6	-12.5	3.3	Aa	M	juvenile	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
31865	18.1	-11.6	3.3	Aa	M	juvenile	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
34525	18.2	-11.4	3.3	Aa	M	juvenile	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33392	19.0	-11.7	3.3	Aa	M	juvenile	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
34499	18.9	-11.9	3.3	Aa	M	juvenile	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33719	17.9	-11.3	3.3	Aa	M	juvenile	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33478	16.8	-13.3	3.3	Aa	F	juvenile	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33172	17.8	-12.4	3.3	Aa	M	juvenile	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
31819	18.0	-12.1	3.4	Aa	M	juvenile	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33311	16.8	-11.9	3.4	Aa	M	juvenile	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
31930	17.8	-15.4	3.4	Aa	M	juvenile	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
34302	17.6	-12.8	3.4	Aa	F	juvenile	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
34228	18.9	-12.1	3.5	Aa	M	juvenile	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33553	18.9	-14.9	3.3	Aa	M	pup	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33342	18.3	-11.5	3.3	Aa	M	pup	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33547	18.8	-11.6	3.3	Aa	F	pup	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
32815	16.5	-12.5	3.4	Aa	M	pup	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
32462	18.6	-11.8	3.4	Aa	F	pup	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
34217	18.6	-14.0	3.1	Aa	M	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
30862	19.6	-12.2	3.1	Aa	M	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
34443	19.3	-13.8	3.1	Aa	M	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
30980	19.5	-14.0	3.1	Aa	M	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
34681	19.0	-13.5	3.1	Aa	M	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
34355	18.5	-13.3	3.1	Aa	M	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
34324	19.6	-12.5	3.1	Aa	M	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
32609	18.9	-11.3	3.2	Aa	M	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
34376	17.2	-12.2	3.2	Aa	M	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
34057	17.0	-14.7	3.2	Aa	M	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
31974	18.3	-12.8	3.2	Aa	M	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
34027	16.9	-14.8	3.2	Aa	M	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP

34627	19.1	-14.2	3.2	Aa	M	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
31450	18.5	-13.3	3.2	Aa	M	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
31851	18.4	-12.8	3.2	Aa	M	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
34672	19.3	-11.5	3.2	Aa	M	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
32840	18.2	-13.2	3.2	Aa	M	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
32483	17.2	-12.2	3.2	Aa	M	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
34496	19.1	-11.0	3.2	Aa	M	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
34616	18.7	-11.8	3.2	Aa	M	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
34059	18.5	-14.6	3.2	Aa	M	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
32527	17.9	-13.0	3.3	Aa	M	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33705	17.3	-11.5	3.3	Aa	M	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
31118	18.7	-11.6	3.3	Aa	M	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
32005	16.6	-12.8	3.3	Aa	M	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33104	19.1	-12.0	3.3	Aa	M	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
34429	17.2	-12.6	3.3	Aa	M	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33388	17.9	-12.7	3.3	Aa	M	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33184	18.5	-12.0	3.3	Aa	M	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33461	19.4	-11.4	3.3	Aa	M	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33307	18.5	-11.5	3.3	Aa	F	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
31463	17.6	-12.6	3.3	Aa	M	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33512	20.0	-11.5	3.3	Aa	M	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
32499	17.6	-11.9	3.4	Aa	F	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
32467	19.2	-12.5	3.4	Aa	M	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33610	17.7	-11.7	3.4	Aa	M	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33421	17.7	-12.0	3.5	Aa	M	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33904	17.5	-12.2	3.6	Aa	M	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33436	17.7	-12.3	3.6	Aa	M	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
32491	18.5	-13.6	3.8	Aa	M	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
32816	16.2	-12.0	3.9	Aa	F	sub-adult	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33882	19.2	-11.8	3.2	Aa	M	NA	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33461	18.7	-11.8	3.2	Aa	M	NA	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33684	19.1	-11.8	3.2	Aa	NA	NA	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
32424	18.0	-11.7	3.3	Aa	M	NA	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33175	17.9	-11.9	3.3	Aa	M	NA	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33652	18.6	-12.4	3.3	Aa	M	NA	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33378	18.3	-11.8	3.3	Aa	M	NA	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
32382	18.4	-11.6	3.3	Aa	M	NA	Tunel I (lower)	-54.82	-68.15	7500-4900 BP

32442	18.0	-12.6	3.3	Aa	F	NA	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33632	19.3	-11.3	3.4	Aa	M	NA	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
32430	18.7	-12.2	3.4	Aa	M	NA	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
32350	17.6	-12.2	3.4	Aa	M	NA	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
32255	18.4	-12.1	3.4	Aa	M	NA	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
XX287	16.2	-12.8	3.5	Aa	M	NA	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33666	17.6	-11.9	3.6	Aa	M	NA	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33666	17.9	-12.2	3.7	Aa	M	NA	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
30422	17.7	-11.6	3.3	NA	NA	NA	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
34437	17.1	-13.3	3.3	NA	NA	NA	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
32816	15.4	-14.5	3.3	NA	NA	NA	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33571	18.5	-11.2	3.4	NA	NA	NA	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
34544	18.2	-11.5	3.4	NA	NA	NA	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
34751	18.6	-11.2	3.2	Of	NA	NA	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33551	18.8	-11.1	3.2	Of	NA	NA	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
30459	19.6	-10.4	3.2	Of	NA	NA	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33458	18.2	-11.8	3.3	Of	NA	NA	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33459	19.1	-11.0	3.3	Of	NA	NA	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
34227	17.7	-12.3	3.5	Of	NA	NA	Tunel I (lower)	-54.82	-68.15	7500-4900 BP
33717	18.4	-10.8	3.5	Of	NA	NA	Tunel I (lower)	-54.82	-68.15	7500-4900 BP

