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Sex-specific diet and rockfish consumption in California sea lions (Zalophus californianus): Insights from molecular scatology

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SEX-SPECIFIC DIET AND ROCKFISH CONSUMPTION IN CALIFORNIA SEA LIONS (*ZALOPHUS CALIFORNIANUS*): INSIGHTS FROM MOLECULAR SCATOLOGY

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ABSTRACT

SEX-SPECIFIC DIET AND ROCKFISH CONSUMPTION IN CALIFORNIA SEA LIONS (*ZALOPHUS CALIFORNIANUS*): INSIGHTS FROM MOLECULAR SCATOLOGY

By Keith M. Hernandez

Molecular diet analysis has the potential to overcome the limitations of traditional methods. I used prey hard parts and molecular methods to examine sex-specific diet trends and rockfish consumption of California sea lions (*Zalophus californianus*, CSL). Fresh scat samples (n=219) were collected from Año Nuevo Island, CA (ANI), during the summers of 2013 and 2014. Prey taxa were identified from fish otoliths and cephalopod beaks recovered from cleaned scats. Sex of the CSL depositing the scat was assigned via multiplex PCR of a CSL microsatellite and a carnivore Y chromosome marker. Prey species also were identified using multiple loci in a Next Generation Sequencing framework. Twenty-two fish and 4 cephalopod taxa were identified from hard parts; additionally, 38 fish and 7 invertebrate taxa were identified from molecular data including 16 rockfish species. Hard parts data overestimated the occurrence of prey with robust hard parts whereas molecular data identified additional taxa that lacked diagnostic hard parts. More scats were assigned to females than males in both years, which may be indicative of greater female use of ANI or an increased presence of non-reproductive females within the Monterey Bay region during summer. Estimates of rockfish consumption in 2013 were similar to previous studies, but fewer rockfish were eaten in 2014 than previously reported. The increased presence of benthic and midwater prey indicated a greater prey base in Monterey Bay compared with previous studies.

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The idea for this project resulted from separate discussions with Dr. Bill Henry and MLML alum Ryan Carle, who introduced me to molecular diet studies and the science of Año Nuevo Island, respectively. This project stems from collaboration between MLML, the Farallon Institute for Advanced Ecosystem Research, the University of California Santa Cruz and Oikonos Ecosystem Knowledge. I am very grateful to Drs. Mike Weise (ONR), Julie Thayer (Farallon Institute) and Jason Hassrick (CDFW) for agreeing to the collaboration. Patricia Morris, Dr. Patrick Robinson and Adam Fox collected the samples for this project. Sample processing would not have been completed without the instruction of Jessie Beck and the assistance of Bonnie Brown, Mason Cole, Jenni Johnson and Sharon Hsu. Heather Robinson (Farallon Institute) identified the hard parts for a companion study. The SWFSC Molecular Ecology lab were an awesome, welcoming group of individuals to learn from and work with; special thanks to Diana, Libby, Cassie, Ellen, Laney and Anthony for your assistance with my research. I would

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INTRODUCTION

Top predators often regulate populations of lower trophic levels. Removing top predators results in less species diversity and greater susceptibility to invasions from non-native species (Ritchie & Johnson 2009). With the shift from single species to ecosystem-based management, understanding the relationships among species becomes necessary to improve management and conservation of target species; inherent in this is knowledge of the trophic relationships among different species. Two often-neglected aspects of trophic investigations are the potential differential impact of males and females on their food resources and the impact of fisheries on the ecological communities in which they occur.

The potential niches of animals can be influenced by physiological and behavioral differences of the sexes. In mammals the energetic demands of males and females are different due to the varied costs of reproduction and mating; females bear the costs of pregnancy and parental care, whereas males tend to allocate more energy towards maximizing reproductive potential. This difference is exaggerated in species that display sexual dimorphism (Fairbairn 1997). Among the pinnipeds (seals and sea lions), pronounced sexual dimorphism is present in all otariids (sea lions and fur seals) and three phocids, the gray seal (*Halichoerus grypus*) and elephant seals (*Mirounga* spp.; Bartholomew 1970). Otariids have a resource defense polygynous mating system, where males control access to a portion of a rookery and can potentially mate with females within their territories (Bartholomew 1970). Thus, males need to be large enough to not only defend their territories from potential competitors, but also sustain themselves

throughout the breeding season. As females are the only sex to provide parental care, females must obtain enough energy to carry a pup to term and provision it until weaning (Costa 1993). The degree of dimorphism varies among species, but in general, male otariids are 2-5 times heavier than females (Lindenfors *et al*. 2002). Given these drastic physiological differences, the ability of males and females to exploit different food resources is possible but has rarely been investigated (Bartholomew 1970).

Coastal fisheries compete for some of the same resources that top predators naturally consume (DeMaster *et al.* 2001). With the passage of the Marine Mammal Protection Act (MMPA) in 1972, most marine mammal populations in US waters have increased; this, coupled with decreasing fish stocks for certain commercially important species, has resulted in increased competition for these limited fisheries resources (Morissette *et al.* 2012). Pinnipeds compete with fisheries (DeMaster *et al.* 1982, Beeson & Hanan 1996, Morissette *et al.* 2012), and within the California Current ecosystem, most of these interactions are attributed to California sea lions (CSL, *Zalophus californianus*; DeMaster *et al.* 1982, Weise & Harvey, 2005). Therefore, it is advantageous to know what percentage of CSL diet is composed of commercially valuable fish species.

California sea lions are an abundant predator in the California Current Ecosystem. Previous studies of CSL diet indicated that they are 'plastic' predators, meaning they target seasonally abundant prey, primarily commercially important fishery species (Lowry *et al.* 1990, 1991, Weise & Harvey, 2008). Whereas sea lion diet is well studied in southern California (Lowry *et al.* 1990, 1991), only a few studies have been conducted

in other parts of their range (i.e. Baja California, Mexico [Orr 1999], and Puget Sound, Washington [Orr *et al.* 2012]). Weise and Harvey (2008) studied sea lion diet from 1997-1999 in Monterey Bay, California, and determined that the diet was dominated by a few species with predictable diet shifts, which occurred with changes in prey density throughout the year. During an El Niño year, CSLs consumed less market squid (*Doryteuthis* [= *Loligo*] *opalescens*) and rockfish (*Sebastes* spp.) and a greater amount of salmon (*Oncorhynchus* spp.), mostly by depredating fish that were already on a fishing line (Weise & Harvey, 2008). Weise (2006) continued CSL diet sampling at Año Nuevo Island, California, from 2001 to 2005; however, the diet data were not presented in the same level of detail, and instead were used to reconstruct percentage mass of three taxa (market squid, rockfish, and sardines [*Sardinops sagax*]). Schooling fishes were still the predominant prey species in all years with changes in composition likely due to oceanographic changes in the California Current (Weise 2006).

The California Current is a highly productive eastern boundary current prone to drastic oceanographic changes. These changes in oceanographic conditions are due to the El Niño-Southern Oscillation (ENSO, McGowan *et al.* 1998) and the Pacific Decadal Oscillation (PDO, Chavez *et al.* 2003). These processes, along with seasonal and annual changes in ocean conditions, have a bottom-up effect on the prey base; during ENSO years, species tend to disperse to more northern waters, likely in search of prey resources, which also move north (McGowan *et al.* 1998). Community changes over longer time scales (20-30 years) are due to a phase shift in the PDO (Chavez *et al.* 2003). Long-term monitoring programs indicated that the diets of higher trophic level predators, such as

seabirds and marine mammals, act as sentinels of prey composition (Ainley *et al.* 1995, Mills *et al.* 2007). Community changes during ENSO years also are reflected in predator diets (Weise & Harvey 2008).

Two fish taxa of particular commercial and ecological importance in the California Current ecosystem are salmon and rockfish. Salmon and rockfishes have historically supported tribal, commercial, and recreational fisheries (Love *et al.* 2002, Love 2011). Long-term commercial fishing operations and periods of little to no regulation have led to a reduction in catches of both taxa, with many salmon stocks and rockfishes considered overfished (Lackey 2002, Love *et al.* 2002, Love 2011). In central California, most salmon taken in fishing operations are Chinook salmon (*Oncorhynchus tshawytscha*) that spawn in the Central Valley of California (Weise & Harvey 2008). Within the Central Valley watershed, there are three genetically distinct spawning groups (termed Evolutionarily Significant Units, ESUs, Waples 1991), two of which are listed under the Endangered Species Act (ESA, Weise & Harvey 2005). The diversity of life history strategies used by rockfishes has resulted in differential rebuilding times of certain species, with longer-lived epibenthic species generally experiencing slower recovery than shallower-water species (Love *et al.* 2002). Whereas scientists and resource managers acknowledge that predators impact the recovery of target species, this is difficult to quantify. Because CSLs are predators of salmon and rockfishes, it would be useful to know which species they are consuming.

The foraging behavior of CSLs affects diet. Studies of predator diets aim to reconstruct the actual diet, and the diet consists of the taxa consumed, the number in

which they were consumed, and estimated lengths and weights of the consumed prey. Tagging studies of CSLs in southern (Melin *et al.* 2008) and central California (Weise *et al.* 2006, 2010) indicated that sea lions foraged to the edge of the shelf break and to approximately 300 m depth (Melin *et al.* 2008). Weise and colleagues (2010) showed that adult males foraged further offshore and dove to greater depths compared with animals (typically sub-adult and adult females) tagged in other studies. Whereas females and males likely used different prey resources, this difference was rarely determined from scat sampling because scats were collected after deposition on land and the sex of the animal was usually unknown. The sex could be determined if researchers made direct observations, or samples were collected at sex-segregated haul-outs (Orr *et al.* 2012). One method to determine the sex of the CSL from which the scat was deposited is to amplify their DNA. Reed and colleagues (1997) amplified a gene on the sex chromosomes of harbor seals (*Phoca vitulina vitulina*) using primers developed to amplify the *SRY* sex-determining gene found in mammals. If one sex was found to consume a particular resource more frequently than the other, this could help to improve our understanding of diet and inform management actions.

Diet of marine mammals has typically been assessed using one of several methodologies. Most researchers analyzed prey hard parts (sagittal otoliths and cephalopod beaks) found in scats of free-ranging individuals or the stomachs of stranded or by-caught animals (Olesiuk 1993). These structures are usually identified to the species level, and allowed for quantification of descriptive metrics (Lance *et al.* 2001). Additionally, prey size was estimated based on linear relationships between otolith or

beak size and total length (Harvey *et al.* 2000). Due to differential digestion rates of hard parts and feeding behavior (e.g., larger prey may not be entirely consumed) however, hard part analysis tended to underestimate the contribution of larger prey species and those with small or non-existent hard parts (Orr & Harvey 2001, Arim & Naya 2003, Sweeney & Harvey 2011). Opportunistic observations of CSL foraging on large prey items also indicated that the head was rarely consumed, which meant that the otoliths of these prey items would not appear in scats or stomach samples (Weise 2005). Also, certain structures cannot be identified to the species level, because they are not distinctive (e.g., otoliths from *Sebastes* spp. and *Oncorhynchus* spp., Lance *et al.* 2001). The use of the all-structure method (Lance *et al.* 2001) has resolved some of these shortcomings. This technique uses additional identifiable structures, such as atlas vertebrae, gill rakers, and elasmobranch denticles or teeth, to identify prey taxa consumed by the predator, and has been useful in resolving the presence of salmonids in the diet (Weise 2000). In addition, the calculated metrics are sensitive to the biases inherent in digestion. Although prey-specific indices have been proposed to reconcile these biases, they are only successful to an extent (Brown *et al.* 2011). Given these biases, researchers have turned to alternative methods for reconstructing marine mammal diets.

Biochemical techniques, such as stable isotope (SI; Kelly 2000, Post 2002) and fatty acid (FA; Budge *et al.* 2006) analyses have been used to obtain long-term trends in carnivore diets. SI analysis determines trophic position based on the principle that predators incorporate into their own tissues heavier isotopes of carbon and nitrogen from the tissues of their prey species. FA analysis operates on a similar principle; dietary fatty

acids from prey species are incorporated into the adipose tissue of the predator, and provide the longest record of diet among the existing techniques (Budge *et al.* 2006). These techniques, however, rely upon large libraries of SI or FA signatures of all potential prey items, which are expensive and logistically difficult to obtain. Whereas SI analysis can infer trophic position and foraging habitat, it is not species specific, with nitrogen signatures reflecting the average trophic level of all prey consumed at the time the tissue was synthesized. In addition, the accuracy of calibration coefficients required for FA analysis has been called into question (Rosen & Tollit 2012).

Modern molecular analysis of fecal DNA incorporates concepts of molecular scatology and DNA barcoding. The DNA barcoding method aims to identify every species based on diagnostic DNA loci (Tautz *et al.* 2002, 2003, Hebert *et al.* 2003, Savolainen *et al.* 2005). Specific loci, such as mitochondrial *16S* or cytochrome c oxidase subunit I (*COI*), function as DNA barcodes due to their high similarity within a species and lesser similarity among species (Savolainen *et al.* 2005, Ward *et al.* 2005). Certain taxa that have undergone recent radiations, however, may not contain enough variability within a barcoding locus to be differentiated at the species level (Moritz & Cicero 2004). Alternative loci that contain diagnostic variable sites would be required to distinguish species in these recently radiated groups (Pearse *et al.* 2007). Molecular scatology uses modified forensic techniques to identify species, sex, and individual from fecal samples for application in field studies of wide-ranging vertebrates (Höss *et al.* 1992, Constable *et al.* 1995, Reed *et al.* 1997). Early work into identifying prey species from fecal or gut DNA often used targeted assays for species of interest, for example,

agricultural pests (Symondson 2002) or commercially important fishery species, such as salmonids (Kvitrud *et al.* 2005). Rapid advances in technology now allow for the identification of multiple taxa in hundreds of samples through Next Generation Sequencing frameworks (NGS, Symondson 2002, Valentini *et al.* 2009, Pompanon *et al.* 2012). Fecal DNA often is degraded, with prey DNA in much lesser quantities than the predator's DNA, and amplification is difficult if a sample is not preserved within 48 hours of deposition (Symondson 2002, Valentini *et al.* 2009, Pompanon *et al.* 2012). This can make difficult the amplification of barcoding loci, which are typically hundreds of base pairs in length. However, previous studies on free-ranging animals have been successful, and the sequencing data were usually highly concordant when compared with a paired data set, such as from prey hard parts (Tollit *et al*. 2009, Pompanon *et al.* 2012). Analysis of prey DNA often results in greater species diversity compared with hard parts, especially for taxa that lack diagnostic hard parts or may not be entirely consumed (Pompanon *et al.* 2012). Disparities, however, appear when trying to compare relative abundances of amplicons (PCR products) to proportion of prey taxa consumed (Deagle *et al.* 2007, 2010). To date, it is not yet possible to accurately estimate the number or size of prey from amplicon data, although some captive feeding studies are beginning to develop correction factors (Thomas *et al.* 2014, 2016). Until these advancements are made, Pompanon and colleagues (2012) recommended reporting relative prey abundances using molecular analysis in conjunction with a technique such as hard parts or stable isotope analysis to get the most comprehensive reconstruction of a predator's diet.

The objectives of this thesis were to: 1) quantify CSL diet during two years, 2) investigate sex-specific diet trends, 3) identify species composition of rockfishes and salmon in CSL diet, 4) compare diet metrics calculated from the hard parts and molecular data to investigate under- and over-representation of prey taxa, and 5) estimate CSL consumption of rockfishes. I hypothesized that CSL diet would not significantly differ between sampling years due to similar oceanographic conditions. Given that CSLs are a sexually dimorphic species, I hypothesized that male CSLs would likely have more offshore and deeper water prey species in their diet than female CSLs. Based on previous research (e.g., Weise & Harvey 2008, Sweeney & Harvey 2011), I hypothesized that sea lions that consumed rockfishes and salmon likely consumed mostly shortbelly rockfish (*S. jordani*) and Chinook salmon (*O. tshawytscha*). I predicted that hard parts data would over-represent cephalopods and under-represent elasmobranchs and teleosts with small otoliths, such as salmon. Finally, I predicted that CSLs that consumed rockfishes did so in similar levels as the last reported estimates (Weise & Harvey 2008), and primarily would have consumed young-of-the-year *S. jordani*.

METHODS

Study site and field methods

Año Nuevo Island (37º 6’ 30” N, 122º 20’ 3” W, ANI) is a small island 32 km northwest of Santa Cruz, California that has historically supported the largest CSL haulout site in the Monterey Bay region (Orr & Poulter 1965). California sea lion scats were collected during the summers (April to August) of 2013 and 2014 from several locations on the island based on the absence of sympatric Steller sea lions (*Eumetopias*

jubatus). Personnel collecting scats travelled around the terraces by crawling to avoid flushing sea lions, nesting Brandt's Cormorants (*Phalacrocorax penicillatus*), and Western Gulls (*Larus occidentalis*). Scats less than 48 hours old were targeted for collection. Scats were collected by hand using inverted plastic bags. Sample bags were labeled with the date and location of collection. Gloves were worn at all times to protect personnel from zoonotic diseases. Scats (n=219) were frozen at -20 ºC at Long Marine Laboratory until transfer to Moss Landing Marine Laboratories where they were stored at -20 ºC until they were processed.

Sample Processing

Before processing, scats were thawed for easier manipulation. Scats were homogenized by hand to ensure they were well mixed; previous studies have noted that DNA distribution in a scat is not homogenous (Deagle *et al.* 2005). Approximately 6 to 15 ml of soft matrix (dependent on the sample's weight) was transferred into labeled plastic tubes and refrozen at -20ºC. The remaining scat was cleaned and processed using the washing machine method (Orr *et al.* 2003). Individual scats were weighed and placed in color-coded mesh bags. Several scats were loaded into a washing machine and washed on the "low" setting. The remaining material (hard parts, sediments, rock, etc.) was transferred into a 500-μm sieve and any remaining fecal material was washed through the sieve. The remaining hard parts were transferred to labeled petri dishes and squid beaks were removed, counted and stored in glass vials containing isopropanol. Hard parts were dried in a food dehydrator until all water evaporated (typically 1-2 days). Hard parts were identified to the lowest taxonomic level possible and enumerated at the Farallon

Institute for Advanced Ecosystem Research using established reference collections and photographic guides (e.g., Clarke 1986, Harvey *et al.* 2000).

Molecular Methods

DNA was extracted from frozen soft matrix using Qiagen® DNA Stool Mini Kits following the manufacturer's instructions for "DNA isolation from larger amounts of stool." To determine that the extractions were proceeding correctly, a preliminary test for DNA presence was conducted by amplifying two sea lion microsatellite primers via PCR and visualization of products on a 1.5% agarose gel. All samples did not amplify marker *Zcw*A05 (Hoffman *et al*. 2007) and contained products of the expected size for marker *Zc*CgDh1.16 (Hernandez-Velazquez *et al.* 2005); this marker was used in downstream tests as a positive control (see Appendix 1 for additional details). Extracted DNA was diluted into ddH_2O at a 1:20 concentration.

Diluted DNA was screened for sea lion sex chromosomes using carnivore *SRY* markers. The sex-determining region of the Y chromosome (*SRY*) was targeted for amplification using primers developed for carnivores (Tablerlet *et al*. 1993, Dallas *et al*. 2000). Initial tests indicated that the expected product size (~70 bp) overlapped with the primer dimer signal. The reverse primer presented by Dallas and colleagues was shifted ~30 bp in the 3' direction to obtain a product approximately 99 bp in length. These primers were multiplexed with the aforementioned microsatellite primer as a positive control and to determine sex of sea lions (Table 1). PCRs were performed on a BioRad S1000^{TM} thermal cycler. Reaction conditions were 95 ºC/2 minutes, 10 cycles of 95 ºC for 30 seconds, 48 ºC for 30 seconds and 72 ºC for 45 seconds, 35 cycles of 89 ºC for 20

seconds, 50 ºC for 30 seconds and 72 ºC for 45 seconds, and a final extension of 72 ºC for 5 minutes. Reactions (15 μl per well) contained 5.81 μl of sterile water, 1.5 μl of GeneAmp®10X PCR buffer II (100 mM Tris-HCl, pH 8.3, 500 mM KCl), 0.9 μl of 25 mM $MgCl_2$, 0.6 μl of 10 mM dNTPs, 1.0 μl of 5 μM each primer, 0.035 μl of 5U/μl AmpliTaq® DNA polymerase, 0.15 μl of 0.1 μg/μl Bovine Serum Albumin (BSA) and 4 μl template DNA. PCR products were visualized on 3.0% agarose gels and scored as female (one band) or male (two bands, Fig. 1).

