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Contrasting the ecological characteristics of powerful domoic acid vectors for the California sea lion: Is market squid one of them?

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Resumen de la tesis que presenta **Ana Filipa Rainha Patrao Da Gama Vieira** como requisito parcial para la obtención del grado de Maestría en Ciencias en Ecología Marina.

Contrastando las características ecológicas de los potentes vectores del ácido domoico para el león marino de California: ¿es el calamar del mercado uno de ellos?

Resumen aprobado por:

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Algunas especies de la diatomea *Pseudo-nitzschia spp.* producen ácido domoico (AD) el cual tiene efecto neurotóxico. Las floraciones de estas diatomeas estan aumentando en severidad y frecuencia al largo de la costa de California, induciendo eventos de mortalidad masiva de leones marinos de California (LMC; *Zalophus californianus*). Examinamos LMC post mortem, y realizamos análisis de contenido estomacal, cuantificación de AD y análisis de isótopos estables (AIS) de carbono (δ^{13}C) y nitrógeno (δ^{15}N) en tejido completo y aminoácidos (AAs) en hígado de LMS y músculo del calamar de mercado (*Doryteuthis opalescens*) para rastrear la acumulación de AD e inferir estrategia de alimentación. Necropsias indicaron que LMC estaban saludables antes de morir y suse estómagos estuvieron llenos de presas. Inicialmente, se sospechóe de intoxicación por AD. AD cuantificado en heces de LMC fueron más altos (153.82 ng mL^{-1}) que en jugo gástrico (19.17 ng mL^{-1}) y en presas consumidas (i.e. calamares; 23 ng mL^{-1}), sugiriendo que la presa tenía relativamente poca toxina. Los estómagos de LMC contenían calamares de mercado intactos de tamaño similar, representando 2.53 ± 1.5% del peso corporal total de LMC (peso húmedo) promedio. Las elipses isotópicas sugieren que los LMC se comportaron como especialistas durante sus últimos días de vida, mientras los calamares eran generalistas. Valores de depredador y presa revelaron una variación moderada en los indicadores tróficos (δ^{15}N$_{Tro-Src}$) y sitios de alimentación (δ^{13}C$_{EAAs}$). Valores bajos de δ^{13}C$_{EAA}$ sugieren que los calamares venían de mar abierto. Las concentraciones de AD e nichos isotópicos entre calamar, anchoveta (*Engraulis mordax*) y sardina (*Sardinops sagax*) sugirieron que los calamares de mercado pueden ser vectores de AD debido a la similitud con el nicho isotópico de anchoveta. Nuestro enfoque multidisciplinario sugiere que AD probablemente no causó la muerte de LMC.

Palabras clave: Ácido domoico (AD), calamar de mercado, análisis de isótopos estables en tejido completo y aminoácidos (AISC-AA), SIA, León marino de California (LMC)

Abstract of the thesis presented **by Ana Filipa Rainha Patrao Da Gama Vieira** as a partial requirement to obtain the Masters of Science degree in Marine Ecology.

Contrasting the ecological characteristics of powerful domoic acid vectors for the California sea lion: Is market squid one of them?

Abstract approved by:

PhD. Rocio Iliana Ruiz-Cooley
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Neurotoxic domoic acid (DA)-producing *Pseudo-nitzschia spp.* diatom blooms are increasing in severity and frequency along the California coast, inducing DA-related mass mortality/stranding events of California Sea Lions (CSL; *Zalophus californianus*). We performed postmortem examinations, stomach content analysis, DA quantification, and δ^{13}C and δ^{15}N stable isotope analyses (SIA) in bulk tissue and compound specific SIA of amino acids (CSIA-AAs) on well-preserved dead CSLs and their prey to track DA accumulation and infer foraging area and trophic position. Necropsy observations indicated overall robust CSL health status based on general body condition and prey-filled stomachs, however, a subset of animals showed myocardial damage. Suspected cause of death was DA-intoxication. Higher average DA levels quantified in CSL feces (153.82 ng mL^{-1}) than gastric juice (19.17 ng mL^{-1}) and consumed prey (i.e. market squid, *Doryteuthis opalescens*; 23 ng mL^{-1}) suggested that the consumed prey contained relatively low DA concentrations. CSL stomachs contained intact market squid of similar size range, representing 2.53 ± 1.5% of total CSL body weight (wet weight) on average. Standard ellipse area (SEA) estimates suggest that CSLs behaved as specialists during their last days of life, while market squid were generalists. AAs δ^{13}C and δ^{15}N values for both predator and prey revealed moderate variation in trophic proxies (δ^{15}N$_{\text{Tro-Src}}$) and foraging sites (δ^{13}C$_{\text{EAAs [Ile, Leu, Phe, Val]}}$). The lower δ^{13}C$_{\text{EAAs}}$ values suggest squid cohorts were migrating from offshore towards the CSL foraging area. Lastly, comparison in DA accumulation and isotopic values between market squid, anchovies (*Engraulis mordax*), sardines (*Sardinops sagax*) suggested that market squid could be a DA vector because their isotopic niches overlap with anchovie from 24 to 52%. Our multidisciplinary approach suggests DA-intoxication was likely not the cause of death.

Keywords: Domoic acid (DA), market squid, compound specific isotope analysis of individual amino acids (CSIA-AA), SIA, California Sea Lion (CSL)

Dedication

To whom searched for fantasy in the world as children, and found it in science as adults.

Because "eu sou da água e a àgua é parte de mim"

Acknowledgements

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Chapter 1. Introduction

The California Current System (CCS) is a highly productive coastal ecosystem that supports a diverse plethora of marine organisms from primary producers to large marine predators (Carretta et al., 2005; Venrick, 2002). At the base of the food web, the occurrence and production of a wide range of diatom species are associated with nutrient availability driven by seasonal upwelling that often induces large microalgae blooms (Checkley & Barth, 2009; Horner et al., 1997; Lelong et al., 2019; Trainer et al., 2000). Anomalous warm climatic events have remarkably impacted the CCS, altered the community of phytoplankton and microbes, with a higher incidence rate and geographic extension of harmful algae blooms (HABs; (Gibble et al., 2021; McCabe et al., 2016). Such HABs can be dominated by the diatom *Pseudo-nitzschia spp.* which can produce domoic acid (DA) (Anderson et al., 2011; Trainer et al., 2000), a glutamate receptor binding neurotoxin that accumulates in filter feeding marine fauna (Lefebvre & Robertson, 2009).

When mammals and seabirds feed on prey contaminated with DA (Bejarano et al., 2008; Gibble et al., 2021; Lefebvre et al., 1999), they can suffer from DA toxicosis leading to several health complications: cognitive and spatial impairment to the temporal lobe, vomiting, diarrhea, coma, and even death (Goldstein et al., 2008; Lefebvre et al., 2001). This potent toxin affects the neurological system of large cetaceans, for example, by decreasing the frequency of singing in humpback whales (*Megaptera novaeangliae*) (Ryan et al., 2019). Common dolphins (*Delphinus delphis*) (Cossaboon et al., 2019) and California sea lions (CSL; *Zalophus californianus*) can exhibit abnormal behaviors after DA ingestion, and often die of acute exposure if the concentrations are high (Gulland et al., 2002; Riva et al., 2009) or from chronic impacts on foraging success and motor skills after repeated DA exposure over their lifetime.

Humans who consume fish and shellfish contaminated with DA can suffer from amnesiac shellfish poisoning (ASP) and experience disorientation, seizures, and uncoordinated movements that may lead to death if high DA concentrations are consumed (Anderson, 2009; Bates et al., 1989; Lefebvre & Robertson, 2010; Todd, 1993). Given the increasing occurrence, geographic range, and toxicity of *Pseudo-nitzschia spp.* blooms, strong efforts to monitor DA outbreaks and rapid shellfisheries closures are taken to prevent the risk of ASP to human populations (Anderson et al., 2001; Trainer et al., 2012). Despite this, unifying monitoring efforts and fishery closures methods with an understanding of which fish or invertebrates can accumulate and transfer DA to higher trophic levels are stronger as either method alone does not provide public health with ample protection from ASP risk (Lefebvre & Robertson, 2009).

DA outbreaks can have devastating consequences in marine wildlife. During the El Niño in 1991, for instance, more than 100 brown pelicans and cormorants were found dead in Monterey Bay, California possibly due to *Pseudo-nitzschia australis* (Fritz et al., 1992; Horner & Postel, 1993). One month later, some people became sick in Washington after consuming razor clams. Shellfish tissue promptly tested resulted in high DA concentrations of the diatom *P. australis* above regulatory limits, causing commercial and recreational closures of razor clams until summer of 1992 (Horner & Postel, 1993). Although no deaths were reported, this was the first case of DA toxicosis on the West Coast of the U.S. (Taylor & Horner, 1994).

The mechanisms that trigger *Pseudo-nitzschia spp.* cells to become toxic are variable. Most studies investigating toxigenic *Pseudo-nitzschia spp.* blooms have found weak correlation between DA and surrounding concentrations of macronutrients (El Niño year) and higher *Pseudo-nitzschia spp.* concentrations with elevated Chla (La Niña) (Trainer, Hickey, et al., 2009), while some blooms seem to survive in high sea surface temperatures (SST) and low nutrients (El Niño) (Trainer, Wells, et al., 2009). The seasonality and strength of coastal upwelling along with strong northwest winds seem to play a key role for triggering DA production (Horner et al., 1997). Some DA outbreaks have been correlated with El Niño events (Wooster et al., 1985). El Niño events of above 19°C may however negatively affect *Pseudo-nitzschia spp.* abundances in the Southern California Bight; decreasing particulate DA concentrations, whereas La Niña events do not (Smith et al., 2018).

For the most part, blooms have been associated with strong upwelling, and complex patterns of coastal circulation that favor retention time and high availability of nutrients in surface waters of Southern California Bight (Anderson et al., 2006; Nezlin et al., 2012). Due to their frequently offshore nature, blooms are often detected first by reports of mass marine mammal strandings from suspected DA toxicosis (Anderson et al., 2016, 2019). In recent years, pervasive DA outbreaks occurred in February and March of 2022 (Anderson, 2022), a period dominated by La Niña cold conditions (Johnson et al. 2022), August to September 2023, and June-August 2023 (Anderson et al., 2023). In early spring 2022, there was a HAB detected in Southern California near the Channel Islands, and 10% of the phytoplankton was represented by *Pseudo-nitzschia spp.* Within a period of a few weeks, strandings of 23 CSL were documented near the area, prompting an alert for possible DA outbreaks.

One of the largest mass marine mammals stranding events in recent history occurred in 2015 during the Large Marine heatwave (2013-2016), where DA killed more than 200 CSLs off the California coast (McCabe et al., 2016). DA concentrations in feces of this pinniped reached over 1,014 ng g^{-1}. This mass mortality event represented one of the largest geographic detections of DA in marine mammals ever recorded

globally (McCabe et al., 2016). Quantifying DA and identifying what prey served as powerful DA vectors for CSL can improve monitoring efforts to better mitigate future outbreaks and marine mammal strandings.

DA vectors are those whose tissue exceeds 20 µg DA g^{-1} (Wekell et al., 2004) and can transfer DA to humans as well as higher trophic level predators in the marine food web (Lefebvre, Bargu, et al., 2002; Lefebvre et al., 1999, 2001); some vectors are important fishery resources. Mussels (*Mytilus edulis*) were responsible for causing sickness to more than 100 people in Prince Edward Island, Canada in 1987 (Todd, 1993), drawing attention to domoic acid for the first time and the naming of a new threat to humans, Amnesic Shellfish Poisoning.

The Dungeness crab (*Metacarcinus* magister) is an important prey and DA vector of CSLs, and its fisheries bring a high amount of revenue each year to California (California Dungeness Crab task Force (CDCTF), 2022; Marien, 1996). Razor clams (*Siliqua patula*) are capable of accumulating DA in their tissues for as long as 6 to 8 months (Wekell et al., 1994). The Pacific whiting (*Merluccius productus*), rockfish (*Sebastes spp*), jack mackerel (*Trachurus symmetricus*), market squid (*Doryteuthis opalescens*), Northern anchovy (*Engraulis mordax*), and Pacific sardine (*Sardinops sagax*) can accumulate and transfer DA to different species marine mammals and seabirds (Akmajian et al., 2017; Lefebvre et al., 1999; Stewart & Yochem, 1991).

Despite that both anchovies and sardines are planktivorous consumers (Rykaczewski, 2009), anchovies represent the most powerful DA vector in the neritic and pelagic zones as they accumulate higher concentrations of DA in viscera than sardines (Bernstein et al., 2021; Lefebvre et al., 1999; Lefebvre, Silver, et al., 2002; McCabe et al., 2016). Anchovies are the preferred prey of CSL because they have the highest fat content among foraging prey species. Market squid are considered suboptimal prey due to their low-fat content, however, they can be key seasonal prey for adult females due to their high winter time abundance (Lowry et al., 2022). In other words, CSL will feed heavily upon anchovies or market squid depending upon availability (Weise & Harvey 2008).

Little information exists on the accumulation and distribution of DA in squid (Lopes et al., 2013). Because squid are opportunistic carnivorous consumers that lack morphological feeding structures to filter feed, market squid should not be DA vectors. However, DA concentrations over the regulatory limit (i.e. 0.37 mg DA (equiv. g1) have been quantified in the stomachs of male market squid (Bargu et al., 2008). Given the increasing occurrence of toxigenic *Pseudo-nitzschia spp.* blooms along the CCS during early spring,

understanding if market squid, a mid-trophic level species, has the potential to accumulate and transfer domoic acid to their predators, is important for understanding the seasonality of CSL mortalities.

Stable isotope analysis (SIA) of δ^{13}C and δ^{15}N in bulk tissue (SIA$_{bulk}$) is an effective approach for investigating trophic relationships as it provides information on the feeding ecology of species, their foraging area, and food web configuration (Adams & Sterner, 2000; Owens, 1988; Peterson & Fry, 1987). The δ^{13}C values typically reflect the isotopic values of phytoplankton given the importance of processes like photosynthesis and carbon fixation for carbon isotope fractionation (Fry & Sherr, 1989; Smith & Epsten, 1971). Therefore, the δ^{13}C values from consumers generally depict the carbon dietary sources in key foraging areas (Kelly, 2000; Smith & Epsten, 1971). The δ^{15}N values in consumers also reflect the nitrogen cycling processes where animals feed (Estep & Vigg, 1985), as well as information from diet (Minagawa & Wada, 1984; Peterson & Fry, 1987). The isotopic difference between consumers tissues and their diet is approximately 0.4 to 1% for δ^{13}C and 3.5 to 4% to for δ^{15}N (Minagawa & Wada, 1984; Peterson & Fry, 1987; Schoeninger & Deniro, 1984).

Examining different types of tissues in animals can provide dietary information over short or long periods of time, depending on tissue turnover rates (period to reach isotopic equilibrium with a new diet) (Fry & Arnold, 1982; Tieszen et al., 1983). For example, in pinnipeds, the liver is a metabolically active tissue that incorporates dietary information over days (Hobson et al., 1996), while muscle tissue in squid can provide information for a few weeks given their quick growth rates (Ruiz-Cooley et al., 2006).

SIA has revealed the foraging strategy and trophic position of key DA vectors for the CSL in Monterey Bay and was important for evaluating DA trophic transfer between prey up to top predators (Bernstein et al., 2021). These authors also differentiated DA accumulation among benthic, coastal, and pelagic habitats and recognized that in costal pelagic zones, pelagic filter feeders were the most powerful DA vectors even during non-bloom periods. Analysis of isotopic niches (δ^{13}C and δ^{15}N) and DA quantification revealed a moderate overlap (40%) between anchovies and sardines indicating that their foraging areas and dietary sources differed by 60%, while DA accumulation was 35 times higher in anchovies (DA = 15.03 ppm) than sardines (DA = 0.42 ppm) (Bernstein et al., 2021). The isotopic niche for market squid from the same locations also overlapped with these planktivorous fish by 50% and 46.23%, respectively, but was associated with minimal DA (0.19 ppm; Bernstein et al., 2021). However, caveats exist when working with SIA in bulk tissue because It is difficult to estimate trophic position (or obtain precise estimates) of animals from different habitats through space and time if isotopic baseline values of primary producers or primary consumers are not also characterized (Post, 2002).

Compound specific isotope analysis (CSIA) of individual amino acids (AA) can separate the trophic effect from baseline values using animal tissue samples (McClelland & Montoya, 2002; McMahon et al., 2010; Popp et al., 2007). The trophic position of animals can be estimated using both trophic- and source-AA δ^{15}N (Chikaraishi et al., 2009; Popp et al., 2007). The δ^{15}N values of the trophic amino acids (Tro-AA), such as arginine (Asx), glutamic acid (Glx), isoleucine (Ile), leucine (Leu), proline (Pro), and valine (Val) experience high isotopic fractionation per trophic position during deamination and transamination reactions (Gaebler et al., 1966; Macko et al., 1987). In contrast source-AAs (Src-AA) δ^{15}N values, such as Lysine (Lys) and Phenylalanine (Phe), experience little to moderate isotopic fractionation per trophic step (Chikaraishi et al., 2009; Gaebler et al., 1966; McClelland & Montoya, 2002) and reflect baseline values of the foraging location of consumers (McClelland & Montoya, 2002). In particular, the δ^{15}N values of phenylalanine (Phe) vary little among some consumers, indicating that this AA is a stable source-AAs (McMahon & McCarthy, 2016).

For δ^{13}C , AAs are classified as Essential AAs (EAA; Ile, Leu, Phe, Val), which reflect primary producers values even if measured from animal tissues because EAA can only be synthesized by primary producers (Larsen et al., 2009; McMahon et al., 2010), therefore, δ^{13}C of EAA measured in animal tissues reflect the primary carbon sources. In contrast, the δ^{13}C from non-essential AAs (NAA) like alanine (Ala), aspartic acid/asparagine (Asp), glutamic acid/glutamine (Glu), glycine (Gly), proline (Pro), and serine (Ser) can be synthesized de novo by the body of consumers, or, obtained through diet, therefore, they provide information from an animal's diet (Hare et al., 1991; Larsen et al., 2009; McMahon et al., 2010).

Hence, the δ^{13}C of EAAs measured in animal tissues could be a powerful indicator of the foraging area where sea lions affected by DA acquired DA from algal blooms. A multidisciplinary approach that includes DA measurements alongside SIA from bulk tissue and individual AAs can be a powerful approach for reconstructing the foraging strategy and habitat associated with predator and prey DA uptake (Ruiz-Cooley et al., In revision).

This study used such a multi-disciplinary and integrative approach to investigate the trophic positioning of an unusual cluster of stranded CSLs along with their presumed prey in the last days of life and assessed the likelihood of a connection to a known DA event. We directly measured DA in biological samples of deceased CSLs and consumed prey (i.e. market squid) to provide novel information of the potential for market squid to accumulate and transfer DA. DA concentrations and isotopic niches were compared between market squid, anchovies, and sardines to evaluate the potential of market squid as a DA vector.

1.1 Objectives

1.1.1 Main objective

To evaluate the potential of market squid (found in dead stranded CSLs) as a DA vector by measuring DA in their tissues and in predator tissues (stranded CSLs), and by measuring stable isotopes in bulk tissue and AA values of δ^{13}C and δ^{15}N to understand foraging strategy, trophic position, and foraging location of predator and prey.

1.1.2 Specific objectives

Quantify the total number and type of prey items that were found in stranded dead CSLs after a possible DA outbreak to calculate what proportion of prey those CSLs consumed.

Evaluate if CSL act as a generalist or specialist during the last days of life using the sizes angles and shapes of Bayesian standard ellipse models from bulk tissue and % overlap of diet.

Compare market squid isotopic niches and estimate trophic position relative to anchovies (and sardines), which are the most powerful pelagic DA vector, using stable isotope analysis of b and c in bulk tissue (SEA$_b$ & SEA$_C$) and a proxy of trophic position from trophic and source AAs.

Chapter 2. Methodology

2.1 Specimen collection

2.1.1 Sea lions

Biological samples were collected from ten stranded deceased CSLs found along the beach in Orange County, California in 2022 from the 16th of February to the 17th of March (Table 1). During that period, *Pseudo-nitzschia spp.* abundance was observed above bloom-level thresholds at regional shore stations, therefore, DA toxicosis was initially considered as a possible cause of death. Moreover, the toxigenic "seriata" size class of *Pseudo-nitzschia* was the dominant group in both Santa Barbara and Catalina Island samples, locations within the Southern California Bight, somewhat removed from the location where the cluster of CSLs washed up in Orange County in February and March (Anderson, 2022). Carcass recovery and necropsies were conducted by personnel at the facilities of the Pacific Marine Mammal Center (PMMC) in California. Sex, age, weight (kg), standard length (cm), maturity and histology were determined for each individual. Fecal samples were collected for DA testing, and liver tissue samples were collected for isotope analyses. Stomach contents were recovered intact from all individuals for further analysis and were transferred in ice.

