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# The Feeding Habits and Selectivity of Siphonophores in Monterey Bay

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# THE FEEDING HABITS AND SELECTIVITY OF SIPHONOPHORES IN MONTEREY BAY

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Alexandra Lapides

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# CALIFORNIA STATE UNIVERSITY MONTEREY BAY

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# DEDICATION

This work is dedicated to the pioneers that made our current ability to explore the ocean possible, be it ships hands, engineers, or scientists.

# ABSTRACT

The Feeding Habits and Selectivity of Siphonophores in Monterey
Bay
by
Alexandra Lapides
A Thesis presented to the faculty of the Department of Marine
Science, California State University Monterey Bay in partial
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Gelatinous zooplankton are historically understudied, and we have much to learn about how they fit into the larger food web. Siphonophores are known to have widely varied diets and to select for a wide variety of prey. In this study I investigated siphonophore feeding habits in Monterey Bay, CA using a long-term remotely operated vehicle video dataset. In addition, I quantified the degree of specialization for each siphonophore-prey pair, and investigated the relationship between genetic distance and specialization differences. I found siphonophores tended to feed upon one prey group and in some cases fed exclusively on one species. Siphonophores also tended to select strongly for one type of prey. I found that more closely related siphonophores tended to show similar selectivity values, but the relationship was weak. Overall, this study upholds that siphonophores are specialists and very selective, and that phylogenetic distance has some positive relationship with selectivity. These findings uphold and expand our knowledge of the midwater food web, allowing us greater model specificity, the enhanced ability to track energy flow and carbon cycling, and greater capacity to manage the midwater ecosystem.

## Acknowledgments

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## Introduction

Cnidarian predators of the Order Siphonophora have worldwide presence and feed upon a variety of prey spanning several trophic levels. Despite their global distribution and ecological importance, we have much to learn about how they fit into the larger pelagic food web. Traditionally gelatinous zooplankton such as siphonophores have been considered a negligible component of food webs, but recent studies suggest the "jelly web" may play a more integral role than initially thought (Robison 2004; Choy et al. 2017; Hays et al. 2018; Thiebot and McInnes 2019). Unfortunately gelatinous zooplankton are difficult to study via traditional methods such as net tows due to their fragility, and feeding data are even more difficult to discern due to destruction or artificial ingestion of other organisms in the net tow. Remotely operated vehicles (ROVs) offer a solution to this problem by allowing scientists to observe organisms *in situ* with relatively minimal disturbance (Choy et al. 2017).

Siphonophores are well suited for an investigation of high-resolution food web links using video data. They are easy to observe *in situ* from an ROV due to their limited swimming ability and cosmopolitan abundance. They react minimally to the presence of ROVs and belong to the "jelly web", allowing exploration of this understudied component of the food web. Additionally, many siphonophores are partially transparent, allowing visual identification of what they have recently eaten.

In the Monterey Bay off the coast of California, siphonophores are generally known to consume a broad variety of prey (Choy et al. 2017; Hetherington et al. 2022). However at the species-by-species level most siphonophores tend to specialize upon one prey type, while only a few are considered generalists (Purcell 1981b; 1981c; Damian-Serrano et al. 2022). Specialized diets may be related to specialization of the siphonophore feeding apparatus

(Damian-Serrano et al. 2021a). These patterns have culminated in predicted and observed feeding guilds for many siphonophores, generally broken down into hard-bodied prey, fish prey, gelatinous prey, or generalists (Damian-Serrano et al. 2021b).

Strong specialization across Siphonophora suggests some form of prey selectivity is likely occurring at the species level. An organism that is not selective (neutral selectivity) feeds on prey at the same rate the prey occurs around it. Positive prey selectivity indicates that an organism is seeking out that specific prey, whereas negative selectivity means that a predator is avoiding that prey relative to its abundance in the environment. If the system is perturbed and prey abundance changes, then understanding the dietary selectivity of a predator would inform predictions of how predators and the overall system may respond. For example, the siphonophore *Nanomia bijuga* selects for krill, which make up 16% of its diet despite only being 0.4% of the individuals observed in a corresponding net tow. Similarly, the siphonophore *Apolemia uvaria* selects for gelatinous zooplankton (13.3% of diet vs 0.2% of individuals in the environment) (Purcell 1981b). As a result, if the abundance of shrimp or gelatinous zooplankton changes in this environment, siphonophore populations will also be affected. Comparisons of net tow data with either visual gut contents or DNA metabarcoding indicate that siphonophores tend to be very selective towards their preferred prey items (Hetherington et al. 2022; Damian-Serrano et al. 2022).

Some dietary patterns may be related to shared ancestry within siphonophore sub-groups. Closely related species have been observed feeding upon similar prey, such as species in the genus *Apolemia* that all feed upon gelatinous prey, or the closely-related *Agalma elegans* and *Agalma okeni* which have both been observed to feed on amphipods (Biggs 1977). Similarly suborders of siphonophores show distinct feeding patterns, with

Cystonectae preferring fish prey, and Calycophorae largely feed upon copepods (Hetherington et al. 2022; Damian-Serrano et al. 2022). Thus, it may be possible that more closely related siphonophores select for more similar prey but this requires investigation across a broader taxonomic range.

This study seeks to characterize the specialization and selectivity of common siphonophores in Monterey Bay using a comprehensive ROV video dataset. In addition, I investigate the generality versus specialization of diets across several siphonophore species, and assess any relationship between selectivity and genetic relatedness. In comparison to previous net-based methods, this approach allows better inclusion of the understudied 'jelly web' and includes some deep-sea species for which literature is sparse. Overall, this study allows greater comprehension of food web linkages, energy flow, and resource partitioning, which informs conservation of the midwater environment.

## Methods

To ascertain siphonophore feeding and abundance, I leveraged the Monterey Bay Aquarium Research Institute's (MBARI's) Video Annotation and Reference System (VARS) database. High definition cameras on MBARI's ROVs capture observations of gelatinous zooplankton and other organisms without removing them from their habitat. Each dive is recorded and reviewed by an in-house suite of expert taxonomists, who identify and annotate each organism seen on video. These annotations are coupled with physical water parameters collected by a SeaBird CTD 19, Seabird 43 O2, and a Wetlabs C-Star Transmissometer (Bellevue, WA). In addition, behaviors such as interactions with other species are noted, including predation events. These video data have been collected in Monterey Bay since

1989 and focus on daytime hours, generally 6:30 am to 6:30 pm. The coverage is year-round and ranges from the surface to 4000 meters depth. As of 2015, there are over 21,000 hours of video recorded and over 4.5 million annotations. For this study, data were confined to Monterey Bay, which I defined as between 35 and 38 degrees Latitude and -121 and -125 degrees Longitude.

