

# Feeding selectivity and niche characteristics in Southern Ocean salps

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## ABSTRACT

Salps are a group of pelagic tunicates with widespread distribution and the potential to strongly impact ecosystem dynamics through their rapid reproduction, carbon export and filtration rates. Like most filter feeders, they are considered to be nonselective in their feeding, although recent reports of differences in the proportions of prey types in salp diets compared to their availability have challenged this paradigm. We investigate the potential for selective feeding and its impacts on salp niche breadth and overlap using scanning electron microscopy of the gut contents of 58 salps from the Southwest Pacific east of New Zealand representing seven species: *Salpa thompsoni*, *Thetys vagina*, *Thalia democratica*, *Salpa fusiformis*, *Ihlea magalhanica*, *Soestia zonaria* and *Pegea confoederata*. We then compare their diet to water column plankton communities via FlowCam and flow cytometry. While most prey types were consumed without preference as expected, dinoflagellates were positively selected across five of the seven salp species regardless of water column prey compositions. Large, spinous diatoms and submicron particles were consistently negatively selected. These selectivities resulted in high niche breadths that still fell short of true generalists, highlighting that selectivity is a spectrum rather than a dichotomy.

**KEYWORDS:** Chatham Rise; Thaliacea; plankton ecology; gelatinous zooplankton; selective feeding

## INTRODUCTION

Salps are a group of gelatinous, filter-feeding pelagic tunicates varying in size from the 4 mm *Thalia democratica* to the  $\geq 30$  cm *Thetys vagina*. Salps can exert strong top-down pressure on planktonic prey populations through their exceptionally high clearance rates on a variety of organisms ranging from submicron bacteria to copepod nauplii (Madin, 1974; Madin and Deibel, 1998). Prey is concentrated through contractions of the salp's outer muscle bands forcing water through a mucous feeding filter that retains prey above a certain size (Madin, 1974). Prey is then slowly drawn through the esophagus and into the stomach for digestion. The cleared water is then passed out of the salp, allowing for locomotion. Unassimilated prey carbon is repackaged into rapidly sinking fecal pellets that are efficiently exported out of the euphotic zone, leading to substantial rates of carbon export (Madin, 1982; Décima *et al.*, 2023; Steinberg *et al.*, 2023). Carbon export is further supplemented by diel vertical migration in select species (Purcell and Madin, 1991; Perissinotto and Pakhomov, 1998; Nishikawa and Tsuda, 2001). These impacts are compounded by some of the fastest growth rates and generation times of any multicellular eukaryotes, thanks to their complex

reproductive cycle containing both asexual solitary and sexual aggregate forms (Heron, 1972; Madin, 1974). This can lead to dense blooms of up to 5003 salps  $m^{-3}$  under ideal conditions (Everett *et al.*, 2011). Unfortunately, most salp studies are opportunistic and sporadic owing to the difficulty in collecting these fragile organisms with standard net tows as well as an inability to predict their presence (Henschke *et al.*, 2016). Consequently, our understanding of their ecological impacts is still poor.

Among filter-feeding plankton such as salps, it is generally understood that the distribution of consumed prey should be roughly equal to and reflect the diversity of the prey field available (i.e. nonselective feeding). This view has been supported by several studies that have found the prey types fed on by salps are roughly proportional to their availability in the water column (Silver, 1975; Vargas and Madin, 2004; Tanimura *et al.*, 2008). From these results, it has been proposed that all salps fill similar niches in their environment and are ecologically equivalent for most purposes (Yount, 1958) such that co-occurring species of salps must strongly compete with one another (Silver, 1975). However, an increasing number of more recent analyses utilizing fatty acid techniques, genetics and *in-situ* feeding observations

have found significant differences between the prey represented in the guts of coexisting salp species and in the ambient water column (von Harbou *et al.*, 2011; Metfies *et al.*, 2014; Dadon-Pilosof *et al.*, 2019; Pauli *et al.*, 2021; Thompson *et al.*, 2023). While the mechanism behind these differences remains unclear, these conflicting results raise the question of whether salps might ingest prey in a manner that is not solely dependent on their availability, in other words, if they display feeding selectivity.

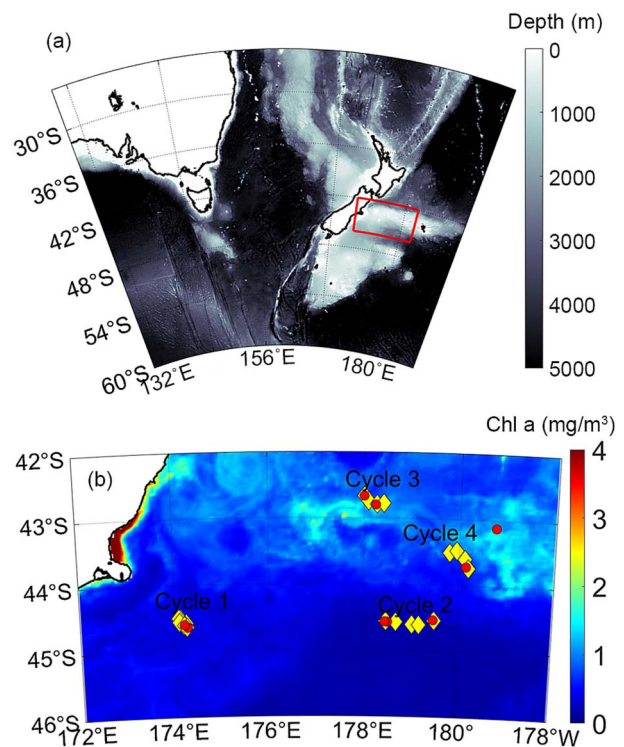
To address these questions, we may consider the salp's food niche as the result of its ingestion of each prey type. The utilization of each specific prey type can be compared to its availability in the environment, allowing one to estimate selection for or against that taxon. The width or breadth of the niche is also informative as broader widths correspond to generalists, as would be expected of nonselective filter-feeders like salps, while narrower ones represent more specialized feeding as is common in raptorial crustaceans or fish (Lakkis, 1994; Katechakis *et al.*, 2004; Shiroza *et al.*, 2022). One may also compare the overlap of the niches of different species to determine similarity in resource usage, the possibility of resource partitioning and the potential for competition. This study acts as a follow-up to Fender *et al.* (2023), which investigated differences in the size spectra, predator-to-prey size ratios and retention efficiency of 58 salps from seven different species collected from the Southwest Pacific using scanning electron microscopy (SEM) analyses of the gut contents. Here, we seek to further compare the taxonomy of ingested prey items to their availability in the ambient water column in order to assess salps' potential for selective feeding and whether selectivity differs between species. We also use this information to provide the first quantifications of salp niche breadth and overlap.