2.1.2 Prey

Specimens from stomach contents were collected in plastic bags, not rinsed with water at any time, and placed in a container to avoid losing any liquid or parts. Each individual prey item was carefully separated out.

Total number of individuals of a given prey item were counted and classified in two categories: complete specimens consisting of a full body, and incomplete specimens: a body part was missing. If squid were encountered, mantle length (cm), and sex were determined, and muscle tissue samples from the mantle were collected from 15 complete individuals for each individual CSL stomach.

Table 1. Biological data recovered from the ten stranded California Sea lions (CSL; mean (1 SD)) and Domoic acid (DA) measurements in predator and prey samples.

CSL ID	Name	Sex	Maturity	Admit Date	Lat & Long	Weight (kg)	SL # (cm)	Prey Items (n)	DA (ng mL^{-1}, SD)		
									CSL	Market Squid	
									Feces	Mantle muscle	Gastric Juice
Z-22-02-22-019	Rubbermaid [A]	Male	Subadult	22-Feb-22	33.5318, -117.77647	49.2	133	59	139	21 (11)**	21 (11)**
Z-22-03-04-027	Urchin [A]	Female	Pup	4-Mar-22	33.53669, -117.78007	19	105	12	156	43*	34*
Z-22-03-07-030	Sand [B]	Male	Subadult	7-Mar-22	33.55948, -117.82065	46.1	147	16	BDL	21 (11)**	12*
Z-22-02-22-020	Gin [C B]	Male	Subadult	22-Feb-22	33.5822, -117.85707	84	116	115	161	21 (11)**	21 (11)**
Z-22-02-16-014	Roy [B]	Male	Yearling	16-Feb-22	33.5826, -117.85757	45.2	127	56	138	22*	24*
Z-22-02-22-021	Tonic [C B]	Male	Subadult	22-Feb-22	33.57949, -117.85269	47.8	158	90	141	21 (11)**	21*
Z-22-03-17-033	Jameson [C]	Male	Yearling	17-Mar-22	33.595298, -117.887135	32.8	128.5	32	BDL	22 (11)**	5.7*
Z-22-02-22-023	Main [A D]	Male	Pup	22-Feb-22	33.54085, -117.78372	33	121	45	BDL	23 (11)**	12*
Z-22-03-05-028	Nose [B]	Male	Subadult	5-Mar-22	33.57186, -117.83860	59.5	147	146	BDL	15*	20*
Z-22-02-17-016	Rye [C]	Male	Subadult	17-Feb-22	33.62893, -117.95783	52.8	140	106	188	21 (11)**	21 (11)**
Average (1SD)						46.94 (17.4)	132.25 (16.1)	67.7 (44.8)	153.82 (19.2)	23 (6.97)	19.17 (7.75)

* CSL standard length = SL; standard deviation = SD.
* Note: A = Laguna Beach; B = Crystal Cove; C = Newport Beach; D = Main Beach.
* Values with ** indicate the use of a less sensitive test kit: min detection limit = 120 ng g-1, values with * indicate the use of a highly sensitive kit: min detection limit = 4.0 ng g-1, see methods for more details.

These tissue samples were kept frozen for DA measurements and SIA. Digestive gland and viscera were the target organs for DA analysis, but if they were not consistently found, muscle from the mantle was collected. Gastric juice possibly derived from CSL and stomach content was also recovered for DA analysis and stored frozen. If fish were found, guts and muscle tissue samples were the target for DA and SIA, respectively.

2.1.3 Potential DA vectors.

Because anchovies and sardines are considered powerful DA vectors (Lefebvre, Silver, et al., 2002), and these planktivorous fish were not available in March 2022 near the Channel Islands, we collected fifteen specimens per species in Bahía de Todos Santos, Mexico in 2023 during a toxic *Pseudo-nitzschia spp.* bloom. This area is part of the southern CCS. Muscle tissue samples were collected from each fish for SIA and trophic position estimates. Prior to dissections, fork length (cm) was measured.

2.2 Domoic acid measurements

Gastric juice (15 mL) was collected from each of the 10 CSL stomachs. A composite sample containing muscle tissue of market squid (n = 15) per CSL stomach was stored in a 50 mL falcon vial and preserved frozen for DA analysis. All samples were analyzed for DA at the Wildlife Algal-toxin Research and Response Network for the U.S. West Coast lab (WARRN-West, Dr. Kathi Lefebvre) Seattle, WA. DA analysis was carried in samples of both gastric juice and squid mantle tissue following the work from Lefebvre et al. (2010, 2016). ABRAXIS® 32 DA ELISA kits were used according to manufacturer's instructions. Briefly, samples were diluted utilizing a sample buffer, where afterwards a 10-point standard curve Amax and a blank were run with each essay. Pre-coated plate wells were washed for 10 min.

After the buffer was removed; sample was added to duplicate wells. An anti-DA-HRP conjugate was added to each well apart from the blank and plate was then incubated for 1hr. After incubation ELx50 and TMB peroxidase substrate was added for a 15 min incubation period. Finally, reaction was terminated with the addition of sulfuric acid and absorbance was measured utilizing a VERSAmax microplate reader. Due to manufacture supply issues related with highly-sensitive test kit (min detection limit = 4.0 ng g^{-1}), some samples were analyzed with a less-sensitive test kit (min detection limit = 120 ng g^{-1}).

2.3   Stable isotope analysis in bulk tissue

Each tissue sample was rinsed with water before being placed in an oven at 50 °C until dry. These samples were lipid extracted using petroleum ether (Ruiz-Cooley et al., 2011) in glass tubes, which were placed in a sonicator for 45 min and two cycles for squid tissue, and 3 cycles for CSL liver tissue. After samples were dried at room temperature, samples were pulverized and homogenized into a fine powder, 1.5 mg of which was then transferred to tin capsules for SIA in bulk tissue at the UC Davis Stable Isotope Facility. A MICRO elemental analyzer and Elementar VisION isotope ratio mass spectrometer were used. Stable isotope ratios are expressed with the following δ-notation:

$$\delta^H X \ = \ [((\ ^H X/ \ ^L X))_{sample} \ - \ (\ ^H X/ \ ^L X)_{standard} \ / \ (\ ^H X/ \ ^L X)_{standard}) \ * \ 1000 \qquad (1)$$

X representing carbon (C) or nitrogen (N), H is the heavy isotope (^{13}C or ^{15}N), and *L* represents the lighter isotope (^{13}C or ^{15}N). The international standards were Vienna Pee Dee Belemnite for C and atmospheric Nitrogen (N_2), and values are expressed in ‰.

2.4   Compound specific isotope of individual amino acids for carbon and nitrogen (CSIA-AA)

A composite sample of 10.5 mg of dry well-homogenized muscle tissue was used for CSIA-AA. Each composite sample was made up of equal weights of pulverized tissue samples of 15 market squid (~0.7 mg per individual squid). CSIA-AA were conducted using Corr et al. (2007) N-acetylmethyl (NACME) method where amino acids were methylated with 1 mL of an acidified methanol solution for one hour. After reagents were evaporated, dichloromethane (DCM) was added to remove excess methanol and water. AA methyl esters were dissolved in 100 μL of ammonium bicarbonate and acetylated in acetic anhydride/anhydrous methanol. Finally, reagents underwent a second evaporation through a stream of nitrogen in an ice bath. An GC-C-IRMS was used for this analysis.

Values of δ^{15}N trophic (Ala, Glx, Leu, lle, Pro, Val) and source AA (Lys and Phe) were obtained from a total of 5 CSLs and market squid found in 10 CSL stomachs and used for trophic proxy. We utilized δ^{15}N from average source-AA as indicators of phytoplankton or Phenylalanine (Phe) because it is an essential-AA and the most stable source because its fractionation was closest to zero and presented close to no variation

among consumers (Chikaraishi et al., 2009; McMahon & McCarthy, 2016). To differentiate between trophic position and foraging location of our CSLs and market squid, we averaged their trophic and source AA δ^{15}N values as a proxy to estimate trophic position:

$$\bar{x}_{i,j} = \text{trophic AA} \sim N\left(\bar{x}_{i,tro`}\ \sigma^2_{tro}\right)$$

$$(2)$$

$$\bar{x}_{i,j} = \text{source AA} \sim N\left(\bar{x}_{i,src`}\ \sigma^2_{src}\right)$$

Where $\bar{x}_{i,j}$ is the average trophic or source AAs. Trophic proxy was estimated utilizing Δ^{15}N for CSL and market squid found in their stomach from the difference between their average trophic and source AA values:

$$\Delta^{15}N_i^{Tro-Src} = \bar{x}_{i,Tro} - \bar{x}_{i,Src`} \qquad (3)$$

Or between one trophic and one source AA:

$$\Delta^{15}N_i^{Glx-Phe} = \bar{x}_{i,Glx} - \bar{x}_{i,Phe`} \qquad (4)$$

Trophic positions were estimated utilizing the following equation (Chikaraishi et al., 2009):

$$\text{Trophic Position (AA Method)} = \frac{\left(\delta^{15}N_{Glx} - \delta^{15}N_{Phe} - 3.4\right)}{7.6 + 1} \qquad (5)$$

Where $\delta^{15}N_{Glx}$ at the top of the equation represents the trophic AA value for glutamic acid (Glx) of an organism and $\delta^{15}N_{Phe}$ represents the source AA value for phenylalanine (Phe) of an organism while -3.4 represents the isotopic difference between Glx & Phe, and 7.6 is the ^{15}N enrichment factor of both Glx and Phe AAs (Chikaraishi et al., 2009). Average essential AA δ^{13}C values (Ile, Leu, Phe, Val) were utilized to gain insights on phytoplankton values from the feeding location where CSLs and market squid fed prior to their death.

2.5 Statistical analysis

2.5.1 Sea lion and prey

Basic statistics (average and one standard deviation (1SD)) for weight and length of CSLs were calculated, and for squid (n = 15) mantle length, $\delta^{13}C$ and $\delta^{15}N$ values (mean, median, SD, range, minimum and maximum values). For the latter, this information derived from 15 squid randomly selected from each CSL stomach.

To gain insights into the foraging strategies of predator and prey, we utilized the package Stable Isotope Bayesian Ellipses (SIBER) version 2.1.9 in R-program version 4.2.1 (Jackson et al., 2011) to conduct a Bayesian ellipses model utilizing the $\delta^{13}C$ and $\delta^{15}N$ from SIA $_{bulk}$ tissue. Using a bivariate equivalent to standard deviation that is determined by Bayesian probabilities, the isotopic niche was determined using Bayesian multivariate standard ellipses. Ellipses were constructed for each independent group (e.g. by animal species or squid from each CSL stomach). Standard ellipse areas (SEA) were calculated for each group of squid collected from different CSL stomachs and for planktivorous fish species considered possible DA vectors to compare the size of their isotopic niches; 10,000 iterations were used to calculate ellipses for market squid per stomach and for market squid with DA vectors from different locations. Those ellipses captured 95% of the data points for each species. The size of each isotopic niche was statistically compared across the posterior probability distribution of the covariance matrix for each prey group by location to yield mean Bayesian standard ellipse areas (SEA$_b$) using a 95% credible interval. Although SEA is a good estimate of population, values tend to be underestimated when sample sizes are small (Jackson et al., 2011). The corrected standard ellipses for sample size are denoted as SEA$_C$ and are not underestimated at low or high sample sizes. To examine differences in diet and habitat of market squid per stomach and market squid with DA vectors from different locations, maximum likelihood estimates % overlap were calculated as a proportion of the non-overlapped area and were expressed as a percent, where complete non-overlapped ellipses are indicated by 0 and complete overlap as 1. Each % overlap proportion reflects specific feeding strategies and use of habitat.

DA concentration expressed as parts per million (ppm; 1 ppm = 1000 ng mL^{-1}) was considered together with the SIA bulk data to associate DA with foraging information.

A simple linear regression model was used to evaluate the relationship between squid mantle length and

δ^{13}C or δ^{15}N value from bulk tissue. A similar model was used to test the relationship between anchovy and sardine fork length with their δ^{13}C or δ^{15}N values. An ANOVA and Tukey Post Hoc Test was conducted to test differences in mantle length, δ^{13}C and δ^{15}N between market squid from different CSL stomachs. Mean confidence interval of δ^{15}N$_{Tro-Phe}$ values for market squid and anchovy were calculated to evaluate trophic differences between locations, and δ^{13}C$_{EAA}$ were utilized to estimate feeding location of CSLs and market squid.

Chapter 3. Results

3.1   Sea lions

The ten analyzed CSL included pups, yearlings and subadults (male and female; Table 1, Fig. A1). All CSLs were described with carcass code of 3 for moderately decomposed, sloughing on fur and or skin, and stomachs distended with squid. Some CSLs were described as having stomach ulcers. Necropsies were unable to determine time of death of all individuals. Since DA is a neurotoxin, one of the best methods of identifying DA exposure in marine mammals is to examine the brain for lesions (Colegrove et al., 2018; Goldstein et al., 2008), but it was not possible due to their decomposition state. Histology findings (Table A1) from one individual presented no overt hippocampal lesions. Acute myocardial damage with/or without mild multifocal nuclear rowing of the heart was found for ZC014 (male/yearling), ZC016 (male/subadult), ZC021 (male/subadult), ZC023 (male/pup), ZC027 (female/pup), and ZC033 (male/yearling) with suspected cause due to DA toxin exposure. The only female CSL (ZC027) was a pup that measured 105 cm and weighed 19 kg, having the lowest weight and length overall. A male/yearling (ZC033) had the lowest weight 32.8 kg (Table 1). Out of the 9 males, only one subadult (ZC020) had the lowest length (i.e. 116 cm), while the lowest weight for males went to ZC033 a yearling with 32.8 kg (Table 1). The highest weighed or length of subadult CSLs was 84 kg and 158 cm for ZC020 and ZC021 respectively (Table 1).

3.2   Prey

All CSL stomachs combined (labeled with the initial letters ZC) were filled with a total of 677 market squid (total complete specimens: 452, total incomplete: 225; See Fig. A2). Parts of five unknown fish were found in 3 CSL stomachs but were not analyzed for DA nor stable isotopes because guts were missing, and a consistent tissue type was not possible to obtain. The subadult male (ZC028) consumed the largest number of squids (n = 146), while the female pup ZC027 consumed the fewer prey items (n = 12) (Table 1). Squid mantle length (MLs) was similar among CSL stomachs. An average of 67.7 ± 44.86 squid were consumed between all CSLs (Table 1). The lowest MLs was found in the male subadult (ZC030), which measured 9.76 ± 1.63 cm, and highest a male subadult (ZC020) that measured 10.88 ± 1.08 cm (Table 2). Market squid found in the male subadults (ZC030 and ZC016) had the lowest minimum (MIN) mantle

length measuring 6 cm, while the female pup (ZC027) and a male subadult (ZC020) had the highest, 9 cm (Table 2). ZC027, ZC033, and ZC028 were the only sea lions that consumed fish (n = 3, n= 1, and n= 1) respectively (Table 1).

Table 2. Biological data and $\delta^{13}C_{bulk}$ & $\delta^{15}N_{bulk}$ values (‰; mean (1 SD)) of market squid collected from each of the ten CSL stomachs. ML = Mantle length.

CSL stomach ID	n	ML Range (cm)		ML mean (cm); (SD)	ML Median (cm)	Weight (mg)	Sex Ratio (F:M)	$\delta^{13}C$	$\delta^{15}N$
		Min	Max						
Z-22-02-22-019	15	8.4	12.5	10.77 (0.89)	10.6	15.36 (4.23)	1:02	-19.01 (0.44)	15.78 (0.36)
Z-22-03-04-027	11	9	12.5	10.38 (1.04)	10.5	9.96 (3.84)	2:02	-18.76 (0.32)	16.07 (0.29)
Z-22-03-07-030	12	6	12	9.77 (1.63)	10	12.58 (5.68)	4:02	-18.83 (0.30)	15.62 (0.26)
Z-22-02-22-020	15	9	13.5	10.89 (1.08)	10.5	23.62 (3.64)	8:06	-19.01 (0.29)	15.48 (0.23)
Z-22-02-16-014	15	8.3	11.5	10.29 (0.84)	10.4	20.64 (3.58)	11:04	-18.80 (0.23)	15.97 (0.30)
Z-22-02-22-021	15	8.5	12.5	10.75 (0.93)	11	26.1 (6.10)	11:04	-19.06 (0.31)	16.01 (0.40)
Z-22-03-17-033	15	6.5	12.5	10.49 (1.45)	10.75	25.01 (7.70)	6:07	-18.88 (0.24)	15.61 (0.54)
Z-22-02-22-023	13	7	12.2	10.07 (1.13)	10.1	17.78 (4.40)	8:05	-18.85 (0.32)	15.74 (0.32)
Z-22-03-05-028	14	7.5	14	10.06 (1.26)	10.2	14.9 (4.18)	4:00	-18.72 (0.30)	15.90 (0.71)
Z-22-02-17-016	17	6	13.3	10.67 (1.12)	11	29.14 (5.94)	10:07	-19.11 (0.31)	15.34 (0.19)

3.2.1 Bulk stable isotope analysis

The $\delta^{13}C$ and $\delta^{15}N$ values from tissue samples of 142 market squid (muscle), 10 CSL (liver), 15 for sardine (muscle), and 15 for anchovy (muscle) were obtained. Each isotope ellipse per CSL stomach comprised 15 market squid, and all squid ellipses overlapped due to relatively similar $\delta^{13}C$ and $\delta^{15}N$ ranges (Fig. 1a); except for squid found in the stomach of ZC014 that had a narrower $\delta^{13}C$ and $\delta^{15}N$ range (Fig. 1a). Squid ellipses that had the highest % overlap were found between ZC027 and ZC014, and ZC030 and ZC023 (Fig. 1b).