Table 1. DNA primers used in the sex assignment assay, including expected size range, annealing temperature (T_a, ºC), sequence and reference. Size ranges are in base pairs (bp).

Primer name	Size range	T_a	Primer Sequence	Reference
*Zc*CgDh1.16	165-170	63	F: CATAACACTCTCCAGTTCCATC R: TAGCAGCAATGTCCCCAATAG	Hernandez-Velasquez *et al.* 2005
*Zcw*A05	96-140	46-48	F: CACTTCACTTCAGCGTCAGTCT R: CTCTTGGCTCCTACAGACATCGT	Hoffman *et al.* 2007
Lut-SRY	70	55	F: GAATCCCCAAATGCAAAACTC R: GGCTTCTGTAAGCATTTTCCA	Modified from Dallas *et al.* 2000*

*The forward primer presented by Dallas was shifted ~20 bp upstream to obtain a larger product size (~99 bp).

To ensure the reproducibility of the assay, a subset of 41 samples was rerun to confirm sex assignments (see Appendix II for additional details). Samples that did not amplify either marker could not be scored as originating from CSLs and were excluded from further analyses. To facilitate Next Generation Sequencing (NGS) analysis, a subset of 192 samples for which sex was determined was selected for prey identification; as the number of samples ranged between one and 19 per sampling day, samples were excluded from days in which more than 10 samples were collected.

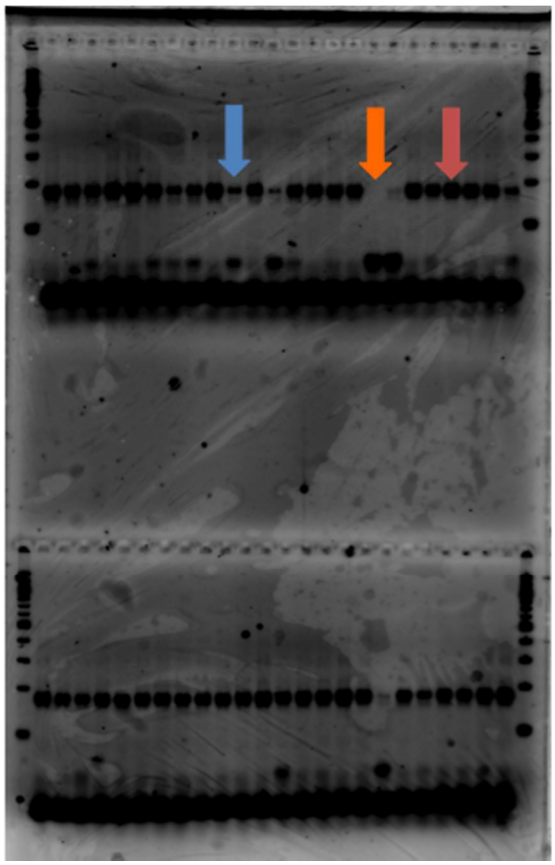

Fig. 1. Representative gel electrophoresis image from the sex assignment assay. An assignment with two bands (blue arrow, top row left) indicates a male CSL and a single band (red arrow, top row right) between 100 and 200 bp indicates a female CSL. Indeterminate assignments (orange arrow, top row middle) either contained a single band below 100 bp or no bands. A standard 1 kb DNA ladder is included in the first and last well of each sample row.

Multiple loci were screened for their ability to distinguish between the rockfishes. Traditional barcoding regions, such as mitochondrial *16S* and cytochrome oxidase c subunit I, do not have the power to distinguish among the different rockfish species (Pearse *et al*. 2007). To resolve this, a panel of 192 Express-Sequence Tag (EST) and double-digest Restriction-Associated DNA (ddRAD) loci (hereafter, rockfish panel) developed from Kelp Rockfish (*Sebastes atrovirens*) and conserved in Cabezon (*Scorpaenichthys marmoratus*) were tested for their ability to discriminate among rockfish species (Baetscher *et al.* unpublished). As these loci display variation between rockfish species and are conserved in Cabezon, these loci should contain interspecific variation that would be useful in discriminating among the rockfish species (Hyde & Vetter 2007). An initial experiment to confirm this assumption was tested on the Illumina MiSeq platform using a modified version of the Genotyping-in-Thousands by sequencing protocol (GT-seq, Campbell *et al.* 2015). The rockfish panel (Table 2) was tested on 48 individuals of known species for 16 species of coastal rockfishes and Cabezon (Table 3). Loci were screened for their ability to differentiate among the 16 rockfish species based on minor allele frequencies of single nucleotide polymorphisms (SNPs) and the panel was narrowed down to 12 loci.

Prey species were identified using a Next Generation Sequencing (NGS) approach. Standard primers recommended for DNA barcoding studies amplify a nearly 700 bp fragment of the 5' end of *COI* (Ivanova *et al.* 2007, Geller *et al.* 2013). Primers were designed to amplify a portion of the 5' region that would be diagnostic for potential

prey taxa and compatible with the rockfish panel for sequencing. *COI* sequences for each of the species identified from hard parts analysis and species found in a previous Monterey Bay study (Weise 2000) were downloaded from GenBank and aligned in Geneious (Kearse *et al.* 2012) to form a local reference database. The local reference database also contained *COI* sequences for potential sources of contamination, including nesting seabirds, sympatric pinnipeds, and humans.

Table 2. Primers used in the *Sebastes* panel including expected product size (bp), the number of variable sites and primer sequences. All loci are from Baetscher et al. (unpublished).

Primer name	Product Size	Variable Sites	Primer Sequence
Sat_EY186501	128	1	F: CGGAGGCAAGTAAGACAGCT R: CTGAGCCTTCTACCACGCAA
Sat_140	101	3	F: TGATGCTTCAACATCTGTGATCT R: TGAGTGAGTTTATACAAGGGTAAACC
Sat_851	129	2	F: AACAAATGGTGAGCCGTGTT R: TGCAGTAACAGATACAGTTATTGTCT
Sat_1458	119	3	F: CTGCTCCAGGTAAGCGTTCA R: TGCGTTAAACAAGTATGCTAGAGC
Sat_1595	116	2	F: TCTAGAAGCTGTCAAAGTGTACTT R: AGCATTATATCACATGCTTGGCA
Sat_1613	129	2	F: TTCATCCAATTGCTGTTGGC R: TGGACGCCGCTGACAATATT
Sat_1748	101	4	F: CCTGCTGATGACATATATGTGGA R: CTACCCCTCTGACAGCCTGA
Sat_2009	113	6	F: CGATTTCAGGTTCCTGGTTTTGT R: TGTAGGAAAAGCACAGACGT
Sat_2157	112	3	F: GTCGGGTCTCCTTCAATGGT R: TTGGTGTTTAAGTAACCAGTGAATT
Sat_2208	114	2	F: AGCCACCAGAAAGAGTTACGT R: TGATGGTGGAGTGGATGATGG
Sat_2468	119	3	F: GCAAAATGGTAATCAAGTGTTGCA R: AGGCATTTTCTTAAAGACTATTCCCA
Sat_2635	117	2	F: GGGTATCTGATTACATTACCTCACA R: TCGTCGACTTTGCTTCTCCT

Table 3. Rockfishes used to test the panel of EST and ddRAD loci for their ability to distinguish among different species. Sample size (*n*) indicates how many individuals of each species were used to test the rockfish panel.

Common name	Scientific name	n
Kelp rockfish	*Sebastes atrovirens*	2
Brown rockfish	*S. auriculatus*	3
Gopher rockfish	*S. carnatus*	3
Copper rockfish	*S. caurinus*	3
Black-and-yellow rockfish	*S. chrysomelas*	3
Widow rockfish	*S. entomelas*	3
Yellowtail rockfish	*S. flavidus*	3
Chilipepper rockfish	*S. goodei*	3
Squarespot rockfish	*S. hopkinsi*	3
Shortbelly rockfish	*S. jordani*	2
Black rockfish	*S. melanops*	3
Blue rockfish	*S. mystinus*	3
Bocaccio	*S. paucispinis*	3
Canary rockfish	*S. pinniger*	3
Stripetail rockfish	*S. saxicola*	3
Olive rockfish	*S. serranoides*	3

Primers were designed using Primer3 (Rozen & Skaltesky 1999), implemented in Geneious. On each alignment (e.g., for fishes and cephalopods) primers amplified a fragment between 100 and 130 bp in length that would be useful for species identification. Primer sequences were tagged with the proprietary Illumina small RNA and Read2 primers for compatibility with the GT-seq method. These primers were included with the rockfish panel to identify non-rockfish prey taxa in the samples. Prey DNA was sequenced using the modified GT-seq protocol, except that the thermal cycler conditions for PCR 1 followed those used by Thomas *et al*. (2014).

Bioinformatics

Raw sequence data were processed in QIIME (Caporaso *et al.* 2010) and Galaxy via the public Galaxy server (http://usegalaxy.org/, Afgan *et al*. 2016) following the workflow provided in Figure 2. Briefly, paired ends of demultiplexed (sequences were assigned to a sample based on a unique barcode) sequences were joined. Primer sequences were trimmed and a Phred score filter of 30 was applied to remove low-quality base calls from the data. The USEARCH quality filter pipeline (usearch_qf, Edgar 2010) was used to filter sequence data, remove chimeric sequences with UCHIME (Edgar *et al*. 2011) and cluster sequences into Operational Taxonomic Units (OTUs); which should equate to species or closely related species groups found in the diet samples. Amplicons that did not meet the percent similarity threshold to match an existing reference sequence were considered *de novo* OTUs. A representative sequence from each OTU was selected for identification and downstream analysis.

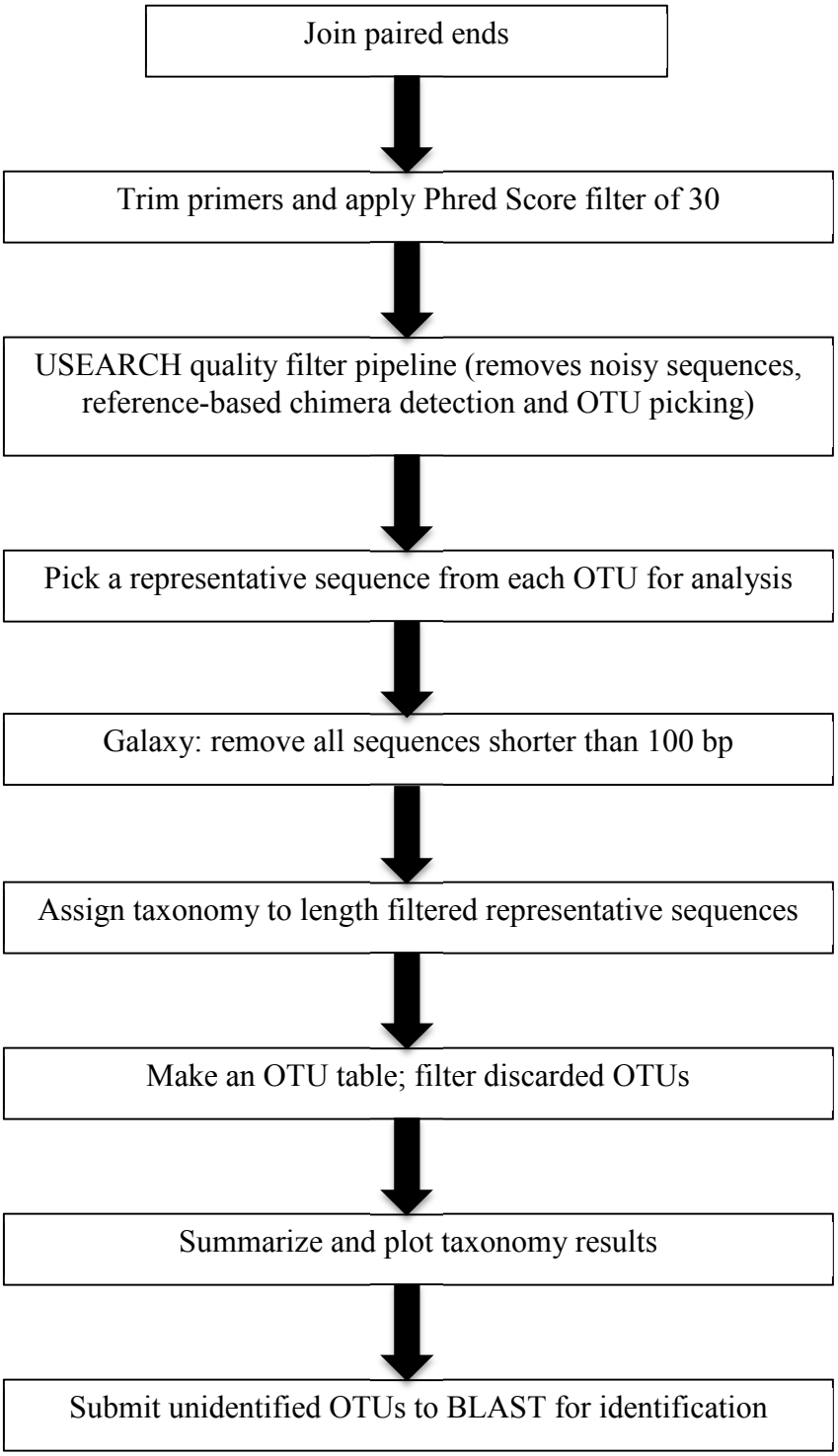

Fig. 2. Flowchart detailing the bioinformatics workflow for processing DNA sequences in QIIME and Galaxy. Unless otherwise indicated, processing steps were accomplished in QIIME.

All representative sequences shorter than 100 bp were removed from the representative set, as this was shorter than the minimum expected product size. Representative sequences were assigned the taxonomic level of greatest confidence by comparison with the reference database using the Basic Local Alignment Search Tool (BLAST, Altschul *et al* 1990) with a sequence similarity of 90% and an E-value score < 1e-20. Taxonomic assignments were collected in an OTU table and summarized. Species level identifications were plotted for each sample to estimate diet composition. *De novo* OTUs were submitted for identification via a BLAST search against the non-redundant nucleotide database.

Diet Description

Sample size sufficiency and prey-specific diet metrics were calculated from the hard parts data. To determine that an adequate number of samples were collected in both years, species accumulation curves were constructed in the R package "vegan" (Oksanen *et al.* 2016). The sample size was deemed sufficient when the slope of the curve reached an asymptote.

Previous researchers have noted that digestion and differential passage time impact reconstructed diet estimates (Harvey 1989, Arim & Naya 2003); as such, published correction factors were applied to counts and measurements of hard parts. Species-specific numeric correction factors (NCFs) were applied to estimates of minimum number of prey consumed; when absent, a general factor of 1.43 was used (Weise & Harvey 2008). When possible, graded length correction factors (gLCFs) were applied to otolith measurements to account for differential digestion. As chitin is

resistant to digestive effects, length correction factors were not applied to cephalopod beak measurements. Published linear relationships between otolith length and estimated prey length and mass were calculated for teleost fish identified to the genus level or lower (Harvey 1989, Harvey *et al.* 2000). As rockfish otoliths were only identified to genus, correction factors and regressions used were based on shortbelly rockfish (*Sebastes jordani*), the most abundant species in previous diet studies (Sweeney & Harvey 2011). Similar equations were used to relate lower rostral length of beaks to estimated mass and dorsal mantle length of cephalopods (Wolff 1982, Clarke 1986, Oxman unpublished data). Specific correction factors and regressions are provided in Appendix III.

The prey-specific diet metrics proposed by Brown and colleagues (2012) were calculated for each sex in each sampling year and cumulatively, across sampling years, to obtain sex-specific and annual values. The percent frequency of occurrence (%FO), which indicated how frequently a prey taxon appeared in the total number of diet samples, was calculated as:

$$\%FO_i = \frac{n_i}{n} \times 100$$

where n_i is the number of scat samples containing prey i and n is the total number of scat samples that contained hard parts. The percent prey-specific number ($\%PN_i$) and prey-specific mass ($\%PM_i$) metrics are both variants of prey-specific abundance based on counts or weights of reconstructed prey, respectively, and can be calculated as:

$$\%PA_i = \frac{\sum_{j=1}^{n} \%A_{ij}}{n_i}$$

where $\%A_{ij}$ is the prey-specific abundance (by counts or weights) of prey *i* in sample *j* and n_i is the number of scat samples containing prey *i* (Brown *et al.* 2012). The minimum number of prey individuals was calculated by taking the greatest number of left or right otoliths (upper and lower beaks for cephalopods).

The percent prey-specific index of relative importance ($\%PSIRI_i$) incorporates these previous metrics to provide a measure of the relative importance of a prey taxon in the diet of the predator and is calculated as:

$$\%PSIRI_i = \frac{\%FO_i \times (\%PN_i + \%PM_i)}{2}$$

For species where $\%PM_i$ could not be calculated (northern lampfish and taxa identified at the family level or higher), a modified $\%PSIRI_i$ containing just $\%FO_i$ and $\%PN_i$ was calculated (Gibble & Harvey 2015).

Diet metrics and sample sufficiency also were calculated for the prey DNA dataset. Sample size sufficiency for the sequencing data was determined by examining rarefaction curves produced by the program QIIME for an asymptote. Frequency of occurrence was calculated for the molecular data using the same equation for the hard parts data. To date, there are no conversion factors published to translate the number of amplicons into per unit mass or number of organisms (Pompanon *et al.* 2012). As such, the prey-specific number and mass metrics cannot be calculated as with hard parts data. However, the prey-specific abundance metric can be used to calculate prey-specific molecular abundance ($\%PMA_i$). Given that rockfish species only could be identified via multiple loci, I also used this equation to account for these multiple loci.

$$\%PMA_i = \frac{\sum_{j=1}^{n} \%(\frac{N}{l})_{ij}}{n_i}$$

In this equation, N is the total number of amplicons attributed to the prey *i* in scat *j*, and *l* is the number of loci used to identify the species. For non-rockfish prey, *l* is always 1 and will not modify the abundance. The value of *l* was determined for rockfishes by comparing OTU tables to a local BLAST search in Geneious against the *Sebastes* panel reference library.

Rockfish consumption model

Sex-specific rockfish consumption was estimated using hard parts data. A variable biomass reconstruction model with correction factors (Joy *et al.* 2006, Sweeney & Harvey 2011) was used to estimate consumption for each sex in each sampling year. The reconstructed biomass consumed can be estimated by the following equation:

$$VBR_i = \frac{f_{ik}\overline{\omega_i}}{\sum_{i=1}^{\omega} f_{ik}\omega_i}$$

where f_{ik} is the number of fish *i* in scat *k*, and ω_i is the average weight of fish species *i*.