## METHODS

### Cruise structure and water column sampling

Data for this project were obtained as part of the 2018 Salp Particle expOrt and Ocean Production (SalpPOOP) cruise conducted from 23 October to 21 November over the Subtropical Convergence east of New Zealand along the Chatham Rise. This cruise consisted of five experimental cycles conducted in a Lagrangian framework, each lasting 3–8 days (Décima *et al.*, 2023), although only the first four cycles are considered in the present work (Fig. 1). Cycle locations were chosen based on historical zooplankton distributions as well as those of fish species known to prey on salps in order to target areas with a high likelihood of salp presence. Each cycle began with and was centered around the deployment of a drifting array that both marked the water mass and carried a variety of *in-situ* incubations. These drifting arrays consisted of a satellite tracker-equipped surface float tethered to a  $3 \times 1$  m holey-socked drogue at 15 m with mesh bags affixed below at various depths to hold experiments.

Daily CTD-Niskin rosette deployments were made to collect water from six to eight depths and provide profiles of water column temperature, salinity, oxygen, photosynthetically active radiation and fluorescence. Water collected from the Niskin bottles was used to characterize the phytoplankton community through high-performance liquid chromatography and size-fractionated chlorophyll *a* (0.2, 2 and 20  $\mu\text{m}$ ). Additionally,



**Fig. 1.** (a) Bathymetry of the region with red box denoting study region. (b) Magnification of study region with color as satellite-derived surface chlorophyll. Circles represent the locations of net deployments for salp collection, whereas diamonds are CTD deployments.

250 mL was collected from the mixed layer (10–12 m) and deep chlorophyll maximum (DCM) when a DCM was present or a second deeper mixed layer sample when not (20–70 m) in 2–three replicates. 2 mL aliquots of these samples were then concentrated by  $\times 25$  via gravity filtration and imaged using the  $\times 10$  objective lens of a FlowCam model VS-IV targeting the larger portion of the phytoplankton community (4–300  $\mu\text{m}$ ). A shipboard Accuri C6 Plus flow cytometer was used to image the smaller *Synechococcus* and phototrophic eukaryotes collected from six depths across the euphotic zone (Stukel *et al.*, 2021) typically spanning 5–100 m, while additional preserved samples from the same casts and depths were later analyzed using a Beckman Coulter CytoFLex S flow cytometer on land to determine the abundance of *Prochlorococcus* and heterotrophic bacteria (Selph, 2021).

### Net deployments and specimen collection

Salps for gut SEM analyses were either collected from a 1 m ring net fitted with a 200  $\mu\text{m}$  mesh and 30 L nonfiltering cod end (68 total deployments), a trapezoidal krill net with a 5 m<sup>2</sup> mouth and 2 mm mesh (15 deployments), or a 0.7 m diameter Bongo net with a 200  $\mu\text{m}$  mesh net (68 deployments). Both the krill net and Bongo nets were equipped with a SCANMAR depth sensor during tows. While the Bongo and krill net were towed obliquely during the day and night to a depth of 200 m, the salp net was deployed as a slow vertical tow during the night to depths of 5–40 m. For salp net tows, which were conducted expressly for live

experiment collection, salps were sorted by species using a plastic ladle into buckets of fresh seawater, while Bongo and krill net catches were first anesthetized with carbonated water. Salps were then measured, identified to species and life stage following Bone (1998) and sexed (Lüskow *et al.*, 2020) before being preserved in 5% formalin <30 minute from the time of collection. When possible, triplicate samples of both life stages across a range of sizes were collected from the first cycle in which that species was encountered.

Depth-stratified estimates of salp abundance were also obtained via a Multiple Opening and Closing Net Environmental Sampling System (MOCNESS) equipped with conductivity, depth, temperature, fluorescence, oxygen, light and transmissivity sensors. Deployments were typically done once during the day and once during the night per cycle, with nine nets sampling various depth intervals down to 2600 m. Since the deployment and recovery process was often several hours long and the catch was of poorer quality than the other nets, MOCNESS samples were not used for any salp experiments, including SEM analyses.

### Imaging and metabarcoding

Once ashore, 58 preserved salps were prepared for SEM and imaged as described in Fender *et al.* (2023). Briefly, each salp had its gut removed, and the contents were imaged using a FEI Nova 400 NanoSEM. Planktonic, nondetrital particles were then outlined in ImageJ (v. 1.52a or 1.53c), sized and sorted into one of 24 classes corresponding to the highest recognizable taxonomic degree. While we were sometimes able to classify larger particles to at least the genus level, degradation due to digestion often meant only functional groups (e.g. centric or pennate diatoms, dinoflagellates, loricate ciliates, etc.) could be confidently identified. Image analysis of FlowCam samples was similarly conducted using VisualSpreadsheet (v. 4.18.5) with particles sorted into one of 28 groups. Since FlowCam was not able to resolve particles smaller than 4  $\mu\text{m}$ , these data were supplemented with flow cytometry data to represent the abundance of prey types below this threshold. The carbon biomass of each particle was then estimated via biovolume using allometric conversions dependent on the functional group of the particle. Fender *et al.* (2023) detail methods for estimating size and carbon biomass. Unless otherwise stated, all water column values are averaged across depths.

Additional *Salpa thompsoni* ( $n = 192$ ), *Soetia zonaria* ( $n = 21$ ), *Pegea confederata* ( $n = 17$ ), *Thetis vagina* ( $n = 1$ ) and *T. democratica* ( $n = 7$ ) were also frozen at sea at  $-80^\circ\text{C}$  for 18S DNA metabarcoding. Guts were later excised, and their contents were extracted in the lab using QIAGEN Power Soil Extraction kits and amplified using PCR conditions following a modified protocol (Piredda *et al.*, 2017). Eukaryotic primers TAREuk454FWD1 were used to amplify the V4 region of the 18S gene, which was subsequently sequenced on an Illumina MiSeq at the Genotoul GeT facility (Toulouse, France).

### Selectivity and niche indices

Here, we define selection as a deviation in the relative contribution of a given prey type in the guts of an organism compared to its availability in the environment (Strom and Loukos, 1998)

and quantify it for each salp species using a modification of Ivlev's Electivity (Ivlev, 1961)

$$E = \frac{p_i - q_i}{p_i + q_i} \quad (1)$$

where  $p_i$  is the mean proportion of the total biomass in the guts of the salp made up of prey type  $i$  and  $q_i$  is the proportion of the total biomass of prey type  $i$  available in the environment. Since there were occasional instances of salp guts with suspiciously low particle counts relative to other comparable guts, we did not wish to bias the mean  $p_i$  for a given species by placing equal weight on salps with non-representative gut contents as may occur when unhealthy or when resting at depth. Therefore, rather than representing  $p_i$  as it is typically defined, we instead use a prey-weighted mean proportion defined as the sum of all prey biomass made up of a given prey type divided by the sum of all prey biomass across all samples of a given salp species. This places greater emphasis on salps with fuller guts under the assumption that they are more representative of normal feeding.