Isotope values from 8 of the 10 sea lions were clustered. CSL isotopic niches did not overlap with squid

(Fig. 1a), and their ellipses had higher δ^{15}N values and a wider range in δ^{13}C values than those of market squid (Fig. 1a) and any other potential prey item. SEA$_b$ of market squid was variable (Fig. 1b), where those found inside two male subadults stomachs (ZC020 and ZC016) had the lowest but in a third (ZC028) had the highest. Both female and male pups (ZC027 and ZC023) had similar SEA$_b$ values.
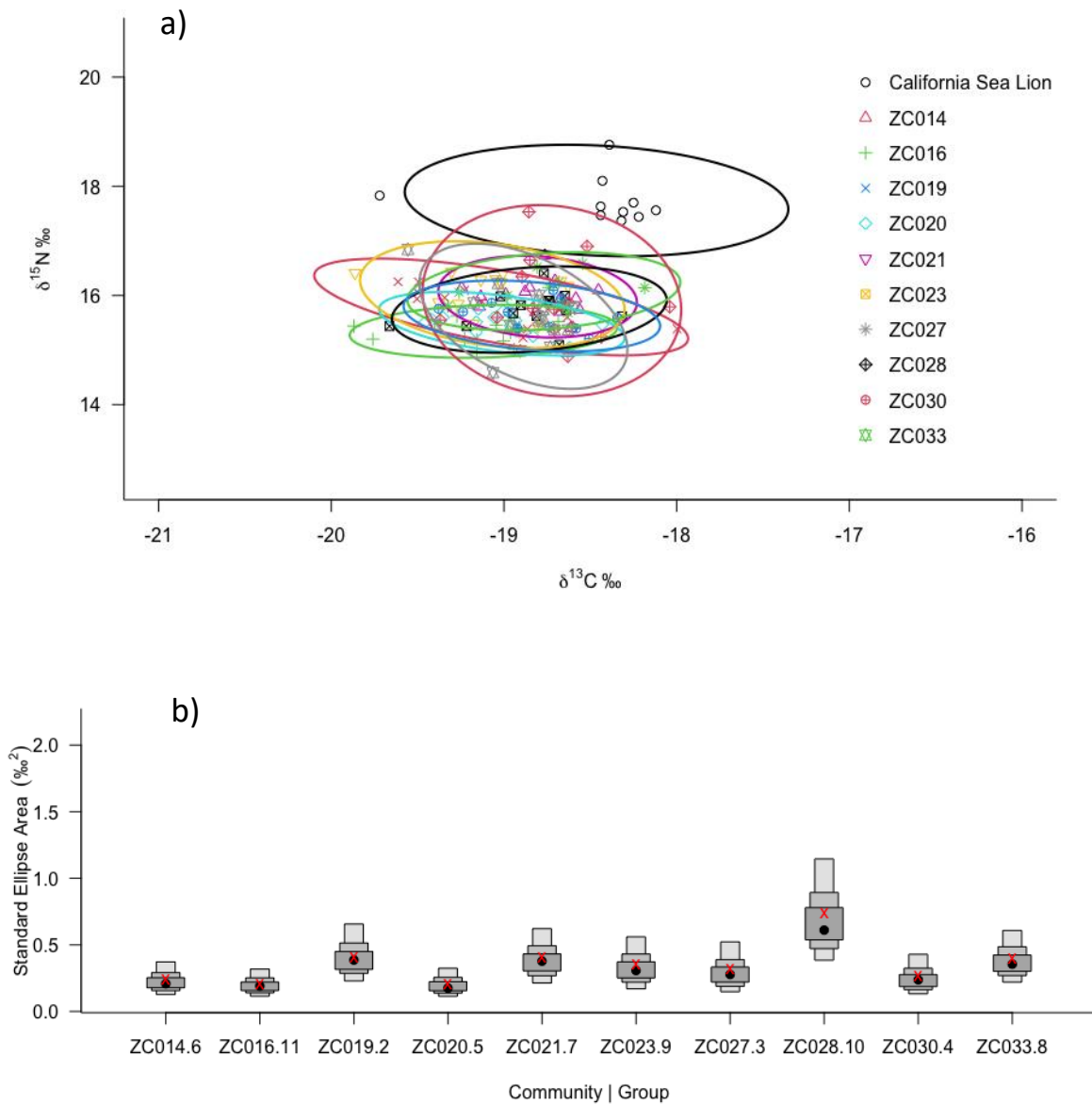
Figure 1. (a) Isotopic niches of analyzed market squid (n = 15) per individual sea lion stomach (n = 10). The standard ellipse for CSLs (O) had the highest δ^{15}N values, and there is only one sea lion with less enriched δ^{13}C values; (b) Market squid mean standard ellipses area (SEA$_b$) and 95% confidence interval associated with Figure. 1a. Black dots represent mean SEA$_b$ after 20,000 iterations and red x's represent ML estimates (SEA$_c$). Shaded density plots represent 50%, 95%, 99% credible intervals. % Overlap = 17% to 66%, % Overlap mean = 44%.
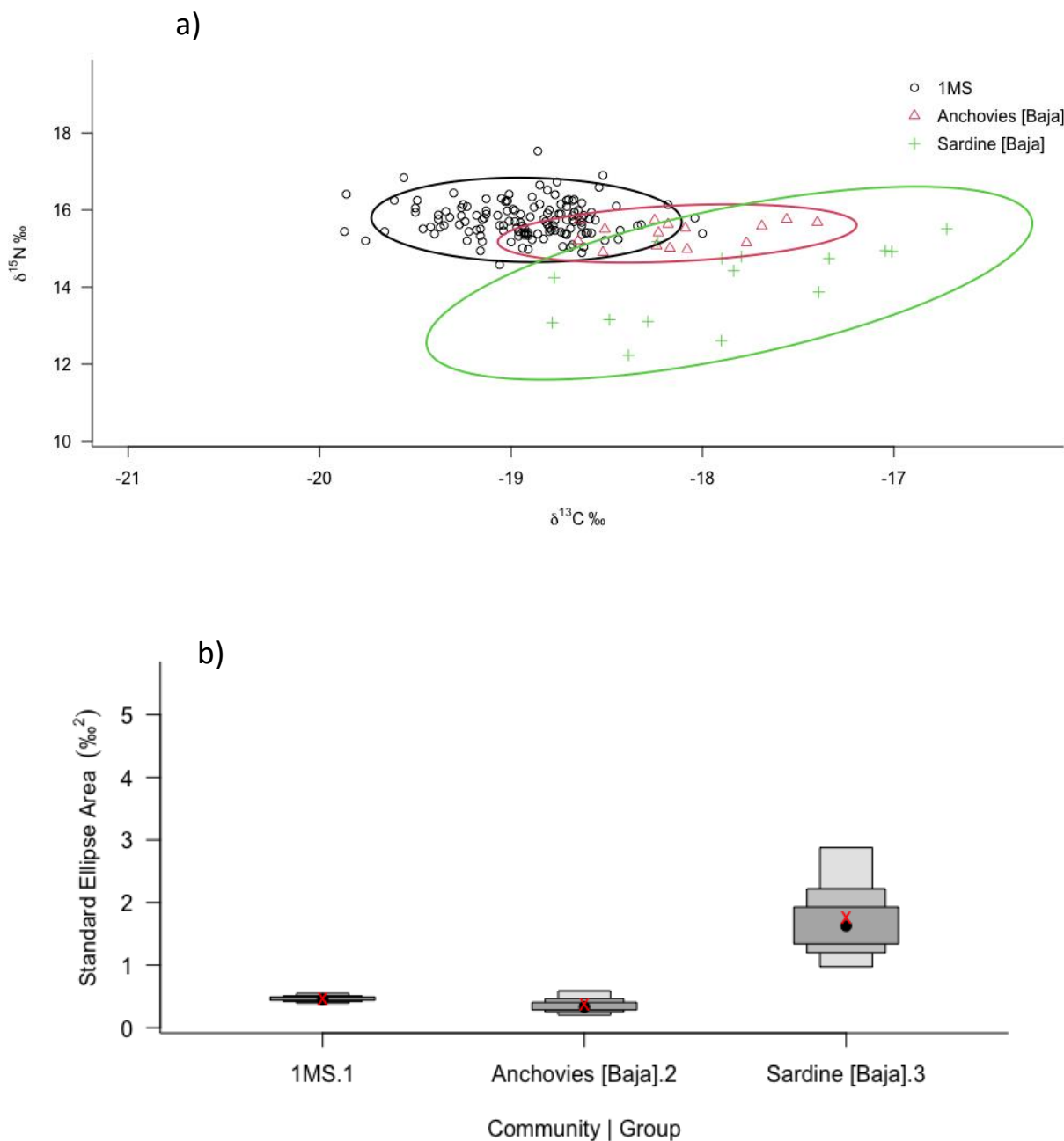
Figure 2. (a) Bulk Bayesian standard ellipses of market squid (o) (n = 142) found in all stomachs of deceased CSL in Orange County, California 2022 compared with anchovies (△) & sardines (+) (n = 15) collected in Ensenada, Baja California, Mexico in 2023 during a toxic *Pseudo-nitzschia* s*pp* bloom; (b) Mean standard ellipses area (SEA$_b$) and 95% confidence interval associated with Figure. 2a. Black dots represent mean SEA$_b$ after 10,000 iterations and red x's represent ML estimates (SEA$_c$). Shaded density plots represent 50%, 95%, 99% credible intervals. Market squid (CSL stomach) and anchovy (Baja) % Overlap = 24.1%.

Paired comparison among squid found in the stomach showed a wide range in percentage overlap, that ranged from 17 to 66% (% overlap mean = 44%; Table 3). Comparison among the SEA$_b$ between anchovy

from Baja California (n = 15) and market squid found in CSL stomachs (n = 142) revealed relatively low % overlap (24.1%) (Fig. 2a, b). Simple linear regression for squid showed mantle length was not statistically significant with either δ^{13}C and δ^{15}N (Fig. A3). Similarly, anchovies showed no relationship between fork length and δ^{13}C (Fig. A4; DF = 13, p-value = 0.467, R² = -0.032), nor δ^{15}N (Fig. A4; DF = 13, p-value = 0.551, R² = -0.046). In contrast, sardine fork length and δ^{13}C were highly associated (Fig. A4; DF = 13, p-value = 0.002, R² = 0.473), but a weak relationship was found between fork length and δ^{15}N (Fig. A4; DF = 13, p-value = 0.073, R² = 0.166).

Table 3. Percent overlap between the isotopic ellipses calculated in SIBER from market squid found in each CSL stomach (from Figure. 1a), and between the potential domoic acid vectors (i.e. market squid and anchovy from Figure 2a). This is the proportion of non-overlapping area between two ellipses *100.

Group 1	Group 2	% Overlap
Market squid from CSL stomach	Market squid from CSL stomachs	% Overlap
ZC019	ZC027	38%
ZC019	ZC030	62%
ZC019	ZC020	42%
ZC019	ZC014	39%
ZC019	ZC021	60%
ZC019	ZC033	46%
ZC019	ZC023	51%
ZC019	ZC028	36%
ZC019	ZC016	27%
ZC027	ZC030	43%
ZC027	ZC020	26%
ZC027	ZC014	66%
ZC027	ZC021	51%
ZC027	ZC033	41%
ZC027	ZC023	58%
ZC027	ZC028	41%
ZC027	ZC016	17%
ZC030	ZC020	60%
ZC030	ZC014	48%
ZC030	ZC021	51%
ZC030	ZC033	50%
ZC030	ZC023	66%
ZC030	ZC028	34%
ZC030	ZC016	40%

ZC020	ZC014	28%
ZC020	ZC021	40%
ZC020	ZC033	40%
ZC020	ZC023	50%
ZC020	ZC028	24%
ZC020	ZC016	58%
ZC014	ZC021	54%
ZC014	ZC033	46%
ZC014	ZC023	57%
ZC014	ZC028	32%
ZC014	ZC016	21%
ZC021	ZC033	57%
ZC021	ZC023	57%
ZC021	ZC028	40%
ZC021	ZC016	29%
ZC033	ZC023	49%
ZC033	ZC028	54%
ZC033	ZC016	30%
ZC023	ZC028	42%
ZC023	ZC016	43%
ZC028	ZC016	19%

The mean values among market squid from the ten CSL stomachs showed significant differences in both $\delta^{13}C$ and $\delta^{15}N$ values (Fig. A5; ANOVA; DF = 9, F-value = 2.69, p-value = 0.006 for $\delta^{13}C$ and DF = 9, F-value = 5.52, p-value = 1.9e-06 for $\delta^{15}N$). Post-hoc Tukey test showed differences in pair ZC028-ZC016 for $\delta^{13}C$ but presented differences in seven pairs for $\delta^{15}N$; ZC027 presenting as most abundant and ZC016 least abundant of the means (Fig. A5; ZC027 Mean = 16.0, ZC016 Mean = 15.3). SEA$_C$ of anchovy and market squid from Monterey Bay were variable but had a greater % overlap (52.7%; Table A2, Fig. A6 b).

3.2.2 Compound specific stable isotope analysis of amino acids (CSIA-AA)

Using CSIA-AA results, CSLs and market squid found in their stomachs presented variable $\delta^{15}N_{Tro-Phe}$ values; with the CSL ZC027 (female/pup) having the second lowest $\delta^{15}N_{Tro-Phe}$ value and ZC014 (male/yearling) the highest (Fig. 3a). CSLs had wider $\delta^{15}N_{Tro-AA}$ averages and less negative $\delta^{13}C_{EAA}$ averages than squid (Figure. 3b).

a)

b)
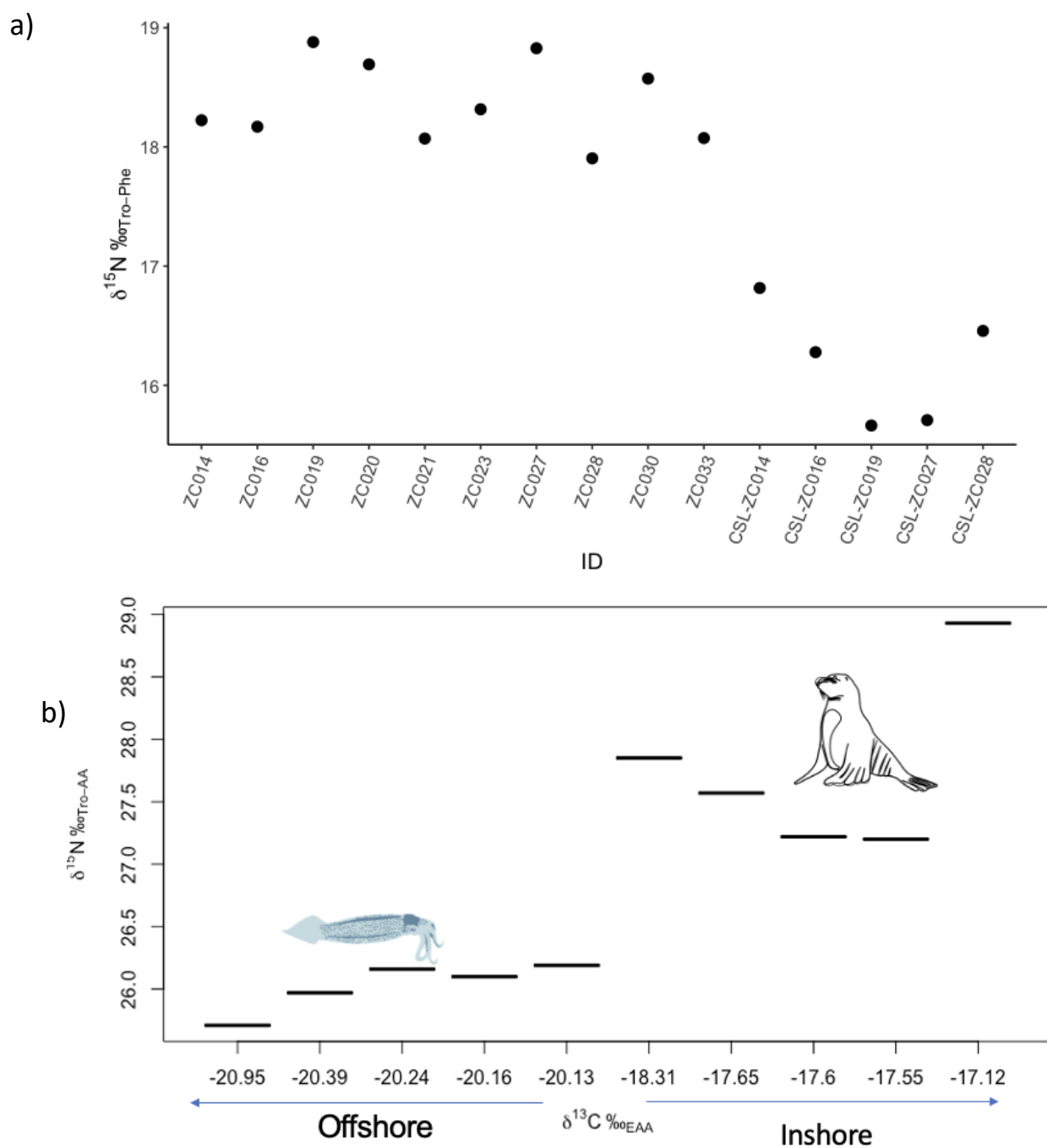
Figure 3. (a) Plot of $\delta^{15}N_{Tro\text{-}Phe\ (source\ AA)}$ of CSL and market squid found in stranded/deceased CSL stomach used as a proxy to estimate trophic position; (b) Box plot of $\delta^{15}N_{Tro\text{-}AA}$ vs. $\delta^{13}C_{EAA}$ values of CSLs and market squid found in CSL stomach.

CSLs had more variable and lower $\delta^{15}N_{Glx}$ values but higher $\delta^{15}N_{Phe}$ values than squid (Fig. A7). $\delta^{15}N_{Glx}$ values of CSLs were lower than squid, but its $\delta^{15}N_{Tro\text{-}AA}$ values were ~1 higher (Fig. A7). $\delta^{15}N_{Phe}$ values from sea lions had a difference of ~2, with less negative $\delta^{13}C_{Phe}$ values for market squid (Fig. 4; Fig. A7). Using both

proxies of trophic position (i.e. $\delta^{15}N_{Tro-Phe}$ and $\delta^{15}N_{Tro-Src}$ values) for the CSLs, the predator had lower proxies than market squid (Fig. A8).
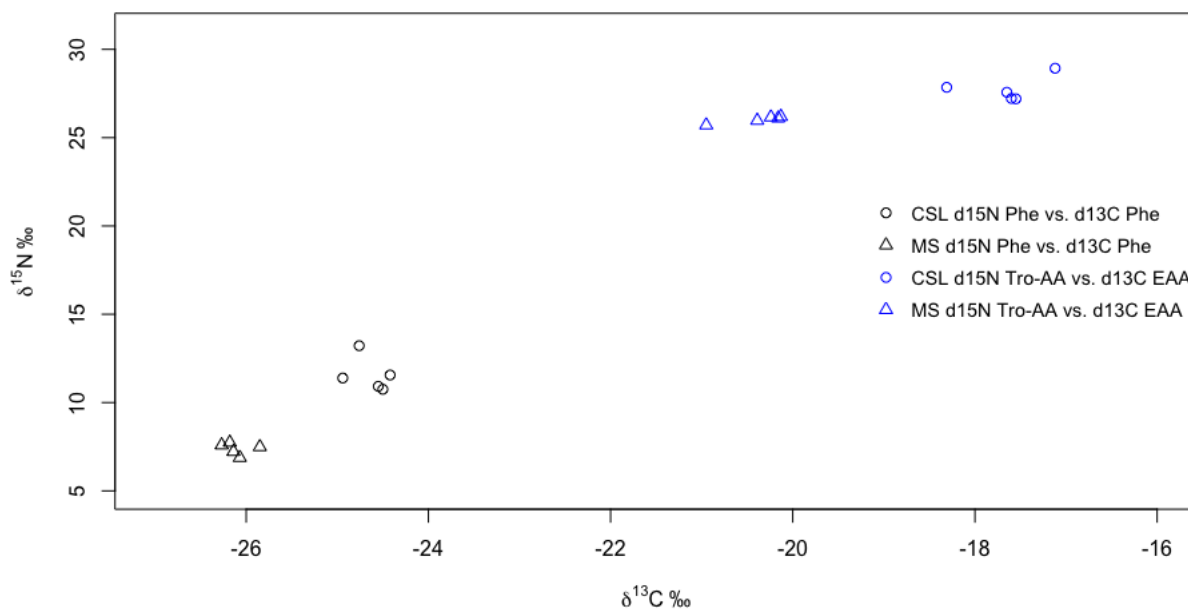
Figure 4. $\delta^{15}N_{Phe}$ vs. $\delta^{13}C_{Phe}$ vs. $\delta^{15}N_{Tro-AA}$ vs. $\delta^{13}C_{EAA}$ of CSL and market squid found in CSL stomach.
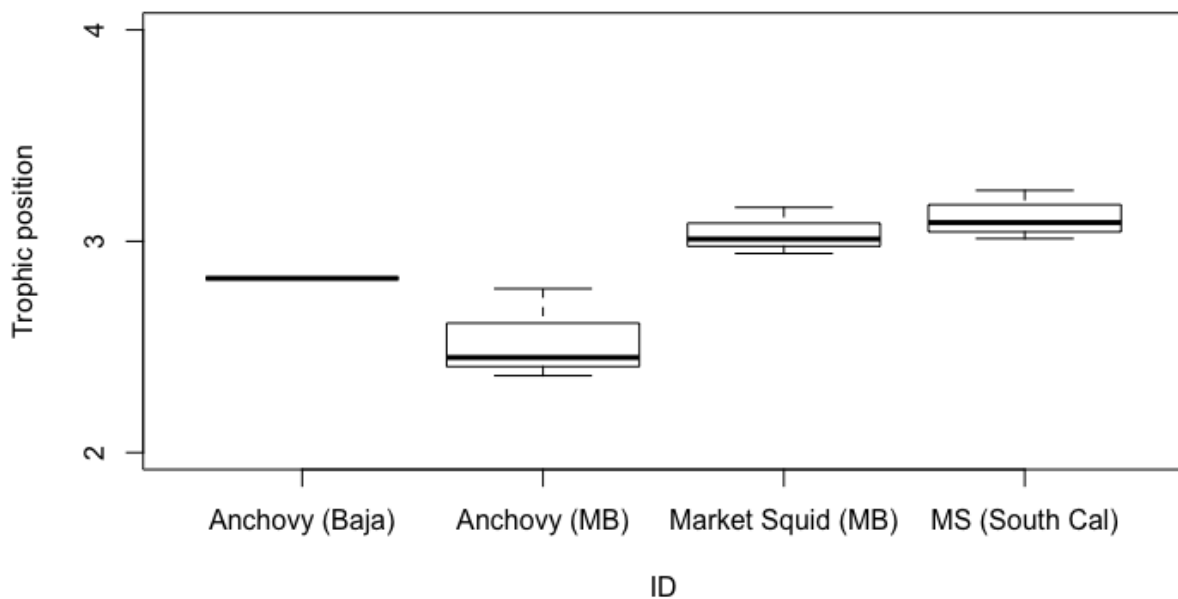
Figure 5. Trophic position of anchovy (found in Monterey Bay in 2018 and Baja California, 2023) and market squid (found in: Monterey Bay, 2018, and Southern California (in stomach of CSLs, 2022)).