To observe siphonophore feeding, I queried the VARS database for every feeding observation of a siphonophore eating another animal. Each observation was accompanied by a framegrab to verify the taxonomic identity of both the predator and their prey. For feeding observations that did not have an associated framegrab and that were not sightings of *Nanomia bijuga*, I reviewed the tapes and took stills from video. Taxonomic identity of both siphonophores and prey were confirmed by experts in the field, and all organisms were identified down to the lowest possible taxonomic category (hereafter ‘concept’). *Nanomia bijuga* feeding observations without associated framegrabs (n=142) were not included in subsequent analyses, due to abundance of routine annotations in the database.

To ascertain the relative degree of feeding specialization for each siphonophore, the Shannon Diversity Index was calculated based on each predator’s diet (Shannon 1948). The Shannon Diversity Index was used because it better captures the richness of concepts in a diet and is less biased towards common species. This analysis was applied twice, once with each prey concept and once with all prey concepts grouped into broad functional groups (Table 1). Note that the list of prey concepts was based on the observed feeding interactions, and the full list of potential prey items includes thousands of other concepts.

Table 1: Individual concepts grouped into functional groups.

| **Functional Group** | **Concepts Within** |
|---|---|
| Gelata | *Bathochordaeus*, *Oikopleura*, Salpida, *Apolemia*, *Forskalia*, *Nanomia bijuga, Aegina*, *Crossota*, *Haliscera conica*, Hydromedusae, Leptothecata, *Mitrocoma cellularia*, Narcomedusae, *Ptychogena lactea, Solmissus, Atolla, Periphylla periphylla*, Cydippida, *Pleurobrachia,* Chaetognatha, *Caecosagitta macrocephala, Poeobius meseres*, Medusae |
| Hard-bodied | Crustacea, Copepoda, Euphausiacea, Lophogastrida, Decapoda, *Eusergestes similis, Petalidium suspiriosum* |
| Fish | Actinopterygii, *Cyclothone*, *Leuroglossus*, Liparidae, Myctophidae, *Tarletonbeania, Careproctus melanurus* |
| Cephalopods | Cephalopoda, *Galiteuthis*, Teuthoidea, Cranchiidae |

To calculate selectivity, I used Strauss' Linear Index (Strauss' Li or simply Li) for each predator-prey combination (Strauss 1979). Strauss' Li is calculated as

$$Li = r_i - p_i, \tag{1}$$

where $r_i$ is the proportion of a single prey type in the siphonophore's diet and $p_i$ is the proportion of that same prey in the siphonophore's environment. For the diet metric $r_i$, I used the feeding data as described above. For the environment metric, $p_i$, the processing was more complex. I queried VARS for every occurrence of each type of siphonophore seen feeding as well as every occurrence of each observed prey. Annotations were constrained to dives that had been fully annotated and fell within the bounds of Monterey Bay as defined above. Given that the annotations to species-level are not perfect, depth outliers at the 97th percentile were removed as they likely represented erroneous entries. For krill, I used the built-in tags of ‘population-quantity 999’ to refer to 50 krill, ‘dense’ to refer to 150 krill, and ‘swarm’ to refer to 400 krill (Rob Sherlock, *pers. comm.*). Data were binned into 50 m depth bins as well as month of the year. The siphonophore predator *Agalma* was removed from the analysis at this stage due to inconsistencies in annotations over time in the VARS database. It should also be noted that for the purpose of this analysis, we assume that every predator can eat every potential prey.

The proportion of prey found in the predator’s environment, $p_i$, was then calculated by taking into account the distributions of predators and prey in respect to depth bin and month of the year. The relative encounter rates between a siphonophore predator and its $i^{\text{th}}$ prey in depth and month bin $j$ is estimated to be:

$$p_i = \frac{\Sigma \frac{n_{ij}}{t_j} \frac{d_j}{\Sigma d} \Sigma t}{\Sigma \frac{n_{pj}}{t_j} \frac{d_j}{\Sigma d} \Sigma t}, \qquad (2)$$

which reduces to:

$$p_i = \frac{\Sigma \frac{n_{ij} d_j}{t_j}}{\Sigma \frac{n_{pj} d_j}{t_j}}. \qquad (3)$$

where $n_{ij}$ is the number of prey $i$ in bin $j$, $n_{pj}$ is the number of all prey in bin $j$, $t_j$ is the time spent in bin $j$ and $\Sigma t$ is the total time spent across all depth and time bins, and $d_j$ is the number of the siphonophore predator in bin $j$ and $\Sigma d$ is the total number of the siphonophore predator across all depth and time bins. The proportion of the predator is included as part of this calculation because it gives a finer and more accurate representation of the predator’s “environment”; high prey abundance in a certain depth bin does not represent a high proportion of a predator’s environment if that predator is not also abundant in the same depth bin. A time correction is also included in this calculation because sampling effort is not equal across all depths and times of year. Depth and time bins where the ROV spent more time surveying are weighted more heavily, as with infinite survey time I would approach the true and accurate proportions of predators and prey per bin. These timing data came from either CTD or navigation data associated with each dive. I only included annotation data for dives and parts of dives that had associated CTD or navigation data. Benthic ROV dives were excluded from this analysis to avoid biasing time corrections from benthic sections of the dive, as this study focused on the midwater environment.

Table 2: Strauss’ Li values broken into 7 equally sized sections to represent a range of negative, neutral, and positive selection scenarios.

| **Strauss’ Li value** | **Selection range represented** |
|---|---|
| -1 to -0.71 | Extreme negative selection |
| -0.71 to -0.43 | Moderate negative selection |
| -0.43 to -0.14 | Slight negative selection |
| -0.14 to 0.14 | Netural selection |
| 0.14 to 0.43 | Slight positive selection |
| 0.43 to 0.71 | Moderate positive selection |
| 0.71 to 1 | Extreme positive selection |

Once Strauss’ Li was calculated for each possible predator-prey pairing, I split the selectivity values into 7 equally-sized sections represented in Table 2. I then did a 2-tailed one-sample T-test for all pairs with sufficient sample size (Strauss 1979). The adjusted sample size for each predator-prey pair was considered to be the denominator of Equation 3. For all calculations, if the predator or prey concept was not species-level, I assumed that the concept represented that taxonomic group itself and its descendants. For example, an observation of a siphonophore eating the shrimp *Eusergestes similis* would also be counted in calculations for that same type of siphonophore eating any Decapoda. This nested analysis did not affect sample size calculations.

To compare the pairwise degree of relatedness to selective patterns, I used a 18s phylogenetic tree of siphonophores to calculate genetic distance between different siphonophore species (Dunn et al. 2005, with additional sequences from Steve Haddock, *pers. comm.*). Genetic distance was calculated as pairwise branch length between every pair of siphonophores. I compared this set of distances to the pairwise Euclidean distance of all predator-prey Li indices for each predator to determine whether genetic distance increased as selective difference increased, and ran a linear regression to verify the trend.