Feeding specialization has been estimated using a variety of metrics with perhaps the most common being Levin's standard niche breadth (Levins, 1968). This index ranges from zero for feeding on a single prey type (i.e. a perfect specialist) to one for equal feeding on all prey types (i.e. a perfect generalist). However, Levin's measure and many of those later derived from it assume all prey types are available at equal abundances. This makes these indices better suited for studies in which the prey field is controlled or unknown, but in scenarios where food resources are not equally abundant, the use of such indices can lead to erroneous conclusions regarding niche breadth as a species with equal proportions of its diet made up by each prey type may actually be exhibiting significant specialization if that is not representative of the true prey community composition (Feinsinger *et al.*, 1981). Since the ambient prey field in our study is known, we instead chose to quantify niche breadth using the Proportional Similarity Index (hereafter  $B_{PSI}$ ) defined as.

$$B_{PSI} = 1 - 0.5 \left( \sum_i |p_i - q_i| \right) \quad (2)$$

Rather than quantifying the breadth of the salp's probability function for resource utilization, this measure instead compares the similarity of that probability function to that of the water column, thereby taking prey availability into account.

The Proportional Similarity Index can also be used to quantify niche overlap between salps, in which case we refer to it as  $O_{PSI}$ , which is analogous to equation 1) with  $p_i$  and  $q_i$  instead representing the prey-weighted mean proportions of a prey type within two salp species. It should be noted that such a simple overlap measure does not necessarily indicate competition because it does not account for the other factors that impact whether that competition is fully realized, namely the abundance of prey as well as other resources. Nevertheless, these calculations are still useful in revealing tendencies in this relationship (Katechakis *et al.*, 2004) such that we view overlap as an appropriate metric for representing the *potential* for competition.

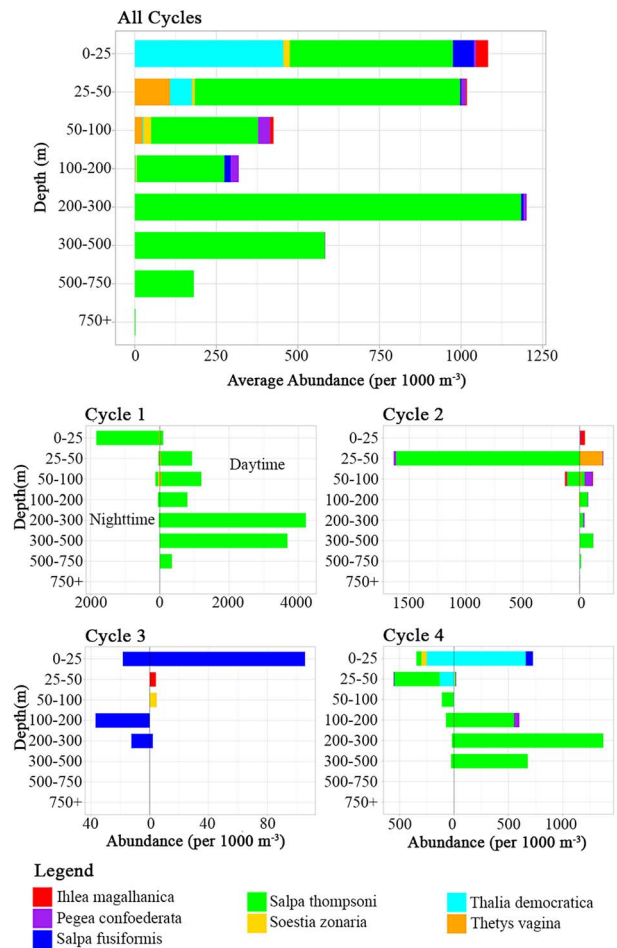
As a complement to our niche-based overlap calculations, we also conducted a principal component analysis (PCA) of the proportions of gut and water column prey taxa using the base R function `prcomp` (R version 4.2.3). Only particles  $>4 \mu\text{m}$  were included in the PCA to prevent visualization from being overly skewed by the salps' exclusion of pico-sized particles relative to their abundance in the water column.

To mitigate differences in taxonomic resolution between the two sampling methods for indices comparing water column and salp gut prey compositions such as  $E$  and  $B_{PSI}$ , prey were reclassified into one of 13 categories roughly targeting the different size-structured functional groups that were abundant across both FlowCam and SEM. For  $O_{PSI}$ , where prey is compared strictly between guts, all 24 particle categories originally identified by SEM were used under the assumption that they are a better representation of the prey species diversity. 95% confidence intervals for all metrics were derived using a Monte Carlo random resampling scheme, although the methods of randomization differed to represent different sources of uncertainty (see [Supplemental Materials](#) for details). Unless otherwise stated, the values presented in the following figures and text represent the means of the 10 000 simulations with lower and upper bounds of the 95% confidence interval (LCI and UCI respectively). We use these confidence intervals to determine significant differences in these means and refer to instances of non-overlap as significant.