Mean confidence intervals presented a difference of ~2 between market squid consumed by CSLs (n = 10) and those from Monterey Bay (n = 3), with anchovies from Monterey Bay (n = 3) presenting with lower

values than market squid from both locations (Fig. A9; Intercept: 2.5 % = 16.2; 97.5 % = 18.4). Anchovies from Monterey Bay had lower trophic positions than those from Baja California, while market squid found in CSL stomachs were relatively similar to those from Monterey Bay (Fig. 5).

3.3 Domoic acid analysis

A male subadult CSL (ZC016) had the highest DA concentrations in feces with 188 ng/mL, while the lowest was ZC014 with 138 ng mL^{-1} (Table 1, Fig. A10). CSLs with feces below detectable DA limits were ZC030, ZC033, ZC023, and ZC028 (Table 1, Fig. A10). All hard part stomach contents of CSLs tested for DA resulted below detectable limits; ZC030 and ZC033 had DA below detectable limits (Table 1, Fig. A10). The highest DA concentration in squid mantle measured with the highly sensitive test kit was 34 ng mL^{-1} in ZC027, while the lowest was 5.7 ng mL^{-1} in ZC033 (Table 1, Fig. A10). Utilizing the same test kit for gastric juices, the highest concentration was also ZC027 measuring 34 ng mL^{-1} while the lowest was ZC033 measuring 5.7 ng mL^{-1}, all consistently yielded low but detectable levels of DA (Table 1, Fig. A10). Both squid mantle and gastric juice tested with the less sensitive testing kit resulted in 21 ± 11 ng mL^{-1} for market squid found in stomachs of ZC019, ZC020, and ZC016 while for gastric juice ZC019, ZC020, and ZC016 were included which all fell beneath the minimum detection limits (Table 1, Fig. A10).

Chapter 4. Discussion

4.1 Sea lions and insights of DA toxicosis

All California sea lions (CSLs) carcasses were of code 3 to late stage 3 because they were moderately decomposed (skin was sloughing and had friable liver) according to previous studies (Dierauf & Gulland, 2001; Ijsseldijk et al., 2019). Le Boeuf et al. (2002) characterized their CSLs carcasses as greater than 2 weeks dead for advanced decomposed carcasses, while moderately decomposed carcasses can be less than 2 weeks dead but greater than a fresh carcass. Considering the results from this previous study, we speculate that our CSLs were dead for a maximum of one week, to a minimum of roughly ~48 hrs considering that tissues and organs from a few individuals exhibit autolysis. Similar histology findings were found in deceased CSLs (Gulland et al., 2002). However, autolysis is not an accurate method of measuring the time of death of a carcass due to environmental conditions, such as temperature, highly affecting the rate of decomposition (Clark et al., 1997). Eight from ten CSLs contained large volcanic stomach ulcers ranging from 1-8 at one time. These ulcers are commonly found in dead marine mammal strandings due to an infection from ascarids (Colegrove et al., 2018; Gardiner, 2011). Overall, body condition from all individuals seems to be in relatively good health, and DA toxicosis was initially considered as the most probable cause of death because a HAB event was underway when CSLs were found stranded/deceased.

Among histology findings (Table A1), five CSLs exhibited mild or moderate myocardial damage, three with multifocal nuclear rowing, and one with karyomegaly of the heart. These lesions have been described in previous records of sea lion strandings caused by DA (Gulland et al., 2002; Lefebvre et al., 2010). Specifically, Gulland et al. (2000) noted that although myocardial lesions were not present for every sea lion in their study, they were in those who died early (within 48 hrs) in a DA bloom. Presence of such cardiac lesions in pinnipeds have been suspected to be the result of DA directly reacting with cardiac glutamate receptors leading to apoptosis of cardiac myocytes (Silvagni et al., 2005; Zabka et al., 2009), which, if sufficiently severe, may lead to death. Lesion consistencies in previous DA marine mammal strandings supported our initial diagnosis of DA as cause of death. However, CSL stomachs were distended with a total of 677 market squid and 5 unknown fish, suggesting a healthy status to successfully catch that number of squid prior to death. The pups consumed 12 and 45 market squids, suggesting that they were in the process of weaning. The typical weight range of a healthy weaning CSL pup is ~26.6 kg (unhealthy = <12.0 kg) (Davis, 2014; DeLong et al., 2017) and yearlings above 25 kg (Greig et al., 2005) with a diet that

normally consists of milk and foraging fish with fewer taxa than adults due to their inexperience (Kastelein et al., 2000; McClatchie, Field, et al., 2016; Orr et al., 2011). The fact that the pups in this study had robust weights and full stomachs suggests these pups were healthy. Subadults consumed, at most, 146 squid per individual, which is inconsistent with a DA toxicosis diagnosis. Typically, stranded marine pinnipeds suspected of DA toxicosis are found with emptied stomachs caused by regurgitation (Goldstein et al., 2008; Silvagni et al., 2005). This does not, however, rule out the possibility of an acute response to DA with immediate respiratory impacts prior to regurgitation.

Additionally, sea lion diets are normally variable, preferring high fatty organisms such as anchovies over those with low fat content such as market squid (Lowry et al., 2022). Young pinnipeds will also typically consume 8 - 15% of their body weight a day, with older individuals consuming less, about 4 - 8% with some variation depending on sexes and seasons (Dierauf & Gulland, 2001; Kastelein et al., 2000). The last meal of the CSLs in this study represented a range of 0.615% to 5.29% of their body weight a day (2.53% ± 1.50% on average), supporting the idea that all individuals were healthy prior to their death. For market squid, the external bodies were intact, i.e. no signs of chewing or degrading. Most internal organs such as testes for males and eggs for females were still visible, apart from finer organs, such as the digestive gland. The 5 unknown fish mostly had soft tissue digested, suggesting that fish were consumed and digested before the invertebrates. This finding could relate to prey tissue type and digestion rates. For instance, after feeding CSLs a diet of 1.4 to 2.3 kg of market squid and three other fish species individually, Hawes (1983) found that market squid was fully digested after 73 – 76 hrs, while anchovy took 48 – 68 hrs. Considering that our squid were intact among all stomachs, it was likely that digestion had not yet set in before CSLs died and provides additional bounds on the temporal window of death, suggesting a fairly rapid event. In other words, CSL death potentially occurred minutes to hours after squid consumption.

4.2 Domoic acid in predator & prey

Although accumulation of DA in CSL feces (138 - 188 ng mL^{-1}) was greater than in gastric juice and market squid mantle, these concentrations were below DA concentrations previously reported for deceased and stranded CSLs due to DA toxicosis (Bargu et al., 2012; Gulland et al., 2002). DA ranged from 200 to 40,000 ng mL^{-1} of DA in feces of pups, yearlings, and subadults who stranded from different causes between 2004-2007 in Monterey Bay, California, reaching as high as 96,800 ng mL^{-1} in animals with acute DA exposure during the same period, particularly during a 2007 DA event (Bargu et al. 2012). While our DA results fall well below DA concentrations for CSL in the literature, it is important to consider the possibility that DA

loads in the animals in our study were higher prior to analysis given that DA levels in feces were relatively higher than in squid tissue and CSL gastric juice. This is underscored by Bowers et al. (2021) who noted DA's stability in feces over short and long periods of time (short term: 8 weeks; long term: 4 years) depending on different sample storage procedures.

The observed similarity of DA concentrations in market squid mantle (15 - 43 ng mL^{-1}) and gastric juice (5.7 - 34 ng mL^{-1}) among all CSLs, suggest that pups, yearlings, and subadults accumulated trace amounts of DA after consuming market squid. Low DA concentrations in stomach content (i.e. 0.3 to 44.0 ng mL^{-1}) have been found in premature pups in San Miguel Island; DA was present in 26% of consumed market squid (Goldstein et al. 2009). This DA concentration range was far lower than the range measured in the stomachs of our three CSL age classes (Table 1). Two previous studies in California report higher concentrations of DA in market squid (190 ng/mL - 370 ng mL^{-1}) (Bargu et al., 2008; Bernstein et al., 2021) than those presented in our study. However, these measurements were lower than DA detected in anchovy viscera, which can reach over 1,175 ug DA g^{-1} (Lefebvre et al., 2001). The presence of DA in market squid tissue as observed in this and previous studies (i.e. Goldstein et al. (2009), Bargu et al. (2008); Bernstein et al. (2021)) supports the potential for market squid to accumulate DA. It is unknown the effect of a continual ingestion of prey with low DA concentrations for sea lions or any other marine mammal. Experimental studies in mice found that DA can accumulate in milk of lactating individuals (Maucher & Ramsdell, 2005). Because DA gross concentration over time could be greater than a single low dose, a continuous exposure of nursing CSL pups to low concentrations of DA in milk could affect their development (Rust et al., 2014). Low DA levels in market squid could produce such risks to pups and yearlings.

4.3 Market squid as prey of CSLs

In southern California, market squid size and abundance are highly influenced by El Niño and La Niña conditions due to changes in upwelling and productivity conditions (Jackson & Domeier, 2003; Zeidberg & Hamner, 2002). The observed low variation in market squid size among CSLs stomachs suggest that squid was abundant during February-March, 2022 and were successfully captured by CSL regardless of sex and maturity. These results are relatively consistent with a previous study in the same overall geographic area where CSL consumed market squid of similar mantle lengths (137 and 118 mm) in winter and spring (Lowry & Carretta, 1999). La Niña conditions present in February-March 2022 could possibly have favored high squid availability and explain the high representation of squid as prey in the stomachs of the ten stranded

sea lions. In contrast, Lowry & Carretta (1999) study occurred during El Niño conditions, underscoring the difficulty in associating specific ecosystem responses with ENSO variability.

Despite similar MLs of market squid found throughout all ten sea lion stomachs, their δ^{13}C and δ^{15}N values in bulk tissue were statistically different (Fig. A3), suggesting that market squid had a different diet, fed in different regions with distinct isotope baseline values or both. Assuming δ^{15}N as the main indicator of trophic position, a higher variability in δ^{15}N range among the squid standard ellipse areas (SEA$_C$) suggests different trophic position, supporting its opportunistic nature. Euphausiids and copepods represent the primary prey for market squid in southern California, and fish to a much lesser extent (Karpov, 1979; Recksiek & Frey, 1978).

Because δ^{13}C values reflect photosynthetic sources of primary producers (Smith and Epstein, 1971), the high overlap in δ^{13}C values among squid SEA$_b$ (Fig. 1a, b) and CSLs suggest feeding in the same overall geographic area. However, the lack of overlap in δ^{13}C values from essential amino acids (AAs) (Fig. 3b) between market squid and CSLs suggests that prey and predator reflected different foraging sites during their last days, and perhaps market squid was a new prey to CSL. This is consistent with reports from fisheries landings in Terminal Island (near Orange County) where commercial landings of 382, 292 pounds of market squid were measured for February 2022 alone (California Department of Fish and Wildlife, n.d.).

Market squid presented high variability in their δ^{15}N$_{Tro-Phe}$ values, further supporting its generalist opportunistic behaviors. Like other squid species capable of adapting to large variations in prey abundance (Hoving et al., 2013), regional and temporal differences in squid diet could explain the variability in squid trophic positions. This is supported by Karpov et al. (1979) who found that differences in prey of market squid are not due to sex or size, but rather to changes in patches of prey and changes in squid behavior in different areas given that their prey seemed to vary by depth and geographic location. In fall of 2022, offshore SST in Southern California were above average while subsurface temperatures were below average (Thompson et al., 2024). Closer to shore, SST and subsurface temperatures were below average in fall at a time when krill abundance dropped and southern copepods increased later in the year (Thompson et al., 2024). The more depleted δ^{13}C$_{EAA}$ in market squid than those from CSLs suggest that market squid were originally offshore and moved nearshore towards CSLs because this offshore-inshore gradient has been found in previous studies using δ^{13}C bulk (Bernstein et al., 2021; Burton & Koch, 1999). To date, there are no reports of these offshore-inshore gradients using δ^{13}C$_{EAA}$ but they are expected. Hence, market squid could have consumed different prey items at varying depths. This phenomenon was witnessed in a study conducted by Karpov (1979) and another by Squires (1957).

For CSL, isotope values for eight of ten individuals were clustered, except ZC027 (f/pup) and ZC028 (m/subadult) which were located within and outside the ellipse border, respectively. The latter could be associated with subadults up to mature males traveling further than females in search of food, while females remain mainly near rookeries throughout most of their life stages (Valenzuela-Toro et al., 2023). Pups dependent on milk remain near rookeries and slowly increase their foraging distance during the weaning stage (Melin et al., 2018). Higher $\delta^{15}N_{bulk}$ values are typically common in milk dependent marine mammals than adults, but dip greatly with the reduction of a solely milk-based diet and increased prey consumption (Mendes et al., 2007; Richards et al., 2002; Ruiz-Cooley et al., 2021). The decrease in $\delta^{15}N_{bulk}$ values in yearlings could be associated with the end of weaning.

Using $\delta^{15}N_{Tro-Src\ AAs}$ values, a moderate variation in trophic proxies was documented among CSLs, which could be associated with moderate differences in diet due to their maturity and experience for capturing prey. In our study, ZC027 (f/pup) presented the second lowest $\delta^{15}N_{Tro-Phe}$ value, while ZC014 (m/yearling) the highest, suggesting that different energetic requirements between CSL pups and yearlings can drastically change their trophic position proxies. This isotopic pattern is consistent with studies in marine mammals using AA $\delta^{15}N$ values, where lower $\delta^{15}N_{Tro-Src}$ values were found in milk dependent dolphins than adults, and higher $\delta^{15}N_{Tro-Src}$ values when calves increased prey consumption (Ruiz-Cooley et al., 2021).

Considering two proxies of trophic position (TP) (i.e. $\delta^{15}N_{Tro-Src}$ or $\delta^{15}N_{Tro-Phe}$), the pattern of variation between CSL and market squid is conflicting (Fig. A8) because market squid had higher TP proxies than CSL (Fig. A8). These inconsistencies have been documented in other marine mammals (Matthews et al., 2020; Ruiz-Cooley et al., 2021) and high trophic level predators (McMahon & McCarthy, 2016), calling for a better understanding of the mechanisms driving isotopic fractionation in AAs. Considering that Phe is an essential-AA and canonical source-AA (McMahon & McCarthy, 2016), using $\delta^{13}C_{Phe}$ values (instead of $\delta^{15}N_{Phe}$) and $\delta^{15}N_{Tro-AAs}$ would be a better indicator of foraging location and trophic position, respectively (Fig. 4). Indeed, our resultant pattern of variation using $\delta^{13}C_{Phe}$ and $\delta^{15}N_{Tro-AAs}$ (Fig. 3b) shows that CSL were ~ 2.6 and 3‰ higher than market squid $\delta^{13}C$ and $\delta^{15}N$ values, respectively, as expected between a predator and prey. This is a key finding that challenges the well-used metrics for determining these traits and supports different foraging regions between predator and prey. Presumably market squid rapidly increased in abundance and availability, becoming a new prey item for CSL during their last days of life (and represented almost entirely our CSL stomachs), and thereby limiting integration of the prey isotopic signature into their tissue.

4.4 Contrasting market squid with anchovy

Market squid, sardines, and anchovies are important in the diets of CSLs (Lowry et al., 2022; Lowry & Carretta, 1999). Anchovies are recognized as the most powerful pelagic DA vector, becoming toxic to CSLs when contaminated with DA (Lefebvre et al., 1999, 2001; Scholin et al., 2000). Comparing the trophic positions and feeding niches of market squid with anchovies can provide insights on whether market squid is a potential DA vector, even a powerful one at that. The SEA_C for market squid consumed by our CSL and that for anchovies from Baja California overlapped 24.1%, while market squid and anchovies from the sites in Monterey Bay in a previous study were found to overlap 52.7% (Bernstein et al., 2021). These overlaps suggest that market squid and anchovies could feed on similar prey items if they fed in the same region. Cailliet et al, (1979) found that market squid made up ~90% of the species composition as by catch when looking in anchovy hauls in Monterey Bay, which supports the idea that both species feed in similar areas. For the isotopic niche of sardines from Monterey Bay, the SEA_C was more similar to anchovies than market squid (Bernstein et al., 2021). In contrast, sardines from Baja California had a more distinctive isotopic niche than anchovies from Baja and market squid from Southern California, suggesting different foraging locations and/or diet. This is reasonable given sardines and anchovies consume phytoplankton and zooplankton but may have different preferences for location and prey size (Van Der Lingen et al., 2006), while market squid are opportunistic feeders, consuming zooplankton and other fish species (Karpov, 1979).

The $\delta^{15}N_{Tro-AAs}$ and $\delta^{15}N_{Tro-Phe}$ results suggest that anchovy vary in trophic position between Baja and Monterey Bay, while the trophic proxy for market squid remains relatively constant across regions. The low % overlap between market squid consumed by CSL and anchovies from Baja California and the variation in anchovy $\delta^{15}N_{Tro-Phe}$ can therefore, in part, be explained by differences in both time (years sampled) and space (different sample locations). Phytoplankton biomass and consumer abundance are affected year to year by low frequency variability in upwelling strength and temperature often associated with basin-scale events, such as El Niño and La Niña (McClatchie, Goericke, et al., 2016). While 2022 was categorized as a weak La Niña year, 2018 and 2023 were considered weak/strong El Niño years, respectively (*NOAA's Climate Prediction Center*, 2023). Interestingly, all three years (2018, 2022, and 2023) were marked by significant changes in the abundance/landings of market squid and anchovy, consequently influenced by changes in prey abundance and distributions (euphausiids and copepods) between years (Leising et al., 2024; Matthews & Ashcraft, 2023; Thompson et al., 2019, 2024). Furthermore, Orange

County and Baja California are different environmentally and oceanographically, further adding variation to the trophic positions and feeding niches of market squid and anchovies (Rosenberger et al., 2009).

Anchovies consume plankton, but most of their energy derives from zooplankton consumption (Van Der Lingen et al., 2006). Both market squid and anchovy prey upon zooplankton as a part of their diet, however, statistically significant differences exist between $\delta^{15}N_{Tro-AA}$ of market squid and anchovy along with variability present in their trophic position estimates between three different locations. This suggests dietary differences between locations and years. Perhaps the higher adaptability of market squid to changes in prey abundance through years and locations over that of anchovies could also play a role. Most of the anchovy dietary input comes from large zooplankton, with mesozooplankton found to maximize anchovy growth. However, the higher variability in trophic position estimates between anchovies from different years and locations suggests their lack of adaptability to changes in favorable food abundances, as opposed to that of market squid, which can adapt quickly and effectively (Van Der Lingen et al., 2006). This is because market squid seem to generally consume a wide range of prey items, with diets shifting in prey type abundance from year to year, location, and depth. As for anchovies, Hill et al. (2015) suggested they were dietary specialists since their diets shifted with decadal time scales, as opposed to market squid, which are generalist opportunistic predators. Market squid and anchovy seem to feed in similar foraging areas and have similar diets, but their diets vary depending on geographic location (Fig. A6 a).

In summary, the assessment suggests that market squid should be considered a viable DA vector as revealed by comparing the $\delta^{13}C$ and $\delta^{15}N$ AA values of squid with the values of the most powerful, known DA vector (i.e. anchovy). Comprehending how DA moves throughout the food web by evaluating differences or similarities in key vector species based on their foraging location and diets can elucidate new insights on how DA is transferred up the food chain to top marine predators.

Chapter 5. Conclusions

DA was found in market squid mantle, gastric juice, and CSL feces, but in low concentration. Therefore, DA was ruled out as the main cause of CSL death. Furthermore, due to differences within CSL and market squid $\delta^{13}C_{Phe}$ and $\delta^{15}N_{Phe}$ values, market squid was identified as a possible new prey for CSLs during February-March of 2022 due to market squid new high availability, but the $\delta^{13}C_{EAA}$ values from squid were not equivalent to those from CSL. Market squid commercial fisheries and market squid spawning both occur year-round, with market squid hatching within 3 to 5 weeks depending on water temperature (California Department of Fish and Wildlife, 2024; Zeidberg et al., 2011, 2012). With 2022 being an especially prolific year for market squid abundance, CSLs possibly encountered high numbers of market squid, resulting in their overabundance in CSL stomachs, but not feeding for a sufficiently long period of time to have reflected squid $\delta^{13}C_{EAA}$ and $\delta^{15}N_{Src-AA}$ values in their tissue. It has been suggested that operational impacts of market squid fisheries are a likely explanation for the cluster of CSL deaths.