Table 6-2 - All compound specific amino acid measurements of $\delta^{13}C$. (AA=Arctocephalus australis, OF= Otraria flavescens)

	$\delta^{13}C$ values of essential amino acids (‰)						$\delta^{13}C$ values of non-essential amino acids (‰)					
spp	Ile	Leu	Lys	Phe	Thr	Val	Gly	Ser	Asp	Glu	Pro	Ala
AA modern												
	-16.9	-25.7	-23.7	-30.7	-6.3	-40.0	3.5	-17.4	-6.8	-29.7	18.6	-23.2
	-14.2	-22.8	-23.3	-17.7	-7.0	-18.5	-3.4	-5.7	-6.9	-20.6	7.2	-16.0
	-13.9	-22.9	-18.0	-23.4	-4.8	-18.4	-4.1	-5.4	-12.1	-13.2	5.3	-16.6
	-18.1	-32.0	-16.7	-24.8	-7.4	-33.3	-17.0	-14.6	-18.7	-20.3	-16.2	-24.3
AVG	**-15.8**	**-25.9**	**-20.4**	**-24.2**	**-6.4**	**-27.6**	**-5.3**	**-10.8**	**-11.1**	**-21.0**	**3.7**	**-20.0**
SD	**2.1**	**4.3**	**3.6**	**5.3**	**1.1**	**10.9**	**8.6**	**6.1**	**5.6**	**6.8**	**14.5**	**4.3**
OF modern												
	-13.6	-23.9	-11.8	-22.7	-4.7	-18.7	-18.6	-2.3	-12.1	-11.7	7.7	-16.3
	-13.2	-22.7	-9.3	-22.1	-3.8	-17.5	-2.0	-4.8	-11.1	-11.6	6.0	-14.9
	-15.3	-24.0	-11.5	-22.7	-7.3	-18.0	-4.7	-3.7	-11.7	-11.5	7.9	-16.2
	-14.0	-22.9	-11.5	-22.0	-2.7	-17.1	-2.5	-4.9	-13.3	-12.1	6.4	-15.2
	-13.3	-22.8	-8.0	-21.2	-6.2	-17.6	-0.9	-5.4	-9.7	-10.5	7.3	-15.6
AVG	**-13.9**	**-23.3**	**-10.4**	**-22.1**	**-4.9**	**-17.8**	**-5.7**	**-4.2**	**-11.6**	**-11.5**	**7.0**	**-15.7**
SD	**0.9**	**0.6**	**1.7**	**0.6**	**1.8**	**0.6**	**7.3**	**1.2**	**1.3**	**0.6**	**0.8**	**0.6**
AA 2200-1600 BP												
	-9.8	-20.0	-10.6	-21.5	1.5	-16.1	-4.8	-4.8	-19.3	-15.6	-14.6	-16.3
	-13.7	-21.7	-8.9	-22.0	6.8	-16.0	-2.0	1.6	-15.4	-18.1	-15.9	-7.7
	-9.0	-20.7	-11.3	-22.0	7.5	-15.8	-2.7	-0.3	-16.6	-18.8	-15.9	-10.0
	-18.1	-21.0	-9.9	-21.8	1.7	-18.3	-4.5	-2.0	-18.6	-15.9	-13.4	-12.5
	-9.0	-19.0	-10.6	-21.5	5.1	-13.2	-1.7	6.5	-16.2	-16.6	-14.4	-8.7
	-7.5	-20.8	-7.2	-21.8	-10.5	-17.7	-7.8	-6.0	-22.3	-15.7	-12.3	-18.3
	-12.3	-16.9	-8.4	-21.1	-3.6	-12.3	-2.2	3.1	-12.8	-13.3	-11.7	-11.2
AVG	**-11.3**	**-20.0**	**-9.6**	**-21.7**	**1.2**	**-15.6**	**-3.7**	**-0.3**	**-17.3**	**-16.3**	**-14.0**	**-12.1**
SD	**3.7**	**1.6**	**1.5**	**0.3**	**6.4**	**2.2**	**2.2**	**4.4**	**3.1**	**1.8**	**1.7**	**3.9**
Of 2200-1600 BP												
	-6.9	-22.3	-9.5	-17.5	-11.4	-7.5	1.3	-9.6	-4.8	-10.4	-7.3	-13.8
	-11.1	-22.9	-10.1	-17.3	-10.3	-22.1	-0.7	-8.8	-7.6	-12.6	-2.4	-15.1
	-11.0	-24.1	-11.4	-17.2	-9.2	-18.8	-2.0	-9.9	-9.6	-14.0	-3.9	-15.3
	-3.2	-21.5	-9.9	-16.6	-9.7	-21.7	1.9	-8.7	-6.8	-11.4	-2.0	-13.2
		-29.4	-15.6	-22.2	-17.8	-8.1	-10.5	-16.8	-31.7	-19.5	-9.1	-17.1
	-6.1	-21.3	-10.3	-16.4	-10.4	-7.4	-1.1	-8.3	-6.9	-11.3	-0.1	-17.2
	-3.4	-19.4		-8.2	-8.8	-9.0	2.9	-8.0	-2.1	-8.9	-8.3	-12.1
	0.7	-17.3	-10.1	-12.5	-5.5	-5.0	8.8	-0.5	2.5	-5.8	10.4	-8.6