## RESULTS

### Salp community distribution

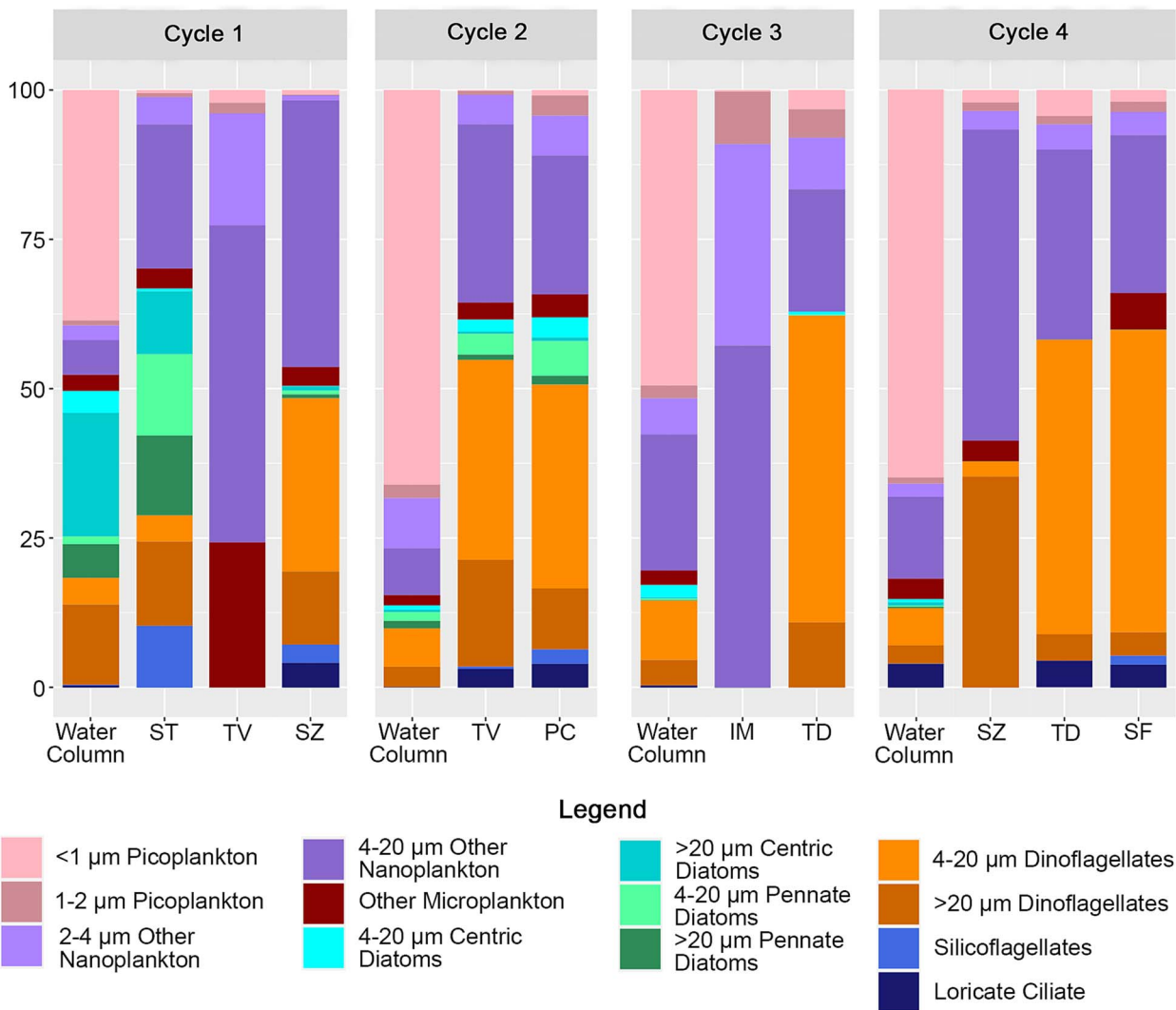
The four Lagrangian cycles presented here broadly represented the subantarctic (Cycles 1 and 2; [Fig. 1](#), [Supplementary Table I](#)) and subtropical (Cycles 3 and 4) influenced waters of the subtropical frontal zone that comprises the Chatham Rise, although there was some evidence of mixing within Cycles 1 and 4 ([Décima et al., 2023](#)). Seven distinct salp species were observed with *T. vagina* found exclusively in the subantarctic cycles, *T. democratica* and *Salpa fusiformis* present only in subtropical conditions, and *S. thompsoni*, *Ihlea magalhanica*, *Soestia zonaria* and *Pegea confoederata* present in both. Almost all cycles exhibiting bloom conditions were dominated by *S. thompsoni* according to vertically averaged MOCNESS catches with 1271 individuals per  $1000 \text{ m}^3$  in Cycle 1, 161 in Cycle 2, 0 in Cycle 3 and 290 in Cycle 4 ([Fig. 2](#)). The abundance of other salp species was always significantly lower with a combined total of 20 individuals per  $1000 \text{ m}^3$  in Cycle 1, 120 in Cycle 2, 50 in Cycle 3 and 201 in Cycle 4. *T. democratica*, the smallest species observed, was restricted to the upper 50 m of the water column. *S. zonaria*, *T. vagina* and *I. magalhanica* were found only within the upper 100 m while *P. confoederata* and *S. fusiformis* were found as deep as 300 m. Only *S. thompsoni* was found in nets deeper than 750 m, which, when coupled with their consistently higher night-time surface presence and daytime presence at depth, presents clear evidence of diel vertical migration as has been reported previously for the species ([Perissinotto and Pakhomov, 1998](#); [Nishikawa and Tsuda, 2001](#); [Lüskow et al., 2020](#)). The only other species to display clear diel patterns was the congeneric *S. fusiformis*, which displayed reverse diel migration, having higher surface abundances during the day as has been observed elsewhere ([Liu et al., 2012](#); [Pascual et al., 2017](#)).



**Fig. 2.** Abundance (organisms per  $1000 \text{ m}^{-3}$ ) at various depth intervals for each of the seven salp species observed averaged over the seven total MOCNESS deployments (top) as well as those made in each cycle (bottom). For the bottom panels, bars extending to the left of the origin represent night-time abundances, whereas those to the right are for daytime abundances.

### Water column community composition

FlowCam images from Cycle 1 were characterized by a variety of phytoplankton species with a predominance of centric and pennate diatoms, such as *Nitzschia* sp., *Thalassiosira* sp., *Proboscia* sp., *Asterionellopsis* sp. and *Chaetoceros* sp. ([Fig. 3](#), [Supplementary Table II](#), [Supplementary Fig. 1](#)). Large dinoflagellate genera such as *Triplos*, *Diplopelta*, *Polykrikos* and *Gyrodinium* as well as the smaller genera *Oxytoxum*, *Gymnodinium* and possibly *Scrippsiella* were also abundant relative to the other cycles. Indeed, Cycle 1 is the only cycle for which FlowCam detected particles larger than  $120 \mu\text{m}$ , with  $\sim 4$  times greater contributions of  $>20 \mu\text{m}$  particles than any of the other three cycles. However, it should be noted that submicron picoplankton, primarily heterotrophic bacteria, still made up more than 39% of total biomass. Cycle 2, while also subantarctic and nutrient-replete, exhibited the largest relative proportion of picoplankton biomass of the four cycles. Compared to Cycle 1, there were far fewer diatoms, with most recognizable large species being Nitzschoid pennate diatoms. In the highly productive subtropical Cycle 3, nanoflagellates made up the largest portion of biomass relative to the other cycles.