Statistical analyses

Prey metrics were analyzed for each hypothesis. Previous researchers (Tollit *et al.* 2007) have noted that %N is the most robust metric to the biases typical of diet data; as such, $\%PN_i$ was used as the dependent variable in appropriate analyses. Community composition data form a matrix where each column is a prey taxon and each row is an independent sample, in this case, a scat sample. Given that a predator can only consume a finite number of prey items, diet matrices often contain many zeros, which skew the data and prevent the use of parametric statistics (Legendre & Legendre 1998, Gotelli &

Ellison 2013). To determine if samples clustered by year and sex, a nonmetric multidimensional scaling plot (NMDS) was constructed, based on the Bray-Curtis dissimilarity distance. A permutational Multivariate Analysis of Variance (PERMANOVA), calculated with the Bray-Curtis dissimilarity distance, was used to test if percent prey-specific number for each species significantly differed between year and sex. A PERMANOVA is a non-parametric analogue to a traditional MANOVA, but is typically resistant to the biases present in community composition data due to the calculation of a dissimilarity matrix between samples (Gotelli & Ellison 2013). To examine potential disparities between the two data sets, Spearman's Rank Correlation Coefficient was calculated (Zar 1996). As the abundance metrics were not directly comparable, %FO was used for these calculations. Kruskal-Wallis tests were used to determine if rockfish consumption as estimated by the VBR model was significantly different between years and sexes. All statistical analyses were conducted in R (R Core Team 2016) and statistical significance was determined relative to $\alpha = 0.05$.

RESULTS

Sample sufficiency and overall hard parts summary

Two hundred-nineteen scats were collected from Año Nuevo Island across both sampling years. Identifiable hard parts were recovered from 214 scats (97.7%). The slope of the cumulative prey curves approached an asymptote at 83 samples in both years (Fig. 3). As 86 scats were collected in 2013 and 133 scats were collected in 2014, we had an adequate sample size to describe the number of taxa in these years.

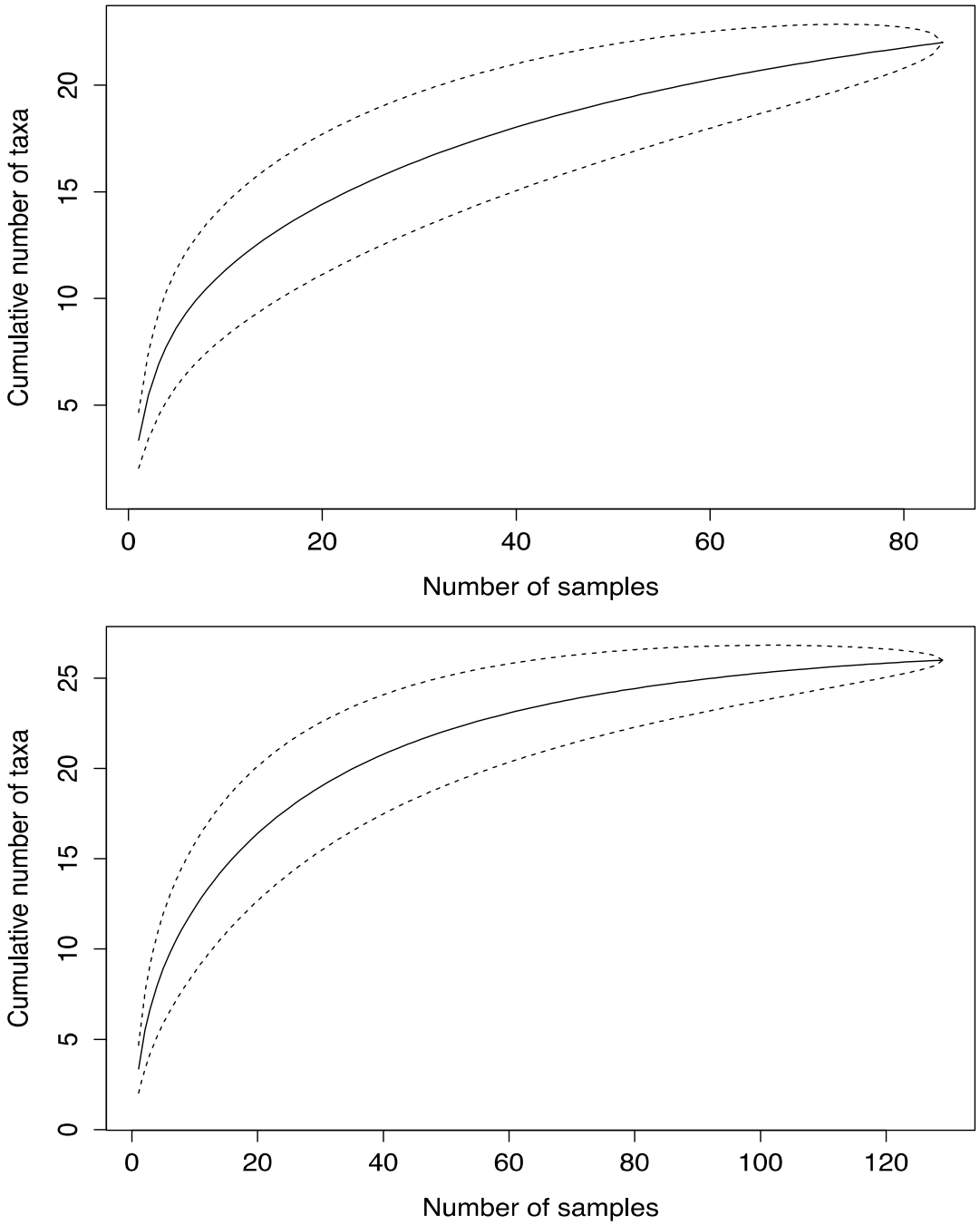

Fig. 3. Cumulative number of taxa (solid line) and 95% confidence intervals (dashed lines) for the diet of *Zalophus californianus* during the summers of 2013 (top) and 2014 (bottom).

California sea lion diet was largely similar in both sampling years based on hard parts data alone. Twenty-two teleost taxa and four cephalopod taxa were identified from hard parts analysis; no hard parts from salmonids or elasmobranchs were found in the scat samples (Table 4).

Table 4. Common names, scientific names, abbreviations, and identification method for prey species identified from California sea lion feces. YOY= young-of-the-year fish. HP = hard parts identification, Mol = molecular identification.

Common name	Scientific name	Abbreviation	Method
Wolf-eel	*Anarrhichthys ocellatus*	Ao	Mol
Sablefish	*Anoplopoma fimbria*	Af	HP, Mol
Spotted Cusk-eel	*Chilara taylori*	Ct	HP
Pacific Sanddab	*Citharichthys sordidus*	Cso	HP, Mol
Unidentified YOY sanddab	*Citharichthys* spp.	Csp	HP
Speckled Sanddab	*Citharichthys stigmaeus*	Cst	HP, Mol
Longfin Sanddab	*Citharichthys xanthostigma*	Cx	Mol
Pacific Herring	*Clupea pallasii*	Cp	HP, Mol
Pacific Saury	*Cololabis saira*	Csa	HP, Mol
Sculpins	Cottidae	Co	HP
Shiner Surfperch	*Cymatogaster aggregata*	Cy	HP
Market squid	*Doryteuthis opalescens*	Do	HP, Mol
Northern Anchovy	*Engraulis mordax*	Em	HP
Pacific Hagfish	*Etmopterus stoutii*	Es	Mol
Walleye Pollock	*Gadus chalcogrammus*	Gc	Mol
White Croaker	*Genyonemus lineatus*	Gl	HP, Mol
Rex Sole	*Glyptocephalus zachirus*	Gz	HP
Boreopacific Armhook Squid	*Gonatopsis borealis*	Gb	Mol
Clawed Armhook squid	*Gonatus onyx*	Go	HP, Mol
Bigfin Eelpout	*Lycodes cortezianus*	Lc	Mol
Slender Sole	*Lyopsetta exilis*	Le	HP, Mol
Pacific Hake	*Merluccius productus*	Mpr	HP, Mol
Dover Sole	*Microstomus pacificus*	Mpa	HP, Mol
Ocean Sunfish	*Mola mola*	Mm	Mol
Red Octopus	*Octopus rubescens*	Oru	HP, Mol
Steelhead	*Oncorhynchus mykiss*	Om	Mol
Chinook Salmon	*Oncorhynchus tshawytscha*	Ot	Mol
Boreal Clubhook Squid	*Onychoteuthis borealijaponica*	Ob	HP, Mol
Robust Clubhook Squid	*Onykia robusta*	Oro	Mol

Table 4. (continued)

English Sole	*Parophrys vetulus*	Pv	HP, Mol
Pacific Pompano	*Peprilus simillimus*	Ps	HP, Mol
Curlfin Sole	*Pleuronichthys decurrens*	Pd	Mol
Flatfishes	Pleuronectidae	Pl	HP
Plainfin Midshipman	*Porichthys notatus*	Pn	HP, Mol
Pacific Sardine	*Sardinops sagax*	Ss	HP
Pacific Mackerel	*Scomber japonicus*	Sja	Mol
Rockfish	*Sebastes* spp.	Seb	HP
Kelp Rockfish	*Sebastes atrovirens*	Sat	Mol
Brown Rockfish	*Sebastes auriculatus*	Sau	Mol
Gopher Rockfish	*Sebastes carnatus*	Scar	Mol
Copper Rockfish	*Sebastes caurinus*	Scau	Mol
Black-and-yellow Rockfish	*Sebastes chrysomelas*	Sch	Mol
Widow Rockfish	*Sebastes entomelas*	Se	Mol
Yellowtail Rockfish	*Sebastes flavidus*	Sf	Mol
Chilipepper Rockfish	*Sebastes goodei*	Sg	Mol
Squarespot Rockfish	*Sebastes hopkinsi*	Sh	Mol
Shortbelly Rockfish	*Sebastes jordani*	Sjo	Mol
Black Rockfish	*Sebastes melanops*	Sme	Mol
Blue Rockfish	*Sebastes mystinus*	Smy	Mol
Bocaccio	*Sebastes paucispinis*	Spa	Mol
Canary Rockfish	*Sebastes pinniger*	Spi	Mol
Stripetail Rockfish	*Sebastes saxicola*	Ssa	Mol
Olive Rockfish	*Sebastes serranoides*	Sse	Mol
Northern Lampfish	*Stenobrachius leucopsarus*	Sl	HP
Krill	*Thysanoessa spinifera*	Ts	Mol
Unidentified cephalopod	Cephalopoda	Uc	HP
Unidentified teleost	Actinopterygii	Ut	HP

Using hard parts, the dominant prey taxon, based on >50% FO across both sampling years, were Pacific Hake (*Merluccius productus*), market squid (*Doryteuthis opalescens*), red octopus (*Octopus rubescens*) and rockfishes (*Sebastes* spp.). Additional overall results based on hard parts analysis alone can be found in Thayer *et al.* (2015).

Molecular sex identification

Positive sex identifications were obtained for a majority of CSLs that deposited scats. Initially, 207 scats (94.5%) for both years were assigned a sex for the CSL that deposited the scat. When samples that had an indeterminate sex assignment (n=12) were re-tested, sex of CSL could be assigned to 218 scats (99.5%, Figure 4, see appendix II for additional details). Of these, more females were identified more than males in both years (73.9% F, 25.6%M, Wilcoxon signed rank test, V = 3, p = 0.5), but it was not significant. Sex assignments were not biased by scat weight (Figure 5, μ_F = 212.6 g, μ_M = 174.1 g; paired t-test, p = 0.14). Males and females were identified throughout the sampling season and both sexes were found in all sampling locations on the island.

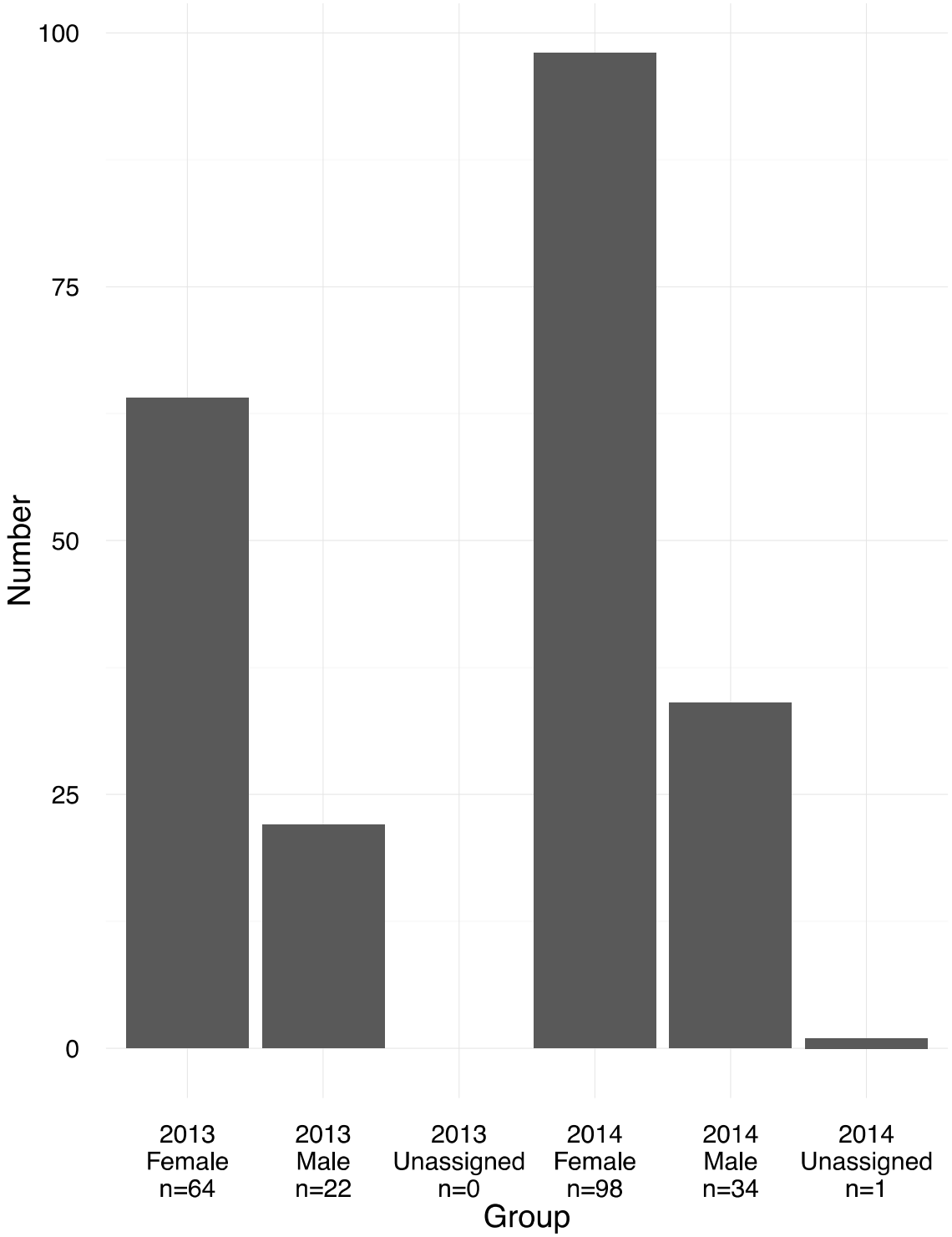

Fig. 4. Summary of scats for *Zalophus californianus* that were assigned to a sex for 2013 and 2014. No samples from 2013 had an indeterminate sex assignment.

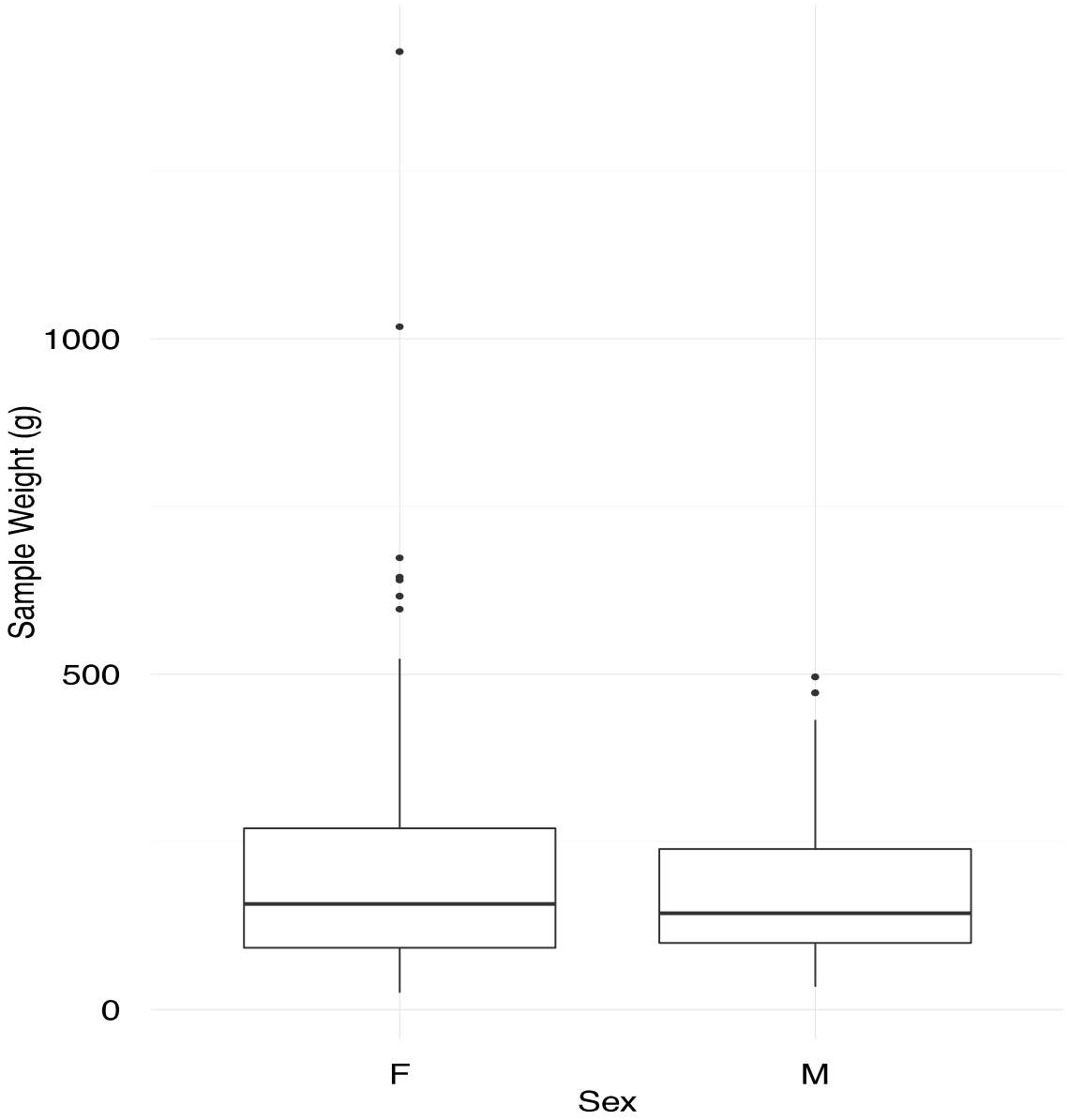

Fig. 5. Boxplot of *Zalophus californianus* sample weights of scats by sex assignment. Black bar is the median weight, box edges are the interquartile range, whiskers represent the 95% confidence intervals, and circles are outliers.

Sex-specific diet: hard parts

For both sexes, the predominant prey items were the same (market squid, red octopus, Pacific Hake, and rockfishes) for both years. In 2013, Pacific Sanddab

(*Citharichthys sordidus*) and Pacific Herring (*Clupea pallasii*) were additionally important prey items for males, but were less important for females (Table 5). The primary cephalopod prey species for females in 2013 was market squid, whereas red octopus was more important for males. Pacific sardine (*Sardinops sagax*) and Rex Sole (*Glyptocephalus zachirus*) increased in importance in 2014 for both sexes (Table 6). The midwater squids *G. onyx* (Go) and *O. borealijaponica* (Ob) decreased in importance between 2013 and 2014.

Table 5. Mean percent frequency of occurrence (%FO), prey-specific number ($\%PN_i$), mass ($\%PM_i$) and index of relative importance ($\%PSIRI_i$) of prey species consumed by male (n=22) and female (n=64) *Zalophus californianus* in 2013 based on hard parts data. Prey taxa are listed in order of decreasing $\%PSIRI_i$ for males.