The use of multidisciplinary approaches, such as stomach content analysis, morphometrics, and $\delta^{13}C$ and $\delta^{15}N$ of bulk SIA and CSIA-AA proved to be a powerful approach to reconstructing marine mammal strandings/deaths, and discriminating individuals that died from DA toxicosis from those that did not. This is especially true when combining $\delta^{13}C$ and $\delta^{15}N$ of bulk SIA and CSIA-AA since, together, they highlight foraging location and similarities/differences in diets between stranded groups while also providing novel understanding of key DA vectors in the case of strandings caused by DA. While market squid were unlikely the cause of this particular cluster of CSL deaths, further studies on DA accumulation in market squid are needed to comprehend how powerful market squid can be as DA vectors. Future DA monitoring in species such as anchovies, sardines, and Dungeness crabs should also incorporate market squid in their monitoring programs because of potential risks marine mammals may exhibit from continual low level DA exposure.

Bibliography

Adams, T. S., & Sterner, R. W. (2000). The effect of dietary nitrogen content on trophic level 15N enrichment. *Limnology and Oceanography*, *45*(3), 601–607. https://doi.org/10.4319/lo.2000.45.3.0601

Akmajian, A. M., Scordino, J. J., & Acevedo-Gutiérrez, A. (2017). Year-round algal toxin exposure in free-ranging sea lions. *Marine Ecology Progress Series*, *583*, 243–248. https://doi.org/10.3354/meps12345

Anderson, C. (2022, March 24). *California HAB Bulletin: February-March 2022 | Southern California Coastal Ocean Observing System*. https://sccoos.org/california-hab-bulletin/february-2022/

Anderson, C. R., Berdalet, E., Kudela, R. M., Cusack, C. K., Silke, J., O'Rourke, E., Dugan, D., McCammon, M., Newton, J. A., Moore, S. K., Paige, K., Ruberg, S., Morrison, J. R., Kirkpatrick, B., Hubbard, K., & Morell, J. (2019). Scaling Up From Regional Case Studies to a Global Harmful Algal Bloom Observing System. *Frontiers in Marine Science*, *6*, 1–24. https://doi.org/10.3389/fmars.2019.00250

Anderson, C. R., Brzezinski, M. A., Washburn, L., & Kudela, R. (2006). Circulation and environmental conditions during a toxigenic *Pseudo-nitzschia australis* bloom in the Santa Barbara Channel, California. *Marine Ecology Progress Series*, *327*, 119–133. https://doi.org/10.3354/meps327119

Anderson, C. R., Kudela, R. M., Benitez-Nelson, C., Sekula-Wood, E., Burrell, C. T., Chao, Y., Langlois, G., Goodman, J., & Siegel, D. A. (2011). Detecting toxic diatom blooms from ocean color and a regional ocean model. *Geophysical Research Letters1*, *38*(4), 1–6. https://doi.org/10.1029/2010GL045858

Anderson, C. R., Kudela, R. M., Kahru, M., Chao, Y., Rosenfeld, L. K., Bahr, F. L., Anderson, D. M., & Norris, T. A. (2016). Initial skill assessment of the California Harmful Algae Risk Mapping (C-HARM) system. *Harmful Algae*, *59*, 1–18. https://doi.org/10.1016/J.HAL.2016.08.006

Anderson, C. R., Smith, J., Kudela, R., Norris, T., Deming, A., Palmer, L., Dover, R., Berman-Kowalewski, M., & Lefebvre, K. (2023). Rapid response to a massive marine mammal stranding event associated with domoic acid poisoning in central to southern California. *IOC Newsletter on Toxic Algae and Algal Blooms*, *73*, 1–4. https://unesdoc.unesco.org/ark:/48223/pf0000389297

Anderson, D. M. (2009). Approaches to monitoring, control and management of harmful algal blooms (HABs). *Ocean & Coastal Management*, *52*(7), 342. https://doi.org/10.1016/J.OCECOAMAN.2009.04.006

Anderson, D. M., Anderson, P., Bricelj, V. M., Cullen, J. J., & Rensel, J. E. J. (2001). *Monitoring and Management Strategies for Harmful Algal Blooms in Coastal Waters* (59th ed.). Intergovernmental Oceanographic Commission Technical Series . https://www.researchgate.net/publication/230561827_Monitoring_and_Management_Strategies_for_Harmful_Algal_Blooms_in_Coastal_Waters

Bargu, S., Goldstein, T., Roberts, K., Li, C., & Gulland, F. (2012). *Pseudo-nitzschia* blooms, domoic acid, and related California sea lion strandings in Monterey Bay, California. *Marine Mammal Science*, *28*(2), 237–253. https://doi.org/10.1111/j.1748-7692.2011.00480.x

Bargu, S., Powell, C. L., Wang, Z., Doucette, G. J., & Silver, M. W. (2008). Note on the Occurrence of *Pseudo-nitzschia australis* and Domoic Acid in Squid from Monterey Bay, CA (USA). *Harmful Algae*, *7*(1), 45–51. https://doi.org/10.1016/J.HAL.2007.05.008

Bates, S. S., Bird, C. J., Freitas, A. S. W. de, Foxall, R., Gilgan, M., Hanic, L. A., Johnson, G. R., McCulloch, A. W., Odense, P., Pocklington, R., Quilliam, M. A., Sim, P. G., Smith, J. C., Rao, D. V. S., Todd, E. C. D., Walter, J. A., & Wright, J. L. C. (1989). Pennate Diatom *Nitzschia pungens* as the Primary Source of Domoic Acid, a Toxin in Shellfish from Eastern Prince Edward Island, Canada. *Canadian Journal of Fisheries and Aquatic Sciences*, *46*(7), 1203–1215. https://doi.org/10.1139/F89-156

Bejarano, A. C., VanDola, F. M., Gulland, F. M., Rowles, T. K., & Schwacke, L. H. (2008). Production and Toxicity of the Marine Biotoxin Domoic Acid and Its Effects on Wildlife: A Review. *Human and Ecological Risk Assessment*, *14*(3), 544–567. https://doi.org/10.1080/10807030802074220

Bernstein, S., Ruiz-Cooley, R. I., Kudela, R., Anderson, C. R., Dunkin, R., & Field, J. C. (2021). Stable Isotope Analysis Reveals Differences in Domoic Acid Accumulation and Feeding Strategies of Key Vectors in a California Hotspot for Outbreaks. *Harmful Algae*, *110*, 102–117. https://doi.org/10.1016/j.hal.2021.102117

Bowers, E. K., Stimmelmayr, R., & Lefebvre, K. A. (2021). Stability of Domoic Acid in 50% Methanol Extracts and Raw Fecal Material from Bowhead Whales (*Balaena mysticetus*). *Marine Drugs*, *19*(8), 423. https://doi.org/10.3390/md19080423

Burton, R. K., & Koch, P. L. (1999). Isotopic tracking of foraging and long-distance migration in northeastern Pacific pinnipeds. *Oecologia*, *119*(4), 578–585. https://doi.org/10.1007/s004420050822

Cailliet, G. M., Karpov, K. A., & Ambrose, D. A. (1979). *Pelagic Assemblage As Determined From Purse Seine And Large Midwater Trawl Catches In Monterey Bay and Their Affinities With The Market Squid,* Loligo opalescens*.: Vol. XX.* https://www.calcofi.com/publications/calcofireports/v20/Vol_20_Cailliet_etal.pdf

California Department of Fish and Wildlife. (n.d.). *Marine Fisheries Data Explorer (MFDE): Custom Queries*. Retrieved June 28, 2024, from https://wildlife.ca.gov/Conservation/Marine/Data-Management-Research/MFDE/Custom-Queries

California Department of Fish and Wildlife. (2024). *California Commercial Market Squid Landing Receipt Data*. https://wildlife.ca.gov/Conservation/Marine/Pelagic/Market-Squid-Landing

California Dungeness Crab task Force (CDCTF). (2022). *October 2022 Recommendations From The California Dungeness Crab Task Force*. https://opc.ca.gov/wp-content/uploads/2009/04/DCTF_LegReport_October2022.pdf

Carretta, J. V., Forney, K. A., Muto, M. M., Barlow, Jay., Baker, J., Hanson, B., Lowry, M. S., Sweetnam, D., Petersen, D., Cordaro, J., & Gutierrez, C. M. (2005). *NOAA Technical Memorandum NMFS: U.S. Pacific Marine Mammal Stock Assessments: 2004*. https://swfsc-publications.fisheries.noaa.gov/publications/TM/SWFSC/NOAA-TM-NMFS-SWFSC-375.PDF

Checkley, D. M., & Barth, J. A. (2009). Patterns and Processes in the California Current System. *Progress in Oceanography*, *83*(1–4), 49–64. https://doi.org/10.1016/J.POCEAN.2009.07.028

Chikaraishi, Y., Ogawa, N. O., Kashiyama, Y., Takano, Y., Suga, H., Tomitani, A., Miyashita, H., Kitazato, H., & Ohkouchi, N. (2009). Determination of aquatic food-web structure based on compound-specific nitrogen isotopic composition of amino acids. *Limnology and Oceanography: Methods*, *7*(11), 740–750. https://doi.org/10.4319/LOM.2009.7.740

Clark, M. A., Worrell, M. B., & Pless, J. E. (1997). Postmortem Changes in Soft Tissues. In *Forensic Taphonomy: The Postmortem Fate of Human Remains* (pp. 151–160). CRC Press LLC. https://edisciplinas.usp.br/pluginfile.php/4551346/mod_resource/content/2/cap%209.pdf

Colegrove, K. M., Burek-Huntington, K. A., Roe, W., & Siebert, U. (2018). Pinnipediae. In *Pathology of Wildlife and Zoo Animals* (pp. 569–592). Elsevier Inc. https://doi.org/10.1016/B978-0-12-805306-5.00023-7

Corr, L. T., Berstan, R., & Evershed, R. P. (2007). Optimisation of derivatisation procedures for the determination of δ13C values of amino acids by gas chromatography/combustion/ isotope ratio mass spectrometry. *Rapid Communications in Mass Spectrometry*, *21*(23), 3759–3771. https://doi.org/10.1002/rcm.3252

Cossaboon, J. M., Hoh, E., Chivers, S. J., Weller, D. W., Danil, K., Maruya, K. A., & Dodder, N. G. (2019). Apex marine predators and ocean health: Proactive screening of halogenated organic contaminants reveals ecosystem indicator species. *Chemosphere*, *221*, 656–664. https://doi.org/10.1016/J.CHEMOSPHERE.2019.01.050

Davis, B. (2014). Effects of weaning age on body composition and growth of *ex situ* California sea lion (*Zalophus californianus*) pups. Master thesis, University of Central Florida. In *Electronic Theses and Dissertations*. https://ucf.digital.flvc.org/islandora/object/ucf%3A50259

DeLong, R. L., Melin, S. R., Laake, J. L., Morris, P., Orr, A. J., & Harris, J. D. (2017). Age- and sex-specific survival of California sea lions (*Zalophus californianus*) at San Miguel Island, California. *Marine Mammal Science*, *33*(4), 1097–1125. https://doi.org/10.1111/mms.12427

Dierauf, L. A., & Gulland, F. M. D. (2001). *CRC Handbook of Marine Mammal Medicine* (Vol. 2). CRC Press. http://fcm.ens.uabc.mx/~yschramm/Mastozoolog%C3%ADa/Libros/crc_handbook_of_marine_mammal_medicine_2nd_ed%20(1).pdf

Estep, M. L. F., & Vigg, S. (1985). Stable Carbon and Nitrogen Isotope Tracers of Trophic Dynamics in Natural Populations and Fisheries of the Lahontan Lake System, Nevada. *Canadian Journal of Fisheries and Aquatic Sciences*, *42*(11), 1712–1719. https://doi.org/10.1139/f85-215

Fritz, L., Quilliam, M. A., Wright, J. L. C., Beale, A. M., & Work, T. M. (1992). An Outbreak of Domoic Acid Poisoning Attributed To The Pennate Diatom *Pseudonitzschia australis*. *Journal of Phycology*, *28*(4), 439–442. https://doi.org/10.1111/J.0022-3646.1992.00439.X

Fry, B., & Arnold, C. (1982). Rapid 13C/12C Turnover During Growth of Brown Shrimp (*Penaeus aztecus*). *Oecologia*, *54*, 200–204. https://link.springer.com/article/10.1007/BF00378393

Fry, B., & Sherr, E. B. (1989). δ13C Measurements as Indicators of Carbon Flow in Marine and Freshwater Ecosystems. In P. W. Rundel, K. A. Nagy, & J. R. Ehleringer (Eds.), *Stable Isotopes in Ecological Research* (Vol. 68, pp. 196–229). Springer. https://doi.org/https://doi.org/10.1007/978-1-4612-3498-2_12

Gaebler, O. H., Vitti, T. G., & Vukmirovich, R. (1966). Isotope Effects In Metabolism Of 14N And 15N From Unlabeled Dietary Proteins. *Canadian Journal of Biochemistry*, *44*(9), 1249–1257. https://doi.org/https://doi.org/10.1139/o66-142

Gardiner, C. H. (2011, January 19). *CASE I: NIAH2010-1 (AFIP 3164221)*. https://www.askjpc.org/wsco/wsc/wsc10/10WSC17.pdf

Gibble, C. M., Kudela, R. M., Knowles, S., Bodenstein, B., & Lefebvre, K. A. (2021). Domoic acid and saxitoxin in seabirds in the United States between 2007 and 2018. *Harmful Algae*, *103*, 101981. https://doi.org/10.1016/J.HAL.2021.101981

Goldstein, T., Mazet, J. A. K., Zabka, T. S., Langlois, G., Colegrove, K. M., Silver, M., Bargu, S., Van Dolah, F., Leighfield, T., Conrad, P. A., Barakos, J., Williams, D. C., Dennison, S., Haulena, M., & Gulland, F. M. D. (2008). Novel symptomatology and changing epidemiology of domoic acid toxicosis in California sea lions (*Zalophus californianus*): An increasing risk to marine mammal health. *Proceedings of the Royal Society B: Biological Sciences*, *275*(1632), 267–276. https://doi.org/10.1098/rspb.2007.1221

Goldstein, T., Zabka, T. S., Delong, R. L., Wheeler, E. A., Ylitalo, G., Bargu, S., Silver, M., Leighfield, T., Dolah, F. Van, Langlois, G., Sidor, I., Dunn, J. L., & Gulland, F. M. D. (2009). The Role of Domoic Acid In Abortion And Premature Parturition Of California Sea Lions (*Zalophus californianus*) On San Miguel Island, California. *Journal of Wildlife Diseases*, *45*(1), 91–108. http://meridian.allenpress.com/jwd/article-pdf/45/1/91/2238212/0090-3558-45_1_91.pdf

Greig, D. J., Gulland, F. M. D., & Kreuder, C. (2005). A Decade of Live California Sea Lion (*Zalophus californianus*) Strandings Along the Central California Coast: Causes and Trends, 1991-2000. *Aquatic Mammals*, *31*(1), 11–22. https://doi.org/10.1578/am.31.1.2005.11

Gulland, F. M. D., Haulena, M., Fauquier, D., Langlois, G., Lander, M. E., Zabka, T., & Duerr, R. (2002). Domoic acid toxicity in Californian sea lions (*Zalophus californianus*): clinical signs, treatment and survival. *The Veterinary Record*, *150*(15), 475–480. https://doi.org/10.1136/vr.150.15.475

Hare, P. E., Fogel, M. L., Stafford Jr, T. W., Mitchell, A. D., & Hoering, T. C. (1991). The Isotopic Composition of Carbon and Nitrogen in Individual Amino Acids Isolated from Modern and Fossil Proteins. *Journal of Archaeological Science*, *18*(3), 277–292. https://doi.org/https://doi.org/10.1016/0305-4403(91)90066-X

Hawes, S. D. (1984). An Evaluation of California Sea Lion Scat Samples as Indicators of Prey Importance. [Master thesis, San Francisco State University Moss Landing Marine Laboratories]. https://scholarworks.calstate.edu/downloads/6d570224h

Hill, A. D., Daly, E. A., & Brodeur, R. D. (2015). Diet variability of forage fishes in the Northern California Current System. *Journal of Marine Systems*, *146*, 121–130. https://doi.org/10.1016/j.jmarsys.2014.08.006

Hobson, K. A., Schell, D. M., Renouf, D., & Noseworthy, E. (1996). Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: implications for dietary reconstructions involving marine mammals. *Canadian Journal of Fisheries and Aquatic Sciences*, *53*(3), 528–533. https://doi.org/https://doi.org/10.1139/f95-209

Horner, R. A., Garrison, D. L., & Plumley, G. F. (1997). Harmful Algal Blooms and Red Tide Problems on the U.S. West Coast. *The American Society of Limnology Oceanography*, *42*(5, part 2), 1076–1088. https://doi.org/https://doi.org/10.4319/lo.1997.42.5_part_2.1076

Horner, R. A., & Postel, J. R. (1993). Toxic diatoms in western Washington waters (U .S . west coast). *Hydrobiologia*, *269*, 197–205. https://link.springer.com/article/10.1007/BF00028018

Hoving, H. J. T., Gilly, W. F., Markaida, U., Benoit-Bird, K. J., -Brown, Z. W., Daniel, P., Field, J. C., Parassenti, L., Liu, B., & Campos, B. (2013). Extreme plasticity in life-history strategy allows a migratory predator (jumbo squid) to cope with a changing climate. *Global Change Biology*, *19*(7), 2089–2103. https://doi.org/10.1111/GCB.12198

Ijsseldijk, L. L., Brownlow, A. C., & Mazzariol, S. (2019). *Best practice on cetacean post mortem investigation and tissue sampling Joint ACCOBAMS and ASCOBANS document*. https://accobams.org/wp-content/uploads/2021/07/Best-practices-on-cetacean-post-mortem-investigation.pdf

Jackson, A. L., Inger, R., Parnell, A. C., & Bearhop, S. (2011). Comparing isotopic niche widths among and within communities: SIBER – Stable Isotope Bayesian Ellipses in R. *Journal of Animal Ecology*, *80*(3), 595–602. https://doi.org/10.1111/J.1365-2656.2011.01806.X

Jackson, G. D., & Domeier, M. L. (2003). The effects of an extraordinary El Niño / La Niña event on the size and growth of the squid *Loligo opalescens* off Southern California. *Marine Biology*, *142*(5), 925–935. https://doi.org/10.1007/S00227-002-1005-4/FIGURES/8

Johnson, G. C., Lumpkin, R., & Johnson, G. C. (2022). State of the Climate in 2022: Global Oceans. *American Meteorological Society*, *104*(9), 149–199. https://doi.org/https://doi.org/10.1175/BAMS-D-23-0076.2

Karpov, K. A. (1979). Prey composition of the market squid, *Loligo opalescens* Berry, in relation to depth and location of capture, size of squid, and sex of spawning squid. *The California Cooperative Oceanic Fisheries Investigations (CalCOFI) Rep*, *XX*, 51–57. https://www.researchgate.net/publication/237454450

Kastelein, R. A., Schooneman, N. M., Vaughan, N., & Wiepkema, P. R. (2000). Food consumption and growth of California sea lions (*Zalophus californianus californianus*). *Zoo Biology*, *19*(2), 143–159. https://doi.org/10.1002/1098-2361(2000)19:2<143::AID-ZOO5>3.0.CO;2-O

Kelly, J. F. (2000). Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. *Canadian Journal of Zoology*, *78*, 1–27. https://doi.org/https://doi.org/10.1139/z99-165

Larsen, T., Taylor, D. L., Leigh, M. B., & O'brien, D. M. (2009). Stable isotope fingerprinting: a novel method for identifying plant, fungal, or bacterial origins of amino acids. *Ecological Society of America*, *90*(12), 3526–3535. https://doi.org/https://doi.org/10.1890/08-1695.1

Le Boeuf, B. J., Giesy, J. P., Kannan, K., Kajiwara, N., Tanabe, S., & Debier, C. (2002). Organochloride pesticides in California sea lions revisited. *BMC Ecology*, *2*(1), 1–8. https://doi.org/10.1186/1472-6785-2-11/FIGURES/3

Lefebvre, K. A., Bargu, S., Kieckhefer, T., & Silver, M. W. (2002). From sanddabs to blue whales: the pervasiveness of domoic acid. *Toxicon*, *40*(7), 971–977. https://doi.org/10.1016/S0041-0101(02)00093-4

Lefebvre, K. A., Dovel, S. L., & Silver, M. W. (2001). Tissue distribution and neurotoxic effects of domoic acid in a prominent vector species, the northern anchovy *Engraulis mordax*. *Marine Biology*, *138*(4), 693–700. https://doi.org/10.1007/S002270000509