AVG	**-5.9**	**-22.3**	**-11.0**	**-16.0**	**-10.4**	**-12.5**	**0.1**	**-8.8**	**-8.4**	**-11.7**	**-2.8**	**-14.1**
SD	**4.3**	**3.6**	**2.1**	**4.1**	**3.5**	**7.1**	**5.5**	**4.4**	**10.1**	**4.0**	**6.2**	**2.8**
AA 7500-4900 BP												
	-9.9	-19.8	-16.9	-16.4	4.0	-14.7	-6.0	2.3	-10.6	-12.2	-15.0	-11.6
	-10.6	-20.8	-14.9	-16.1	3.6	-14.7	-1.9	6.2	-9.7	-11.4	-13.9	-11.5
	-11.4	-20.5		-16.6	0.6	-13.2	-3.6	3.7	-10.4	-12.6	-15.4	-13.7
	-9.7	-18.7	-16.5	-16.3	6.2	-13.2	-6.5	2.6	-13.4	-13.1	-16.2	-14.1
	-9.6	-21.6	-14.2	-17.0	2.1	-14.8	-5.2	3.3	-11.1	-13.4	-15.1	-11.8
	-11.9	-23.1	-14.2	-19.2	2.1	-16.6	-6.4	6.1	-13.1	-13.6	-14.3	-12.3
	-9.7	-19.2		-17.4	5.4	-13.6	-4.3	8.5	-12.3	-13.4	-14.0	-9.9
	-12.0	-20.6	-14.3	-17.7	3.0	-14.9	-5.7	5.0	-11.7	-13.5	-13.4	-13.8
	-10.1	-19.0		-15.2	3.6	-14.4	-4.7	5.5	-11.6	-12.8	-14.4	-11.5
AVG	**-10.5**	**-20.4**	**-15.2**	**-16.9**	**3.4**	**-14.5**	**-4.9**	**4.8**	**-11.6**	**-12.9**	**-14.6**	**-12.2**
SD	**1.0**	**1.4**	**1.2**	**1.1**	**1.7**	**1.1**	**1.5**	**2.0**	**1.2**	**0.7**	**0.9**	**1.4**
Of 7500-4900 BP												
	-8.7	-25.0	-13.6	-22.6	-12.5	-16.2	-10.8	-20.3	-20.5	-15.6	1.0	-17.5
	-10.7	-22.5	-9.1	-17.9	-4.1	-12.5	-8.7	-8.7	-9.7	-8.7	1.2	-12.9
	-10.9	-22.0	-8.9	-17.2	-9.4	-10.9	-6.0	-13.1	-7.5	-8.9	1.0	-13.7
	-11.1	-23.2	-9.3	-16.6	-9.3	-10.8	-9.2	-13.5	-9.2	-9.9	-1.0	-13.1
	-10.0	-25.0		-16.4	-13.8	-11.8	-7.4	-17.4	-8.6	-10.7	-3.4	-13.9
	-16.7	-27.2	-14.6	-20.1	-20.7	-15.5	-4.2	-24.0	-17.3	-15.3	-1.4	-14.0
	-14.1	-26.5	-14.8	-19.6	-15.7	-14.2	-5.0	-18.3	-10.6	-12.4	1.7	-15.1
AVG	**-11.7**	**-24.5**	**-11.7**	**-18.6**	**-12.2**	**-13.1**	**-7.4**	**-16.5**	**-11.9**	**-11.6**	**-0.1**	**-14.3**
SD	**2.7**	**2.0**	**2.9**	**2.3**	**5.3**	**2.2**	**2.4**	**5.1**	**5.0**	**2.9**	**1.9**	**1.6**
Shag 7500-1600 BP												
	-8.9	-20.9		-14.7	-12.6	-9.1	4.5	-11.2	-2.6	-15.5	-16.9	-12.7
	-5.1	-21.3	-8.7	-12.8	-14.2	-7.7	13.5	-9.3	0.0	-12.6	-13.3	-12.0
	-6.9	-21.3	-7.7	-13.2	-15.8	-9.6	11.4	-11.0	-1.0	-15.5	-15.9	-11.4
	-8.9	-24.6	-10.6	-16.1	-15.3	-11.9	9.4	-21.7	-6.6	-21.0	-20.4	-13.2
	-8.6	-22.6	-9.8	-15.0	-19.7	-10.2	9.9	-12.5	-4.0	-20.0	-20.7	-14.1
	-5.3	-20.8	-10.5	-13.2	-16.4	-8.6	9.3	-14.5	-2.6	-16.6	-21.1	-12.0
	-7.0	-21.8	-8.9	-12.8	-17.2	-7.4	6.3	-15.5	-4.0	-17.6	-23.1	-14.4
	-8.2	-22.2	-9.6	-13.2	-19.4	-8.9	8.7	-13.7	-5.0	-18.7	-22.7	-12.7
AVG	**-7.4**	**-21.9**	**-9.4**	**-13.9**	**-16.3**	**-9.2**	**9.1**	**-13.7**	**-3.2**	**-17.2**	**-19.3**	**-12.8**
SD	**1.5**	**1.2**	**1.0**	**1.2**	**2.4**	**1.4**	**2.8**	**3.8**	**2.1**	**2.7**	**3.5**	**1.0**
Kelp Modern												
	-7.5	-17.5	-7.9	-17.3	7.1	-9.1	-1.0	17.1	-5.3	-8.5	-7.3	-7.4