Taxon	%FO		%PN		%PM		%PSIRI	
	F	M	F	M	F	M	F	M
Pacific Sanddab	14.1	4.5	8.3	51.6	2.4	100	16.9	2580.8
Pacific Hake	68.8	40.9	24.4	42.7	22.9	54.1	313.7	1174.6
Pacific Herring	1.6	9.1	23.2	43.6	ND	50.7	12.4	1109.7
Red Octopus	62.5	50	33.8	43.4	21.0	46.0	386.2	1024.3
Rockfishes	56.3	45.5	32.2	54.5	15	35.4	269.6	983.9
Market Squid	76.6	40.9	30.0	27.9	67.1	53.8	1044.9	776.1
English Sole	0	4.5	0	41	0	35.6	0	732.7
Squid (Ob)	28.1	4.5	6.2	16.7	4.1	73.6	26.7	615.9
Northern Anchovy	7.8	4.5	30.9	55.9	5.4	6.6	87.7	186.3
Pacific Sardine	1.6	4.5	7.2	16.5	1.2	17.8	5.0	149.3
Squid (Go)	25	18.2	17.4	11.7	4.1	9.0	48.3	62.2
Teleost	32.8	40.9	13.9	21.9	ND	ND	23.3	31.4
Sculpins	1.6	9.1	2.4	13.1	ND	ND	1.9	11.1
Flatfishes	1.6	4.5	1.4	2	ND	ND	1.5	3.3
Rex Sole	4.7	0	12.4	0	10.8	0	69.5	0
Northern Lampfish	1.6	0	100	0	ND	0	50.8	0
White Croaker	6.3	0	4.9	0	2.2	0	8.6	0
Cephalopod	12.5	0	4.1	0	ND	0	8.3	0
Shiner Surfperch	3.1	0	2.6	0	1.8	0	3.8	0
Spotted Cusk-eel	1.6	0	2.7	0	0.5	0	1.4	0
Speckled Sanddab	1.6	0	2.1	0	0.6	0	1.3	0
Pacific Saury	1.6	0	1.2	0	0.1	0	0.8	0

Table 6. Mean percent frequency of occurrence (%FO), prey-specific number ($\%PN_i$), mass ($\%PM_i$) and index of relative importance ($\%PSIRI_i$) of prey species consumed by male (n=34) and female (n=98) *Zalophus californianus* in 2014 based on hard parts data. Prey taxa are listed in order of decreasing $\%PSIRI_i$ for males.

Taxon	%FO		%PN		%PM		%PSIRI	
	F	M	F	M	F	M	F	M
Pacific Sardine	8.2	17.7	53.9	63.5	25.6	61.0	693.4	1946.2
Market Squid	73.5	64.7	29.8	35.8	31.6	32.4	507.3	611.3
Rex Sole	5.1	8.8	9.4	20.9	14.9	40.2	72.3	425.3
Pacific Hake	67.4	61.8	30.8	29.6	24.9	26.4	416.9	421.6
Rockfishes	55.1	47.1	28.9	30.2	16.3	18.4	263.3	301.7
Dover Sole	4.1	5.9	16.3	21.7	26.9	15.8	221.9	174.2
Red Octopus	56.1	47.1	27.8	19.6	11.7	6.9	190.9	91.1
Pacific Sanddab	7.1	17.7	15.4	16.6	4.4	7.1	37.7	67.5
Speckled Sanddab	2.0	5.9	4.9	13.5	0.9	3.7	3.2	28.0
Northern Lampfish	1.0	11.8	2.2	27.9	ND	ND	1.6	19.8
Teleost	19.4	20.6	22.9	17.9	ND	ND	21.1	19.3
Cephalopod	26.5	26.5	22.6	10.1	ND	ND	24.6	18.3
Northern Anchovy	4.1	5.9	12.8	35.3	4.5	0.7	30.5	14.6
Squid (Go)	6.1	11.8	12.5	9.4	1.5	1.3	12.5	12.2
Squid (Ob)	3.1	2.9	7.4	2.6	1.4	5.5	6.7	8.7
Slender Sole	2.0	5.9	24.5	5.9	8.9	1.9	109.9	8.4
Shiner Surfperch	3.1	5.9	5.5	6.1	3.3	1.6	10.7	7.8
Pacific Saury	0	5.9	0	4.7	0	2.0	0	7.7
Pacific Herring	1.0	2.9	17.6	3.2	12.9	2.7	114.9	5.8
Plainfin Midshipman	1.0	2.9	8.6	3	3.3	0.4	14.8	2.0
Sablefish	2.0	0	20.2	0	33.7	0	340.0	0
White Croaker	5.1	0	11.1	0	6.2	0	37.2	0
YOY Sanddab	11.2	0	20.6	0	ND	0	15.9	0
Flatfishes	2.0	0	26.9	0	ND	0	14.5	0
English Sole	1.0	0	5.7	0	4.6	0	13.4	0
Pacific Pompano	1.0	0	19.4	0	ND	0	10.2	0

Females in general had greater prey species richness (cumulative number of taxa consumed) in both years (2013: S_F=21, S_M=14; 2014: S_F=25, S_M=20), and females in 2014 had a greater Shannon diversity measure than the other year-sex groups (H'_{F14} =

3.04), but this was not significantly different between the sexes or sampling years (Kruskal-Wallis $\chi^2 = 3$, $p = 0.3916$ for both tests). These differences may also be due to the larger number of samples collected in 2014 than 2013.

Of the most frequently consumed prey, Pacific Hake had the greatest average lengths in both years, with mean lengths of 26.8 ± 8.1 cm SD in 2013 and 19.5 ± 9.6 cm SD in 2014 (Figure 6, 7). Market squid had mean dorsal mantle lengths of 11.7 ± 1.5 cm SD in 2013 and 11.2 ± 1.8 cm SD in 2014. The estimated lengths of red octopus were nearly unimodal, with average dorsal mantle lengths of 2.08 ± 0.05 cm SD in both years. Rockfishes consumed by CSLs had average lengths of 12.5 ± 5.2 cm SD in 2013 and 11.4 ± 5.7 cm SD in 2014.

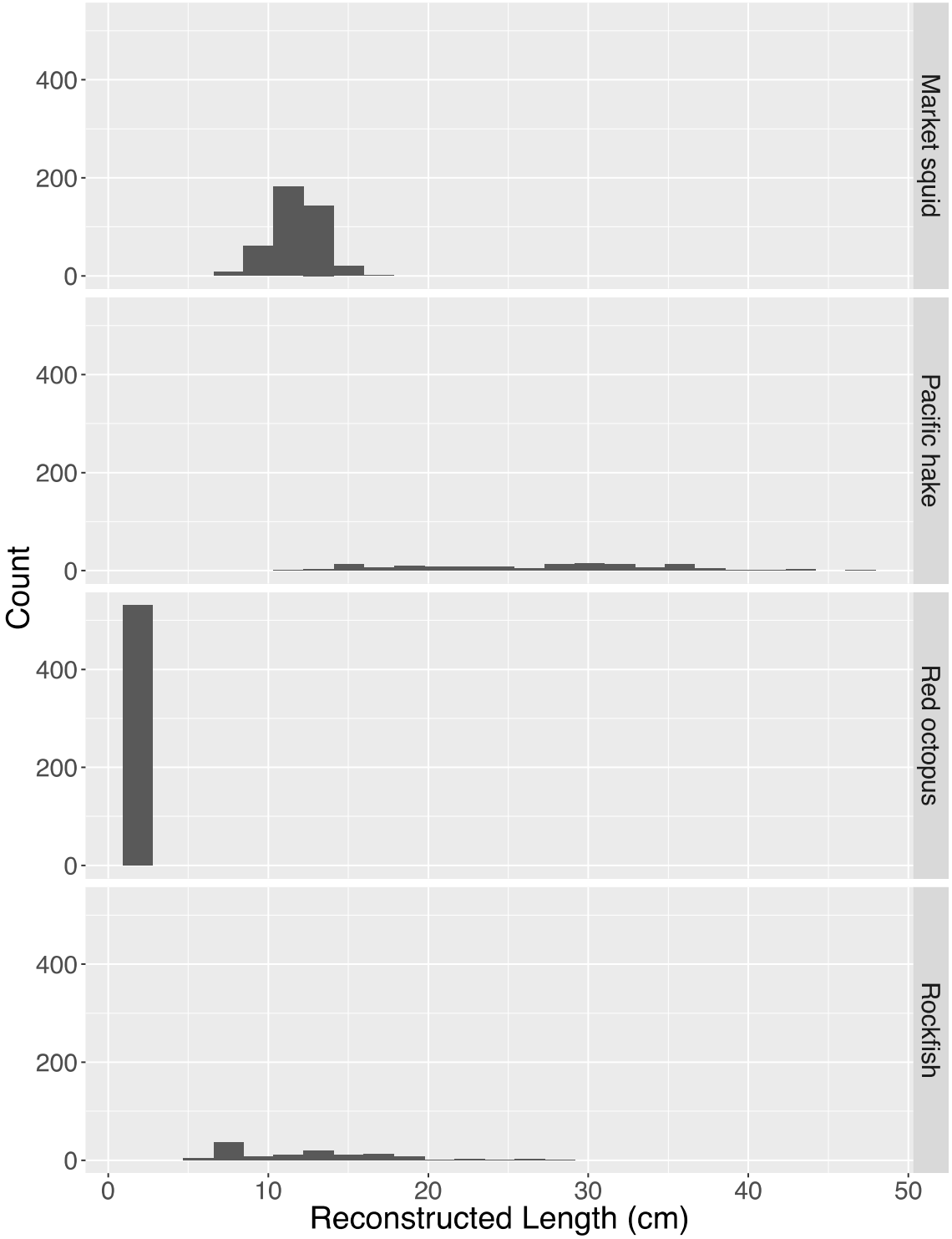

Fig. 6. Histograms of reconstructed lengths (cm) for the four taxa most frequently consumed by *Zalophus californianus* in 2013.

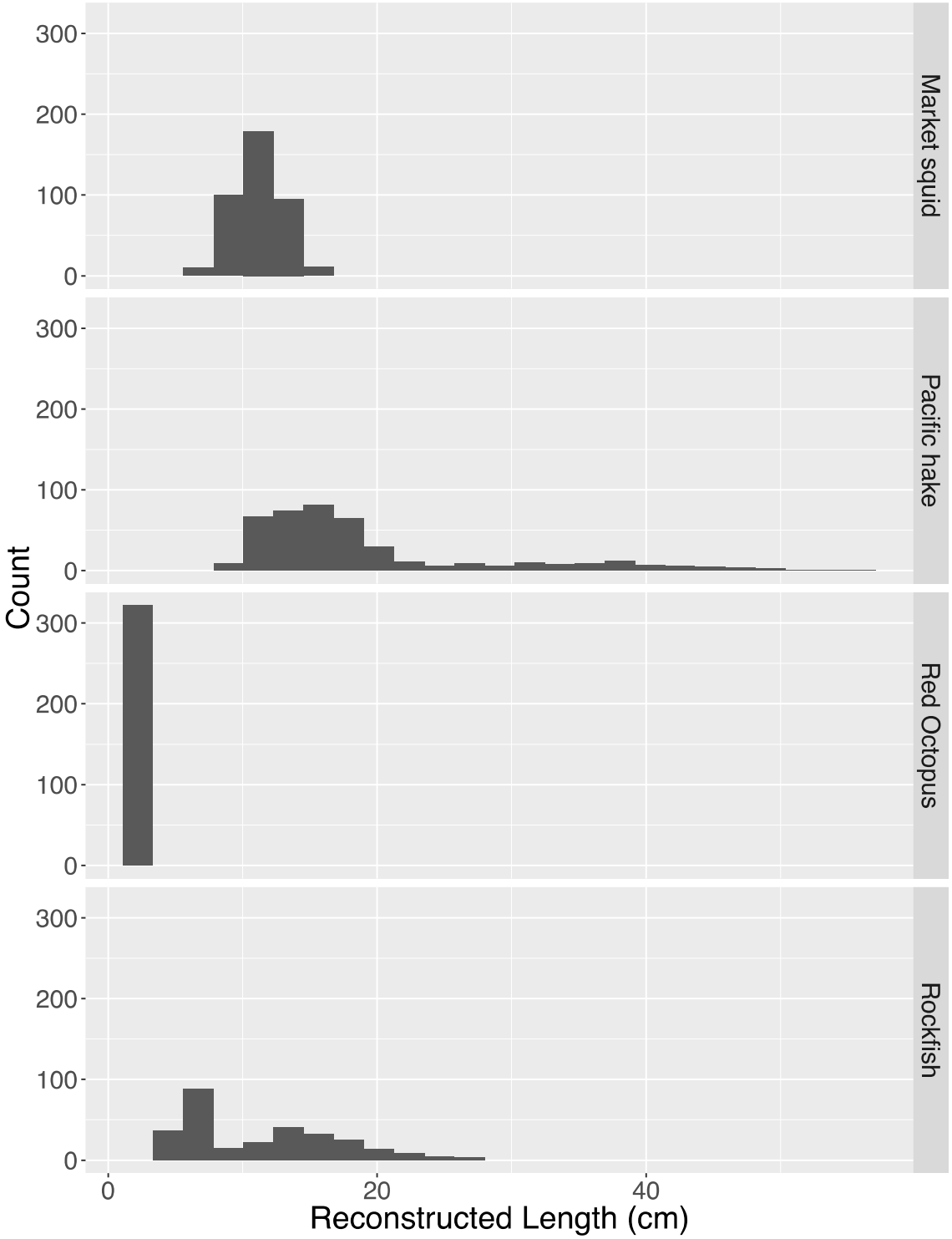

Fig. 7. Histograms of reconstructed lengths (cm) for the four taxa most frequently consumed by *Zalophus californianus* in 2014.

Nonmetric multidimensional scaling of pairwise sample comparisons indicated that samples clustered into their respective year and sex groups (Figure 8). The most dissimilar groups were males in 2014 and females in 2013. Females in 2014 were most similar to males in 2014, but also similar to males in 2013. Analysis of the pairwise dissimilarity matrix with a PERMANOVA indicated that percent prey-specific number was significantly different between year, sex, and a year*sex interaction term (sum of all partial R^2 = 65.8%, all $p < 0.001$), however, year explained the most variation in the data (partial R^2 = 46.9%, Table 7).

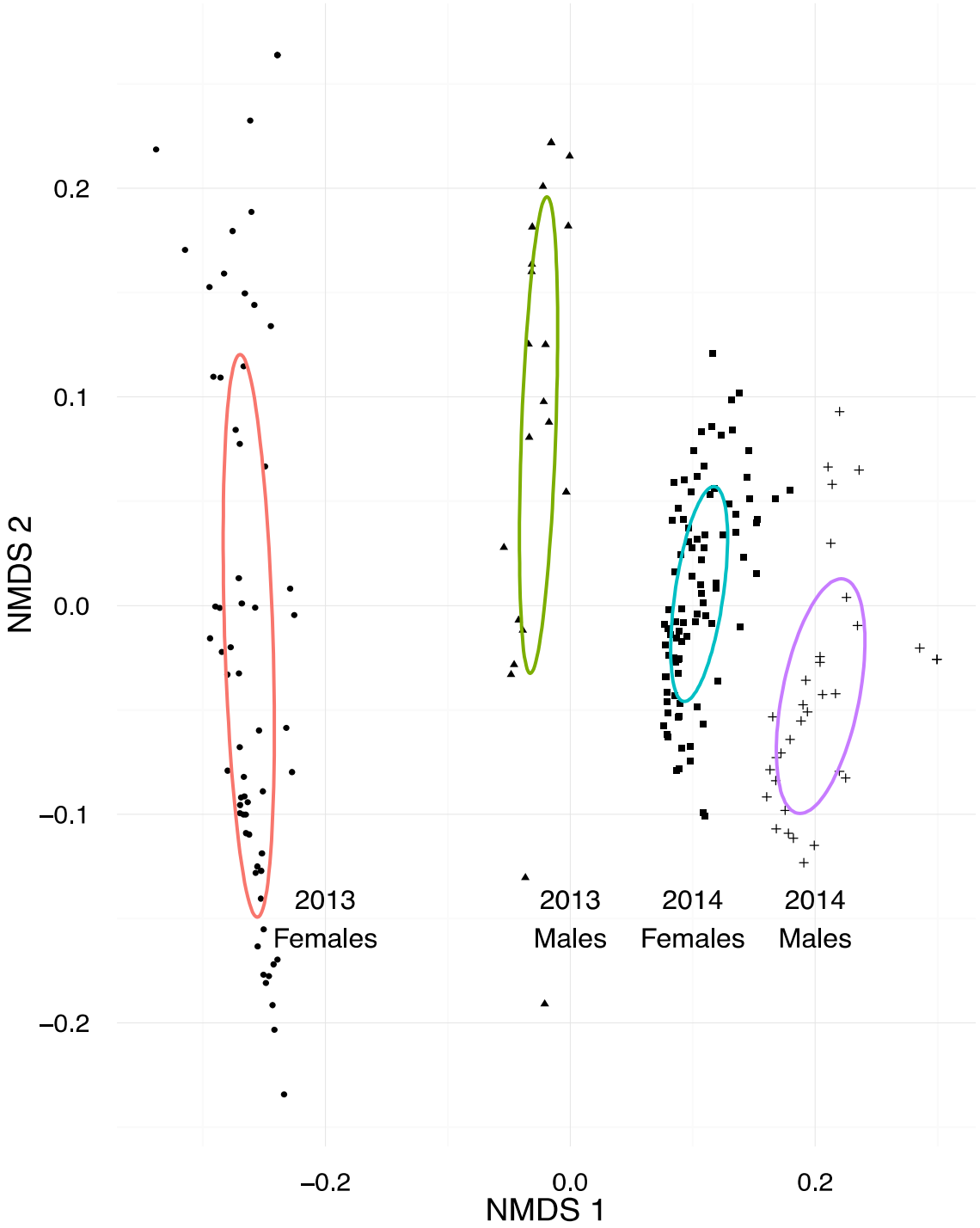

Fig. 8. Nonmetric multidimensional scaling (NMDS) plot of *Zalophus californianus* scat samples based on the Bray-Curtis dissimilarity measure. Circles are 2013 females,

triangles are 2013 males, squares are 2014 females and plus signs are 2014 males. Colored ellipses represent the 95% confidence limit for each group.

Table 7. Summary of PERMANOVA results, including degrees of freedom (df), sum of squares, pseudo-F values, partial R^2 values and p-values, based on 1000 iterations.

Factor	df	Sum of Squares	Mean Squares	Pseudo-F	R^2	P-values
Year	1	9.43	9.43	286.87	0.469	<0.001
Sex	1	1.93	1.93	58.62	0.095	<0.001
Year*Sex	1	1.87	1.87	56.87	0.093	<0.001
Residuals	209	6.87	0.03		0.342	
Total	212	20.1			1	

Sex-specific diet: prey DNA

Prey DNA was amplified from all samples selected for analysis, with sequences from a total of 54,593,699 amplicons recovered from both sequencing runs. Filtering and OTU selection reduced this to a total of 30,399 OTUs. Taxonomic identification via BLAST against the local reference database identified 38 fish and 7 invertebrate taxa, including 16 rockfish species (Table 4). No DNA sequences were recovered from seabirds or humans, although a small percentage of OTUs (<1%) were assigned to CSLs. Unidentified OTUs (<1% all OTUs) queried by BLAST on the NCBI nr database returned no result or had a positive hit for a prey taxon in the local reference database, insects, or arachnids. As such, they were not included in further results.

The Next Generation Sequencing runs improved the identification of rockfishes in CSL diet and allowed for the identification of salmonids despite the lack of hard

parts present in fecal samples. All rockfish species in the reference library were detected in DNA recovered from scat samples, although amplicon abundance attributed to each species varied (Figure 9). The majority of scats contained DNA from bocaccio (*Sebastes paucispinis*), shortbelly (*S. jordani*), chilipepper (*S. goodei*), kelp (*S. atrovirens*), canary (*S. pinniger*), stripetail (*S. saxicola*) and olive (*S. serranoides*) rockfishes, whereas the fewest amount of amplicons were attributed to black-and-yellow (*S. chrysomelas*) and yellowtail (*S. flavidus*) rockfishes. Two salmonids were identified from DNA sequences, Chinook Salmon and Steelhead; both species occurred in low abundances (<7% FO).