## Results

Using MBARI's VARS dataset, I collected novel data about siphonophore feeding. I collected 155 distinct observations of 14 siphonophore species feeding on 36 different types of prey, for a total of 504 potential predator-prey pairs. Five species of siphonophore had only one feeding observation each (Fig. 1). The number of observations varied widely between predators, with *Bathyphysa conifera* only observed 5 times in our total dataset (feeding or non-feeding), and *Nanomia bijuga* observed 112,921 times. Overall, this represents a broad dataset with which to investigate siphonophore feeding behavior in Monterey Bay.

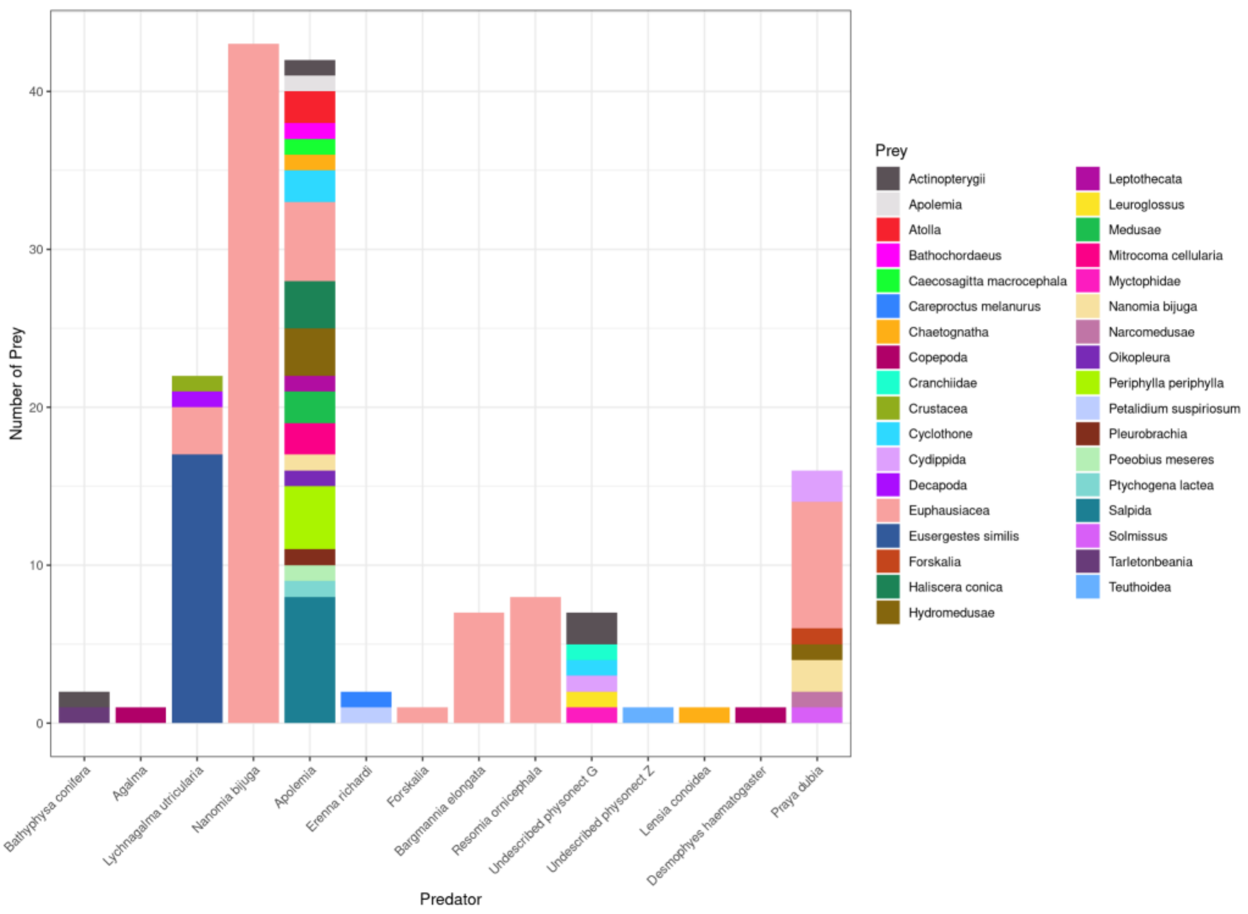

Figure 1: number of feeding observations on each prey type, per siphonophore predator, by prey type.

Table 3: Shannon diversity indices for all siphonophore feeding observations at the lowest taxonomic level and those same observations grouped to larger categories. Siphonophores are sorted as Cystonects, followed by Physonects, followed by Calycophorans, in rough phylogenetic order.

| **Predator** | **Individual Concepts** | **Functional Groups** |
|---|---|---|
| *Bathyphysa conifera* | 0.69 | 0 |
| *Agalma* | 0 | 0 |
| *Lychnagalma utricularia* | 0.90 | 0.18 |
| *Nanomia bijuga* | 0 | 0 |
| *Apolemia* | 2.73 | 0.61 |
| *Erenna richardi* | 0.69 | 0.69 |
| *Forskalia* | 0 | 0 |
| *Bargmannia elongata* | 0 | 0 |
| *Resomia ornicephala* | 0 | 0 |
| Undescribed Physonect G | 1.75 | 0.80 |
| Undescribed Physonect Z | 0 | 0 |
| *Lensia conoidea* | 0 | 0 |
| *Desmophyes haematogaster* | 0 | 0 |
| *Praya dubia* | 1.56 | 0.69 |

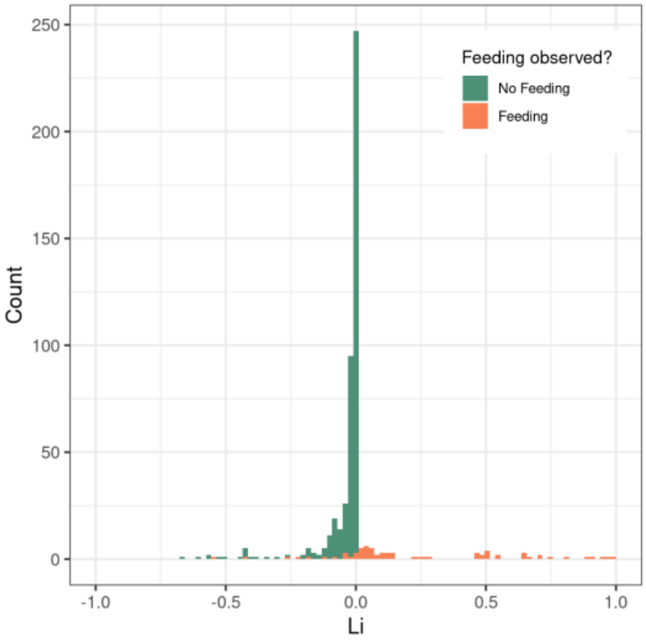

Figure 2: Distribution of selectivity values (Strauss' Li) values for all potential predator-prey pairs in the dataset, colored by whether feeding observations existed for a particular predator-prey pair.