Lefebvre, K. A., Powell, C. L., Busman, M., Doucette, G. J., Moeller, P. D. R., Silver, J. B., Miller, P. E., Hughes, M. P., Singaram, S., Silver, M. W., & Tjeerdema, R. S. (1999). Detection of Domoic Acid in Northern Anchovies and California Sea Lions Associated with an Unusual Mortality Event †. *Natural Toxins*, *7*(3), 85–92. https://doi.org/10.1002/(sici)1522-7189(199905/06)7:3<85::aid-nt39>3.0.co;2-q

Lefebvre, K. A., Quakenbush, L., Frame, E., Huntington, K. B., Sheffield, G., Stimmelmayr, R., Bryan, A., Kendrick, P., Ziel, H., Goldstein, T., Snyder, J. A., Gelatt, T., Gulland, F., Dickerson, B., & Gill, V. (2016). Prevalence of algal toxins in Alaskan marine mammals foraging in a changing arctic and subarctic environment. *Harmful Algae*, *55*, 13–24. https://doi.org/10.1016/J.HAL.2016.01.007

Lefebvre, K. A., & Robertson, A. (2009). Domoic acid and human exposure risks: A review. *Toxicon*, *56*, 218–230. https://doi.org/10.1016/j.toxicon.2009.05.034

Lefebvre, K. A., & Robertson, A. (2010). Domoic acid and human exposure risks: a review. *Toxicon : Official Journal of the International Society on Toxinology*, *56*(2), 218–230. https://doi.org/10.1016/J.TOXICON.2009.05.034

Lefebvre, K. A., Robertson, A., Frame, E. R., Colegrove, K. M., Nance, S., Baugh, K. A., Wiedenhoft, H., & Gulland, F. M. D. (2010). Clinical signs and histopathology associated with domoic acid poisoning in northern fur seals (*Callorhinus ursinus*) and comparison of toxin detection methods. *Harmful Algae*, *9*(4), 374–383. https://doi.org/10.1016/j.hal.2010.01.007

Lefebvre, K. A., Silver, M. W., Coale, S. L., & Tjeerdema, R. S. (2002). Domoic acid in planktivorous fish in relation to toxic *Pseudo-nitzschia* cell densities. *Marine Biology*, *140*(3), 625–631. https://doi.org/10.1007/s00227-001-0713-5

Leising, A., Hunsicker, M., Tolimieri, N., Williams, G., & Harley, A. (2024). *2023-2024 California Current Ecosystem Status Report A report of the NOAA California Current Integrated Ecosystem Assessment Team (CCIEA) to the Pacific Fishery Management Council*. https://www.pcouncil.org/documents/2024/02/agenda-item-h-1-a-cciea-team-report-1-2023-2024-california-current-ecosystem-status-report-electronic-only.pdf/

Lelong, A., Hégaret, H., Soudant, P., Bates, S. S., Lelong, A. L., Le`ne, H. É., Le`ne, L., & Garet, H. É. (2019). *Pseudo-nitzschia* (Bacillariophyceae) species, domoic acid and amnesic shellfish poisoning: revisiting previous paradigms. *Phycology*, *51*, 168–216. https://doi.org/10.2216/11-37.1

Lopes, V. M., Lopes, A. R., Costa, P., & Rosa, R. (2013). Cephalopods as vectors of harmful algal bloom toxins in marine food webs. *Marine Drugs*, *11*(9), 3381–3409. https://doi.org/10.3390/md11093381

Lowry, M. S., & Carretta, J. V. (1999). Market squid (*Loligo opalescens*) in the diet of California Sea Lions (*Zalophus californianus*) in southern California (1981-1995). In *The California Cooperative Oceanic Fisheries Investigations (CalCOFI) Rep* (Vol. 40). https://calcofi.com/publications/calcofireports/v40/Vol_40_Lowry___Carretta.pdf

Lowry, M. S., Nehasil, S. E., & Moore, J. E. (2022). Spatio-temporal diet variability of the California sea lion *Zalophus californianus* in the southern California Current Ecosystem. *Marine Ecology Progress Series*, *692*, 1–21. https://doi.org/10.3354/meps14096

Macko, S. A., Fogel ESTEP, M. L., Hare, P. E., & Hoering, T. C. (1987). Isotopic fractionation of nitrogen and carbon in the synthesis of amino acids by microorganisms. *Chemical Geology (Isotope Geoscience Section)*, *65*(1), 79–92. https://doi.org/https://doi.org/10.1016/0168-9622(87)90064-9

Marien, K. (1996). Establishing Tolerable Dungeness Crab (*Cancer magister*) and Razor Clam (*Siliqua patula*) Domoic Acid Contaminant Levels. *Environmental Health Perspectives*, *104*(11), 1230–1236. https://doi.org/10.1289/ehp.104-1469507

Matthews, C. J. D., Ruiz-Cooley, R. I., Pomerleau, C., & Ferguson, S. H. (2020). Amino acid δ15N underestimation of cetacean trophic positions highlights limited understanding of isotopic fractionation in higher marine consumers. *Ecology and Evolution*, *10*(7), 3450–3462. https://doi.org/10.1002/ece3.6142

Matthews, K., & Ashcraft, S. (2023). *Staff Summary for August 22-23, 2023 Item No. 20: White Seabass Fishery Management Plan 2021–2022 Annual Review report*. https://nrm.dfg.ca.gov/FileHandler.ashx?DocumentID=214913

Maucher, J. M., & Ramsdell, J. S. (2005). Domoic acid transfer to milk: Evaluation of a potential route of neonatal exposure. *Environmental Health Perspectives*, *113*(4), 461–464. https://doi.org/10.1289/EHP.7649/ASSET/C5CF7781-5393-4F98-8A4B-57DB9562FCDD/ASSETS/GRAPHIC/EHP0113-000461F6.JPG

McCabe, R. M., Hickey, B. M., Kudela, R. M., Lefebvre, K. A., Adams, N. G., Bill, B. D., Gulland, F. M. D., Thomson, R. E., Cochlan, W. P., & Trainer, V. L. (2016). An unprecedented coastwide toxic algal bloom linked to anomalous ocean conditions. *Geophysical Research Letters*, *43*(19), 10,366-10,376. https://doi.org/10.1002/2016GL070023

McClatchie, S., Field, J., Thompson, A. R., Gerrodette, T., Lowry, M., Fiedler, P. C., Watson, W., Nieto, K. M., & Vetter, R. D. (2016). Food limitation of sea lion pups and the decline of forage off central and southern California. *The Royal Society Publishing*. https://doi.org/10.1098/rsos.150628

McClatchie, S., Goericke, R., Leising, A., Auth, T. D., Bjorkstedt, E., Robertson, R. R., Brodeur, R. D., Du, X., Daly, E. A., Morgan, C. A., Chavez, F. P., Debich, A. J., Hildebrand, J., Field, J., Sakuma, K., Jacox, M. G., Kahru, M., Kudela, R., Anderson, C., … Jahncke, J. (2016). State of the California Current 2015-16: comparisons with the 1997-98 El Niño. *The California Cooperative Oceanic Fisheries Investigations (CalCOFI) Rep*, *57*, 1–57. https://www.researchgate.net/publication/311734121_State_Of_The_California_Current_2015-16_Comparisons_With_The_1997-98_El_Nino

McClelland, J. W., & Montoya, J. P. (2002). Trophic relationships and the nitrogen isotopic composition of amino acids in plankton. *Ecology*, *83*(8), 2173–2180. https://doi.org/10.1890/0012-9658(2002)083[2173:TRATNI]2.0.CO;2

McMahon, K. W., Fogel, M. L., Elsdon, T. S., & Thorrold, S. R. (2010). Carbon isotope fractionation of amino acids in fish muscle reflects biosynthesis and isotopic routing from dietary protein. *Journal of Animal Ecology*, *79*(5), 1132–1141. https://doi.org/10.1111/J.1365-2656.2010.01722.X

McMahon, K. W., & McCarthy, M. D. (2016). Embracing variability in amino acid δ15N fractionation: mechanisms, implications, and applications for trophic ecology. *Ecosphere*, *7*(12), 1–26. https://doi.org/10.1002/ECS2.1511

Melin, S. R., Trillmich, F., & Gamboa, D. A. (2018). California, Galapagos, And Japanese Sea Lions. *Encyclopedia of Marine Mammals*, 153–157. https://doi.org/10.1016/b978-0-12-804327-1.00003-0

Mendes, S., Newton, J., Reid, R. J., Zuur, A. F., & Pierce, G. J. (2007). Stable carbon and nitrogen isotope ratio profiling of sperm whale teeth reveals ontogenetic movements and trophic ecology. *Population Ecology*, *151*, 605–615. https://doi.org/10.1007/s00442-006-0612-z

Minagawa, M., & Wada, E. (1984). Stepwise enrichment of δ15N along food chains: Further evidence and the relation between δ15N and animal age. *Geochimica et Cosmochimica Acta*, *48*(5), 1135–1140. https://doi.org/10.1016/0016-7037(84)90204-7

Nezlin, N. P., Sutula, M. A., Stumpf, R. P., & Sengupta, A. (2012). Phytoplankton blooms detected by SeaWiFS along the central and southern California coast. *Journal of Geophysical Research: Oceans*, *117*(7). https://doi.org/10.1029/2011JC007773

NOAA's   Climate   Prediction   Center.   (2023). https://origin.cpc.ncep.noaa.gov/products/analysis_monitoring/ensostuff/ONI_v5.php

Orr, A. J., van Blaricom, G. R., de Long, R. L., Cruz-Escalona, V. H., & Newsome, S. D. (2011). Intraspecific comparison of diet of California sea lions (*Zalophus californianus*) assessed using fecal and stable isotope analyses. *Canadian Journal of Zoology*, *89*(2), 109–122. https://doi.org/10.1139/Z10-101

Owens, N. J. P. (1988). Natural Variations in 15N in the Marine Environment. *Advances In Marine Biology*, *24*, 389–451. https://doi.org/https://doi.org/10.1016/S0065-2881(08)60077-2

Peterson, B. J., & Fry, B. (1987). Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics*, *18*(1), 293–320. https://doi.org/https://doi.org/10.1146/annurev.es.18.110187.001453

Popp, B. N., Graham, B. S., Olson, R. J., Hannides, C. C. S., Lott, M. J., Ló Pez-Ibarra, G. A., Galván-Magaña, F., & Fry, B. (2007). Insight into the Trophic Ecology of Yellowfin Tuna, *Thunnus albacares*, from Compound-Specific Nitrogen Isotope Analysis of Proteinaceous Amino Acids. *Terrestrial Ecology*, *1*, 173–190. https://doi.org/https://doi.org/10.1016/S1936-7961(07)01012-3

Post, D. M. (2002). Using Stable Isotopes To estimate Trophic Position: Models, Methods, and Assumptions. *Ecology*, *83*(3), 703–718. https://sci-hub.se/https://doi.org/10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2

Recksiek, C. W., & Frey, H. W. (1978). Biological, Oceanographic, and Acoustic Aspects of The Market Squid, *Loligo opalescens* Berry. *Fish Bulletin*, *169*, 1–186. https://escholarship.org/uc/item/1dz5j9cf

Richards, M. P., Mays, S., & Fuller, B. T. (2002). Stable carbon and nitrogen isotope values of bone and teeth reflect weaning age at the Medieval Wharram Percy site, Yorkshire, UK. *American Journal of Physical Anthropology*, *119*(3), 205–210. https://doi.org/10.1002/AJPA.10124

Riva, G. T. de la, Johnson, C. K., Gulland, F. M. D., Langlois, G. W., Heyning, J. E., Rowles, T. K., & Mazet, J. A. K. (2009). Association of an unusual marine mammal mortality event with *Pseudo-nitzschia spp.* blooms ALONG the southern California coastline. *Journal of Wildlife Diseases*, *45*(1), 109–121. https://doi.org/10.7589/0090-3558-45.1.109

Rosenberger, K. J., Hamilton, P. E., & Xu, J. (2009). Coastal ocean transport patterns in the central Southern California Bight. *Geological Society of America Special Papers*, *454*, 193–226. https://doi.org/10.1130/2009.2454(3.3)

Ruiz-Cooley, R. I., Anderson, C. R., Kudela, R., Dunkin, R., & Field, J. (n.d.). (In review) Perturbations in a pelagic food web during the NE Pacific Large Marine Heatwave and persistent harmful diatom bloom. In *Harmful Algae*.

Ruiz-Cooley, R. I., Garcia, K. Y., & Hetherington, E. D. (2011). Effects of lipid removal and preservatives on carbon and nitrogen stable isotope ratios of squid tissues: Implications for ecological studies. *Journal of Experimental Marine Biology and Ecology*, *1*(407), 101–107. https://doi.org/10.1016/J.JEMBE.2011.07.002

Ruiz-Cooley, R. I., Gerrodette, T., Chivers, S. J., & Danil, K. (2021). Cooperative feeding in common dolphins as suggested by ontogenetic patterns in δ15N bulk and amino acids. *Journal of Animal Ecology*, *90*(6), 1583–1595. https://doi.org/10.1111/1365-2656.13478

Ruiz-Cooley, R. I., Markaida, U., Gendron, D., & Aguíñiga, S. (2006). Stable isotopes in jumbo squid (*Dosidicus gigas*) beaks to estimate its trophic position: comparison between stomach contents and stable isotopes. *Journal of the Marine Biological Association of the United Kingdom*, *86*(2), 437–445. https://doi.org/10.1017/S0025315406013324

Rust, L., Gulland, F., Frame, E., & Lefebvre, K. (2014). Domoic acid in milk of free living California marine mammals indicates lactational exposure occurs. *Marine Mammal Science*, *30*(3), 1–8. https://doi.org/10.1111/mms.12117

Ryan, J. P., Cline, D. E., Joseph, J. E., Margolina, T., Santora, J. A., Kudela, R. M., Chavez, F. P., Pennington, J. T., Wahl, C., Michisaki, R., Benoit-Bird, K., Forney, K. A., Stimpert, A. K., DeVogelaere, A., Black, N., & Fischer, M. (2019). Humpback whale song occurrence reflects ecosystem variability in feeding and migratory habitat of the northeast Pacific. *PLOS ONE*, *14*(9), e0222456. https://doi.org/10.1371/JOURNAL.PONE.0222456

Rykaczewski, R. R. (2009). Influence of Oceanographic Variability on the Planktonic Prey and Growth of Sardine and Anchovy in the California Current Ecosystem [Technical Report, UC San Diego Scripps Institution of Oceanography]. In *UC San Diego Scripps Institution of Oceanography Report*. https://escholarship.org/uc/item/307453xf

Schoeninger, M. J., & Deniro, M. J. (1984). Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. *Geochimica et Cosmochimica Acta*, *48*(4), 625–639. https://doi.org/https://doi.org/10.1016/0016-7037(84)90091-7

Scholin, C. A., Gulland, F., Doucette, G. J., Benson, S., Busman, M., Chavez, F. P., Cordarok, J., DeLong, R., Vogelaere, A. De, Harvey, J., Haulena, M., Lefebvrel, K., Lipscomb, T., Loscutoff, S., Lowenstine, L. J., Marin III, R., Miller, P. E., McLellan, W. A., Moeller, P. D. R., … Dolah, F. V. M. (2000). Mortality of sea lions along the central California coast linked to a toxic diatom bloom. *Nature*, *403*(6765), 80–84. https://doi.org/10.1038/47481

Silvagni, P. A., Lowenstine, L. J., Spraker, T., Lipscomb, T. P., & D Gulland, F. M. (2005). Pathology of Domoic Acid Toxicity in California Sea Lions (*Zalophus californianus*). *Vet Pathol*, *42*(2), 184–191. https://doi.org/10.1354/vp.42-2-184

Smith, B. N., & Epsten, S. (1971). Two Categories of 13C/12C Ratios for Higher Plants. *Plant Physiology*, *47*(3), 380–384. https://doi.org/https://doi.org/10.1104/pp.47.3.380

Smith, J., Connell, P., Evans, R. H., Gellene, A. G., Howard, M. D. A., Jones, B. H., Kaveggia, S., Palmer, L., Schnetzer, A., Seegers, B. N., Seubert, E. L., Tatters, A. O., & Caron, D. A. (2018). A decade and a half of *Pseudo-nitzschia spp.* and domoic acid along the coast of southern California. *Harmful Algae*, *79*, 87–104. https://doi.org/10.1016/J.HAL.2018.07.007

Squires, H. J. (1957). Squid, Illex illecebrosus (LeSueur), in the Newfoundland Fishing Area. *Journal of the Fisheries Research Board of Canada*, *14*(5), 693–728. https://doi.org/10.1139/F57-028

Stewart, B. S., & Yochem, P. K. (1991). Seasonal and Annual Variability in the Diet of California Sea Lions *Zalophus califomlanus* at San Nicolas. *Fishery Bulletin*, *89*(2), 331–336. https://www.researchgate.net/publication/240628418

Taylor, F. J. R., & Horner, R. A. (1994). Review of the marine environment and Biota of strait of Georgia, Puget sound and Juan De Fuca Strait: Red Tides and Other Problems With Harmful Algal Blooms in Pacific Northwest Coastal Waters. In R. C. H. Wilson, R. J. Beamishz, F. Aitkens, & J. Bell (Eds.), *Proceedings of the BC/ Washington Symposium on the Marine Environment* (pp. 175–186). Canadian Technical Report of Fisheries and Aquatic Science. https://www.eopugetsound.org/sites/default/files/features/resources/ProceedingsMarineEnvSymp1994Optimized.pdf

Thompson, A. R., Schroeder, I. D., Bograd, S., Hazen, E., Jacox, M., Leising, A., Wells, B., Largier, J., Fisher, J., Jacobson, K., Zeman, S., Bjorkstedt, E., Robertson, R., Kahru, M., Goericke, R., Peabody, C., Baumgartner, T., Lavaniegos, B., Miranda, L., & Melin, S. (2019). State of the California Current 2018-19: A novel anchovy regime and a new marine heatwave? *The California Cooperative Oceanic Fisheries Investigations (CalCOFI) Rep*, 1–65. https://www.researchgate.net/publication/338902228

Thompson, A. R., Swalethorp, R., Alksne, M., Santora, J. A., Hazen, E. L., Leising, A., Satterthwaite, E., Sydeman, W. J., Anderson, C. R., Auth, T. D., Baumann-Pickering, S., Baumgardner, T., Bjorkstedt, E. P., Bograd, S. J., Bowlin, N. M., Burke, B. J., Daly, E. A., Dewar, H., Field, J. C., … Wells, B. (2024). State of the California Current Ecosystem report in 2022: a tale of two La Niñas. *Frontiers in Marine Science*, *11*, 1294011. https://doi.org/10.3389/FMARS.2024.1294011/BIBTEX

Tieszen, L. L., Boutton, T. W., Tesdahl, K. G., & Slade, N. A. (1983). Fractionation and turnover of stable carbon isotopes in animal tissues: Implications for δ13C analysis of diet. *Oecologia*, *57*(1–2), 32–37. https://doi.org/10.1007/BF00379558

Todd, E. C. D. (1993). Domoic Acid and Amnesic Shellfish Poisoning - A Review. *Journal of Food Protection*, *56*(I), 69–83. https://doi.org/10.4315/0362-028X-56.1.69

Trainer, V. L., Adams, N. G., Bill, B. D., Stehr, C. M., Wekell, J. C., Moeller, P., Busman, M., & Woodruff, D. (2000). Domoic acid production near California coastal upwelling zones, June 1998. *Limnology and Oceanography*, *45*(8), 1818–1833. https://doi.org/10.4319/lo.2000.45.8.1818

Trainer, V. L., Hickey, B. M., Lessard, E. J., Cochlan, W. P., Trick, C. G., Wells, M. L., MacFadyen, A., & Moore, S. K. (2009). Variability of *Pseudo-nitzschia* and domoic acid in the Juan de Fuca eddy region and its adjacent shelves. *Limnology and Oceanography*, *54*(1), 289–308. https://doi.org/10.4319/LO.2009.54.1.0289

Trainer, V. L., Stephen S. Bates, S. S., Lundholm, N., Thessen, A. E., Cochlan, W. P., Adams, N. G., & Trick, C. G. (2012). *Pseudo-nitzschia* physiological ecology, phylogeny, toxicity, monitoring and impacts on ecosystem health. *Harmful Algae*, 271–300. https://doi.org/0.1016/j.hal.2011.10.025

Trainer, V. L., Wells, M. L., Cochlan, W. P., Trick, C. G., Bill, B. D., Batgh, K. A., Beall, B. F., Herndon, J., & Lundholm, N. (2009). An ecological study of a massive bloom of toxigenic *Pseudo-nitzschia cuspidata* off the Washington State coast. *Limnology and Oceanography*, *54*(5), 1461–1474. https://doi.org/10.4319/lo.2009.54.5.1461