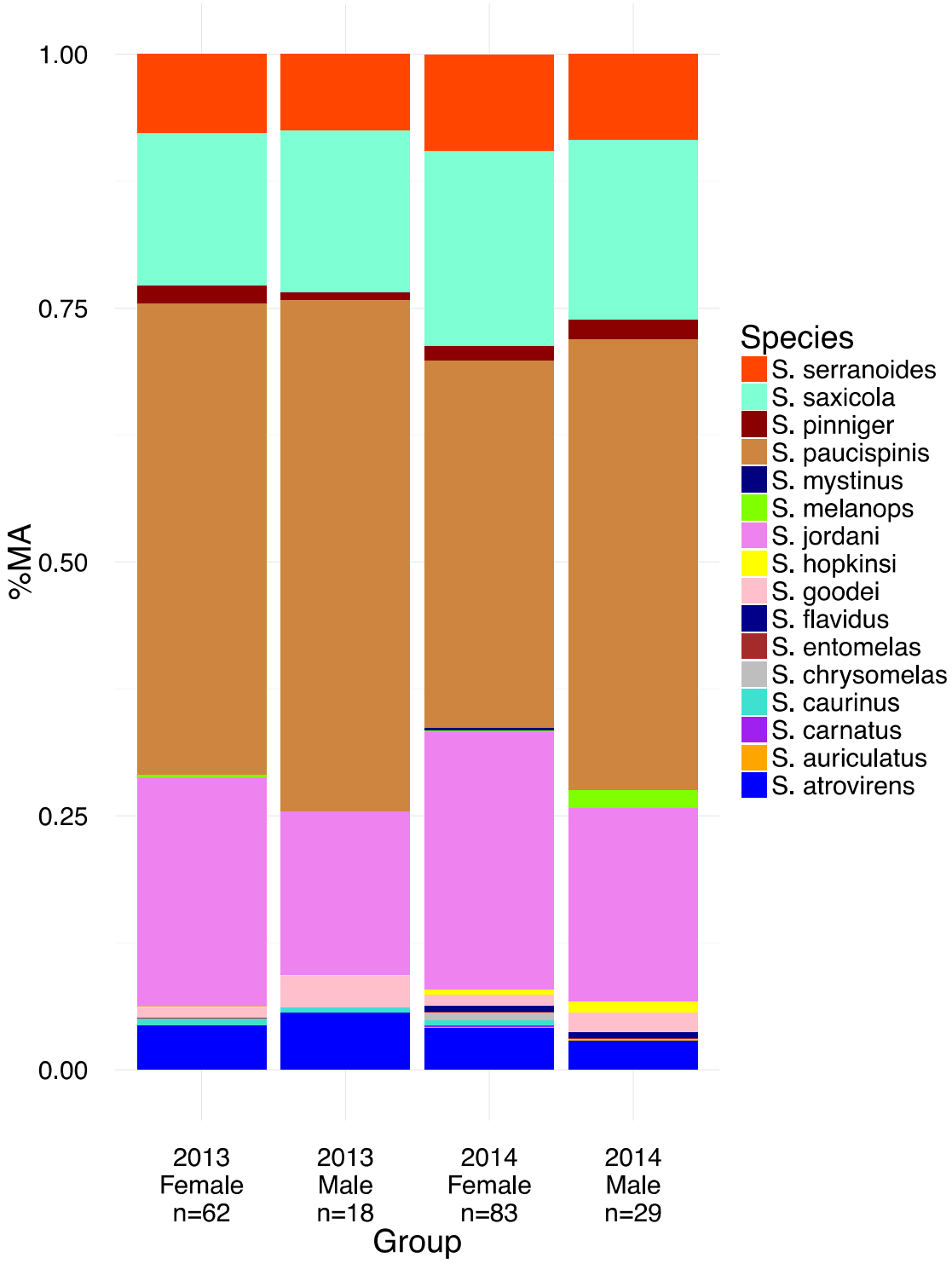

Fig. 9. Composition of rockfishes consumed by each year–sex group, normalized to 100%.

Similar to the hard parts data, the predominant taxa (based on >50% FO) included market squid, Pacific Hake, and rockfishes. In 2013, sablefish, the krill *Thysanoessa spinifera*, and the midwater squids *Gonatus onyx* and *Onychoteuthis borealijaponica* also occurred in greater than 50% of samples (Table 8). In 2014, sablefish, *G. onyx*, *O. borealijaponica*, Longfin Sanddab, *Citharichthys xanthostigma*, and Dover Sole, *Microstomus pacificus*, also were found in a majority of samples (Table 9). Notably, no amplicons were attributed to red octopus in 2013 and %FO was <15% in 2014 whereas red octopus were important in the diet when using hard parts. This likely reflects long passage times for octopus beaks through the CSL digestive tract or the fragility of octopus DNA to digestion.

Prey array indices were calculated from the %FO molecular data. The Shannon Diversity metric was greater for both sexes in 2014 having a greater diversity metric ($H'_{F14} = 3.34$, $H'_{M14} = 3.32$) than in 2013 ($H'_{F13} = 3.15$, $H'_{M13} = 3.13$); and females had a greater diversity metric than males in both years. Similarly, females had greater species richness values ($S_{F13} = 33$ species, $S_{F14} = 40$ species) than males for both years ($S_{M13} = 30$ species, $S_{M14} = 38$ species). Array indices were not significantly different between years or sexes (Kruskal–Wallis $\chi^2 = 3$, p-value = 0.3916 for both tests). Array indices were not compared between methods because they were not calculated using the same metric. When %FO data were compared between methods, years and sexes, the importance of rockfishes, Pacific Hake, and market squid as prey species was apparent

(Figure 10). Cephalopod occurrence was less in the molecular data set compared with the hard parts data set.

Table 8. Mean percent frequency of occurrence (%FO) and both corrected ($\%PMA_{ic}$) and uncorrected ($\%PMA_i$) prey-specific molecular abundance of prey species consumed by male (n=22) and female (n=64) *Zalophus californianus* in 2013 based on molecular data. Refer to Table 4 for abbreviations.

	%FO			$\%PMA_i$			$\%PMA_{ic}$		
Species	Overall	F	M	Overall	F	M	Overall	F	M
Bocaccio	100	100	100	32.4	31.3	36.5	31.4	30.0	36.3
Squid (Ob)	100	100	100	10.8	12.0	6.6	22.9	25.4	14.2
Rockfish (Sjo)	100	100	100	19.8	20.7	16.9	13.9	14.6	11.6
Rockfish (Ssa)	100	100	100	16.0	15.7	17.3	10.1	9.7	11.5
Rockfish (Sse)	100	100	100	11.6	11.6	11.7	5.1	5.03	5.4
Rockfish (Sat)	100	100	100	3.2	3.0	3.9	3.1	2.8	4.1
Rockfish (Sg)	98.8	98.4	100	1.3	1.0	2.4	1.0	0.7	2.3
Pacific Hake	93.8	98.4	77.8	0.5	0.4	0.8	1.9	1.8	2.8
Sablefish	91.3	93.6	83.3	2.1	2.2	1.4	5.5	5.4	5.9
Rockfish (Spi)	80	80.7	77.8	0.8	0.9	0.4	0.9	1.1	0.6
Market Squid	78.8	80.7	72.2	0.5	0.3	1.1	1.3	0.9	2.8
Rockfish (Sh)	73.8	79.0	55.6	0.0	0.0	0.0	0.0	0.0	0.0
Krill	66.3	70.9	50	0.3	0.3	0.5	1.0	0.9	1.4
Rockfish (Scau)	66.3	70.9	50	0.3	0.3	0.1	0.4	0.4	0.3
Rockfish (Sau)	60	62.9	50	0.0	0.0	0.0	0.0	0.0	0.0
Squid (Go)	53.8	58.1	38.9	0.0	0.0	0.0	0.1	0.1	0.0
Rockfish (Se)	53.8	56.5	44.4	0.1	0.1	0.0	0.05	0.1	0.0
Walleye Pollock	45	53.2	16.7	0.0	0.0	0.0	0.1	0.1	0.0
Sanddab (Cx)	42.5	40.3	50	1.3	1.7	0.2	2.8	3.6	0.5
Dover Sole	40	40.3	38.9	1.1	0.4	3.5	3.2	1.6	9.1
Rockfish (Scar)	33.8	30.7	44.4	0.0	0.0	0.0	0.0	0.0	0.0
Rockfish (Smy)	30	32.3	22.2	0.0	0.0	0.0	0.0	0.0	0.0
Wolf-eel	16.3	17.7	11.1	0.2	0.2	0.3	0.7	0.7	0.8
Pacific Saury	10	8.1	16.7	0.4	0.1	0.0	0.1	0.1	0.1
Pacific Sanddab	6.3	6.5	5.6	0.3	0.4	0.0	0.6	0.8	0.0
Squid (Gb)	6.3	6.5	5.6	0.0	0.0	0.0	0.0	0.0	0.0
Slender Sole	5	4.8	5.6	0.2	0.0	0.8	0.5	0.0	2.1
Squid (Oro)	3.8	4.8	0	0.0	0.0	0	0.2	0.2	0
Pacific Herring	2.5	1.6	5.6	0.1	0.0	0.2	0.6	0.0	1.1
Black Rockfish	2.5	3.2	0	0.0	0.0	0	0.1	0.1	0
Steelhead	2.5	1.6	5.6	0.0	0.0	0.0	0.0	0.0	0.0
Curlfin Sole	1.3	1.6	0	0.0	0.0	0	0.1	0.1	0
Pacific Pompano	1.3	0	5.6	0.0	0	0.0	0.0	0	0.0
Teleost (Pn)	1.3	1.6	0	0.0	0.0	0	0.0	0.0	0

White Croaker	0	0	0	0	0	0	0	0	0
Pacific Mackerel	0	0	0	0	0	0	0	0	0
Rockfish (Sf)	0	0	0	0	0	0	0	0	0

Table 9. Mean percent frequency of occurrence (%FO) and both corrected ($\%PMA_{ic}$) and uncorrected ($\%PMA_i$) prey-specific molecular abundance of prey species consumed by male (n=34) and female (n=98) *Zalophus californianus* in 2014 based on molecular data. Refer to Table 4 for abbreviations.

	%FO			$\%PMA_i$			$\%PMA_{ic}$		
Species	Overall	F	M	Overall	F	M	Overall	F	M
Bocaccio	100	100	100	31.3	29.1	37.7	28.5	26.1	35.6
Rockfish (Sjo)	100	100	100	21.7	22.7	18.8	17.6	18.4	15.3
Rockfish (Ssa)	100	100	100	18.8	18.8	18.8	13.9	13.9	14.2
Rockfish (Sse)	100	100	100	13.4	13.6	12.8	6.8	6.9	6.8
Kelp Rockfish	100	100	100	2.3	2.5	1.8	2.8	3.0	2.3
Rockfish (Sg)	96.4	97.6	93.1	1.1	0.9	1.4	0.9	0.8	1.5
Longfin Sanddab	91.1	91.6	89.7	3.1	2.3	5.3	7.1	4.6	14.2
Rockfish (Sh)	91.1	91.6	89.7	0.2	0.2	0.4	0.5	0.3	0.9
Pacific Hake	80.4	84.3	68.9	1.4	1.5	1.1	5.5	6.1	3.1
Rockfish (Spi)	79.5	83.1	68.9	0.8	0.8	1.1	1.2	1.1	1.5
Market Squid	78.6	80.7	72.4	0.9	1.2	0.3	3.8	4.5	1.5
Dover Sole	74.1	78.3	62.1	2.0	2.5	0.4	4.7	5.3	2.3
Squid (Ob)	73.2	75.9	65.5	2.8	3.4	0.5	6.0	7.2	2.1
Widow Rockfish	73.2	75.9	65.5	0.0	0.0	0.1	0.0	0.0	0.1
Black Rockfish	67.9	68.7	65.5	0.2	0.1	0.8	0.4	0.1	1.4
Sablefish	66.1	63.9	72.4	1.6	1.9	0.7	4.7	5.4	2.7
Copper Rockfish	63.4	63.9	62.1	0.2	0.2	0.0	0.3	0.4	0.0
Gopher Rockfish	61.6	67.5	44.8	0.1	0.1	0.0	0.1	0.1	0.0
Squid (Go)	46.4	49.4	37.9	0.3	0.3	0.1	1.3	1.6	0.2
Pacific Pompano	45.5	48.2	37.9	0.9	1.2	0.0	2.1	2.7	0.0
Brown Rockfish	40.2	37.4	48.3	0.0	0.0	0.0	0.0	0.0	0.1
Krill	39.3	42.2	31.0	0.3	0.2	0.4	1.0	0.9	1.3
Blue Rockfish	36.6	38.6	31.0	0.1	0.1	0.0	0.2	0.2	0.0
Rockfish (Sch)	28.6	28.9	27.6	0.2	0.2	0.0	0.4	0.6	0.1
Rockfish (Sf)	27.7	30.1	20.7	0.1	0.1	0.1	0.5	0.4	0.5
Walleye Pollock	27.7	30.1	20.7	0.1	0.1	0.1	0.3	0.3	0.4
Slender Sole	23.2	24.1	20.7	0.3	0.4	0.0	0.5	0.7	0.0
Squid (Oro)	11.6	13.3	6.9	0.0	0.0	0.0	0.0	0.0	0.0
Squid (Gb)	9.8	13.3	0	0.3	0.3	0	0.9	0.9	0
Pacific Sanddab	8.0	6.0	13.8	0.0	0.0	0.1	0.1	0.1	0.1
Ocean Sunfish	5.4	7.2	0	0.0	0.0	0	0.0	0.0	0
Pacific Saury	4.5	3.6	6.9	0.1	0.0	0.1	0.1	0.0	0.3
Pacific Hagfish	3.6	4.8	0	0.5	0.5	0	0.9	0.9	0

English Sole	3.6	3.6	3.5	0.1	0.1	0.0	0.2	0.3	0.0
White Croaker	3.6	2.4	6.9	0.0	0.1	0.0	0.1	0.1	0.0
Bigfin Eelpout	2.7	2.4	3.5	0.1	0.0	0.3	0.7	0.0	1.9
Chinook Salmon	2.7	1.2	6.9	0.0	0.0	0.0	0.2	0.1	0.3

Table 9. (continued)

Steelhead	2.7	2.4	3.5	0.0	0.0	0.0	0.0	0.0	0.0
Wolf-eel	2.7	2.4	3.5	0.0	0.0	0.0	0.1	0.2	0.0
Pacific Mackerel	0.9	0	3.5	0.0	0	0.0	0.0	0	0.0
Teleost (Pn)	0.9	0	3.5	0.0	0	0.0	0.0	0	0.0
Speckled Sanddab	0.9	1.2	0	1.1	1.1	0	3.9	3.9	0
Pacific Herring	0	0	0	0	0	0	0	0	0
Red Octopus	0	0	0	0	0	0	0	0	0
Curlfin Sole	0	0	0	0	0	0	0	0	0

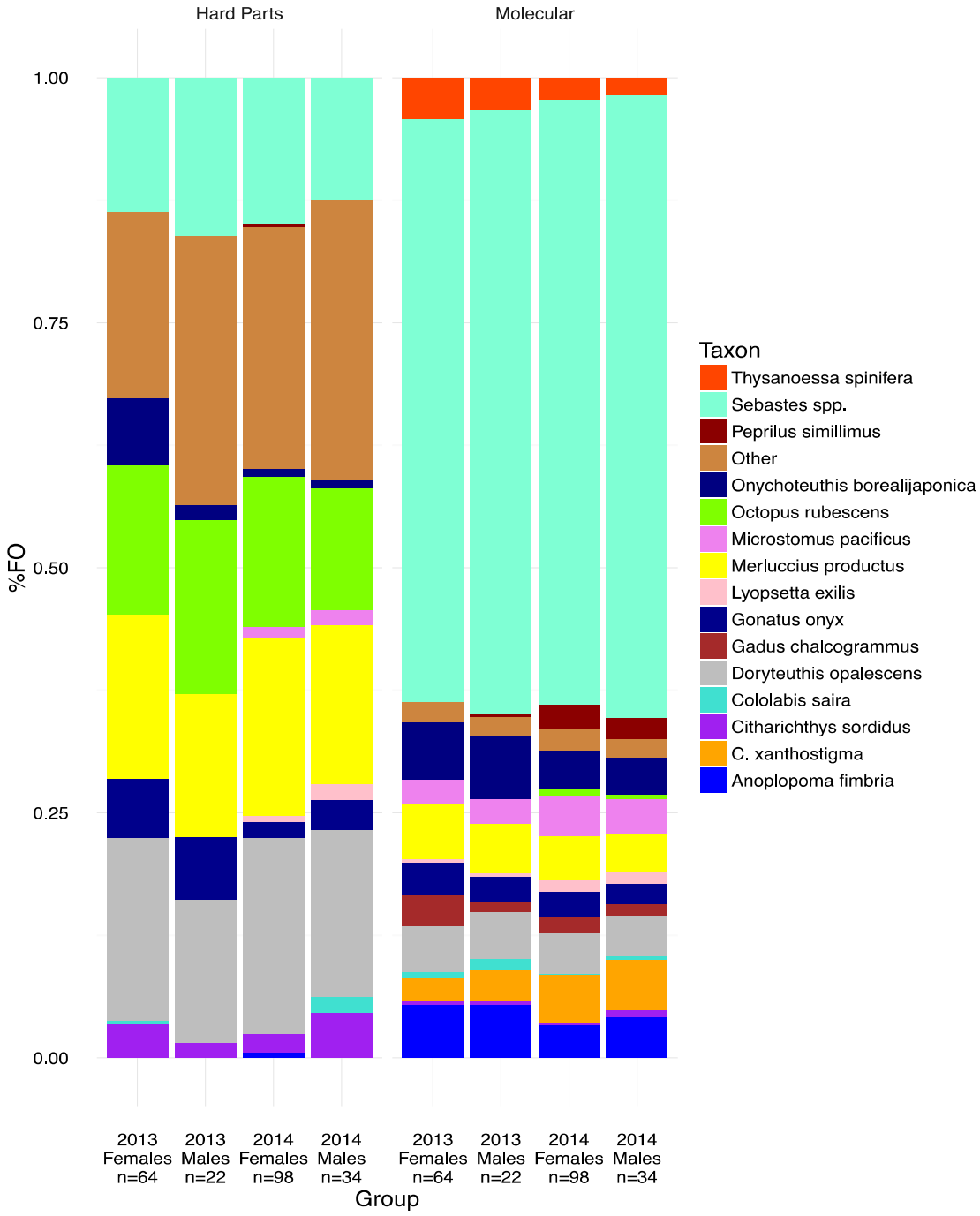

Fig. 10. Percent frequency of occurrence of prey, normalized to 100%, found in *Zalophus californianus* scats for males and females in each year for each method. Taxa identified above the species level and those that comprised less than 5% FO for a majority of groups were collapsed into an "Other" Category. Rockfishes also were collapsed into *Sebastes* spp. for comparison between methods.

Comparison of hard parts and molecular results

Prey frequency of occurrence data were compared between methods using separate Spearman's rank correlation coefficients for each year and sex group. For all groups, the ranks of prey %FO data were significantly different between methods ($p>0.05$). Across years and sexes, cephalopods and Pacific Hake had greater %FO in the hard parts data set than the molecular data set, except for *Onychoteuthis borealijaponica*, which had greater %FO in the molecular data set. The molecular data identified additional taxa not found in the hard parts data, while also emphasizing the importance of rockfishes to CSLs (Figure 10).