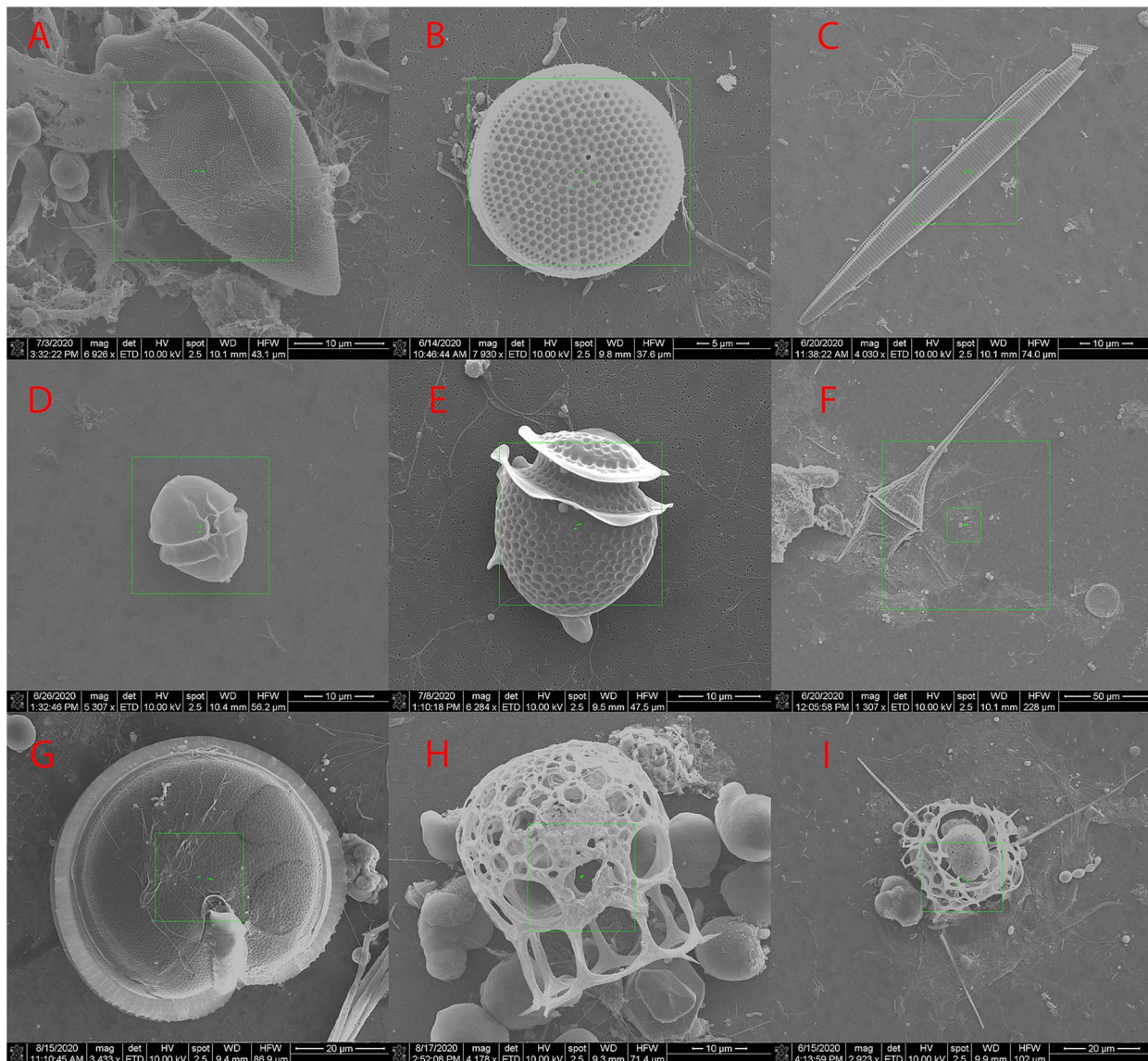
**Fig. 3.** Relative proportion of biomass in the ambient water columns and guts of salps made up by each of 13 prey types from each of the four cycles, averaged by species. TD = *Thalia democratica*, PC = *P. confoederata*, SZ = *S. zonaria*, TV = *T. vagina*, ST = *S. thompsoni*, SF = *S. fusiformis*, IM = *I. magalhanica*.

While dinoflagellates were still common; here they were mostly smaller species such as *Gymnodinium* sp. and/or *Scrippsiella* sp. The subtropical Cycle 4 was similar to Cycle 2 with respect to the dominance of picoplankton over larger groups but with far fewer diatoms and  $\sim 5$  times more loricate ciliates than any other cycle.

### Salp dietary composition

The relative contributions of each prey type to the salp guts were clearly influenced by the prey field available and were highly variable between species. For example, while *S. thompsoni* displayed fairly similar relative proportions to the  $>2 \mu\text{m}$  portion of the water column of Cycle 1 (Fig. 3), its gut contained far more silicoflagellates, no loricate ciliates, more pennate diatoms as opposed to the centric species which dominated the water column and more nanoflagellates. *S. zonaria* from Cycle 1 was even more different relative to the ambient environment, exhibiting guts with relatively few diatoms but far more small dinoflagellates

and large nanoflagellates. One of the most abundant objects observed in the guts of all salps were  $\sim 1\text{--}20 \mu\text{m}$  unidentifiable spherical particles. Given their abundance, broad range in size and importance in constructing complete size spectra comparable to the water column, we assume this category comprises the various nondescript nanoplankton found in the FlowCam and the flow cytometer (Fender *et al.*, 2023). Regardless of species, the main contributors to the gut contents of most salps were nanoflagellates and dinoflagellates, while the contribution of picoplankton to salp gut biomass was consistently low. Each of the other species observed in the water column was also found in the guts of at least one species of salp (Fig. 4, Supplementary Fig. 2), with the notable exceptions of *Chaetoceros* sp., *Proboscia* sp., *Polykrikos* sp., *Gyrodinium* sp and coccolithophores. While numerically rare, there were signs of salps ingesting zooplankton, including tintinnid lorica, *Dictyocysta* sp. and polycystine radiolarians (Fig. 4H–I). Nothing related to larger zooplankton (e.g. copepod body parts) was found.