	-4.3	-20.1	-7.3	-17.4	6.1	-12.2	-0.6	8.4	-8.2	-9.2	-8.7	-19.5
	-8.2	-21.2	-8.4	-16.9	2.5	-10.6	-5.5	2.4	-5.4	-9.0	-6.9	-14.9
	-12.9	-24.7	-9.8	-19.0	-1.8	-15.1	-13.0	-2.4	-6.0	-14.0	-11.8	-17.4
AVG	**-8.2**	**-20.9**	**-8.4**	**-17.7**	**3.5**	**-11.8**	**-5.0**	**6.4**	**-6.2**	**-10.2**	**-8.7**	**-14.8**
SD	**3.5**	**3.0**	**1.1**	**0.9**	**4.0**	**2.6**	**5.7**	**8.4**	**1.3**	**2.5**	**2.2**	**5.3**
Squid Modern												
	-17.3	-18.0	-15.0	-23.0	-7.2	-18.5	0.8	-0.1	-0.8	-4.9	-13.2	-14.1
	-14.1	-17.6	-13.1	-22.0	-5.8	-17.9	-1.4	-0.4	5.5	-2.0	-13.1	-14.1
	-15.8	-18.1	-14.5	-23.1	-7.1	-17.8	1.0	-0.7	14.3	-1.7	-14.2	-12.8
	-16.5	-16.8	-13.6	-22.9	-6.5	-17.6	1.0	-0.1	16.0	-1.7	-13.7	-13.0
AVG	**-15.9**	**-17.6**	**-14.1**	**-22.8**	**-6.7**	**-18.0**	**0.3**	**-0.3**	**8.8**	**-2.6**	**-13.6**	**-13.5**
SD	**1.4**	**0.6**	**0.9**	**0.5**	**0.6**	**0.4**	**1.2**	**0.3**	**7.8**	**1.6**	**0.5**	**0.7**
Fish 7500-4900 BP												
	-16.3	-28.2	-15.1	-20.9	-4.5	-25.8	-2.7	8.0	0.2	-2.4	-11.6	-16.6
	-16.1	-27.3	-19.5	-25.7	-6.7	-26.0	-1.7	8.4	-5.6	-11.6	-12.4	-17.0
	-16.4	-28.1	-16.2	-19.1	-17.0	-24.0	-2.0	7.3	0.7	-6.3	-10.8	-17.2
	-11.5	-32.4	-14.5	-21.7	-1.7	-24.3	6.8	9.2	-4.6	-7.9	-11.6	-15.4
	-12.6	-31.0	-16.4	-19.8	-2.8	-25.2	-2.9	10.8	-4.6	-12.5	-11.7	-19.8
	-11.4	-31.5	-14.4	-21.3	-4.0	-24.7	8.4	10.5	-7.1	-9.0	-12.5	-16.2
	-12.3	-31.8	-15.6	-20.9	-2.4	-24.4	1.7	10.7	-6.6	-7.8	-13.3	-17.8
	-12.7	-30.7	-15.3	-19.6	-1.0	-23.7	-3.1	12.1	-5.6	-7.4	-12.6	-19.0
AVG	**-13.7**	**-30.1**	**-15.9**	**-21.1**	**-5.0**	**-24.8**	**0.6**	**9.6**	**-4.2**	**-8.1**	**-12.1**	**-17.4**
SD	**2.2**	**2.0**	**1.6**	**2.1**	**5.2**	**0.8**	**4.6**	**1.7**	**3.0**	**3.1**	**0.8**	**1.4**

Table 6-3 – All compound specific amino acid measurements of $\delta^{15}N$ (‰). (AA=Arctocephalus australis, OF= Otraria flavescens)