Rockfish consumption

The VBR model indicated rockfish consumption was a small proportion of overall biomass consumed by CSLs (Figure 11). In 2013, rockfishes constituted an estimated 9.7 ± 24.1% biomass of female diet and 16.8 ± 32.8% of the biomass of male diet. Similarly, in 2014 rockfishes constituted 7.0 ± 19.7% of the biomass of female diet and 7.9 ± 23.9% of the biomass of males. Rockfish consumption was not significantly different between years (Kruskal-Wallis $\chi^2 = 0.20556$, $p = 0.6503$) or between sexes (Kruskal-Wallis $\chi^2 = 0.53899$, $p = 0.4629$). The composition of rockfish species consumed by CSLs compared with different time series reflected temporal and geographic changes in the rockfish community (Figure 12). Compared with pelagic rockfish cruises, CSLs consumed less shortbelly rockfish and more bocaccio. Rockfish species composition was dissimilar between a coastal monitoring program that monitors the effectives of marine

protected areas (Starr *et al*. 2015) and sea lions. However, the presence of primarily nearshore rockfish species in CSL diet, such as kelp and olive rockfish, indicated that occasional foraging occurred in nearshore environments.

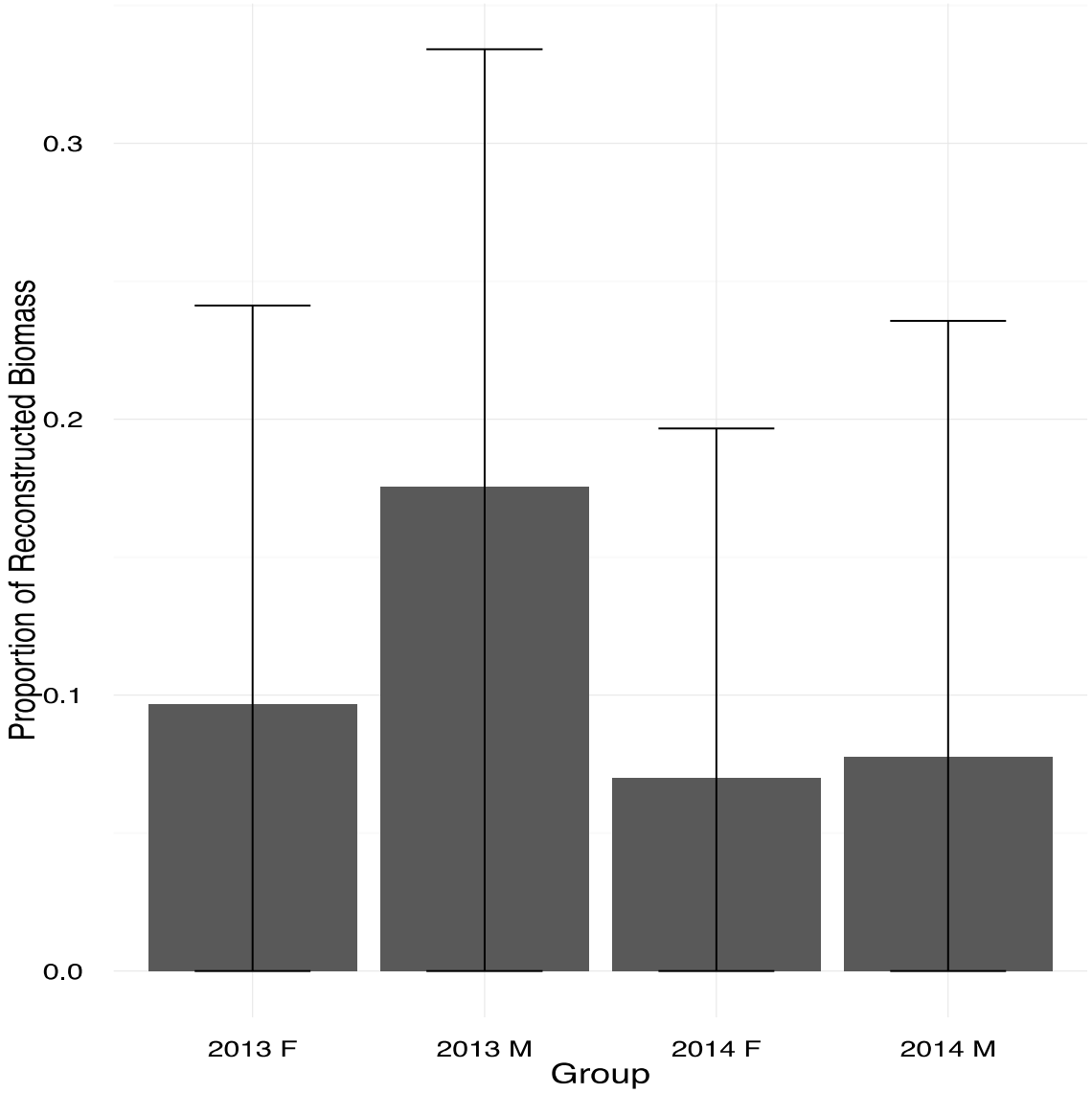

Fig. 11. Mean proportion of reconstructed biomass of rockfish consumed by male and female *Zalophus californianus* in 2013 and 2014, based on a Variable Biomass Reconstruction model with correction factors. Error bars are the standard deviation of the mean.

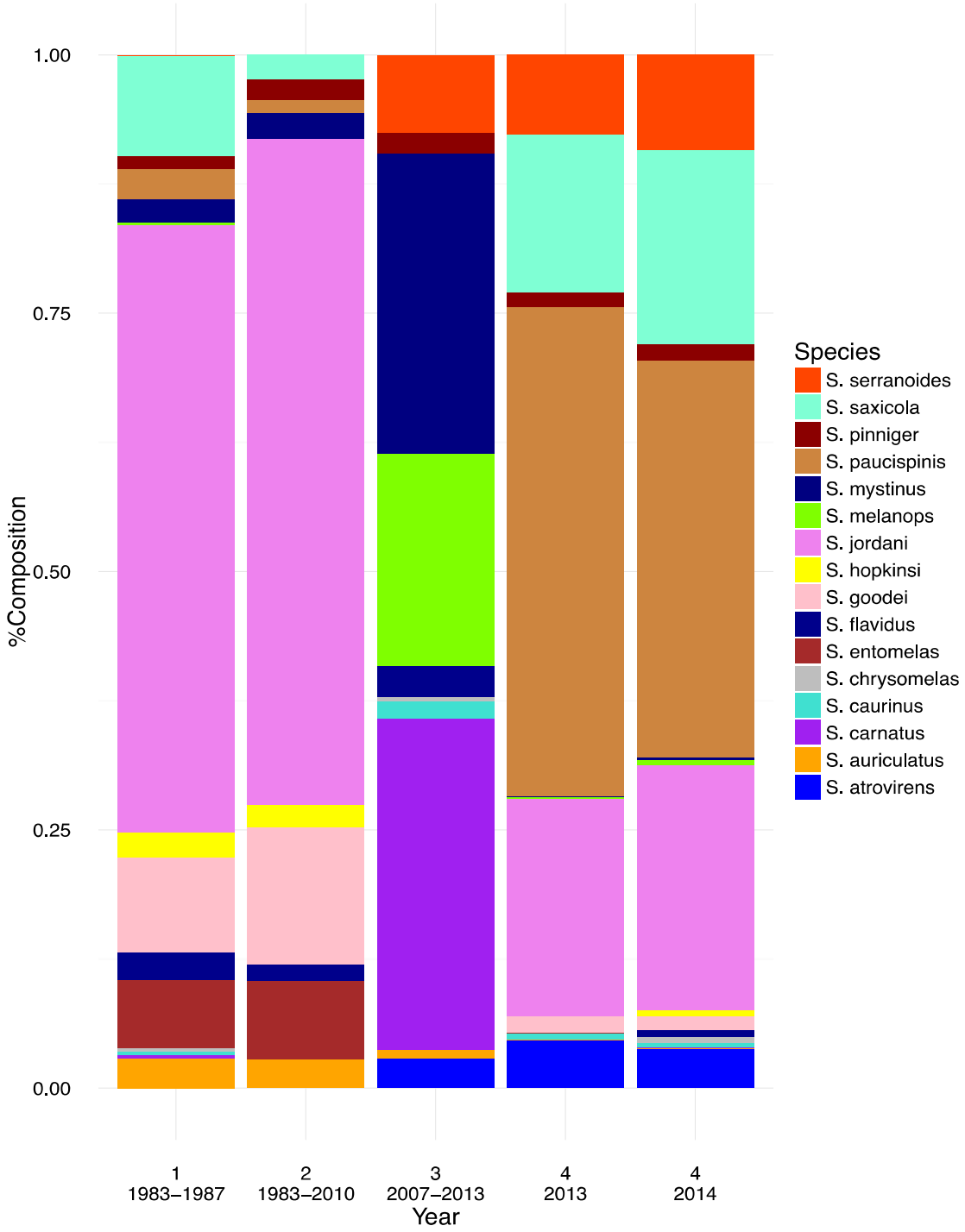

Fig. 12. Percent composition of rockfishes, normalized to 100%, found in time series of rockfish community studies and *Zalophus californianus* scats around central California. Years denote study period and numbers refer to references: 1) Wyllie Echeverria *et al*. (1990), 2) Ralston *et al*. (2013), 3) Starr *et al*. 2015, 4) This study.

The composition of rockfishes in CSL diet also was compared to commercial landings of rockfishes (Figure 13). Commercial landings in 2013 and 2014 were greatest for widow (*S. entomelas*) and chilipepper rockfish (*S. goodei*), whereas CSLs primarily consumed bocaccio and shortbelly rockfish.

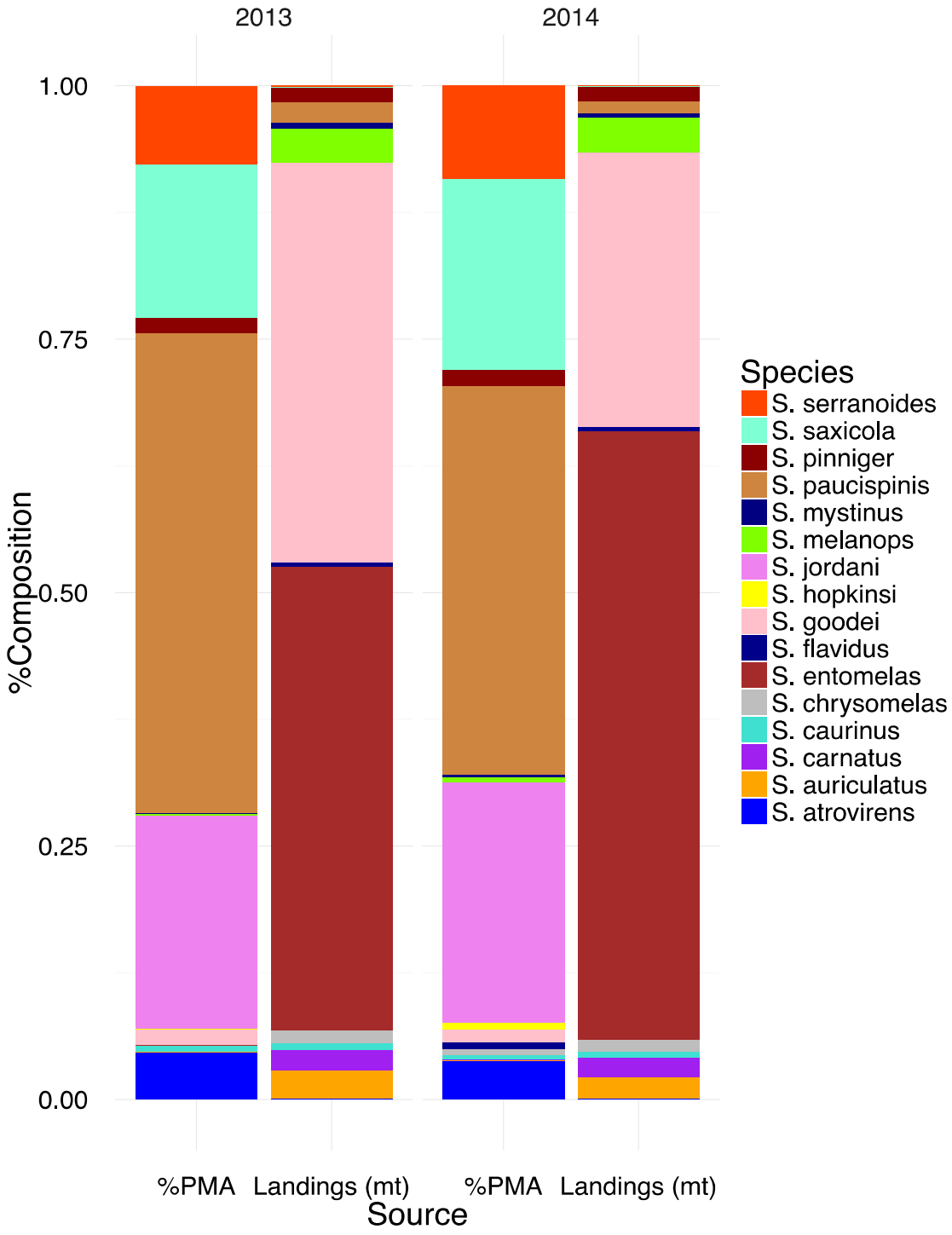

Fig. 13. Percent composition of rockfishes, normalized to 100%, based on percent amplicon abundance (%PMA) in DNA recovered from *Zalophus californianus* scats and central California commercial landings data (mt).

DISCUSSION

Overall diet trends

Previous researchers have noted that CSLs throughout their range are generalist, plastic predators (Lowry *et al*. 1990, 1991, Weise & Harvey 2008, Orr *et al*. 2012; Figure 14). This study supports this observation, as CSLs in 2013 and 2014 consumed dozens of fish and cephalopod species, but only several species were consumed with regularity in high abundances. Using two techniques, I was able to identify several species not previously recorded as prey of CSLs. These include the midwater squid *Onykia robusta* and several fishes: wolf-eel *Anarrhichthys ocellatus*, Walleye Pollock, *Gadus chalcogrammus*, Ocean Sunfish *Mola mola*, and Curlfin Sole *Pleuronichthys decurrens*. Additionally, several taxa not previously recorded as prey of CSLs in Monterey Bay were recorded here, including the midwater squid *Gonatopsis borealis*, and two fishes: the Longfin Sanddab (*Citharichthys xanthostigma*), and the Bigfin Eelpout (*Lycodes cortezianus*).

The diversity of prey species found in this study can be used to infer habitat use by CSLs in Monterey Bay. Prey species can be roughly grouped into habitat ranges: pelagic, midwater, and benthic. CSLs are typically considered epipelagic foragers given their diving ability (maximum dive depth ~300 m, Melin *et al*. 2008, Weise *et al*. 2010). Additionally, it is likely energetically less expensive to target pelagic prey species that school, such as market squid and juvenile rockfishes.

Similarly, it is energetically less expensive for sea lions to dive shallower than their maximum depth.

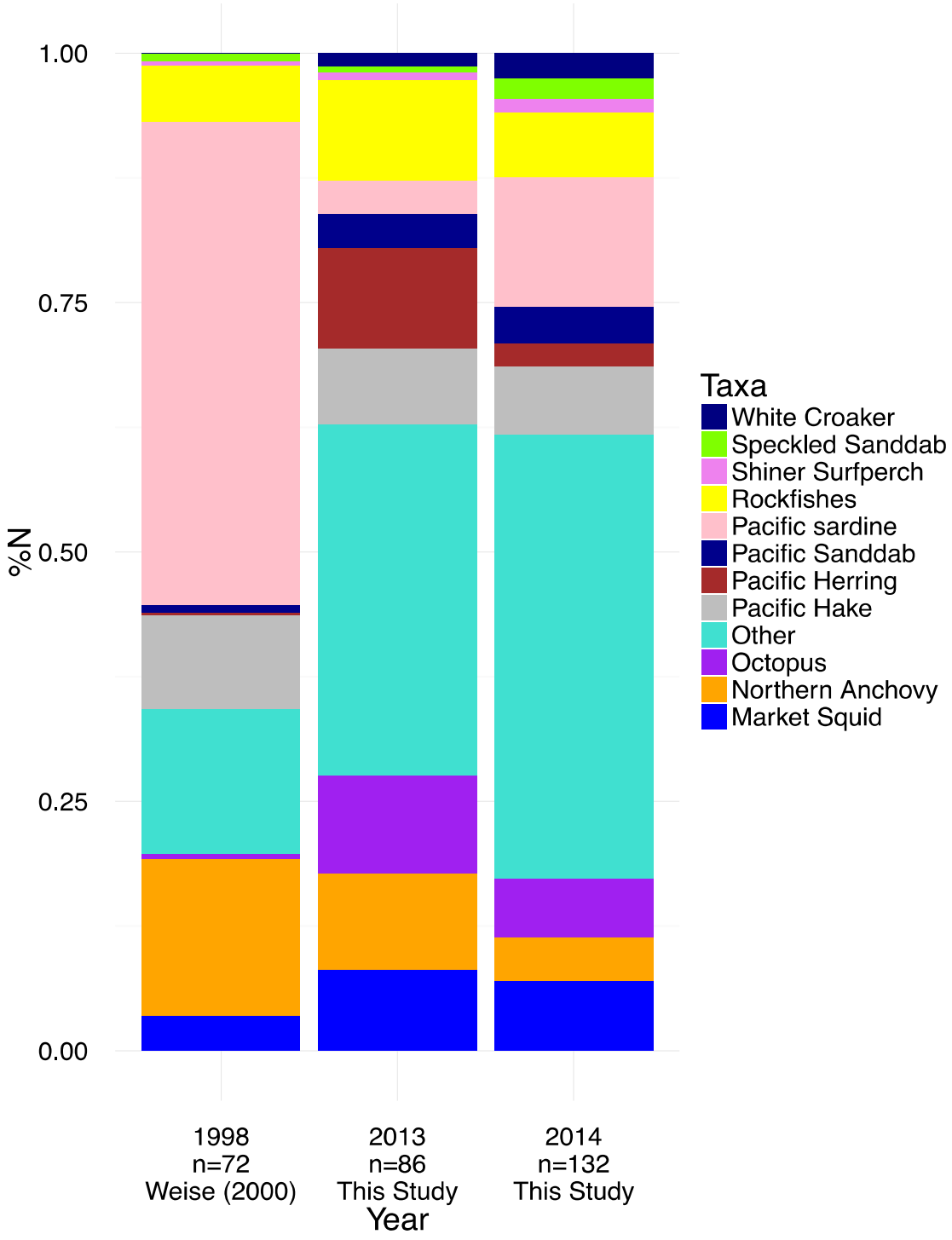

Fig. 14. Percent number of prey identified from hard parts, normalized to 100%, found in *Zalophus californianus* scats collected around Monterey Bay. Prey taxa that were

absent in any year were grouped into an "Other" category. 1998 data are from Weise (2000).

This may explain why benthic associated shallow water prey, such as flatfishes and perch-like fishes were consistently recorded in their diet (Lowry *et al.* 1990, 1991, Weise & Harvey 2008). Whereas adult sablefish are benthic in deep water, juvenile and young-of-the-year individuals are found at shallower depths, thus making them available to CSLs as prey (Love 2011). As the reconstructed lengths of sablefish based on otolith measurements were in the 30 cm range, this confirms that CSLs consumed juvenile sablefish. Several midwater prey species, including the squids *Onykia robusta*, *Onychoteuthis borealijaponica*, and *Gonatus onyx* and the myctophid *Stenobrachius leucopsarus*, were recorded in the diet in this study. Previous investigators (Lowry *et al.* 1990, 1991) have noted myctophids and midwater squids in CSL diet. Given that CSLs are relatively shallow divers compared with other pinnipeds, the consumption of midwater prey likely occurs when these prey species undergo daily vertical migrations.