Table 4: Predator, Prey, Li, p values, $r_i$, and $p_i$ for all predator-prey pairs with both sufficient sample sizes in feeding and environmental data and also sufficient Li values to assume normality and apply statistics, sorted by Li value.

| **Predator** | **Prey** | **Li** | **p** | $r_i$ | $p_i$ |
|---|---|---|---|---|---|
| *Lychnagalma utricularia* | Decapoda | 0.75 | << 0.001 | 0.783 | 0.034 |
| *Lychnagalma utricularia* | *Eusergestes similis* | 0.71 | << 0.001 | 0.739 | 0.031 |
| *Apolemia* | Gelata | 0.29 | << 0.001 | 0.810 | 0.522 |
| *Praya dubia* | Gelata | 0.25 | 0.049 | 0.5 | 0.254 |
| *Apolemia* | Salpida | 0.15 | 0.014 | 0.190 | 0.042 |
| *Apolemia* | *Periphylla periphylla* | 0.09 | 0.038 | 0.095 | 0.001 |
| *Apolemia* | Leptothecata | 0.08 | 0.089 | 0.095 | 0.018 |
| *Apolemia* | Euphausiacea | -0.12 | 0.017 | 0.119 | 0.238 |
| *Praya dubia* | Euphausiacea | -0.19 | 0.133 | 0.5 | 0.688 |
| *Praya dubia* | Crustacea | -0.23 | 0.069 | 0.5 | 0.727 |
| *Apolemia* | Crustacea | -0.27 | << 0.001 | 0.119 | 0.388 |

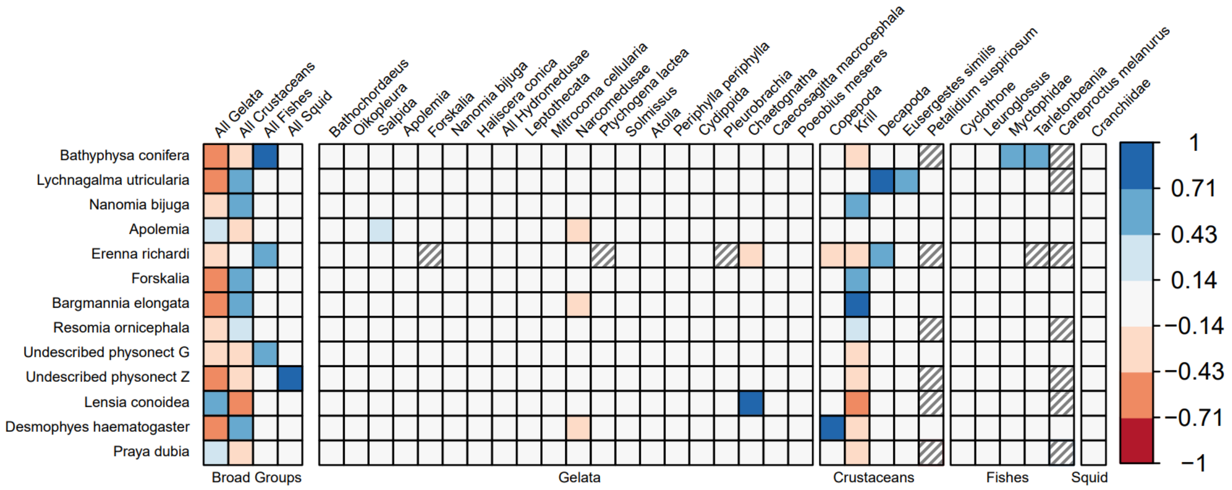

Figure 3: Matrix of Selectivity (Strauss' Li) indices by predator-prey pair. Siphonophores are sorted in broad phylogenetic groups (Cystonects, then Physonects, then Calycophorans). Functional groups are listed on the left and their corresponding components are listed in chunks on the right. Cells are colored so that bluer values indicate more positive selection and redder values indicate more negative selection, and white indicates neutral selection. The breaks are represented more fully in Table 2. Hatched cells had no environmental overlap between predator and prey, so selectivity calculation would not be biologically meaningful. Most predator-prey pairs did not have sufficient sample sizes to test for significance; those that did are listed in Table 4.

Siphonophore species tended to show high specialization in their feeding and tended to select for one prey concept or prey type. Most species fed upon only one prey type, indicated by zero Shannon Diversity Index (Table 3). The highest individual Shannon Diversity Index belonged to *Apolemia* at 2.73, although when prey items were grouped into larger categories *Apolemia* reported a Shannon Diversity Index in the middle of the group.

Overall, 88% (444) of 504 potential predator-prey pairs had neutral Li selectivity values between -0.14 and 0.14 (Fig. 2). Thirty predator-prey pairs had positive selection higher than 0.14 (6%), and of those, 7 predator-prey pairs had positive selection of 0.71 or higher (1%). Conversely, 30 predator-prey pairs had negative selection lower than -0.14 (6%) and none had negative selection of -0.71 or lower. Only 11 predator-prey pairs had sufficient sample size and selectivity indices to assume normality and apply statistical analysis. Of those, 6 were significantly positively selective ($p < 0.05$), 3 were significantly negatively selective, and 2 were not significantly selective (Table 4). Generally, organisms only showed strong selection for a single prey type (hard-bodied, gelata, fish, or squid), and otherwise selection was relatively neutral. The only exception to this pattern was *Erenna richardi*, which selected for decapods and fishes (Fig. 3). Overall this indicates that these results generally agree with the broader literature.

### Species - by - species patterns

Of the siphonophore predators in this dataset, four stood out as having especially significant or notable feeding patterns. These siphonophores are discussed in more detail below.

#### *Lychnagalma utricularia*

The physonect *Lychnagalma utricularia* showed strong selection for decapods due to feeding with very significant positive selection on the shrimp *Eusergestes similis* (n=17, p = <<0.0001) and occasionally krill (n=3). The depth distribution of *Lychnagalma utricularia* is relatively narrow and falls squarely inside the maximum of *Eusergestes similis*'s distribution

(Fig. 4).