Valenzuela-Toro, A. M., Costa, D. P., Mehta, R., Pyenson, N. D., & Koch, P. L. (2023). Unexpected decadal density-dependent shifts in California sea lion size, morphology, and foraging niche. *Current Biology*, *33*(10), 2111–2119. https://doi.org/10.1016/j.cub.2023.04.026

Van Der Lingen, C. D., Hutchings, L., & Field, J. G. (2006). Comparative trophodynamics of anchovy *Engraulis encrasicolus* and sardine *Sardinops sagax* in the southern Benguela: Are species alternations between small pelagic fish trophodynamically mediated? *African Journal of Marine Science*, *28*(3–4), 465–477. https://doi.org/10.2989/18142320609504199

Venrick, E. L. (2002). Floral patterns in the California Current System off southern California: 1990-1996. *Journal of Marine Research*, *60*(1), 171–189. https://doi.org/10.1357/002224002762341294

Weise, M. J., & Harvey, J. T. (2008). Temporal variability in ocean climate and California sea lion diet and biomass consumption: implications for fisheries management. *Marine Ecology Progress Series*, *373*, 157–172. https://doi.org/10.3354/MEPS07737

Wekell, J. C., Gauglitz, E. J., Bamett, H. J., Hatfield, C. L., Simons, D., & Ayres, D. (1994). Occurrence Of Domoic Acid In Washington State Razor Clams (*Siliqua patula*) During 1991-1993. *Natural Toxins*, *2*(4), 197–205. https://doi.org/10.1002/nt.2620020408

Wekell, J. C., Hurst, J., & Lefebvre, K. A. (2004). The origin of the regulatory limits for PSP and ASP toxins in shellfish. *Article in Journal of Shellfish Research*, *23*(3), 927–930. https://www.researchgate.net/publication/285809374

Wooster, W. S., Fluharty, D. L., Washington Sea Grant Program., & International Recruitment Investigations in the Subarctic (Group). (1985). *El Niño North : Niño effects in the Eastern Subarctic Pacific Ocean*. Washington Sea Grant Program, University of Washington. https://repository.library.noaa.gov/view/noaa/45825

Zabka, T. S., Goldstein, T., Cross, C., Mueller, R. W., Kreuder-Johnson, C., Gill, S., & Gulland, F. M. D. (2009). Characterization of a degenerative cardiomyopathy associated with domoic acid toxicity in california sea lions (*Zalophus californianus*). *Veterinary Pathology*, *46*(1), 105–119. https://doi.org/10.1354/VP.46-1-105/ASSET/IMAGES/LARGE/10.1354_VP.46-1-105-FIG12.JPEG

Zeidberg, L. D., Butler, J. L., Ramon, D., Cossio, A., Stierhoff, K. L., & Henry, A. (2012). Estimation of spawning habitats of market squid (*Doryteuthis opalescens*) from field surveys of eggs off Central and Southern California. *Marine Ecology*, *33*(3), 326–336. https://doi.org/10.1111/j.1439-0485.2011.00498.x

Zeidberg, L. D., & Hamner, W. M. (2002). Distribution of squid paralarvae, *Loligo opalescens* (Cephalopoda: Myopsida), in the Southern California Bight in the three years following the 1997-1998 El Niño. *Marine Biology*, *141*(1), 111–122. https://doi.org/10.1007/s00227-002-0813-x

Zeidberg, L. D., Isaac, G., Widmer, C. L., Neumeister, H., & Gilly, W. F. (2011). Egg capsule hatch rate and incubation duration of the California market squid, *Doryteuthis* (=*Loligo*) *opalescens*: Insights from laboratory manipulations. *Marine Ecology*, *32*(4), 468–479. https://doi.org/10.1111/j.1439-0485.2011.00445.x

Appendix

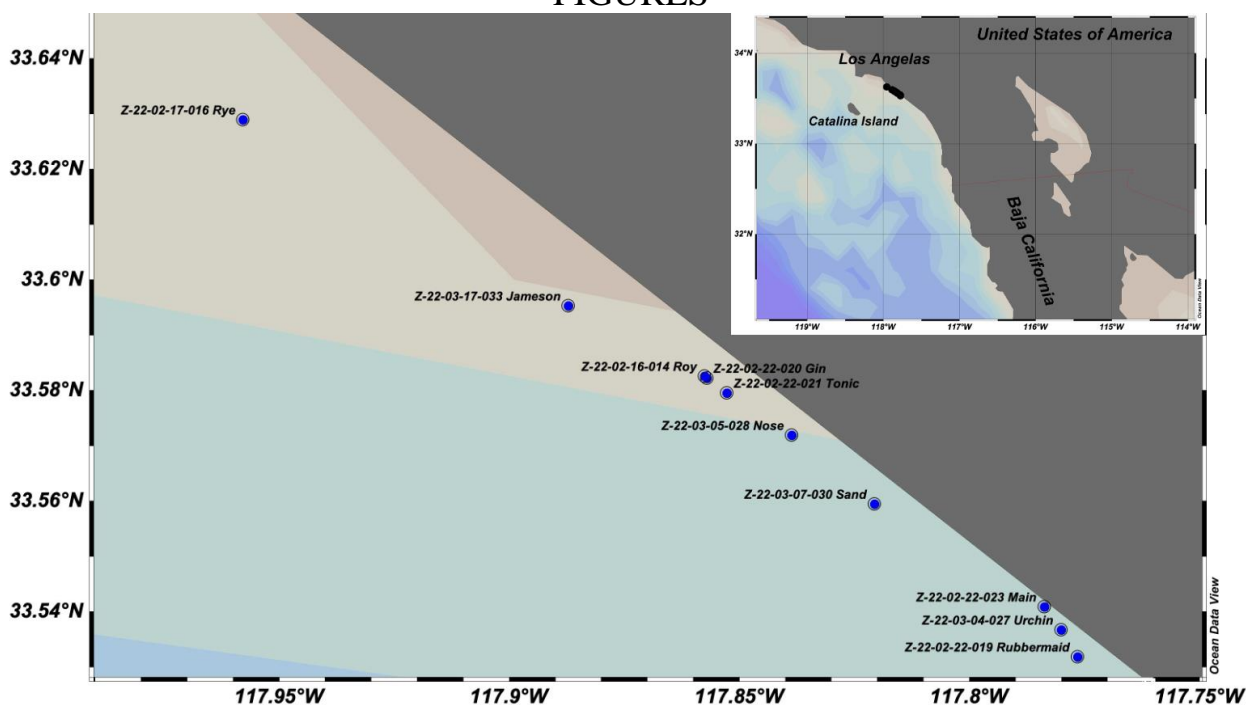**Table A1.** Full histology for CSLs (n = 10) found stranded from the 16th of February - 17th of March in Orange County, California in 2022.

Full Histology Findings				
CSL ID	**Sex**	**Maturity**	**Histology Significant Findings**	**Histology Cause of Death**
Z-22-02-22-019	Male	Subadult	Liver: Hepatitis, necro suppurative, mild, multifocal random and periportal, subacute; most of tissues too autolyzed to determine definitively	Hepatitis (R/O secondary to bacterial sepsis vs other); possible suspect DA but feces BDL for DA and no changes in heart; brain not submitted due to decomp
Z-22-03-04-027	Female	Pup	Heart: Mild, multifocal, acute myocellular swelling and karyomegaly (degeneration, presumed) and multifocal nuclear rowing	Suspect DA vs other (no significant underlying disease detected)
Z-22-03-07-030	Male	Subadult	N/A	N/A
Z-22-02-22-020	Male	Subadult	No significant findings (NSF); most of tissues too autolyzed to determine definitively	Unknown
Z-22-02-16-014	Male	Yearling	Heart: Mild multifocal nuclear rowing and karyomegaly	Suspect DA vs other (no overt lesions in hippocampus, DA was identified in the feces. Heart changes very mild and non-specific, could be an early indication of myocardial damage due to toxin exposure)
Z-22-02-22-021	Male	Subadult	Heart: Mild to moderate multifocal acute myocardial degeneration and diffuse edema; brain not submitted due to decomp; most of tissues too autolyzed to determine definitively	Suspect DA vs other (no significant underlying disease detected)
Z-22-03-17-033	Male	Yearling	Lung: Mild multifocal chronic histiocytic and lymphocytic pneumonia with few intralesional nematode *Metastrongylus* (*Parafilaroides decorus*). Heart: Moderate multifocal myocardial nuclear karyomegaly	Suspect DA vs. Other (brain was not submitted)
Z-22-02-22-023	Male	Pup	Heart: Mild multifocal nuclear rowing and karyomegaly	Suspect DA (Heart changes, while very mild and non-specific, could be an early indication of myocardial damage due to toxin exposure)

Z-22-03-05-028	Male	Subadult	N/A	N/A
Z-22-02-17-016	Male	Subadult	Heart: Minimal regional (IV septum) equivocal acute myocardial degeneration and mild diffuse edema (R/O suspect DA vs other); brain not submitted due to decomp; most of tissues too autolyzed to determine definitively	Suspect DA vs other (no significant underlying disease detected)

Table A2. Isotopic niche metrics controlled for location. Ellipses areas (SEA), ellipses corrected for sample size (SEA$_C$).

	Market squid (from CSL Stomach)	Anchovy (Baja)	Sardine (Baja)	Market Squid (MB)	Anchovy (MB)	Sardine (MB)
SEA	0.464	0.355	1.63	0.953	0.608	0.377
SEA$_C$	0.468	0.383	1.76	0.990	0.621	0.440

FIGURES

Figure A1. Site of deceased CSLs (n = 10) found stranded from the 16th of February - 17th of March in Orange County, California in 2022. Strandings took place during harmful DA bloom.

a)

b)