In this study, market squid, Pacific hake, and rockfishes were the most frequently consumed prey taxa in both the molecular and hard parts data sets, which highlights the importance of these species to CSLs. The importance of these taxa to upper trophic level consumers in Monterey Bay has been known since Morejohn *et al.* (1978) studied the diet of nearly 130 species of fish, seabirds and marine mammals. Red octopus, Pacific sanddab, Pacific herring, and Pacific sardine also were important prey for CSLs based on

a single method, for a single sex, or by number. These taxa also were found in a previous investigation of CSL diet in Monterey Bay between 1997 and 1998, but in different abundances (Weise 2000). This reflects the effect of El Niño conditions on the prey base in the summer of 1998 and the sensitivities and biases of the different methods (Weise & Harvey 2008). In ENSO years, species distributions tend to move northwards and their predators also must adjust their foraging ranges or consume these new prey species to meet their energetic needs. In contrast, 2013 was considered a highly productive year, with greater upwelling levels and corresponding increases in the abundances of prey species positively associated with upwelling such as rockfishes and market squid and lesser abundances of schooling fishes such as Pacific Sardine and Northern Anchovy (Leising *et al*. 2014). Whereas the majority of the California Current ecosystem transitioned to a less productive, warm-water state in 2014, certain areas within central California, including Monterey Bay, remained productive (Leising *et al*. 2015). Within Monterey Bay, upwelling was above average and the abundances of rockfishes and market squid were greater compared with other taxa surveyed as part of CalCOFI efforts (Leising *et al*. 2015). The PERMANOVA results supported these observations as year explained more variation in the diet than sex. Similarly, the residuals explained greater than 30% of the variation in the diet data. This means that additional factors not included in the PERMANOVA model account for the remaining variation in diet data not explained by year, sex, or the interaction of these factors. Given how the California Current system underwent an oceanographic shift in 2014, an oceanographic index may account for a portion of this variance. Changes in oceanographic states are determined

based on indices that measure conditions, such as the Pacific Decadal Oscillation or the Multivariate ENSO Index (MEI) for ENSO (McGowan *et al*. 1998, Chavez *et al*. 2003). Including one of these indices or environmental covariates, such as sea surface temperature, in models may explain additional variation in the diet.

Sex-specific diet

The use of molecular scatological techniques allowed me to explicitly investigate if there were sex-specific diet trends in CSLs. Previous studies of CSL foraging ecology have inferred the potential for sexual segregation in diet based on differential diving ability and habitat use by tagged animals (Melin *et al*. 2008, Weise *et al*. 2010). Although I did not find explicit sexual segregation in prey consumed by CSLs, there were some trends present in the diet. In both years and both methods, female CSLs had greater prey species richness than males. This means that females consumed additional taxa not taken by males or it could be a sampling artifact of having approximately three times as many scats attributed to females than males. Frequency of occurrence data indicated that females consumed smaller, more benthic-associated prey species not consumed by males. This could be explained either by the greater energetic demands of males, thus males eating larger or more energy rich prey, or the coastal, benthic foraging by female CSLs close to shore. Given that adult male CSLs are 3.5 times as heavy as female CSLs (Lindenfors *et al*. 2002), males may be preferentially targeting prey species that have a greater energetic value with the minimal amount of effort. The alternative explanation is that females consumed nearshore benthic species near Año Nuevo Island. The species that were eaten primarily by females and to a lesser extent by males are smaller species

such as Spotted Cusk-eel (*Chilara taylori*), Plainfin Midshipman (*Porichthys notatus*), White Croaker (*Genyonemus lineatus*), and Shiner Surfperch (*Cymatogaster aggregata*). These species generally have a lesser caloric content compared with the more frequently consumed species such as market squid and rockfishes (Sildwell 1980, Perez & Mooney 1986). Whereas males did not consume these species in 2013, they did so in varying abundances in 2014. Given the low caloric value of these species, males may opportunistically consume these species to supplement their energetic needs when they are unable to consume enough of their primary prey species.

The time of sample collection may have also prevented examination of sex-specific differences in diet. Samples were collected in the summer, when most reproductive age females and males would be at the breeding colonies in the Channel Islands (Antonelis & Fiscus 1980). As such, the samples may have been deposited by sub-adult males and females, which are of a similar size and would therefore not exhibit sexual segregation in foraging. If fecal sex hormone levels were significantly different among age classes, then future researchers could incorporate hormone analysis in intraspecific diet studies to consider the effect of age on diet differences.

In studies of intraspecific differences among taxa, the presence of sexual segregation is correlated with a greater degree of sexual dimorphism (Ralls 1977, Fairbairn 1997). Taxa that experience sexual segregation in foraging tend to segregate by foraging habitat (Staniland 2005), have different morphology (Rand 1952), or consume different prey species (Selander 1966). Whereas sexual size dimorphism is present in all otariids and some phocids, the degree of dimorphism varies by species (Fairbairn 1997)

and investigations of sexual segregation in other aspects of life history are difficult outside of the breeding season. Krüger and colleagues (2014) proposed that sexual dimorphism evolved in pinnipeds as a response to foraging niche separation, not in response to a polygynous mating system. Sexual segregation via habitat use is present in several species that have a large degree of sexual size dimorphism, such as elephant seals (Lewis *et al*. 2006), southern sea lions (*Otaria flavescens*, Baylis *et al*. 2016), and Antarctic fur seals (*Arctocephalus gazella*, Staniland & Robinson 2008). Baylis and colleagues (2016) also investigated sexual segregation in southern sea lions via stable isotope analysis of whiskers; however, they found a high degree of isotopic niche overlap between males and females. As such, they suggested that individual choices of prey consumed had a larger influence on sexual segregation in this species (Baylis *et al*. 2016). Given that the degree of sexual dimorphism in CSLs is smaller than in other otariids, such as the southern sea lion and Antarctic fur seal, it stands to reason that there would be a smaller degree of sexual segregation in foraging behavior. This is supported by the PERMANOVA results in this study, which indicated that year had a greater influence on diet differences than sex. Similarly, the NMDS plot shows the separation of samples by year and sex. While the NMDS axes do not contain factor loadings as in principal components analysis, it stands to reason that NMDS 1, which explains the most variation in the data, is associated with year. The additional axes in the NMDS therefore are associated with sex, the interaction of year and sex, and residual variation not explained by these three factors. Further study of sexual segregation in the foraging behavior of

other dimorphic pinnipeds should pair a diet technique with archival tags to understand whether sexual segregation is better explained by habitat use or diet composition.

Comparison of results from hard parts and molecular data

The integration of multiple techniques to study predator diets allows for a broader understanding of their resource use and niche breadth. In this study, I paired relatively new methods, molecular scatology and metagenetic prey identification, with the more established analysis of prey hard parts to better understand CSL diet. Whereas hard parts analysis has been used for decades to study marine mammal diets, it over-represents taxa with hard parts that have long passage times and underrepresents soft bodied and large prey items, and those with fragile structures that may not survive digestion (Arim & Naya 2003). Metagenetic techniques can theoretically identify any species consumed by the predator if enough template DNA is present in the sample, but the technique has yet to be widely implemented due to technical requirements and the inability to convert amplicon amounts to number or biomass of prey (Pompanon *et al*. 2012). Spearman rank correlation coefficient analyses from this study found significant differences in reconstructed diet between methods, which indicated that the occurrence of certain taxa significantly differ between methods. In particular, hard parts analysis had greater %FO for cephalopods and fish that have sturdy otoliths, such as Pacific Hake (Figure 10).

In this study, there were some differences in reconstructed diet using each method. As expected, metagenetic techniques greatly increased the number of identified species in CSL diet for both sexes in both years, nearly doubling the number of species recorded in the diet in both years, due in no small part to the ability to distinguish

rockfishes to the species level. Additionally, prey taxa that were traditionally underrepresented in hard parts data, such as salmonids and agnathans, were recorded in this study. One of the notable differences in results of the methods was the importance of cephalopods. Red octopus, which was a significant prey item in the hard parts data, was less abundant in the molecular data. Many captive feeding studies have noted that cephalopod beaks are retained in the gut for longer than otoliths (Orr & Harvey 2001, Sweeney & Harvey 2011). In contrast, DNA degrades quickly in scats and often is not recovered if a scat is not preserved within 48 hrs (Pompanon *et al*. 2012). Given the drastic differences in the occurrence of octopus in the data sets, it is likely that octopus were consumed greater than 3 days before the scat was excreted. An alternative explanation is that octopus DNA may be difficult to recover from scats or remained unassigned during bioinformatic processing. However, if octopus DNA was present but had too much sequence variation to be confidently assigned to the *Octopus rubescens* reference, it could have been assigned to a higher taxonomic level. As this did not occur, it is more likely that octopus DNA is fragile relative to the DNA of other cephalopods in scat samples. Conversely, the midwater squids *Gonatopsis borealis* and *Onykia robusta* were present in the molecular data but did not have beaks present in the hard parts data. This likely reflects excretion of these beaks before the animal came ashore. While there were unidentified cephalopod beaks in the hard parts data, the beaks of *G. borealis* and *O. robusta* have distinctive characteristics that differentiate them from the other squids recovered from CSL scat samples.

Another major difference between the methods was the difference in the frequency of certain fish taxa. For example, sablefish, which was only found in the scats of female CSLs in 2014, was more prevalent in the molecular data sets from both years. Different abundances of fish species between hard parts and molecular data reflects otoliths that were either dissolved within the digestive tract, excreted at sea, or broken during cleaning. Alternatively, these prey species may have been consumed when the animal was at sea and the DNA may have been excreted before the animal returned to shore, whereas the hard part was retained in the gut longer. This highlights the benefit of pairing these techniques.

The use of molecular scatology not only allowed for sex assignment, but also provided insight to CSL presence at ANI. Previous researchers have suggested ANI was a male-dominated site (Orr & Poulter 1965). However, this study found a predominance of females in both sampling years. This may reflect changing population demographics relative to Orr & Poluter (1965); with a growing population, more individuals of both sexes will disperse to additional haul outs throughout their range. Alternatively, this could also reflect differential on-shore defecation rates that would bias scat recovery. Given that adult females with pups are typically restricted to the rookeries on the Channel Islands during the summer, the presence of females at ANI during this study may be indicative of juvenile or non-reproductive females foraging near Monterey Bay. This may not be unexpected because Monterey Bay tends to have greater productivity compared with waters off southern California, and as such would provide better foraging opportunities (Leising *et al*. 2015). Given the difficulty in distinguishing adult females

and sub-adult animals based on morphology alone, future census efforts may consider incorporating molecular scatological techniques to estimate sex ratios at haul out sites.

Efficacy of molecular techniques in predator diet studies

The molecular methodologies presented in this study represent a step towards incorporating molecular techniques with an established technique, hard parts analysis to study pinniped diets. This is the first study of CSL diet that assigned the sex of the animal to the scat deposited, which allowed for the calculation of sex-specific diet metrics. Additionally, this is the first study of CSL diet using metagenetic-based techniques. Previous pinniped diet studies that inferred potential sex differences relied on sample collection at sex-segregated haul out sites (e.g., Baylis *et al*. 2016); however, not all pinnipeds have sex-segregated haul outs. The sex assignment assay developed for this study is straightforward and does not require specialized reagents for a successful reaction and has comparable assignment success to other assays. Matejusová and colleagues (2013) developed a real-time PCR assay with Taqman chemistry that was able to assign scats to pinniped species and sex through the use of markers for interphotoreceptor retinoid-binding protein (*IRBP*) and *ZFX/ZFY*, respectively. Although they tested a smaller number of scats, they also had high assignment accuracy (>90%) for both species and sex. The inclusion of a species-specific autosomal marker is a necessity for field studies that incorporate molecular sex identification. Not only does this provide a positive control for females, it is also useful for sites used by multiple species, such as Año Nuevo Island. Whereas CSLs are the predominant species present on ANI in the summer, the island also is frequently used by northern elephant seals, and to a lesser

extent by Steller sea lions and northern fur seals (*Callorhinus ursinus*, Orr & Poulter 1965). For the purpose of this study, the use of a CSL-specific microsatellite marker was sufficient, however, future researchers may consider the use of a species-specific marker, such as *IRBP* or mitochondrial *16S* (Masland *et al*. 2010).

The metagenetic techniques used in this study provided a framework for future studies. The use of the barcoding marker *COI* allowed for the identification of fish and invertebrate prey taxon from CSL scats. A frequently noted issue with DNA amplification from scats is the low template levels of prey DNA relative to predator DNA. Many published studies to date incorporate a blocking primer to prevent the amplification of the predator DNA (Tollit *et al*. 2009). Using the fish and cephalopod *COI* primers developed for this study in a GT-seq framework resulted in minimal amplification of CSL *COI* sequences. The amplification of crustacean and non-target invertebrate taxa (insects and arachnids), however, indicated that the *COI* primers used in this study could be redesigned to improve specificity to fish and cephalopods. Given the prevalence of the krill *T. spinifera* in the amplicon data of this study, and the occasional presence of pelagic red crab (*Pleuroncodes planipes*) in CSL scats from southern California haul outs (Lowry *et al*. 1990, 1991), an alternative would be to design a crustacean-specific *COI* primer set and improve the specificity of the cephalopod *COI* primers.

The local reference library assembled for this study may have biased some of the taxonomic assignments. Prey reference libraries are typically informed *a priori* based on community composition in the study system or previous diet studies (e.g., Shehzad *et al*.

2012). The reference library used in this study was informed in this manner, incorporating results from hard parts identification, a previous study of CSL diet in Monterey Bay (Weise 2000), and previous pinniped diet studies in central California (e.g., Gibble & Harvey 2015). However, because the reference library sequences were also included in the multiple sequence alignments used for the short *COI* primer design, the primers are more likely to preferentially amplify those taxa as opposed to other taxa that were not expected in the diet. In order to avoid this potential bias in future studies, primers should be designed with a larger set of sequences with a greater taxonomic coverage than what is included in the reference library.

One challenge of employing a multi-locus approach is the lack of a comprehensive, existing bioinformatics pipeline to streamline analysis. Given the massive amount of information generated in genomic barcoding studies (on the order of millions of sequences totaling hundreds of GB of sequence files), efficient data processing pipelines and sufficient computing resources are a necessity. Pipeline development is hampered in part by the inconsistency in processing pipelines used in previous predator diet studies. Many investigators used custom-built pipelines or multiple programs to accomplish the various processing steps necessary to filter the data to a representative fraction that can be analyzed. Additionally, many researchers often use one or two primer sets to amplify prey taxa (Shehzad *et al*. 2012, Bowles & Trites 2013) from a few dozen samples; I used 14 primer sets on 192 samples. In this study, I primarily used QIIME and dependencies therein, although it was necessary to use the open source Galaxy server to accomplish length-based sequence filtering. Whereas data

processing was relatively straightforward in QIIME, I was unable to use existing default parameters or the downstream diversity analyses, as these are optimized for microbial *16S* datasets with a relatively well-understood phylogeny. For those just learning bioinformatic methods, this can present a steep learning curve that may discourage future molecular investigations. Documentation of processing pipelines should be encouraged, as this will provide an existing analytical framework for future studies.

In an effort to reduce the amplicon data set to a representative set via OTU selection, it was possible that certain taxa consumed infrequently and in low abundances did not appear in the representative set of OTUs. In this study, OTU selection parameters were chosen based on a combination of settings used in a previous study of pinniped diet (Thomas *et al*. 2014) and following recommended standards for processing metagenetic data generated on Illumina platforms (Caporaso *et al*. 2012, Bokulich *et al*. 2013, T. Campbell pers. comm.). In this study, more than a dozen fish taxa were removed from the data set once quality and length-based filtering were applied to the representative set including two identified via hard parts, Shiner Surfperch and Pacific Sardine. Given the low abundance of these species their removal from the final representative OTU set was unlikely to impact the overall trends reported in this study. The number of OTUs generated in this study was in excess of 30,000 for both sequencing runs, which was greater than expected. Upon examination of OTU tables generated by QIIME, multiple OTUs were assigned to a single species. This reflects intraspecific sequence variation in excess of the clustering parameters used in the OTU selection process. The recommended threshold for species level OTU identification in QIIME is 97% using the

RDP classifier (Caporaso *et al*. 2010); in this study, I used the 90% similarity threshold in BLAST following Thomas *et al*. (2014). Future researchers could investigate the relationship between the clustering parameter and the accuracy of taxonomic assignments. An additional field of study that could provide guidelines for OTU selection and filtering is eDNA (environmental DNA) research, as these investigations also use barcoding markers to identify taxa from samples with low template amounts (Goldberg *et al*. 2016).

Targeted taxon-specific DNA-based studies were the norm before the shift towards metagenomic methods based on DNA barcoding (Symondson 2002). This study incorporated a taxon-specific component to identify rockfishes present in CSL scat samples. The twelve-marker panel used in this study allowed me to distinguish between 16 species of coastal rockfishes, including those from recently diverged species, such as the Kelp/Copper/Gopher/Black-and-Yellow and Yellowtail/Olive complexes. Given the difficulty in distinguishing rockfishes with traditional barcoding regions (Pearse *et al*. 2007), the use of this panel presents a tool for future studies of rockfish communities. One aspect of the rockfish panel to be aware of is the potential for incorrect species assignment among the recently diverged complexes. This could explain the low frequency of amplicons assigned to Yellowtail and Black-and-Yellow rockfishes across both sequencing runs. An alternative explanation is that juvenile recruitment for these species was less in both sampling years than their closely related sister species. Additionally, the rockfish panel likely underrepresents the diversity of *Sebastes* species consumed by CSLs. The 16 species included in the reference library were preferentially

chosen based on their abundances in the previous survey efforts (Wyllie Echverria *et al*. 1990, Ralston *et al*. 2013) and prevalence in the coastal environment. However, as with other prey taxa, sequence quality filtering removed at least one additional rockfish species, Yelloweye Rockfish (*S. ruberrimus*), from the final OTU dataset.

Consumption of rockfishes

Regardless of method used to reconstruct CSL diet, rockfishes represented an important component of the diet. Weise and Harvey (2008) suggested that CSLs were primarily consuming Shortbelly Rockfish, and in an earlier study, Weise (2000) suggested Bocaccio was the predominant rockfish species consumed. This study demonstrated that not only are both of these species consumed by CSLs with regularity, but CSLs also consumed a vast array of the coastal rockfishes present within the Monterey Bay. The species chosen for the reference library were selected in part based of their abundances in juvenile rockfish surveys conducted by the National Marine Fisheries Service (Wyllie Echeverria 1990, Ralston *et al*. 2013). Comparison of rockfish species composition in CSL diet to juvenile rockfish surveys, as well as to the nearshore monitoring of the California Collaborative Fisheries Research Program (CCFRP, Starr *et al*. 2015), indicated that CSLs were eating rockfish species relative to their abundance in certain habitats. In nearly 30 years of juvenile rockfish surveys, Shortbelly Rockfish was the primary species found in trawls; in contrast, CSLs primarily consumed Bocaccio, followed by Shortbelly Rockfish, in the two years of this study. These species are likely consumed by CSLs in offshore environments. The presence of similar rockfish species in

CSL diet and the CCFRP data indicated that CSLs consumed certain nearshore species, such as Olive and Kelp Rockfish, but ate less of other nearshore species, such as those in the Kelp/Copper/Gopher/Black-and-Yellow complex, along with Blue and Black Rockfish. In contrast, species such as Stripetail and Chilipepper rockfish likely occurs at sea, as these species are rarely recorded in CCFRP monitoring efforts but are found in juvenile rockfish survey trawls. Based on comparison of rockfish composition of CSL diet to commercial landings data, CSLs primarily consumed species not targeted by commercial fisheries. In both years, Widow and Chilipepper Rockfish had the greatest landings in fisheries data, but were of minimal importance in CSL diet. It is within reason to assume that CSLs may consume additional rockfish species that were not included in the rockfish reference library. Future studies using these methods could attempt to include additional species in the reference library, with the understanding that additional markers may be required to successfully distinguish among these additional species, especially if they are recently diverged (Hyde & Vetter 2007, Pearse *et al.* 2007).