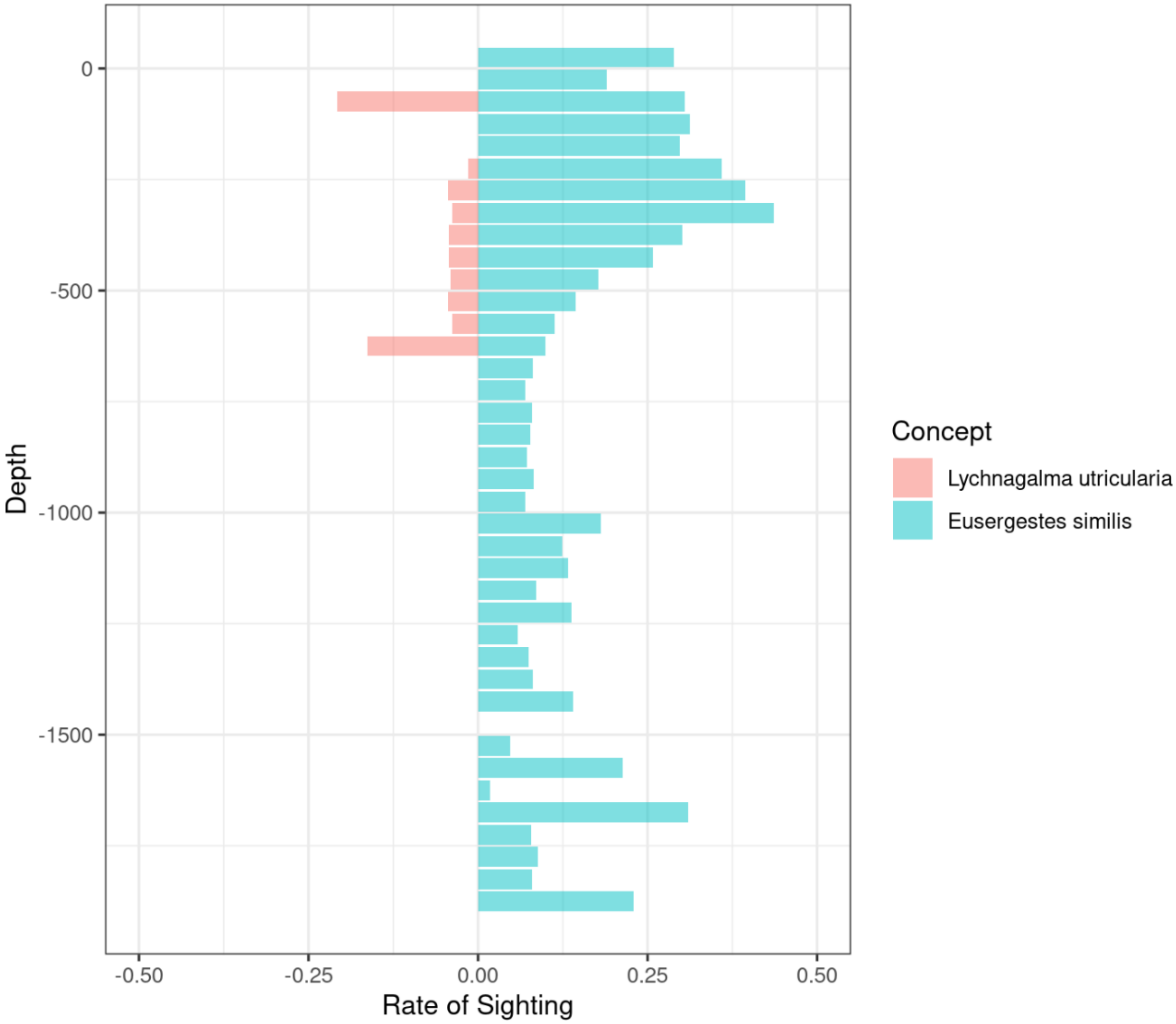

Figure 4: *Lychnagalma utricularia* vs *Eusergestes similis* distribution by depth, integrated over any seasonal patterns. Data were binned by 50m depth bins before being corrected by amount of time spent per bin.

<u>*Apolemia*</u>

*Apolemia* had the most varied diet of any siphonophore in this study. *Apolemia* fed on a variety of gelata (13 different types observed in this study, 2.6 times greater variety than the next most common gelata consumer, *Praya dubia*). This variety explained the collapse of the Shannon Diversity Index when applied at the prey group level for *Apolemia*: while *Apolemia* fed upon many kinds of gelata, it still specialized on gelata. Notably, *Apolemia* also fed upon the larvaceans *Bathochordaeus* and *Oikopleura*, as well as upon salps. *Apolemia* were also significantly selective for the coronate scyphozoan *Periphylla periphylla* ($p = 0.04$), and significantly selective for gelata as a functional group ($p << 0.001$). Although there were multiple observations of *Apolemia* feeding on krill, *Apolemia* were still significantly negatively selective for krill ($p = 0.008$) and for Crustacea in general ($p << 0.001$). *Apolemia* were not significantly positively selective for Leptothecates ($p = 0.09$). It was also observed feeding upon the fish *Cyclothone*.

Of all prey items, *Apolemia* fed with the most positive selectivity on salps ($n = 8$, $p = 0.014$) (Fig. 3). I assumed that each salp capture was a separate event even if the salp captured was actually a colony, since in both cases each salp must be captured by the predator. *Apolemia* and salps both have broad (and overlapping) depth distributions and show marked similarity in the shape of their distributions (Fig. 5). In the first 1000 m, both *Apolemia* and salps show high abundance at the surface, followed by a local minimum at roughly 250 m, followed by increased abundance at roughly 500 m.