	Ala	Gly	Thr	Ser	Val	Leu	Ileu	Pro	Asp	Glu	Phe	Tyr	Lys
AA 7500-4900 BP													
3632 AVG	26.8	14.5	-17.6		28.5	26.7	26.4	27.0	22.9	27.6	15.7	11.3	
1930 AVG	26.3	17.7	-15.6	15.2		25.6		25.0	22.9	26.7	14.0	11.4	
3635 AVG	26.0	15.8	-18.4	14.3	27.4	26.2		25.6	23.2	27.3	15.7	13.4	
0169 AVG	27.2	16.0	-17.5	17.3	29.1	27.6		27.5	26.5	30.9	17.4	12.8	
3533 AVG	21.2	17.3	-25.0	13.6	26.2	26.6	23.7	27.4	23.4	25.8	14.5	10.6	
3786 AVG	24.2	10.4	-21.1	12.4	24.0	23.6	23.8	23.0	20.4	24.0	13.0	9.7	
7288 AVG	19.3	14.9	-23.7	13.0	24.2	27.2		25.6	21.7	25.6	12.2	10.0	
2609 AVG	21.5	14.8	-20.1	14.2	24.1	27.4		26.6	22.4	25.8	12.5	11.3	
3719 AVG	22.2	13.8	-16.3	14.2	23.8	24.7	24.2	23.5	20.7	24.0	13.8	11.6	
Avg	**23.9**	**15.0**	**-19.5**	**14.3**	**25.9**	**26.2**	**24.5**	**25.7**	**22.7**	**26.4**	**14.3**	**11.3**	
SD	**2.9**	**2.2**	**3.3**	**1.5**	**2.2**	**1.3**	**1.3**	**1.6**	**1.8**	**2.1**	**1.7**	**1.2**	
AA 2200-1600 BP													
5880 AVG	21.7	11.7	-14.0	13.1	22.2	22.3	22.3	23.9	20.5	23.8	13.2	9.0	11.4
2527 AVG	23.6	9.9	-18.2	11.4	24.6	23.4	23.6	25.7	20.3	24.6	12.4	7.4	12.5
0194 AVG	23.1	13.2	-19.7	14.4	25.2	25.2	25.4	27.7	22.6	26.3	14.1	10.4	13.4
0195 AVG	27.0	10.7	-20.1	11.9	27.2	26.8		25.8	22.2	26.7	12.5	10.0	10.8
7702 AVG	21.8	9.8	-24.0	13.2	25.9	25.3	25.0	25.4	21.6	26.1	11.7	9.6	12.5
2421 AVG	25.1	8.6	-18.2	12.2	27.2	25.5	26.4	25.3	21.7	25.9	14.0	8.8	11.8
6957 AVG	24.0	15.5	-25.9	16.4	26.8	26.1	25.3	27.9	23.5	26.7	15.6	12.1	16.5
2349 AVG	23.7	10.4	-18.5	12.9	25.3	25.5	25.1	26.2	21.8	26.0	14.1	10.2	12.0
Avg	**23.7**	**11.2**	**-19.8**	**13.2**	**25.5**	**25.0**	**24.7**	**26.0**	**21.8**	**25.7**	**13.4**	**9.7**	**12.6**
SD	**1.7**	**2.2**	**3.7**	**1.6**	**1.7**	**1.5**	**1.4**	**1.3**	**1.0**	**1.0**	**1.3**	**1.4**	**1.8**
AA modern													
BCAP2 AVG	25.5	12.9	-16.2	14.3	26.2	26.6	26.1	25.9	22.6	26.9	14.9	9.4	11.0
A1315 AVG	26.6	13.0	-19.0	14.7	28.6	27.5	26.6	26.6	24.3	28.1	15.8	13.1	13.8
2858 AVG	22.5	9.2	-19.5	14.1	24.2	23.3		23.0	19.4	22.7	12.8	9.8	9.2
Avg	**24.9**	**11.7**	**-18.2**	**14.4**	**26.3**	**25.8**	**26.4**	**25.1**	**22.1**	**25.9**	**14.5**	**10.8**	**11.4**
SD	**2.1**	**2.2**	**1.8**	**0.3**	**2.2**	**2.2**	**0.4**	**1.9**	**2.5**	**2.8**	**1.5**	**2.1**	**2.3**
OF modern													
OF-FLA1 AVG	25.8	13.8	-23.4	16.8	28.4	27.8	25.1	27.5	24.2	28.0	14.3	10.9	12.3
OF-L2 AVG	28.0	16.0	-20.4	18.0	29.9	28.8	26.1	29.0	27.4	30.5	14.6	12.3	13.2
OF-MLA AVG	24.3	10.9	-12.5	14.6	24.4	24.7		23.9	20.7	24.5	15.7	9.8	11.0
OF-L4 AVG	25.6	11.4	-18.7	11.7	23.8	24.7	23.6	25.1	20.3	24.8	13.9	9.9	10.1
OF-R AVG	25.0	12.1	-17.4	12.2	24.8	25.1	26.2	25.8	21.0	25.3	12.4	9.0	11.3

Avg	**25.7**	**12.8**	**-18.5**	**14.7**	**26.3**	**26.2**	**25.3**	**26.3**	**22.7**	**26.6**	**14.2**	**10.4**	**11.6**
SD	**1.4**	**2.1**	**4.0**	**2.8**	**2.7**	**2.0**	**1.2**	**2.0**	**3.1**	**2.6**	**1.2**	**1.3**	**1.2**
Fish 7500-4900 BP													
IMI-08	28.8	14.9			30.4	28.2	26.7	25.1	27.8	28.9	13.2		12.9
IMI-10	28.4	15.5	-11.1	11.7	27.1	29.2		25.7	28.7	30.1			12.0
86284	30.5	13.5	-13.7		26.7	27.1		23.8	24.1	28.2	13.8		11.8
85520	30.3	11.3	-11.4	8.5	25.7	25.9	25.5	24.2	24.2	28.3			9.7
Mac-SH-01	30.3	13.5	-7.5		26.8	24.8		23.6	23.2	28.5	11.1		11.7
Mac-Sh-03	27.5	11.6	-8.8		23.2	21.8		21.8	19.6	25.4			9.8
Mac-SH-05	28.3	13.8			23.3			23.3	21.2	25.7			9.9
86439	27.2	14.4						22.1					
Avg	**28.9**	**13.6**	**-10.5**	**10.1**	**26.2**	**26.2**	**26.1**	**23.7**	**24.1**	**27.9**	**12.7**		**11.1**
SD	**1.3**	**1.5**	**2.4**	**2.3**	**2.5**	**2.7**	**0.9**	**1.4**	**3.3**	**1.7**	**1.4**		**1.3**
Shag 7500-1600 BP													
Ka_Ph_229	27.6	15.8			28.9	28.1	20.8	5.2	30.7	27.5	30.5	29.6	24.7
Ka_Ph_434	27.4	15.9	-7.2	18.4	27.9	27.1	21.5		31.0	26.4	30.6	27.4	14.4
Sh_Ph_329	26.1	14.7	-10.4	16.2	27.2	25.8	23.0	2.1	29.4	26.0	28.9	27.5	22.6
Sh_Ph_106	25.6	12.4	-13.3		27.1	25.1	24.0	1.6	28.7	25.7	28.5	27.2	18.3
BC_Ph_3	22.2	11.1			24.2	21.8			25.9	22.3	23.8		
BC_Ph_2	24.7	12.4			26.9	23.4	27.4	-1.6	28.1	24.4	27.3	27.2	20.7
Tun_Ph_862	23.3	11.9			24.2	21.3	17.3		26.1	22.1	24.7	24.1	20.9
Tun_Ph_694	25.7	14.2	-0.2	17.7	27.9	25.2	27.0	-0.3	30.1	25.3	28.0	29.2	21.4
Avg	**25.3**	**13.5**	**-7.8**	**17.4**	**26.8**	**24.7**	**23.0**	**1.4**	**28.8**	**25.0**	**27.8**		**20.5**
SD	**1.9**	**1.8**	**5.6**	**1.1**	**1.7**	**2.4**	**3.6**	**2.6**	**1.9**	**1.9**	**2.5**		**3.3**