The VBR results indicated that rockfishes constituted between 7 and 16% of the biomass consumed by CSLs. These results reflect that, whereas sea lions frequently consume rockfishes, their contribution to the overall biomass was less than may be expected due to consumption of small individuals. Adult rockfishes tend to be spiny, which makes consumption difficult. In contrast, juvenile rockfishes have less developed spines, therefore, they would be easier to consume (Love *et al.* 2002). In addition, younger fish may be easier to exploit if they school for protection before they recruit to their adult habitats. The VBR results presented in this study did not attempt to compute

species-specific biomass estimates because estimated lengths could not be assigned to species, which would have required the genetic identification of the otolith.

In this study, CSLs were confirmed to consume at least 16 species of rockfishes. Rockfishes are present in a variety of habitats in the California Current Ecosystem, from the intertidal to the continental shelf and slope (Love *et al*. 2002). Juveniles, however, tend to be found in shallower waters and different habitats than adults. As otoliths were not genetically identified to species, I was unable to determine if CSLs ate rockfish species of different sizes. However, given that average lengths of rockfish were estimated at 12.5 cm in 2013 and 11.4 cm in 2014, it is likely that CSLs primarily consumed juveniles across species. This is consistent with previous studies of CSL diet (Lowry *et al*. 1990, 1991, Weise 2000).

Sea lions primarily consumed bocaccio, shortbelly, stripetail and olive rockfish during this study; while all these species have pelagic larvae and recruit to nearshore environments, they exhibit slightly different life histories as they age. Bocaccio and shortbelly rockfish move offshore as they age, and are frequently found at depths around 200 m in central California (Love *et al*. 2002). Given the potential dive capabilities of CSLs and that juveniles of these species are found in schools at depths shallower than where adults are found, it is likely that CSLs were targeting schools of juveniles in shallower offshore habitats. Stripetails occur over a wider range of depths as they age (25-547 m), but are also common around 200 m depth (Love *et al*. 2002). Unlike boccacio and shortbelly, stripetails tend to be found in low relief habitat once they recruit. As adult stripetails were found at the deepest parts of their depth range, CSLs that

consumed juvenile stripetail rockfish likely did so near the seafloor at shallow depths. In contrast, olive rockfishes tend to be found in shallower waters (up to 172 m) and are associated with high relief substrates (Love *et al*. 2002). As such, CSLs that ate olive rockfish likely did so in nearshore, high relief environments, such as kelp beds. Given that CSLs are consuming juvenile rockfishes and CSLs are constrained in their potential dive capabilities, CSLs may consume other rockfish species that recruit to shallower water before moving to deep water as adults. Future researchers may want to include those species with longer rebuilding times (the estimated time it takes for a stock to recover from overfishing), such as cowcod (*S. levis*) and yelloweye rockfish (*S. ruberrimus*) to determine if CSLs consume these species.

Previous researchers suggested that shortbelly rockfish was the dominant species consumed by CSLs (Weise & Harvey 2008). In contrast, the results of my study indicated that there is no single species that is frequently occurs in CSL diet. This could either be due to consumption of these rockfishes or assignment errors during bioinformatic processing. Of the species included in the reference library, five are members of clades that are recently diverged: yellowtail and olive form one clade, whereas the other clade is composed of kelp, copper, gopher and black-and-yellow rockfish (Hyde & Vetter 2007). Given this information, incorrect assignment of the other species in the reference library is unlikely. While incorrect assignment is more likely among the members of these clades, the inclusion of multiple loci should decrease assignment error. Therefore, it is more likely that CSLs do consume multiple species of rockfishes. If there is uncertainty of assignment between these recently diverged species,

taxonomic assignment could be limited to a clade-level identification instead of a species-level identification.

California sea lions as sentinels

Predator diets often are studied to infer changes in the ecosystems they inhabit. Marine predators, including pinnipeds, are studied to infer changes in the community composition of the prey they consume (Ainley *et al*. 1995, Melin *et al*. 2012). Changes in the prey base are indicative of changes in oceanographic conditions. The shift in oceanographic conditions in 2014 resulted in greater species richness in CalCOFI surveys (Leising *et al*. 2015); this was similarly reflected in CSL diet, as prey species richness was greater for both sexes in 2014 than 2013. Surveys of CSL pups at the major Channel Island rookeries are routinely included in CalCOFI reports as the success of cohorts is closely tied to the foraging success of the mother, which in turn is dependent on the oceanographic conditions influencing the recruitment and distribution of prey species (Melin *et al*. 2012). Continued monitoring of foraging grounds, such as Monterey Bay, can be incorporated in ecosystem surveys to provide additional understanding of community changes over time.

The techniques used in my study can be incorporated in future monitoring efforts to improve our understanding of the role of CSLs in the California Current Ecosystem. Molecular scatology can be incorporated in census efforts to understand the changing demographics of CSLs at haul outs in the Monterey Bay region. These molecular techniques can be incorporated and refined in future diet studies to examine sex-based differences in diet in other seasons and sampling years. Although I did not find explicit

sexual segregation in the diet of male and female CSLs, it is possible that there could be greater differences in prey species consumed at other times of the year as prey communities become less diverse. Weise (2000) found that CSL scats sampled in spring had the greatest prey diversity compared with other seasons. With fewer prey species present in the community at other times of the year, the potential for overlap in prey species consumed increases. Additionally, prey DNA analysis can provide information about juvenile rockfish abundance in the California Current. Although rockfish were sampled by NOAA in California waters annually, these efforts were limited to the summer. CSL scats can be collected for a fraction of the cost of operating a research vessel and would allow for studies of the coastal rockfish community throughout the year. In summary, the techniques used in my study can complement existing efforts that study the communities in the California Current and further our understanding of intraspecific differences in California sea lions.

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APPENDICES

Appendix I: Test of DNA presence in *Zalophus californianus* scats

To confirm that DNA extractions from California sea lion (*Zalophus californianus*) scats contained DNA, a subset of extracted samples were tested for the presence of sea lion DNA. Two microsatellite markers (*Zc*CgDh1.16 [Hernandez-Velazquez *et al.* 2005] and *Zcw*A05 [Hoffman *et al*. 2007]) were tested on eight fecal DNA samples. Reactions (15 μl per sample) contained 6.81 μl of sterile water, 1.5 μl of GeneAmp®10X PCR buffer II (100 mM Tris-HCl, pH 8.3, 500 mM KCl), 0.9 μl of 25 mM $MgCl_2$, 0.6 μl of 10 mM dNTPs, 1.0 μl of 5 μM each primer, 0.035 μl of 5U/μl AmpliTaq® DNA polymerase, 0.15 μl of 0.1 μg/μl Bovine Serum Albumin (BSA) and 4 μl template DNA. Reactions were run on a BioRad S1000^{TM} thermal cycler with the following conditions: 95ºC for 2 minutes, 10 cycles of 95ºC for 15 seconds, 53ºC for 15 seconds and 72ºC for 45 seconds, 25 cycles of 89ºC for 15 seconds, 55ºC for 15 seconds and 72ºC for 45 seconds, a final extension at 72ºC for 5 minutes. PCR products were

visualized on a 1.5% agarose gel with a standard 1 kb DNA ladder and scored as a positive amplification if a band appeared between 100 and 200 bp. All samples amplified with marker *Zc*CgDh1.16 and failed with marker *Zcw*A05 (Fig. A1.1).

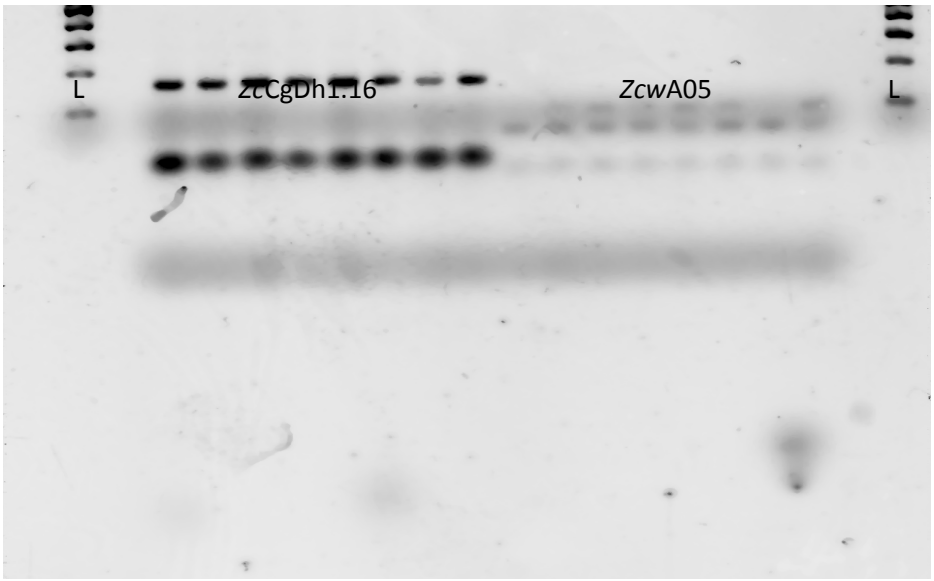

Fig. A1.1. Gel electrophoresis image of the test for DNA presence from *Zalophus californianus* extractions. The same eight samples were tested with each primer set. A standard 1 kb DNA ladder is included in the first and last well of the gel.

Appendix II: Reproducibility test for *Zalophus californianus* sex assignment from fecal DNA

To confirm that the results of sex identification assay were valid, as well as to re-test samples that had an unconfirmed sex assignment (n=12), a group of samples were retested with the sex identification assay. The mix of samples included the aforementioned 12 with indeterminate sex assignment, as well as samples assigned as male and female California sea lions (n=29), elephant seal (*Mirounga angustirostris*) (n=4) samples and three NTCs of water. Reactions (15 μl per well) contained 5.81 μl of sterile water, 1.5 μl of GeneAmp®10X PCR buffer II (100 mM Tris-HCl, pH 8.3, 500 mM KCl), 0.9 μl of 25 mM $MgCl_2$, 0.6 μl of 10 mM dNTPs, 1.0 μl of 5 μM each primer, 0.035 μl of 5U/μl AmpliTaq® DNA polymerase, 0.15 μl of 0.1 μg/μl Bovine Serum Albumin (BSA) and 4 μl template DNA. PCR was run on a BioRad® S1000 thermal cycler with the following protocol: 95 ºC/2 minutes, 10 cycles of 95 ºC for 30 seconds, 48 ºC for 30 seconds and 72 ºC for 45 seconds, 35 cycles of 89ºC for 20 seconds, 50 ºC for 30 seconds and 72 ºC for 45 seconds, and a final extension of 72 ºC for 5 minutes. Products were loaded onto a 3% agarose gel run at 155V for 90 minutes and scored following the established protocol (males have 2 bands at ~155 and 99 bp; females have a single band at ~155 bp and indeterminate samples contain a single band at 99 bp or no bands at all). All samples except one were assigned a sex (Fig A2.1). Agreement between original scores and the second scoring was high, with questionable assignments

confirmed as male or female by JC Garza. The single sample (14-0722-10) was excluded from further analysis as it consistently could not be assigned a sex nor confirmed as a California sea lion.

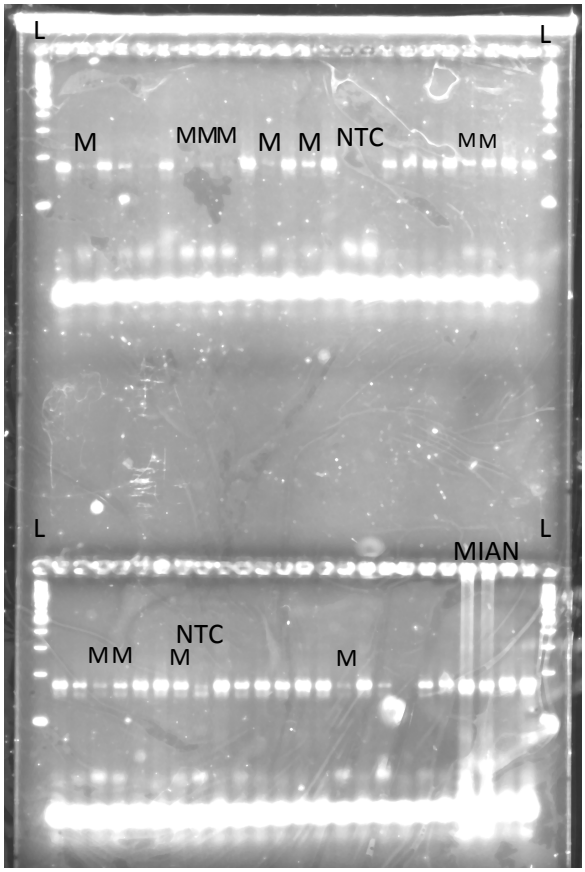

Fig. A2.1. Gel electrophoresis image of the reproducibility assay for the *Zalophus californianus* sex assignment assay. All samples that had an indeterminate assignment were re-tested along with positively sexed samples (scores indicated for indeterminate samples). Three NTCs containing sterile water were included. The last four samples in the second row are from two northern elephant seals (MIAN). A 1 kb DNA ladder is included in the first and last well of each row.

Appendix III: Correction factors and regression equations used to estimate number and mass of prey items consumed by *Zalophus californianus* during the study period.

Table A3.1. Correction factors applied to estimates of prey number and length from otolith and beak length. When species-specific numbers were not available, a numeric correction factor (NCF) for a closely related species was used; failing that, a correction factor of 1.43 (Orr & Harvey 2001) was applied. Grade-specific length correction factors (gLCFs) were used when possible; otherwise, average length correction factors (aLCF) were used. Correction factors were not applied to measurements of cephalopod beaks. Original references are provided except for species that lack correction factors. Grade 1 = low level of erosion, grade 2 = moderate level of erosion, and grade 3 = high level of erosion (Sweeney & Harvey 2011).

Species	NCF	aLCF	gLCF		Reference
			Grade	Factor	
Anoplopoma fimbria	1.43	NA	NA		NA
Chilara taylori	1.3 (for *M. productus*)	NA	NA		Orr & Harvey (2001)
Citharichthys sordidus	2.13	1.15	1	1.01	Phillips & Harvey (2009)
			2	1.10	
			3	1.26	
C. stigmaeus	1.07	1.1	NA		Phillips (2005)
Clupea pallasi	1.3	1.22	NA		Orr & Harvey
Cololabis saira	1.43	NA	NA		NA
Cymatogaster aggregata	1.7	1.49	NA		Bowen (2000); Harvey (1989)
Doryteuthis opalescens	1.1	1.00, 1.06	NA		Sweeney & Harvey (2011)
Engraulis mordax	2.2	1.30	1	1.3	Sweeney & Harvey (2011)
			2	1.48	
			3	1.70	
Genyonemus lineatus	1.43	NA	NA		NA
Glyptocephalus zachirus	1.3	1.36	NA		Harvey (1989)
Gonatus onyx	NA	NA	NA		NA
Lyopsetta exilis	1.43	NA	NA		NA

Merluccius productus	1.3	1.52	1	1.06	Orr & Harvey (2001); Sweeney & Harvey (2011)
			2	1.56	
			3	2.08	
Microstomus pacificus	1.2	1.25	NA		Harvey (1989)
Octopus rubescens	1.2	NA	NA		Bowen (2000)
Onychoteuthis borealijaponicus	NA	NA	NA		NA
Parophrys vetulus	4.1	1.31	NA		Harvey (1989)
Peprilus simillimus	1.43	NA	NA		NA
Porichthys notatus	1.3	NA	NA		NA
Sardinops sagax	3.0	1.09	1	1.04	Sweeney & Harvey (2011)
			2	1.12	
			3	1.35	
Sebastes jordani	1.46	1.34	1	1.06	Sweeney & Harvey (2011)
			2	1.21	
			3	1.56	
Stenobrachius leucopsarus	1.43	NA	NA		NA

Table A3.2. Regressions used to estimate prey length (SL = standard length in cm for fishes, DML = dorsal mantle length in mm for cephalopods) and mass (g) from measurements of otoliths (ventral length, VL) and beaks (lower rostral length, LRL or upper rostral length, URL). When species-specific regressions are not available, a mean mass, based on average fish length is reported instead.

Species	Length Regression	Mass Regression	Reference
Anoplopoma fimbria	SL = 5.28*(VL) +1.62	$M = 0.0163*(SL)^{2.902}$	Harvey *et al.* (2000)
Chilara taylori	SL = 2.51*(VL) + 2.15	$M = 0.0004*(SL)^{3.761}$	Harvey *et al.* (2000)
Citharichthys sordidus	SL = 2.87*(VL) + 3.29	$M = 0.0352*(SL)^{2.710}$	Harvey *et al.* (2000)
C. stigmaeus	SL = 3.2*(OL) – 0.3	$M = 8.12800*(SL)^{0.26}$	Harvey (1987)
Clupea pallasii	SL = 5.24*(VL) – 1.85	$M = 0.0044*(SL)^{3.398}$	Harvey *et al.* (2000)
Cololabis saira	ND	$\mu = 21.4$ g	FishBase
Cymatogaster aggregata	SL = 1.74*(VL) – 0.52	$M = 0.0100*(SL)^{3.515}$	Harvey *et al.* (2000)
Doryteuthis opalescens	DML = 607.8*(LRL) + 32.4 DML = 542.7*(URL) + 42.2	ln(M) = [ln(LRL)*1.4] + 6.0 ln(M) = [ln(URL)*1.21] + 5.7	Wolff (1984)
Engraulis mordax	SL = 2.280*(VL) + 0.85	$M = 0.0485*(SL)^{2.413}$	Harvey *et al.* (2000)
Genyonemus lineatus	SL = 1.52*(VL) + 4.66	$M = 0.0550*(SL)^{2.700}$	Harvey *et al.* (2000)
Glyptocephalus zachirus	SL = 4.80*(VL) – 2.50	$M = 0.0238*(SL)^{2.692}$	Harvey *et al.* (2000)

Gonatus onyx	DML = 12.82 + 190.2*(LRL) DML = 15.22 + 181.5*(URL)	ln(M) = 4.99 + 2.13*ln(LRL) ln(M) = 4.69 + 1.93*ln(URL)	Wolff (1984)
Lyopsetta exilis	SL = 3.37*(VL) + 1.08	$M = 0.0058*(VL)^{3.293}$	Harvey *et al.* (2000)
Merluccius productus	SL = 2.04*(VL) + 0.96	$M = 0.0081*(SL)^{2.966}$	Harvey *et al.* (2000)
Microstomus pacificus	SL = 3.72*(VL) + 6.97	$M = 0.0094*(SL)^{3.092}$	Harvey *et al.* (2000)
Octopus rubescens	DML = 5.08(LRL) + 18.671	M = 0.415(DML) + 32.44	Oxman (Unpublished data)
Onychoteuthis borealijaponicus	DML = -28.9 + 61.0*(LRL)	ln(M) = 0.576 + 3.00*ln(LRL)	Clarke (1986)
Parophyrs vetulus	SL = 3.82*(VL) – 2.76	$M = 0.0163*(SL)^{2.939}$	Harvey *et al.* (2000)
Peprilus simillimus	SL = 0.1*(LVL) + 11.2 SL = 1.1*(RVL) + 6.7	$\mu = 28.3$ g	Harvey (1987)
Porichthys notatus	SL = 2.80*(VL) – 2.59	$M = 0.0207*(SL)^{2.916}$	Harvey *et al.* (2000)
Sardinops sagax	SL = 6.108*(VL) – 1.618	$M = 0.007*(VL)^{2.758}$	Sweeney (2008)
Sebastes jordani	SL = 1.689*(VL) + 1.095	$M = 2.136*(VL)^{1.219}$	Phillips (2005)
Stenobrachius leucopsarus	SL = 46.63*(OH) – 0.829	$M = 0.00000656*(SL)^{3.121}$	Sinclair *et al.* (2015)