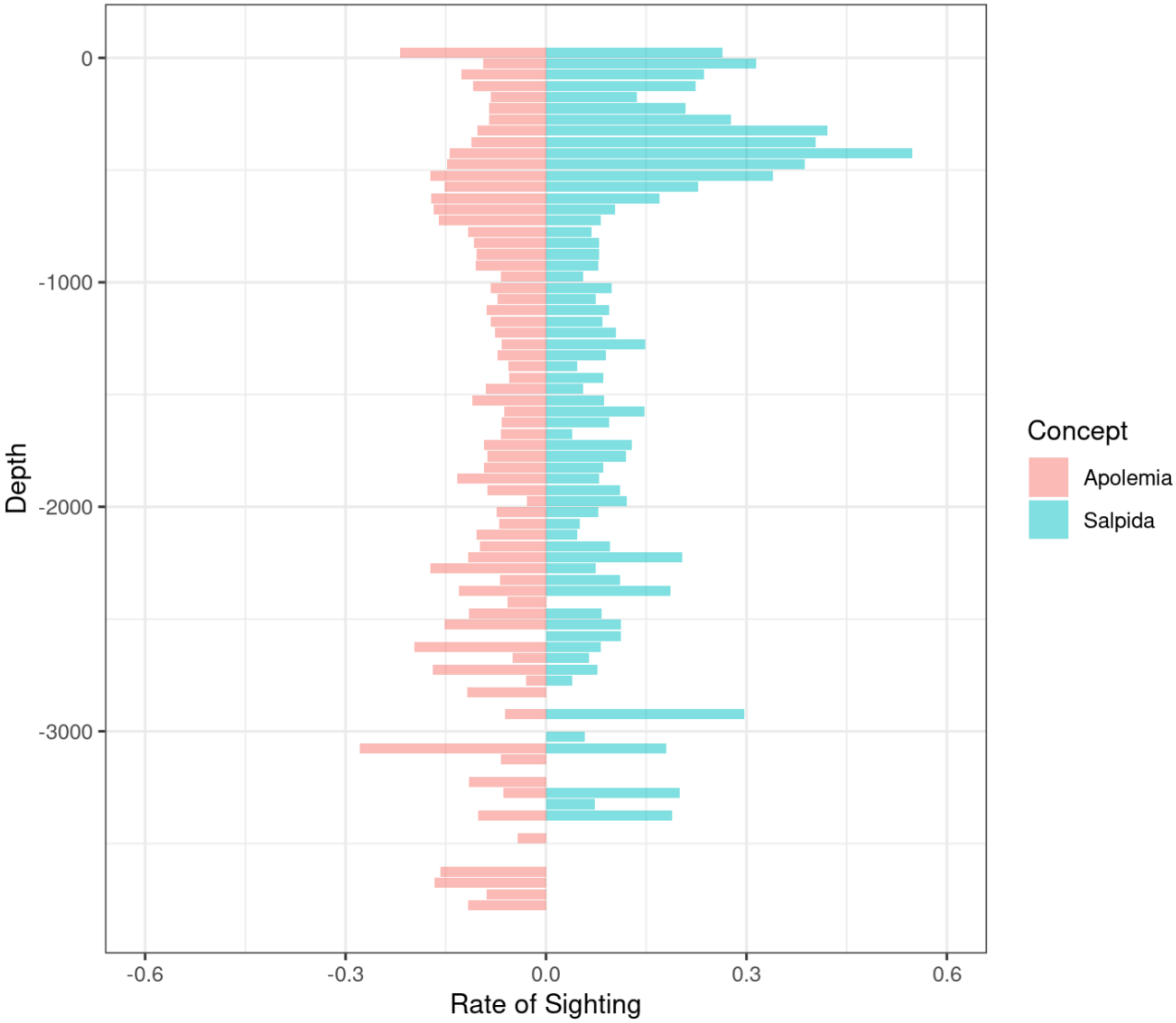

Figure 5: *Apolemia* and Salpida ranges by depth, integrated over time of year. The binning procedure here is the same as for figure 4.

_Nanomia bijuga_

While in the literature *Nanomia bijuga* is known to eat a variety of foods (copepods, decapod larvae, shrimp, chaetognaths, gelata, stomatopods, amphipods), in our dataset *Nanomia bijuga* fed exclusively or almost exclusively on krill (Purcell 1981b; Damian-Serrano et al. 2022). I excluded one *Nanomia bijuga* + *Aegina* observation from this dataset; since narcomedusae are known to prey on gelatinous species, the identity of the predator could not be determined with confidence. *Nanomia bijuga*'s specialization on krill is so strong that it is impossible to have a large enough dataset to test for significance (as selective signal increases, the sample size needed to assume normality trends towards infinity).

### *Praya dubia*

The Calycophoran *Praya dubia* was the only other siphonophore with significant sample size to test for significance in any of its predator-prey pairs. It is significantly positively selective for gelata ($p = 0.027$), and significantly negatively selective for Crustacea ($p = 0.037$). It is not significantly negatively selective for krill ($p = 0.071$). *Praya dubia* was observed feeding upon medusae, other siphonophores, cydippids, and krill.

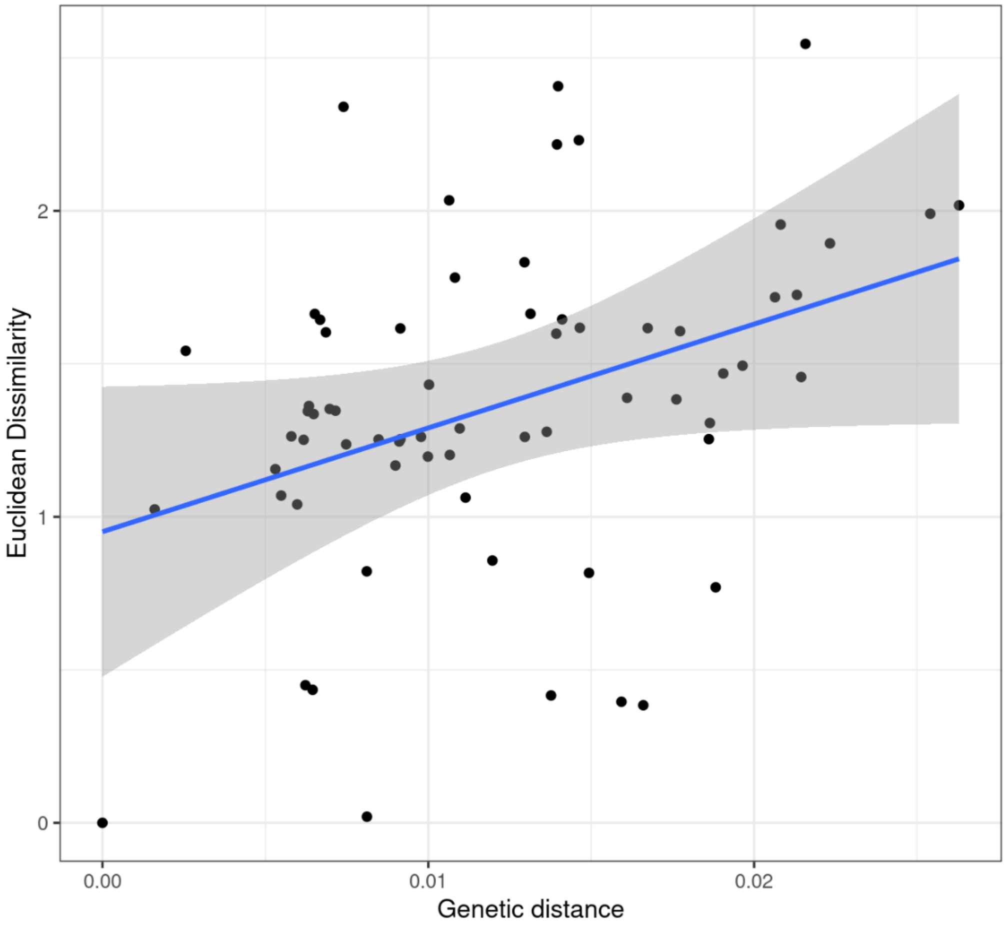

Figure 6: Pairwise Euclidean dissimilarity of Li values plotted against genetic distance of predators, with linear regression and 95% confidence interval indicated.