Table 6-4 – Bulk δ2H of organisms from the Beagle Channel from different time periods.

	Sample ID	δ^2H (‰)	% H	% C	C/H Ratio
Fur seal (7500-4900 BP)	33786	-2.7	6.0	43.1	7.2
	33632	52.7	5.4	42.8	8.0
	30169	63.4	2.9	31.6	10.9
	33652	76.6	5.3	43.3	8.2
	32609	66.1	5.3	20.4	3.9
	33719	11.5	5.1	28.5	5.6
	31930	-3.8	6.7	44.0	6.6
	33553	36.5	4.2	39.2	9.4
	32288	23.0	7.2	43.3	6.0
	33565	37.4	7.0	41.3	5.9
	Average	**36.1**	**5.5**	**37.7**	**7.1**
Fur seal (2200-1600 BP)					
	37702	19.2	4.3	73.9	17.3
	42439	38.0	4.6	37.2	8.1
	36597	11.9	5.6	80.7	14.4
	40194	93.9	5.7	37.0	6.5
	35880	19.4	2.9	78.1	26.8
	42421	87.1	7.0	34.7	5.0
	Average	**44.9**	**5.0**	**56.9**	**13.0**
Fur seal (Modern)					
	BCAP2	45.9	5.9	40.0	6.8
	A1315	42.2	9.5	42.4	4.5
	RNP-2858	28.2	5.5	36.1	6.5
	Average	**38.8**	**7.0**	**39.5**	**5.9**
Sea lion (7500-4900 BP)					
	OF34227	42.0	4.9	35.0	7.1
	OF33458	50.0	4.2	38.5	9.2
	OF33551	52.6	5.2	40.0	7.7
	OF34751	61.5	5.2	38.5	7.4
	OF33717	100.0	4.8	35.4	7.4
	OF30459	46.8	5.3	38.6	7.3
	OF33459	30.5	5.2	39.6	7.6
	Average	**54.8**	**5.0**	**37.9**	**7.7**
Sea lion (2200-1600 BP)					
	H28_7571C17	16.9	3.2	34.8	10.7
	H28_77B	20.1	1.9	0.1	0.1
	H34_PB3100	-46.2	1.9	0.1	0.1
	H28_101C1	5.1	1.7	0.1	0.1
	BCIIV968	77.3	4.1	30.9	7.5
	KAIAII9037	-45.0	2.1	34.3	16.0
	STAT107	-32.1	0.7	25.2	38.4
	Average	**-0.5**	**2.2**	**17.9**	**10.4**
Sea lion (Modern)					
	MD-OF-FLA1	61.5	5.1	36.6	7.2
	MD-OF-L2	71.6	4.9	36.6	7.4

	MD-OF-L4	68.4	5.6	36.3	6.5
	MD-OF-MLA	73.3	4.2	36.8	8.8
	MD-OF-R	66.4	5.5	39.5	7.1
	Average	**68.2**	**5.1**	**37.2**	**7.4**
Kelp					
	Tres Amigos	-59.2	3.8	31.4	8.2
	TH-Kelp1	-76.1	3.7	26.4	7.1
	Kelp1	-71.2	3.7	23.8	6.4
	Kelp2	-65.4	3.6	26.8	7.4
	Average	**-68.0**	**3.7**	**27.1**	**7.3**
Squid					
	Squid1	-97.9	6.2	42.4	6.8
	Squid2	-87.2	6.1	43.4	7.1
	Squid3	-93.8	6.3	44.7	7.0
	Squid4	-80.8	5.9	43.1	7.3
	Squid5	-93.1	6.1	40.2	6.6
	Average	**-90.6**	**6.1**	**42.8**	**7.0**

Table 6-5 - All compound specific amino acid measurements of δ^2H (‰). (AA=Arctocephalus australis, OF= Otraria flavescens)