Figure A2. . Pictures of (a) CSL M/Subadult & F/Pup and (b) M/Subadult stomach on left and F/Pup organs on right.
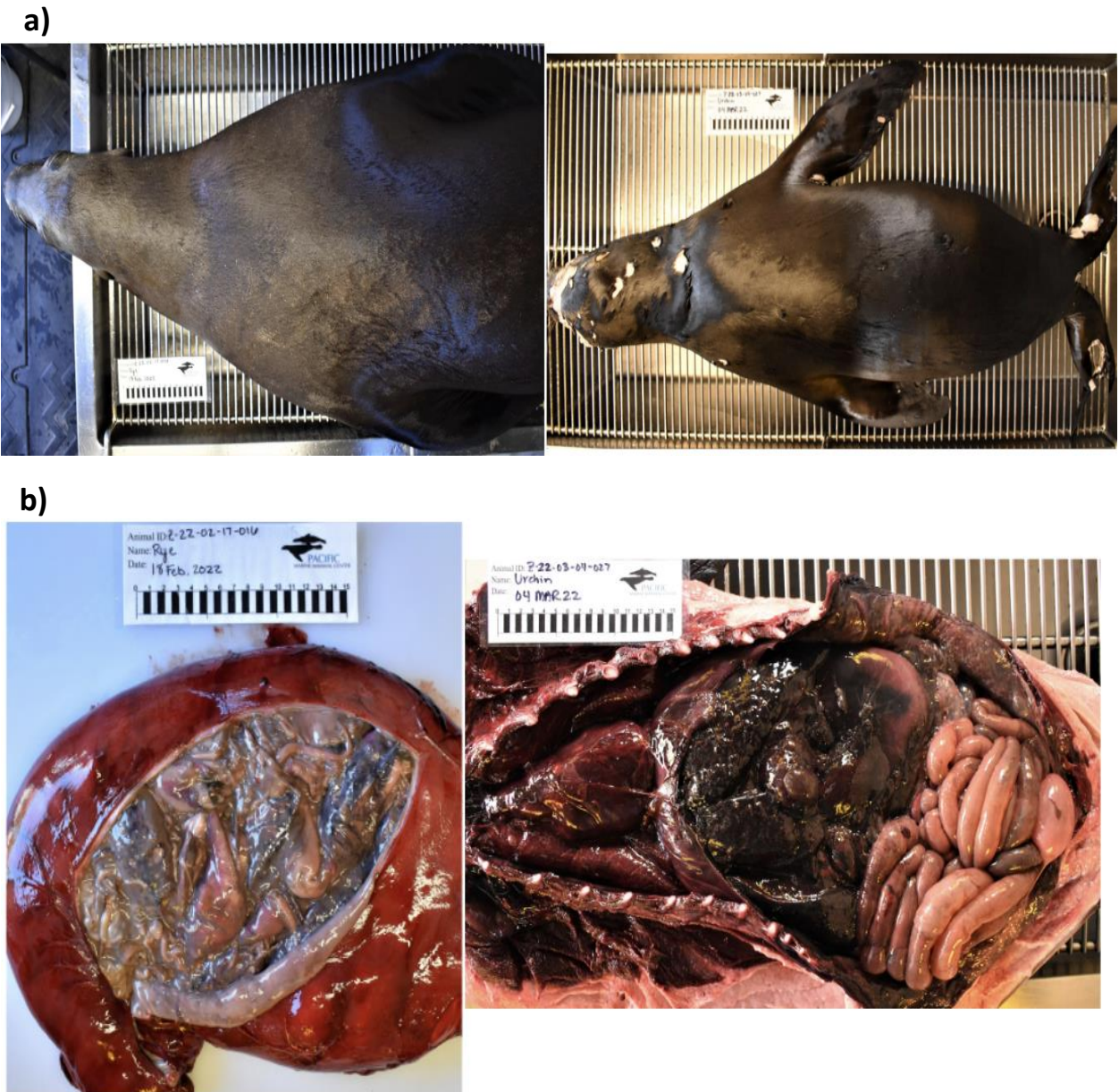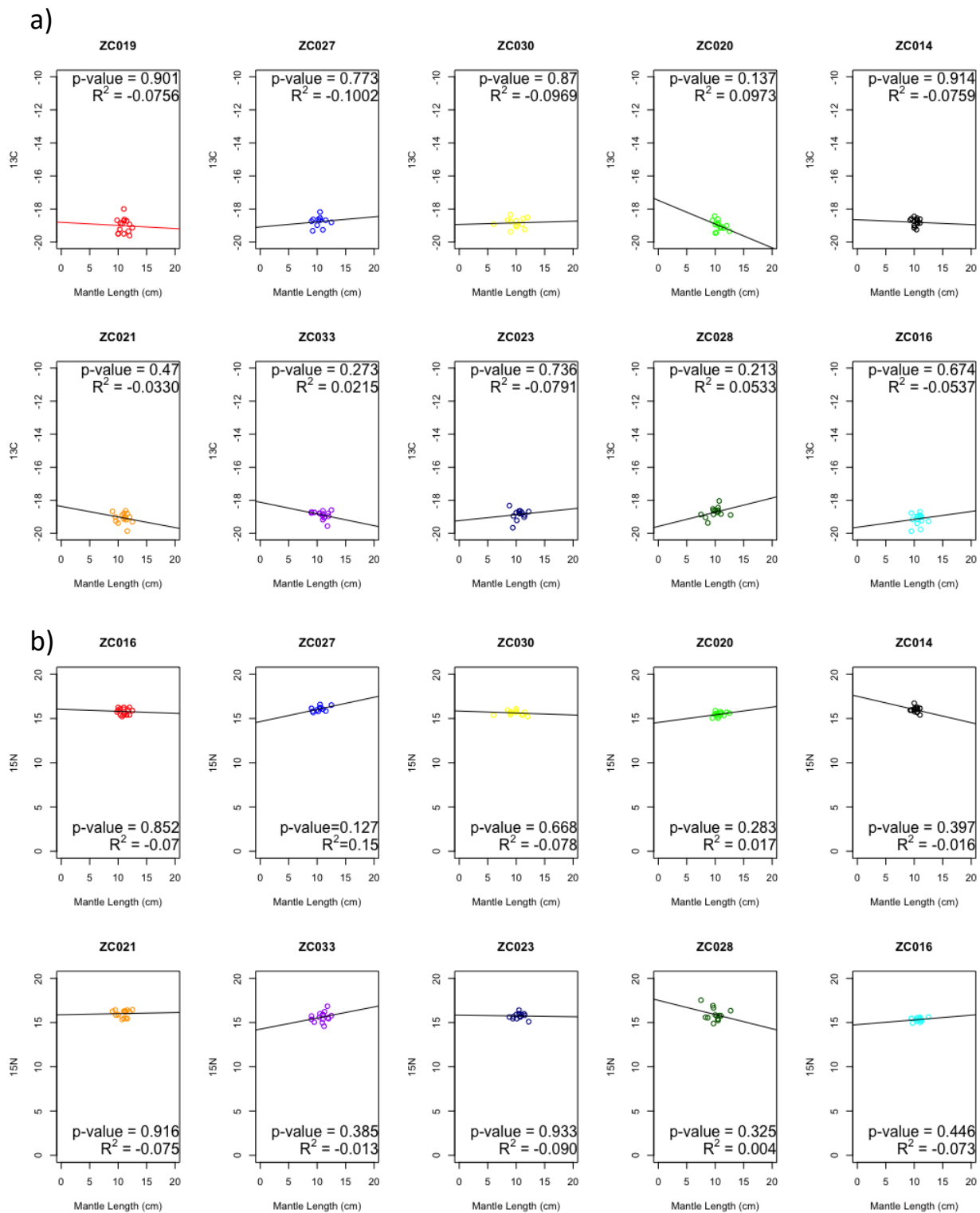
Figure A3. Simple linear regression of market squid mantle length (cm) vs. (a) δ^{13}C and (b) δ^{15}N found inside CSL stomach in 2022.
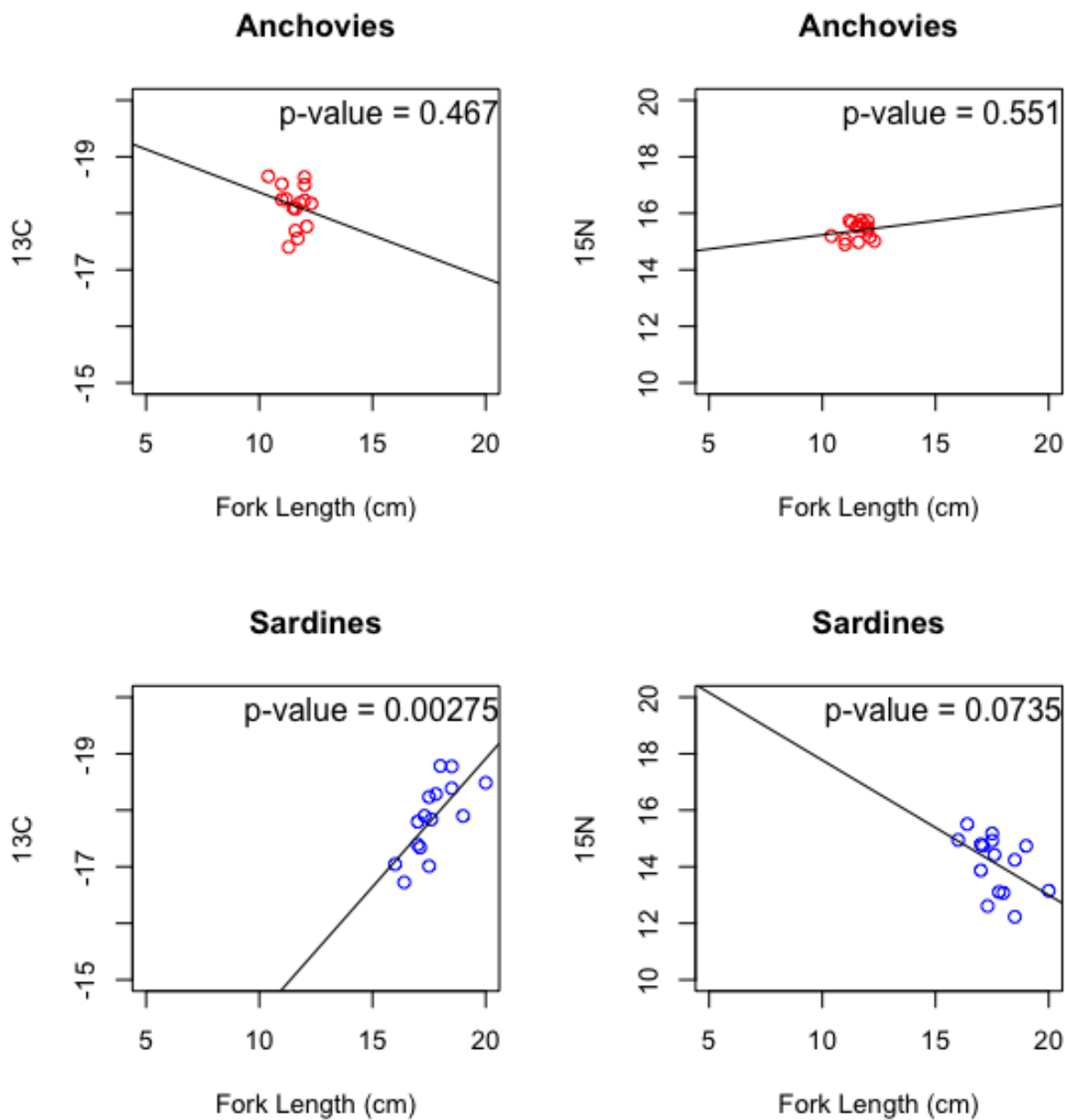
Figure A4. Simple linear regression of δ^{13}C and δ^{15}N vs. anchovy and sardine fork length (cm) collected in Ensenada, Mexico in 2023 during a toxic *Pseudo-nitzschia spp* bloom.
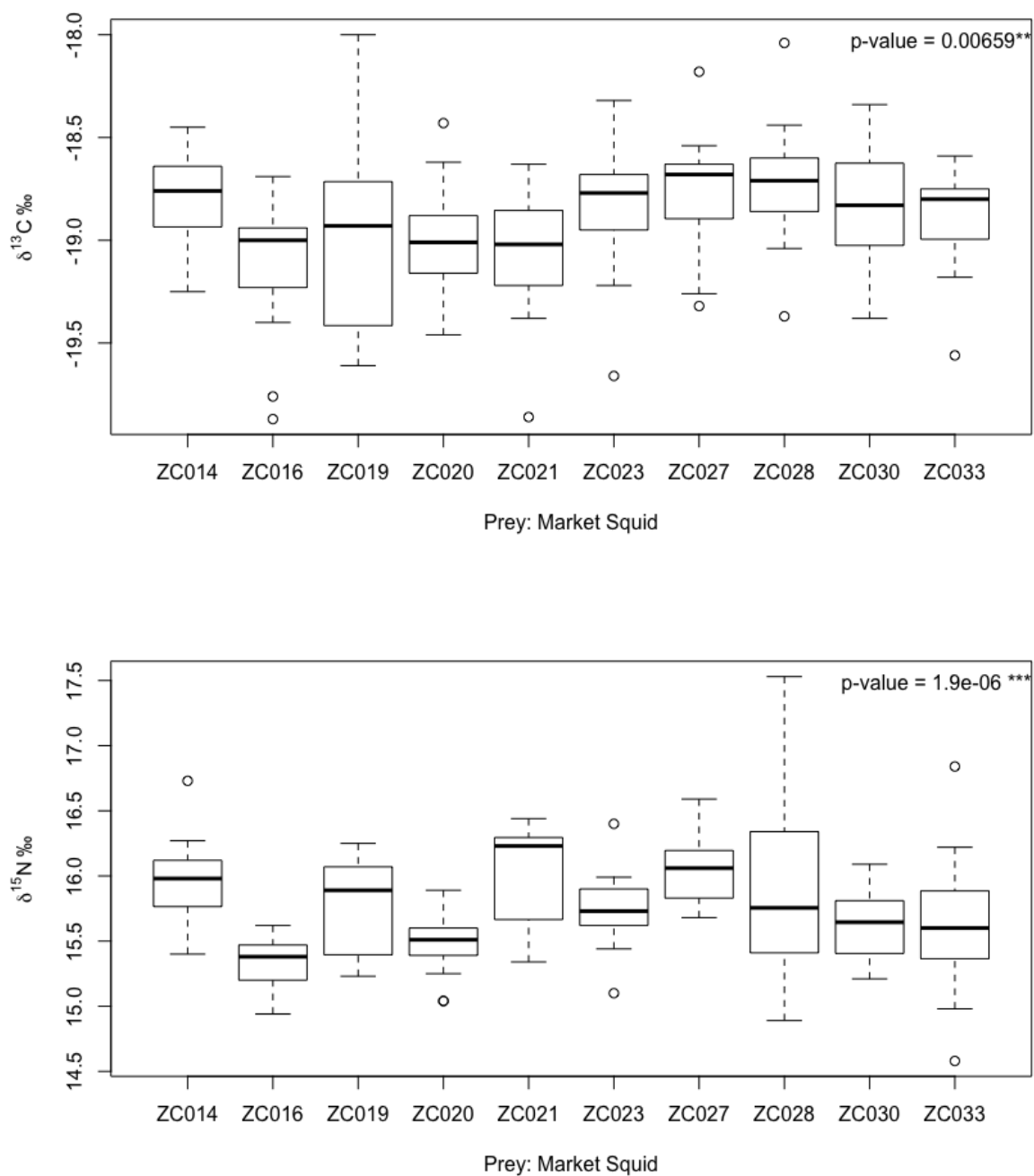
Figure A5. One-way ANOVA of market squid mantle length (cm) vs. $\delta^{13}C$ and $\delta^{15}N$. Tukey test: 13C showed statistically significant difference in comparison: ZC028-ZC016; 15N showed statistically significant difference in comparisons: ZC027-ZC020, ZC021-ZC020, ZC027-ZC016, ZC028-ZC016, ZC021-ZC016, ZC020-ZC014, ZC016-ZC014.
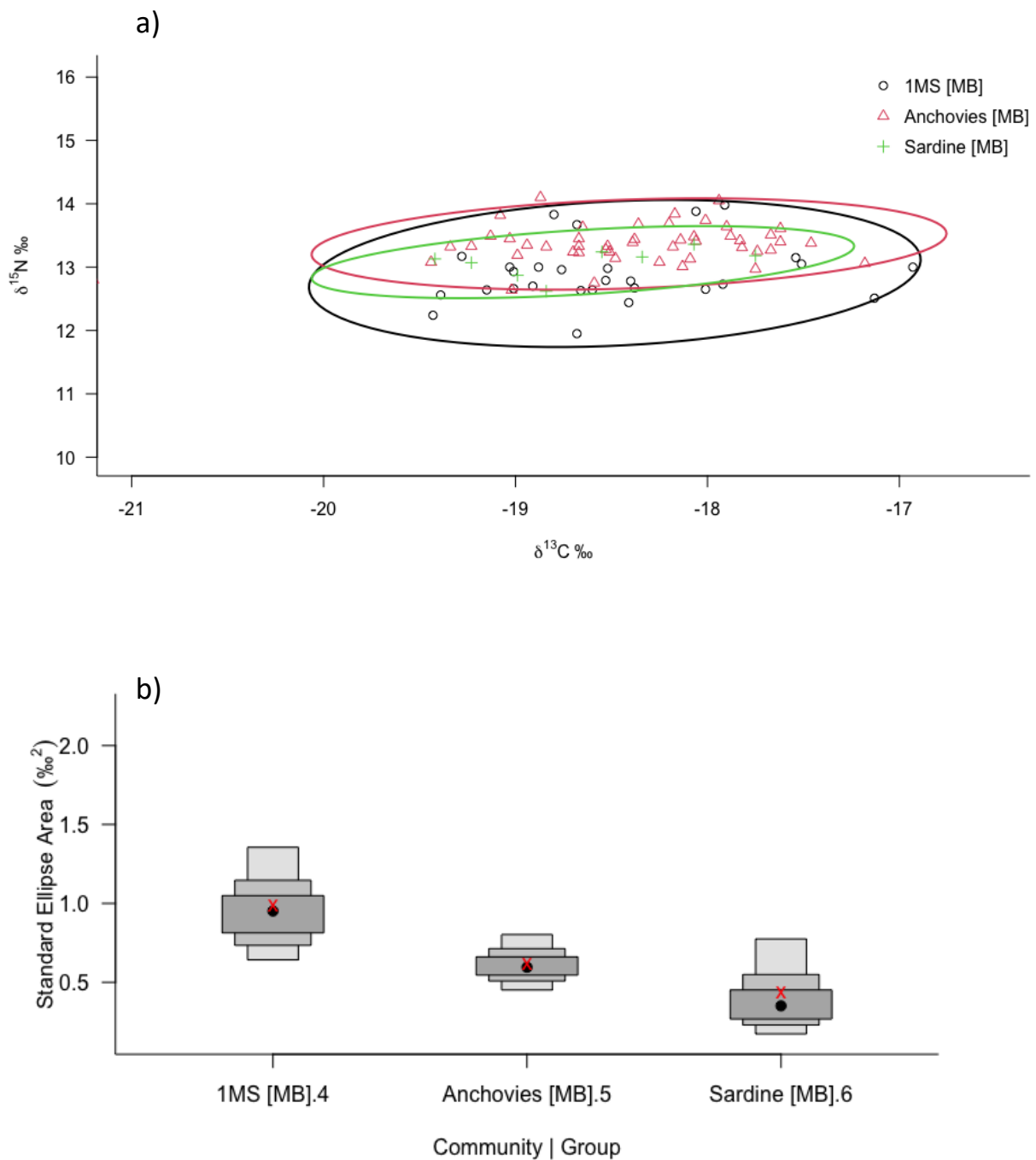
Figure A6. Bulk Bayesian (a) standard ellipses of market squid (o) (n = 28), anchovies (△) (n = 48), sardines (+) (n = 8) collected in Monterey Bay, California in 2018 (Bernstein et al, 2021); (b) Mean standard ellipses area (SEA$_b$) and 95% confidence interval associated with Figure A6a Black dots represent mean SEA$_b$ after 10,000 iterations and red x's represent ML estimates (SEA$_c$). Shaded density plots represent 50%, 95%, 99% credible intervals. Market squid (MB) and anchovy (MB) % Overlap = 52.7%.
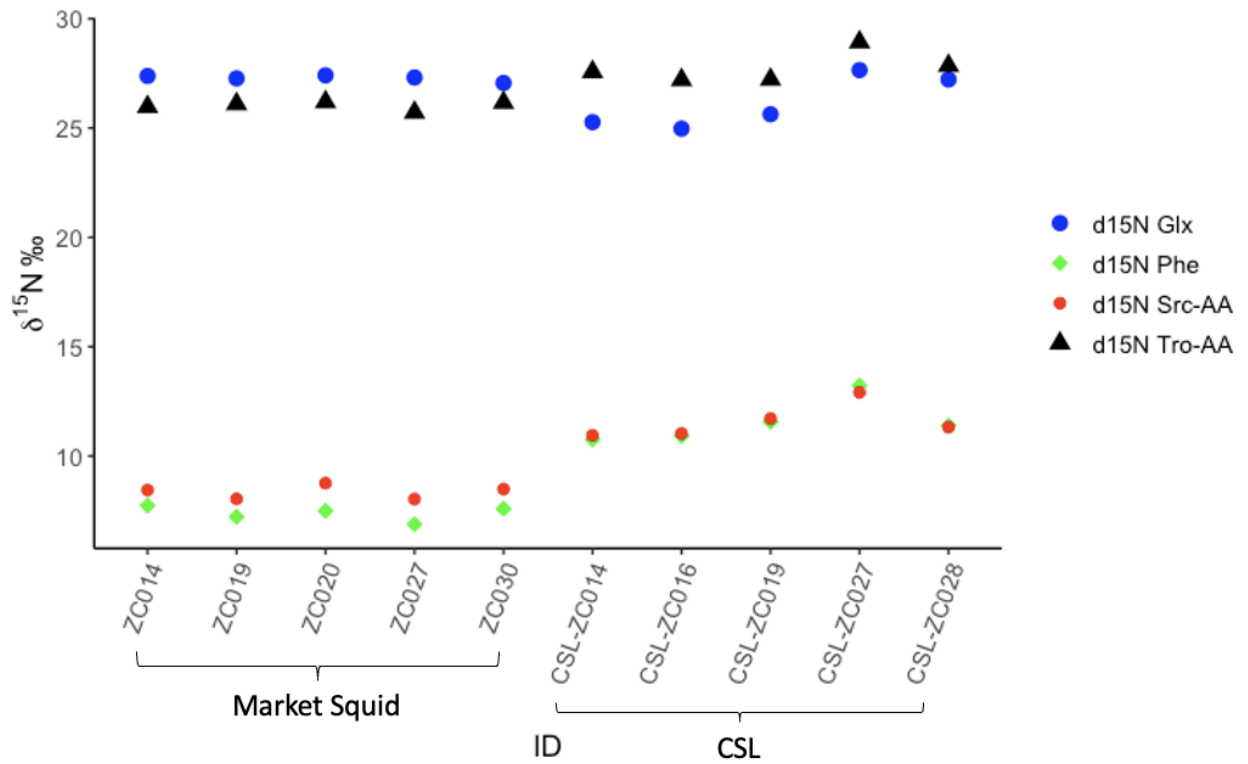
Figure A7. Utilization of $\delta^{15}N_{Glx}$ compared to $\delta^{15}N$ of CSLs and market squid to showcase glutamic acid as best trophic AA value to calculate trophic position.
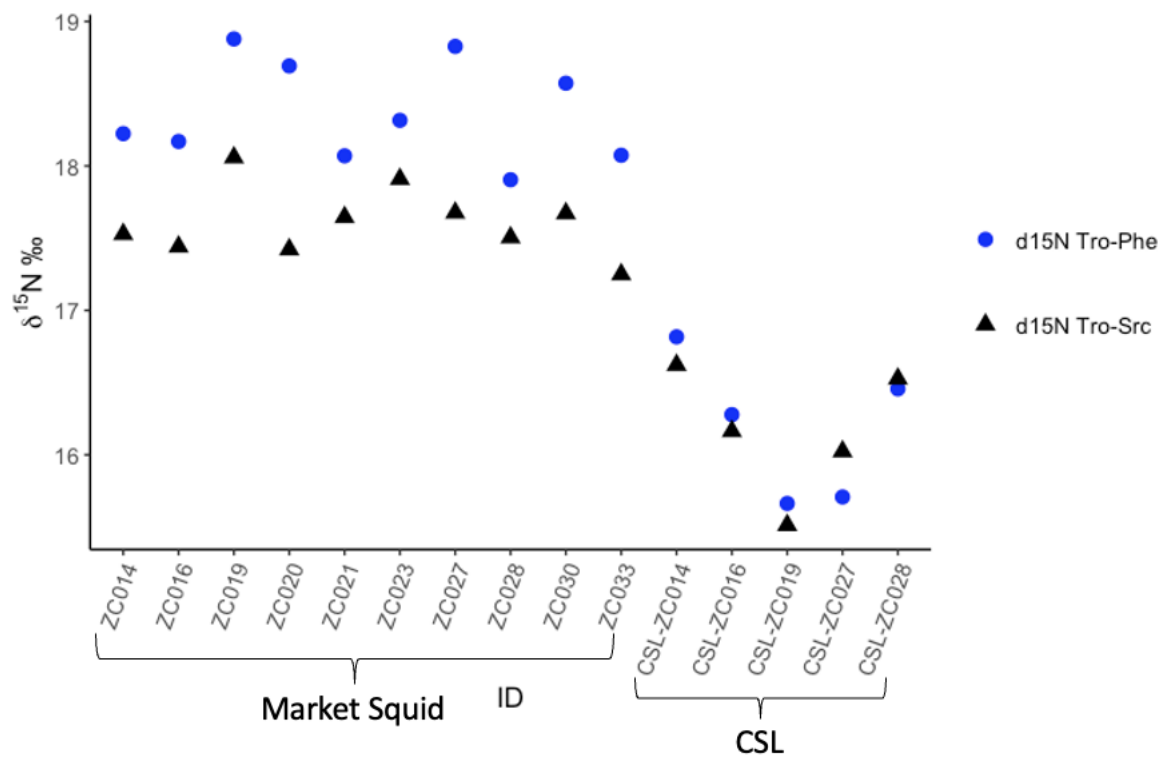
Figure A8. Utilization of $\delta^{15}N_{Tro-Phe}$ or $\delta^{15}N$ as proxies of trophic position for CSLs and market squid found in CSL stomachs from Orange County, California 2022.
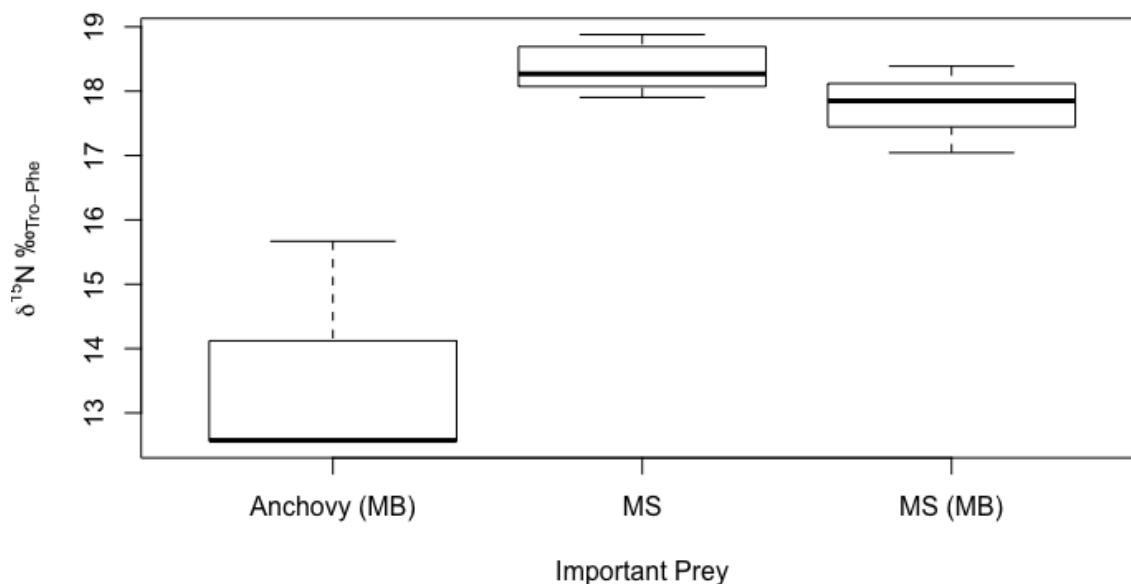
Figure A9. Boxplot of $\delta^{15}N_{Tro\text{-}Phe}$ vs. different prey items from 2 different locations (CSL stomach in 2022; Monterey Bay in 2018; and Bahía de Todos Santos, Ensenada, Baja California, Mexico in 2023).
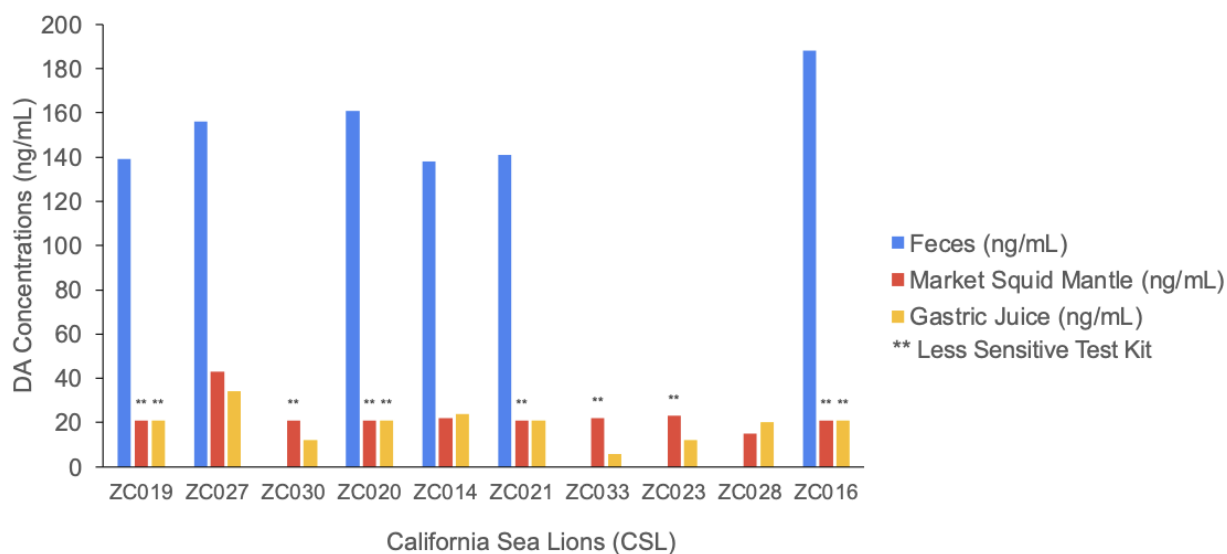
Figure A10. Domoic acid (DA; ng mL^{-1}) results in feces from California Sea Lions and market squid, and gastric juice. Values with ** indicate the use of a less sensitive test kit: min detection limit = 120 ng g^{-1}, values * indicate the use of a highly sensitive kit (min detection limit = 4.0 ng g^{-1})).