### Genetic results

The potential similarity between selectivity for closely related species motivated a test of phylogenetic relatedness as a function of selectivity difference. As genetic distance increased, difference in selectivity also increased ($p = 0.001$, Fig. 6). However, the variance in this trend was notable and the R squared value was low (0.13). Notable outliers include *Nanomia bijuga* and *Forskalia*, which are not the most closely related but showed extremely similar selective trends, and *Bargmannia elongata* and *Lensia conidea* which are not the furthest related but show extremely distinct selective trends.

## Discussion

In this study, I found that siphonophores had very specialized feeding habits, with many feeding upon only one type of prey. Even when observed feeding upon more than one species, they are nearly always restricted to one prey group. A clear example of this is *Bathyphysa conifera*, whose Shannon Diversity Index collapses to zero once prey are binned by group: although it has been observed feeding upon multiple types of fish, its diet was still exclusively fish. Although most siphonophore species have specialized diets, as a group they have been observed to feed upon a variety of prey spanning multiple trophic levels, indicating that they have complex ties to the “jelly web” as well as the food web at large (Damian-Serrano et al. 2022).

This high degree of specialization is somewhat to be expected in pelagic habitats. In a model that investigated specialization for sessile organisms, specialists outcompeted generalists in low-disturbance environments and in environments where most species were specialists (Büchi and Vuilleumier 2014). In birds, assemblages of species with similar degrees of specialization tended to co-occur (Julliard et al. 2006). Both these patterns (low-disturbance environment and high number of specialists) are present in the pelagic, suggesting that specialization is a way to partition resources in a relatively homogeneous environment.

Because siphonophores tend to show strong positive selection towards relatively few prey items, most siphonophore-prey pairs tested showed neutral Li indices. The distribution of Li values for siphonophore-prey pairs showed low kurtosis, indicating that siphonophores generally prefer to feed upon the things that are in their direct and immediate environment. Ostensibly, these prey are the least energetically expensive to capture due to already being commonplace. It should be noted that I assume every siphonophore predator can eat every observed type of prey, but this may not be true: some of what seems to be neutral or negative selection may be the inability of the siphonophore to consume the prey, either due to body size or limitations of the nematocyst morphology. For example, *Resomia* cannot catch fish because it has Crustacea-specialized nematocysts, which seek to entangle prey rather than piercing the epidermis to deliver venom (Damian-Serrano et al. 2021a, Alejandro Damian-Serrano, *pers. comm.*).

### Species-by-species patterns

#### *Lychnagalma utricularia*

In this study, *Lychnagalma utricularia* was observed to be feeding upon *Eusergestes similis* as well as krill. Additionally, *L. utricularia* was significantly positively selective for *Eusergestes similis.* These findings concur with the literature, where *L. utricularia* has returned both euphausiid and sergestid shrimp sequences from gut content metabarcoding (Damian-Serrano et al. 2022). Additionally, *Lychnagalma utricularia* has extremely specialized tentillum for prey capture, featuring floating golden lures and lacking an adhesive terminal filament (Pugh and Harbison 1986). These lures are likely specialized for capturing *Eusergestes similis.*

#### *Apolemia*

The *Apolemia* in this study were observed to feed upon a wide variety of gelatinous, hard bodied, and fish prey. Notably, *Apolemia* fed upon larvaceans as well as salps. These chordates are all key regulators of carbon cycling to the deep sea and siphonophores feeding upon them may regulate or modulate the amount of carbon exported (Damian-Serrano et al. 2022). These results are consistent with the broader literature. For example, Purcell (1981c) observed *Apolemia uvaria* to feed upon gelata, hard-bodied prey, and fish, including cladocerans, molluscs, and copepods.

However, when broken down by species, *Apolemia* shows different feeding tendencies. *Apolemia rubriversa* feeds upon copepods and salps, *Apolemia lanosa* feeds upon only copepods, and an undescribed *Apolemia* species eats ctenophores, larvaceans, mysids, and euphausiids. This indicates that spatially coexisting *Apolemia* species may niche partition by feeding upon different prey types (Damian-Serrano et al. 2022). It is possible that the seemingly wide variety of prey that *Apolemia* is observed to eat in this study would show stricter patterns if broken down into species-level categories.

*Apolemia uvaria*, and *Apolemia lanosa* have been predicted to be gelatinous specialists from their tentilla morphology (Damian-Serrano et al. 2021a). Here I demonstrate that *Apolemia* feeds upon a wide variety of gelata and shows significant positive selection for both gelata and salps. These results are consistent with Purcell, who found that *Apolemia uvaria* selects positively for gelata. However, Purcell also found that *A. uvaria* selects positively for chaetognaths and for shrimp (Purcell 1981b). I found no positive selection for chaetognaths and significant negative selection for both krill and crustaceans more generally. It is possible that these differences are due to differences in environment; this study focused on the mesopelagic while previous data focused on the epipelagic. Other studies suggest siphonophore food webs in the epipelagic may differ from those in the mesopelagic: mesopelagic webs are more specialized and less nested (with narrower feeding niches) (Hetherington et al. 2022).

### *Nanomia bijuga*

*Nanomia bijuga* in this study were observed to eat exclusively krill. In gut content metabarcoding, mesopelagic *N. bijuga* returns both krill and stomatopod DNA sequences (Damian-Serrano et al. 2022). Like with *Apolemia*, shallow *Nanomia* specimens have different feeding patterns than those observed from the mesopelagic. In shallow water, they fed upon copepods and hyperiid amphipods, and showed positive selection for copepods, decapod larvae, shrimp, and chaetognaths (Purcell 1981b; Hetherington et al. 2022; Damian-Serrano et al. 2022). As with *Apolemia*, these patterns may be due to differences in food web structure in the mesopelagic and epipelagic zones (Hetherington et al. 2022).