	δ^2H values of essential amino acids (‰)						δ^2H values of non-essential amino acids (‰)					
	Ileu	Leu	Lys	Phe	Thr	Val	Gly	Ser	Asp	Glu	Pro	Ala
Kelp (Modern)												
dH_TH-Kelp1	-180.2	-94.2	-131.0	-92.2	-218.5	-3.2	-57.2	-141.8	17.2	-31.2	-51.0	14.3
dH_Kelp-Har1	-236.4	-34.9	-160.2	-106.1	-251.8	6.0	-71.3	-170.7	60.9	16.1	-39.1	89.6
dH_Kelp-Har2	-240.5	-66.3	-132.8	-140.2	-196.7	-38.9	-22.8	-92.6	49.4	-11.8	-60.6	37.8
dH_3Amigos	-182.5	-1.3	-142.0	-74.1	-147.4	27.9	58.0	-29.9	79.1	30.1	-15.3	119.4
avg	**-209.9**	**-49.2**	**-141.5**	**-103.2**	**-203.6**	**-2.0**	**-23.3**	**-108.7**	**51.6**	**0.8**	**-41.5**	**65.3**
sd	**33.0**	**40.1**	**13.3**	**28.0**	**43.8**	**27.8**	**57.9**	**61.7**	**26.0**	**27.5**	**19.5**	**47.9**
Squid (Modern)												
dH_Squid-1		-82.5	-117.8	-107.4	96.3	-62.3	92.1	-0.9	-62.6	-30.2	353.7	92.7
dH_Squid-3	-151.3	-58.0	-113.3	-233.3	142.1	-43.2	113.9	18.4	72.7	53.9	455.7	145.2
dH_Squid-2	-149.8	-81.1	-248.1	-131.8	143.6	-74.4	76.1	-28.2	16.6	12.5	475.7	112.5
avg	**-150.6**	**-73.9**	**-159.7**	**-157.5**	**127.3**	**-60.0**	**94.0**	**-3.5**	**8.9**	**12.0**	**428.4**	**116.8**
sd	**1.1**	**13.7**	**76.6**	**66.7**	**26.9**	**15.8**	**19.0**	**23.4**	**68.0**	**42.1**	**65.4**	**26.5**
Fur seals (Modern)												
2858	-110.4	-94.7	29.8	-98.0	153.5	-84.7	8.1	2.5	-48.5	-18.0	535.0	21.3
A1315	-134.4	-100.2	-189.4	-93.4	117.2	-87.6	3.8	-38.8	-66.7	-25.7	508.1	22.1
BCAP2		-65.1	-94.1	-80.1	215.9	-58.7	19.2	37.7	-82.7	-0.4	499.9	52.4
Md-Aa_LJ	-206.0	-91.3		-153.3	-111.7	-196.0	-14.4	219.3	26.6	-26.8	291.2	
avg	**-150.3**	**-87.8**	**-84.5**	**-106.2**	**93.7**	**-106.8**	**4.2**	**55.2**	**-42.8**	**-17.7**	**458.5**	**31.9**
sd	**49.7**	**15.6**	**109.9**	**32.3**	**142.9**	**60.9**	**14.0**	**113.8**	**48.4**	**12.2**	**112.6**	**17.7**
Sea Lions (Modern)												
OF-R	-100.2	-63.8	-146.6	-46.5	176.0	-70.3	23.4	0.3	-12.2	-7.3	634.3	28.2
OF-FLA1	-60.5	-53.7				10.6	22.8	35.7	30.3	44.0	574.9	63.9
OF-MLA	-82.7	-71.5	-139.8	-69.4	198.8	-78.8	25.4	35.8	-74.4	-5.5	580.1	33.3
OF-L2		-57.5				5.7	36.2		12.3	50.2	639.5	58.0
OF-L4	-2.2	-67.2	-150.4	-96.6	206.0	-51.4	16.9	42.5	-76.0	8.5	584.9	54.8
avg	**-61.4**	**-62.8**	**-145.6**	**-70.8**	**193.6**	**-36.8**	**24.9**	**28.6**	**-24.0**	**18.0**	**602.8**	**47.7**
sd	**42.7**	**7.2**	**5.4**	**25.0**	**15.7**	**42.3**	**7.0**	**19.1**	**49.1**	**27.4**	**31.4**	**15.8**
Sea Lions (7500-4900 BP)												
Of_33458	-169.4	-117.2	-82.0	-109.4	77.5	-177.2	20.1	227.3	-177.2	3.2	677.4	-24.6
Of_33459	-182.6	-124.2	-98.2	-142.8	31.7	-188.8	15.5	184.6	-188.8	-5.5	567.7	-23.4
Of_33551	-223.3	-106.0	-206.6	-133.3	81.7	-175.2	31.6	198.5	-175.2	-8.8	664.1	-37.5
Of_33717	-250.1	-115.4	-94.0	-103.3	40.1	-197.8	32.8	180.6	-197.8	-4.8	940.0	-15.9
Of_33754	-189.5	-116.5	-94.7	-117.0	92.6	-223.9	27.9	211.1	-223.9	-14.9	644.1	-32.5