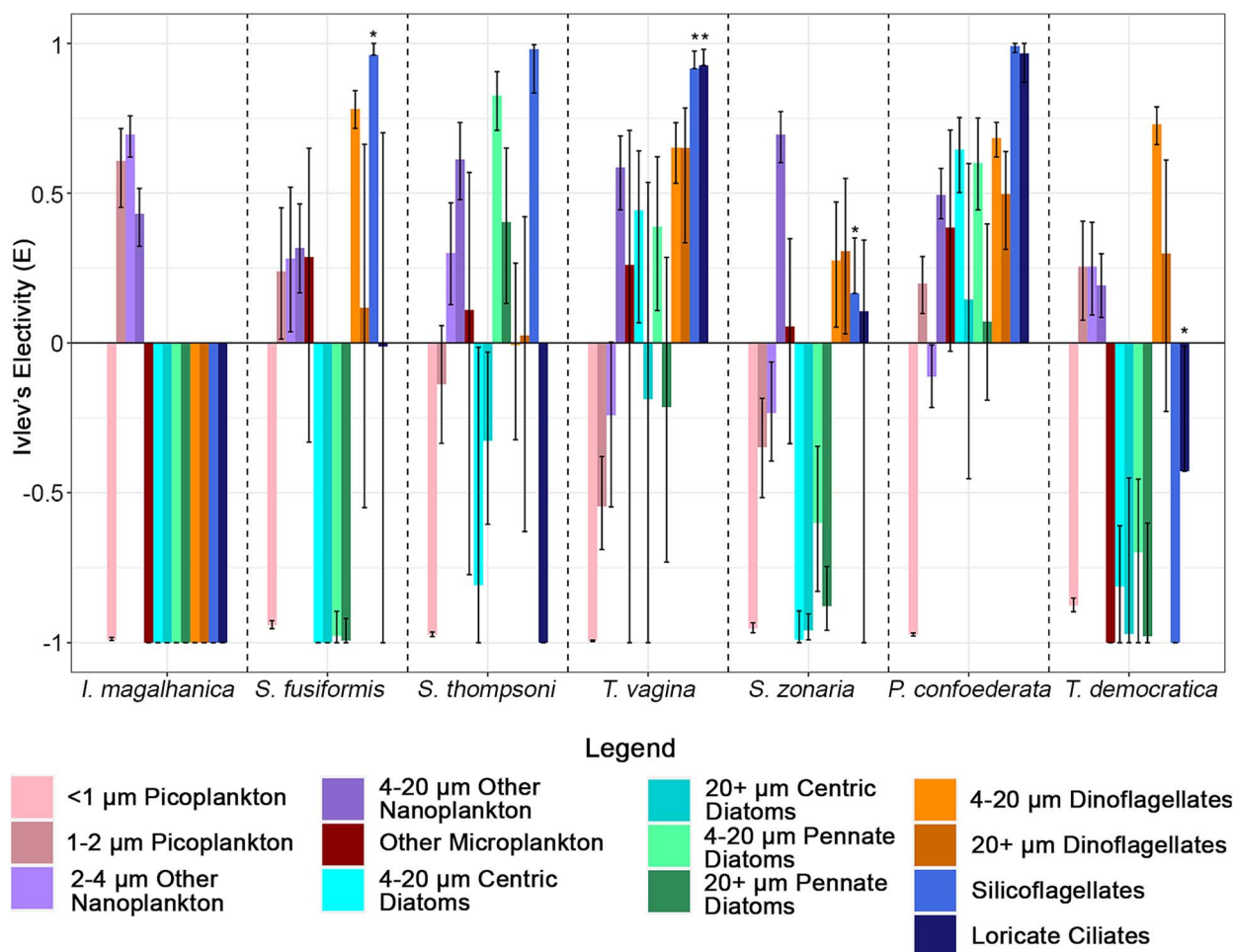


**Fig. 4.** Composite of example SEM images taken from various salp stomachs. (a) dinoflagellate *Prorocentrum dentratum* (b) centric diatom *Thalassiosira* sp. (c) Nitzschoid pennate diatom (d) small dinoflagellate, possibly *Scrippsiella* sp. (e) Dinoflagellate *Dinophysis* sp. (f) Dinoflagellate *Tripos* sp. (g) Dinoflagellate *Diplopelta* sp. (h) Loricate ciliate *Dictyocysta* sp. (i) Unidentified polycystine radiolarian. Note the difference in scale as represented by the scale bar in the bottom right of each image.

### Feeding selectivity

We quantified selection for or against each of the 13 shared prey types identified across salp guts and the water column using Ivlev's Electivity (E). All salps shared a strong negative selection for  $<1 \mu\text{m}$  picoplankton, with *T. democratica* having the least negative and *I. magalhanica* the most negative (Fig. 5; Table I).  $1\text{--}2 \mu\text{m}$  picoplankton, however, were positively selected in *I. magalhanica*, *S. fusiformis*, *P. confoederata* and *T. democratica*; neutrally selected in *S. thompsoni*; and negatively selected in *T. vagina* and *S. zonaria*. For the larger prey types, most species positively selected at least one of the size classes of dinoflagellates. *I. magalhanica* and *S. thompsoni* were exceptions as the former did not ingest dinoflagellates and the latter was neutral. Most species showed strong positive selection for silicoflagellates, although we caution that lower confidence intervals often could not be

determined for these values given their rarity in the water column. Therefore, with the exception of *S. thompsoni* and *P. confoederata*, it is unclear whether these selectivities are significantly different from neutral. Small centric and pennate diatoms were only positively selected in *T. vagina* and *P. confoederata*, while *S. thompsoni* positively selected both large and small pennates but against centrals. All other species exhibited E values for diatoms less than or not significantly different from zero, with the most negative selection being for large pennates in *S. fusiformis*. Other unidentified nanoplankton-sized particles were also generally positively selected, with *T. vagina* exhibiting neutral selection for smaller nanoplankton while *P. confoederata* and *S. zonaria* were the only species to negatively select small nanoflagellates. Other microplankton were never positively selected in any of the salp species. Finally, *T. vagina* and *P. confoederata* positively selected



**Fig. 5.** Ivlev's Electivity for each of the seven salp species averaged across individuals, where the origin represents neutral selection at a value of zero. Electivities  $>0$  indicate selection for a given prey type (represented by color), while values below represent selection against. Error bars represent 95% CIs, although some prey types were so rare in the water column that the LCI could not be determined. These cases are denoted by asterisks.

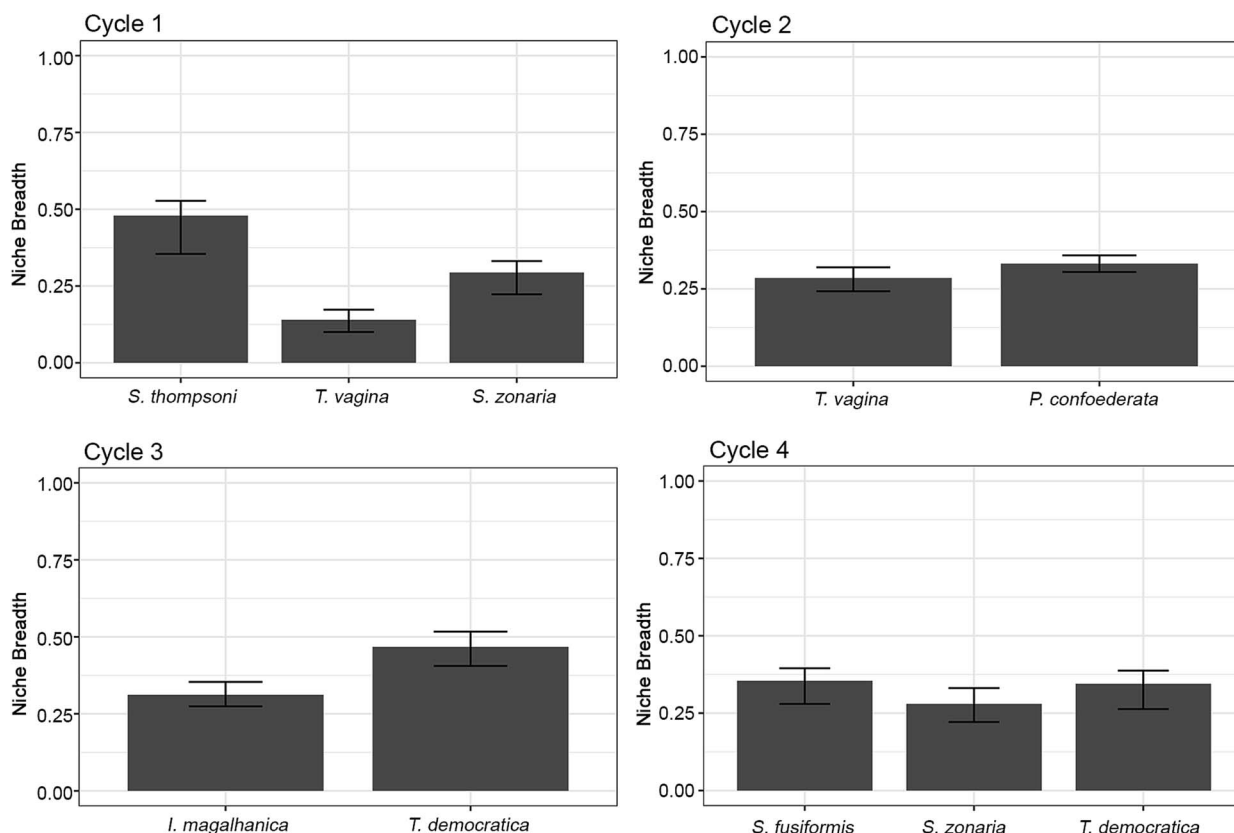
loricate ciliates (albeit with an unknown LCI for the former for the same reason as for silicoflagellates), while they were neutrally selected in *S. fusiformis* and *S. zonaria* and negatively selected in *I. magalhanica*, *S. thompsoni* and *T. democratica*.