### Other species

Some of our selectivity results were consistent with previous studies, but some differed from earlier findings. I observed *Bargmannia elongata* to feed exclusively on krill and to positively select for krill, which aligns with krill and ostracod prey DNA sequences recovered from *B. elongata* gastrozooids (Damian-Serrano et al. 2022). This also corroborates Damian-Serrano et al.'s prediction of *B. elgonata*'s feeding guild to be large crustaceans (Damian-Serrano et al. 2021a). *Erenna richardi* was observed to eat both the shrimp *Petalidium suspiriosum* and the fish *Careproctus melanurus*, giving it positive selection for both decapods and fishes. This result is partially congruent with *E. richardi*'s predicted feeding guild of fishes; *E. richardi*'s close relative *E. laciniata* is also predicted to feed upon fishes due to tentilla morphology (Damian-Serrano et al. 2021a). In contrast to the positive selection for krill shown here, *Forskalia* sp. has otherwise been found to positively select for copepods and decapod larvae and negatively select for krill (Purcell 1981b; Hetherington et al. 2022; Damian-Serrano et al. 2022). In addition, *Forskalia* has been observed feeding upon a variety of prey including fish, mollusks, copepods, and sergestid shrimp; it is possible that the limited number of feeding observations in this study (n = 1) inhibited a more accurate representation of *Forskalia*'s feeding habits (Biggs 1977; Damian-Serrano et al. 2022). Finally, *Resomia ornicephala* also fed exclusively on and selected for krill, but its relatives *Resomia dunni* and *Resomia persica* are predicted to be generalists from tentilla morphology (Damian-Serrano et al. 2021a). This discrepancy may again be due to depth: *Resomia ornicephala* lives notably shallower in the water column than *R. dunni* or *R. persica* (Pugh and Haddock 2010). Generally, physonects showed some positive selection towards krill, but this was not true in all cases.

For the Calycophorans, *Desmophyes haematogaster* was predicted to feed upon small crustaceans and *D. haematogaster*'s relative *D. annectens* has returned copepod DNA from its gut (Damian-Serrano et al. 2021a, 2022). In this study, I observed *D. haematogaster* to consume a copepod and to select for copepods which is in full alignment with previous studies. *Praya dubia* consumed a wide variety of gelata as well as krill and had slight positive selection for gelata. *Praya dubia*'s relative *Praya retiuclata* is predicted to feed upon gelata (Damian-Serrano et al. 2021a). Finally, in this study, I only observed *Lensia conoidea* to consume a chaetognath, but they have previously returned copepod DNA and have been predicted to feed upon small crustaceans (Damian-Serrano et al. 2021a, 2022). While calycophorans have been observed to eat copepods, we saw no selection towards copepods in this study or any feeding trends for calycophorans more generally. The lack of copepod selection may be due to methodological bias, discussed below.

Cystonects are well known fish eaters and select strongly for fish (Purcell 1981b; Hetherington et al. 2022; Damian-Serrano et al. 2022). In this study, I observed *Bathyphysa conifera* to feed upon and select for exclusively fish, as expected for its suborder.

### Genetic results

While genetic distance had a significant effect on dissimilarity of prey specialization, the strength of the trend was slight and the variance high. Thus, while genetic distance is likely correlated with prey specialization, other, likely more significant drivers are at play. Siphonophores in particular show intense plasticity in their evolutionary trajectories, with generalists becoming specialists, specialists becoming generalists, and even specialists on one prey evolving directly into specialists on another prey (Damian-Serrano et al. 2021b). Siphonophore food webs are also less nested and more specialized at depth (Hetherington et al. 2022). Differences in selectivity are therefore likely driven by environmental niche partitioning, with closely related and spatially-overlapping species adapting to fill different niches as a function of available niche space and competition in their environment (McNaughton 1978; Blaber 1986).

### Caveats and future directions

Although the results in this study come from a large dataset and generally agree with previous studies, there are a few caveats to note. Video data offer us a unique perspective into the pelagic community that is especially suitable for observation of gelatinous zooplankton, but can impact the perceived abundances of other organisms. For example, small animals such as ostracods and copepods do not show up well on video, which likely explains why they have been reported as prey of multiple siphonophore species in the literature but were almost absent as prey in this study. Similarly, animals that avoid the light or motion of the

ROV, such as squids or fishes, are likely underrepresented in the VARS dataset (Benoit-Bird et al. 2023). This underrepresentation would artificially inflate the selectivity values reported for fishes and squid, as they would be observed less than they actually occur in the environment (dampening the $p_i$ metric), but they would be observed just as frequently once they were captured and killed by siphonophores (leaving the $r_i$ metric unchanged). The inverse is also possible, where animals that are attracted to the lights or motion of the ROV would be overrepresented in the dataset. Additionally, I only applied population corrections (population-quantity-999, dense, and swarm tags) to krill due to previous work confirming these corrections as generally valid across the dataset (Rob Sherlock, *pers. comm.*). I did not apply any population corrections to other organisms, even though population-quantity tags existed for many organisms. Overall, this means these population estimates should be taken as the lowest possible abundances of the organisms observed in this study.

To calculate the proportion of prey in a predator's environment it was necessary to consider changes in abundance of predators and prey in space and time. A complicating factor when investigating selectivity over the course of a year is that it is difficult to distinguish true changes in abundance from changes in communities that are caused simply by water masses bringing different gelatinous zooplankton across the same sampling location, and some researchers have concluded that what appears to be seasonal changes of abundance are really just due to horizontal advection of water masses (Mackie et al. 1988). Given our opportunistic data, I cannot confidently isolate which process is occurring, so these results should be interpreted as a snapshot of feeding behaviors within the greater Monterey Bay region specifically.

Krill was the most commonly consumed prey in this study, likely due to the common presence and high caloric value of this prey item creating a large niche space for krill feeding, specialization, and selection. Significant change or loss in krill abundance is likely to affect all species that feed upon krill, but specifically those which are specialized to feed upon krill at a higher proportion than it is observed in the prey field. These species include *Forskalia* spp.*, Bargmannia elongata, Resomia ornicephala,* and *Nanomia bijuga*. Of particular note is the common siphonophore *Nanomia bijuga*, which associates with the deep scattering layer, potentially to track krill populations (Mackie et al. 1988). Further research is needed to investigate the movement of *Nanomia bijuga* and krill with higher temporal resolution to determine whether any prey tracking is occurring.

## Conclusion

Overall, this study reports in detail the feeding habits of siphonophores in Monterey Bay, leveraging a massive dataset to make new inferences about the pelagic food web. All siphonophores in this study showed strong prey preferences, generally exclusive to one prey item or prey type, but not always correspondingly strong selectivity. For siphonophore+prey pairs that showed strong selective signals, the results were generally consistent with the literature. I also reported on selective patterns for 10 siphonophore species for the first time (*Lensia conoidea, Desmophyes haematogaster, Praya dubia, Bathyphysa conifera, Lychnagalma utricularia, Erenna richardi, Bargmannia elongata, Resomia ornicephala,* Undescribed Physonect G, and Undescribed Physonect Z). The use of video data in this study offers a novel perspective of feeding in siphonophores, complementing existing gut content, metabarcoding, and stable isotope analyses. The findings presented allow us to create more accurate food web models, better understand energy flow, and link disparate organisms.

Overall, this expands our functional knowledge of the midwater environment and strengthens application towards environmental management efforts.

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# CALIFORNIA STATE UNIVERSITY MONTEREY BAY

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Thesis of Alexandra Lapides:

# THE FEEDING HABITS AND SELECTIVITY OF SIPHONOPHORES IN MONTEREY BAY

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