Of_34227	-207.8	-117.3	-79.1	-161.0	-18.4	-218.4	30.2	191.0	-218.4	-17.8	663.4	-11.0
Of_30459	-240.2	-124.8	-79.3	-168.3	8.5	-226.8	24.5	187.7	-226.8	-24.0	568.0	-28.4
avg	**-209.0**	**-117.3**	**-104.8**	**-133.6**	**44.8**	**-201.2**	**26.1**	**197.3**	**-201.2**	**-10.4**	**675.0**	**-24.8**
sd	**30.3**	**6.2**	**45.6**	**25.2**	**41.3**	**21.9**	**6.4**	**16.7**	**21.9**	**9.2**	**125.5**	**9.2**
Fur seals (2200-1600 BP)												
2439mid	-138.3	-142.1	-62.4	152.6		-142.3	-28.8		43.5	-30.1	376.7	-55.1
0194mid	-179.6	-160.8	-74.6	221.6		-138.8	-47.5		29.6	-47.3	586.7	-58.1
5880mid	-127.2	-152.6	-93.2	77.5		-135.6	-44.2		18.3	-54.2	291.4	-56.1
6957mid	-185.4	-138.2	-114.4	-0.6		-140.8	-75.7		-81.0	-58.8	291.7	-70.9
2421mid	-127.4	-106.6	-92.0	210.8		-90.2	-3.1		-21.9	-21.6	556.2	6.1
0195mid	-59.9	-99.8	-76.8	336.0		-82.0	-12.3		15.6	7.9	578.2	20.6
7702mid	-157.7	-132.9	-118.2	-43.8		-122.8	-22.5		-50.1	-46.9	363.1	-16.2
2522mid	-157.6	-125.6	-107.8	67.9		-104.6	-30.8		-14.6	-30.0	560.0	-20.2
avg	**-141.7**	**-132.3**	**-92.4**	**127.7**		**-119.6**	**-33.1**		**-7.6**	**-35.1**	**450.5**	**-31.2**
sd	**39.6**	**21.1**	**20.2**	**126.3**		**24.2**	**22.7**		**42.5**	**21.7**	**131.8**	**33.6**
Fur seals (7500-4900 BP)												
3632old	-185.4	-110.0	-69.5	-73.5	-51.5	-184.8	44.8	236.0	101.5	-77.9	443.0	-18.9
1930old	-190.1	-105.3	-91.0	-123.5	-5.0	-171.8	14.6	219.9	89.9	-75.6	383.4	16.7
3635old	-196.2	-107.6	-86.9	-69.6	-31.6	-174.0	15.0	208.1	88.7	-76.7	358.1	-9.5
0169old	-196.9	-117.2	-52.2	-97.3	-16.6	-193.1	16.6	233.3	128.9	-49.7	564.1	-36.3
3876old	-143.4	-134.0	-116.4	-24.7		-121.6	-5.1		-349.3	-196.9	340.0	-8.8
3533old		-92.3	-98.2	123.4		-22.0	6.0		-298.9	-199.8	407.0	54.7
2609old		-94.4	-97.9	127.1		-34.7	37.7		-292.4	-173.5	473.0	48.1
7288old	-170.5	-111.3	-93.0	122.4		-55.9	4.4		-301.6	-172.9	478.2	50.2
3719old		-93.8	-80.2	105.9		-52.5	20.5		-278.9	-171.4	344.4	42.4
avg	**-180.4**	**-107.3**	**-87.3**	**10.0**	**-26.2**	**-112.3**	**17.2**	**224.3**	**-123.6**	**-132.7**	**421.3**	**15.4**
sd	**20.5**	**13.3**	**18.4**	**107.4**	**20.1**	**70.8**	**15.8**	**12.9**	**215.3**	**60.9**	**74.9**	**34.7**

Figure 6-1 – A comparison of bulk δ^2H values to C/H ratios. Individuals with C/H ratios >20 were excluded.

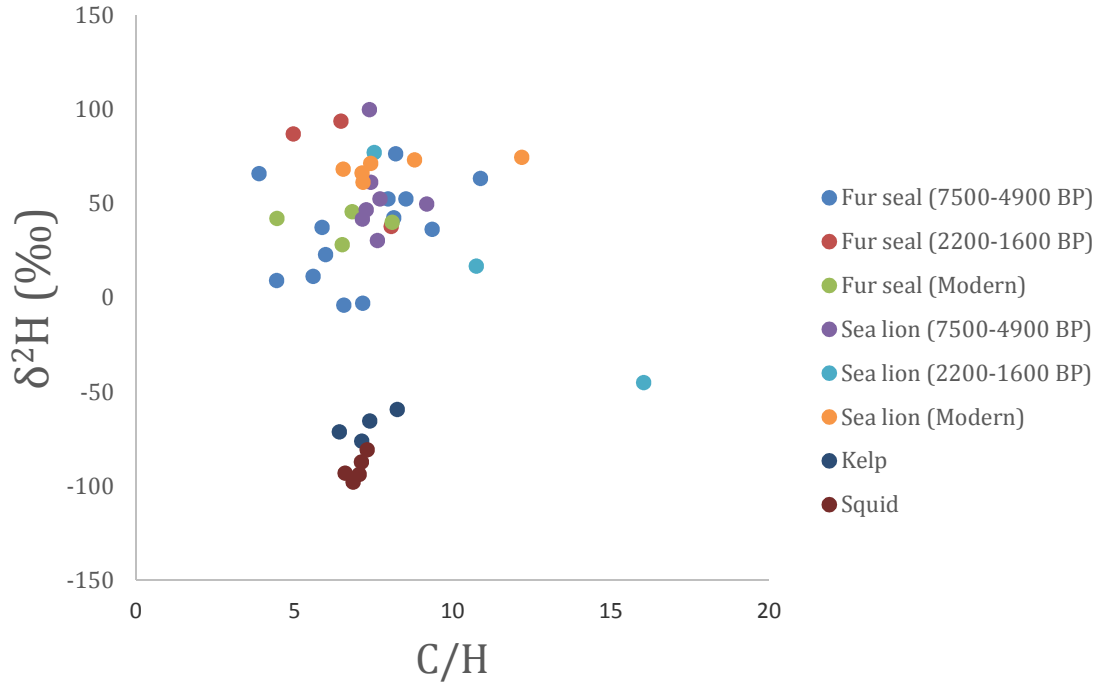

Figure 6-2 – PCA of essential amino acids of d2H from different time periods. High overlap suggests little difference between different time periods. PC axes are essentially the same when species are grouped together in the same type of analysis.