### Niche breadth and overlap

To quantify the degree of dietary specialization between species, we calculated niche breadth ( $B_{PSI}$ ), which is interpreted such that lower values represent less similarity between gut contents and the water column and, therefore, greater specialization, while values closer to one represent generalist feeding. Niche breadth values were broadly similar between cycles, with most  $B_{PSI}$  values falling between 0.28 and 0.48 (Fig. 6; LCI = 0.22, UCI = 0.53). Within cycles, however, salps collected from the same water mass often displayed differing niche breadths from their peers. For example, the salps of Cycle 1 ranged from the highest  $B_{PSI}$  observed of 0.48 (0.35, 0.53) for *S. thompsoni* to a more moderate 0.29 (0.22, 0.33) in *S. zonaria* to the lowest niche breadth observed of 0.14 (0.10, 0.17) in *T. vagina*. While the niche breadths of *T. vagina* and *P. confoederata* from Cycle 2 were fairly similar, *T. democratica* from Cycle 3 also displayed

a high  $B_{PSI}$  of 0.47 (0.41, 0.52), much higher than that of the co-occurring *I. magalhanica* at only 0.31 (0.27, 0.35). Finally, the niche breadths of the salps of Cycle 4 were nearly the same at 0.35 (0.28, 0.40) for *S. fusiformis*, 0.35 (0.26, 0.39) for *T. democratica* and 0.28 (0.22, 0.33) for *S. zonaria*. It is also interesting to note that all three of the salp species collected from multiple cycles (*T. vagina*, *S. zonaria* and *T. democratica*) had substantially different  $B_{PSI}$  values in each, although only a single individual of *T. vagina* was collected from each cycle.

$O_{PSI}$ , similar to its use for niche breadth, quantifies the degree of similarity between two utilization functions, although rather than comparing that of a single salp to that of the water column we now apply it to two different salp species. An  $O_{PSI}$  of 0 therefore denotes no overlap in the niche space occupied by the two species while a value of 1 denotes perfect overlap. We found the greatest overlap between the niches of *T. democratica* and *S. fusiformis* at 0.89 (0.80, 0.92) and the lowest between *I. magalhanica* and every other salp species with the exception of *S. zonaria* (Fig. 7). *S. thompsoni* also had particularly low overlap with most other species, ranging from 0.14 (0.12, 0.18) with *I. magalhanica* up to 0.54 (0.45, 0.61) with *P. confoederata*. Nearly



**Fig. 6.** PSI niche breadth for the feeding of each salp species that was collected from each cycle on the 13 standard prey types. Error bars represent 95% CI.



**Fig. 7.** PSI niche overlap of each salp species averaged across individuals, where a value of 1 represents perfect dietary overlap. TD = *Thalia democratica*, PC = *P. confoederata*, SZ = *S. zonaria*, TV = *T. vagina*, ST = *S. thompsoni*, SF = *S. fusiformis*, IM = *I. magalhanica*.

all other salp pairings had moderately high overlaps of 0.59–0.74 (0.50–0.78).

While our implementation of  $O_{PSI}$  allows for the comparison of salp guts between species pairings that were not actually observed, the interpretation of those pairings is complicated by the differences in the ambient prey field within the cycles from which they were collected (Fig. 3). This is to say that the

low  $O_{PSI}$  overlap between *I. magalhanica* and *S. thompsoni*, for example, could simply be because they were collected from different water masses representative of different prey fields. For this reason, we further supplemented our quantitative niche overlap indices with a PCA of the relative proportions of gut biomass for each salp species as well as that of the water column (Fig. 8). Overall, salps cluster by species more than they do by cycle and exhibit substantial similarity in gut content makeup, mirroring  $O_{PSI}$ . *I. magalhanica* shows little overlap with the other species, echoing the dissimilarity of its guts compared to both the *T. democratica* of its own cycle as well as those of the other salps, while *S. thompsoni* exhibits remarkable variability among individuals. Similarly to  $O_{PSI}$ , *T. democratica* and *S. fusiformis* cluster closely together regardless of whether the individuals were collected from Cycles 3 or 4, with most individuals of one species appearing more similar to those of the other than they are to the ambient water column of the cycle from which they were collected.

## DISCUSSION

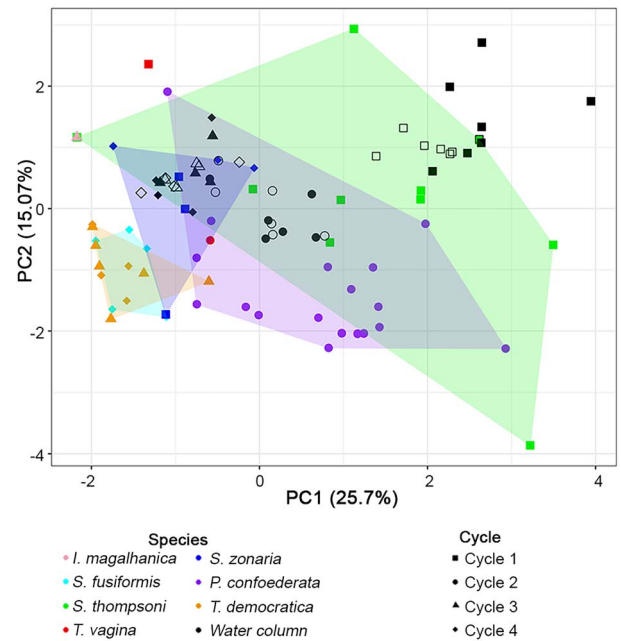
### Mechanisms of selection

Of the 91 combinations of salp species and prey type considered here, we find evidence of selection in ~75% of them, including some selection that differs between salp species (Fig. 5). This may seem surprising given the lack of any immediately apparent mechanisms for selection relative to something like a

**Table 1:** Ivlev's Electivity of each of the seven salp species for the 13 prey categories.

Prey Type	<i>I. magalhannica</i>	<i>S. fusiformis</i>	<i>S. thompsoni</i>	<i>T. vagina</i>	<i>S. zonaria</i>	<i>P. confederata</i>	<i>T. democratica</i>
<1 $\mu$ m Picoplankton	-0.989 (-0.993, -0.982)	-0.941 (-0.953, -0.926)	-0.973 (-0.998, -0.964)	-0.994 (-0.996, -0.991)	-0.952 (-0.967, -0.934)	-0.973 (-0.977, -0.967)	-0.876 (-0.896, -0.851)
1-2 $\mu$ m Picoplankton	0.608 (0.453, 0.716)	0.239 (0.013, 0.451)	-0.138 (-0.335, 0.058)	-0.545 (-0.689, -0.379)	-0.348 (-0.516, -0.185)	0.198 (0.098, 0.288)	0.255 (0.076, 0.406)
2-4 $\mu$ m Nanoplankton	0.696 (0.621, 0.758)	0.282 (0.037, 0.52)	0.3 (0.128, 0.468)	-0.241 (-0.546, 0.003)	-0.234 (-0.395, -0.064)	-0.113 (-0.215, -0.007)	0.255 (0.093, 0.403)
4-20 $\mu$ m Nanoplankton	0.431 (0.323, 0.516)	0.317 (0.167, 0.464)	0.613 (0.478, 0.736)	0.586 (0.444, 0.691)	0.696 (0.602, 0.772)	0.494 (0.415, 0.583)	0.192 (0.085, 0.298)
>20 $\mu$ m Microplankton	-1 (-1, -1)	0.287 (-0.331, 0.65)	0.11 (-0.773, 0.569)	0.261 (-1, 0.71)	0.055 (-0.336, 0.348)	0.385 (-0.028, 0.711)	-1 (-1, -1)
4-20 $\mu$ m Centric diatoms	-1 (-1, -1)	-1 (-1, -1)	-0.808 (-1, -0.014)	0.443 (0.067, 0.641)	-0.991 (-1, -0.894)	0.646 (0.502, 0.753)	-0.812 (-1, -0.61)
>20 $\mu$ m Centric diatoms	-1 (-1, -1)	-1 (-1, -1)	-0.326 (-0.605, -0.031)	-0.188 (-1, 0.536)	-0.959 (-0.99, -0.904)	0.145 (-0.453, 0.599)	-0.971 (-1, -0.451)
4-20 $\mu$ m Pennate diatoms	-1 (-1, -1)	-0.977 (-1, -0.895)	0.826 (0.71, 0.906)	0.388 (0.108, 0.622)	-0.6 (-0.828, -0.345)	0.601 (0.444, 0.751)	-0.698 (-1, -0.455)
>20 $\mu$ m Pennate diatoms	-1 (-1, -1)	-0.993 (-1, -0.919)	0.403 (0.132, 0.651)	-0.214 (-0.731, 0.286)	-0.878 (-0.958, -0.746)	0.071 (-0.191, 0.397)	-0.978 (-1, -0.601)
4-20 $\mu$ m Dinoflagellates	-1 (-1, -1)	0.781 (0.717, 0.842)	-0.008 (-0.323, 0.267)	0.652 (0.533, 0.735)	0.275 (0.053, 0.47)	0.684 (0.621, 0.736)	0.73 (0.662, 0.788)
>20 $\mu$ m Dinoflagellates	-1 (-1, -1)	0.118 (-0.549, 0.663)	0.025 (-0.629, 0.421)	0.651 (0.334, 0.784)	0.306 (0.03, 0.549)	0.497 (0.312, 0.639)	0.298 (-0.229, 0.611)
Silicoflagellates	-1 (NA, -1)	0.961 (NA, 1)	0.981 (0.835, 0.996)	0.916 (NA, 0.974)	0.165 (NA, 0.35)	0.991 (0.97, 1)	-1 (NA, -1)
Loricata ciliates	-1 (NA, -1)	-0.012 (-1, 0.702)	-1 (-1, -1)	0.927 (NA, 0.98)	0.106 (-1, 0.343)	0.967 (0.87, 1)	-0.428 (NA, -0.011)

Values >0 represent a greater proportion of the given prey type in the gut contents than its availability in the water column (i.e. positive selection) and vice versa for lower values (negative selection). Means with lower and upper confidence intervals are presented.



**Fig. 8.** PCA of the relative proportions of prey items in the salp guts vs FlowCam observations, excluding <math>< 4 \mu\text{m}</math> prey. For salps, color represents species with polygons bounding each. For FlowCam observations, filled or open shapes represent whether the sample was taken in the mixed layer (empty) or at the DCM (filled). Cycle is represented by shape for both.

raptorial copepod, but there is growing evidence for selectivity among various filter feeders. For example, oysters and mussels show preference on the basis of algal shape and surface properties (Bougrier *et al.*, 1997; Rosa *et al.*, 2013), benthic ascidians exhibit variable clearance rates and capture efficiencies for different types of prey (Dadon-Pilosof *et al.*, 2017; Jacobi *et al.*, 2021) and sponges show preference for cyanobacteria over other types of picoplankton, detritus, and DOC (McMurray *et al.*, 2016). Within the pelagic tunicates, doliolids seem to preferentially graze on dinoflagellates and diatoms (Pond and Sargent, 1998; Walters *et al.*, 2019; Frischer *et al.*, 2021), such that some have suggested diatom abundance may drive doliolid blooms (Sutherland and Thompson, 2022). They have also been observed to arrest the gill cilia responsible for their feeding currents in response to undesirably large particles detected via sensory hair cells, and pyrosomes possess similar mechanoreceptive organs (Caicci *et al.*, 2013). Appendicularians have been known to exclude >30  $\mu\text{m}$  particles as in *Fritillaria borealis* (Flood, 2003) as well as exhibit reduced ingestion of spinous prey such as foraminifera and exceedingly long prey such as *Trichodesmium* (Alldredge, 1976). Indeed, Conley *et al.* (2018) propose four mechanisms for selection among mucous mesh filter feeders: prey size, shape, chemical composition and behavior on the part of the grazer, each of which helps explain the trends in selection we find here.

Prey size, and by extension shape, is perhaps the most obvious and well-studied mechanism of selection in filter feeders. Successful capture is largely determined by mesh spacing, as by sieving mechanisms, particles larger than the mesh are efficiently retained while those smaller typically are not. It therefore

comes as little surprise that every species of salp investigated here displayed strong negative selection for submicron picoplankton as most microscopic investigations of their mesh spacing have found it to be  $\sim 1 \mu\text{m}$ , although the minimum dimensions in salps of different species vary by up to a factor of 5 (Silver and Bruland, 1981; Bone *et al.*, 2000, 2003). Various studies on the retention efficiency and clearance rates for plastic beads of known size, as well as natural prey assemblages note sharp declines for particles smaller than 1–4  $\mu\text{m}$  (Harbison and McAlister, 1979; Kremer and Madin, 1992), with some even reporting differences based on salp species and/or size (Caron *et al.*, 1989; Stukel *et al.*, 2021; Fender *et al.*, 2023). Indeed, we find here that while still strongly negatively selected, submicron particles had the highest electivity in *T. democratica*, a species that others have reported to be particularly efficient at retaining bacteria compared to co-occurring relatives (Vargas and Madin, 2004). However, it remains unclear whether this ability stems from taxonomic affiliation or smaller average size (e.g. our specimens averaged  $12.6 \pm 2.8 \text{ mm}$ ,  $n = 8$ ).

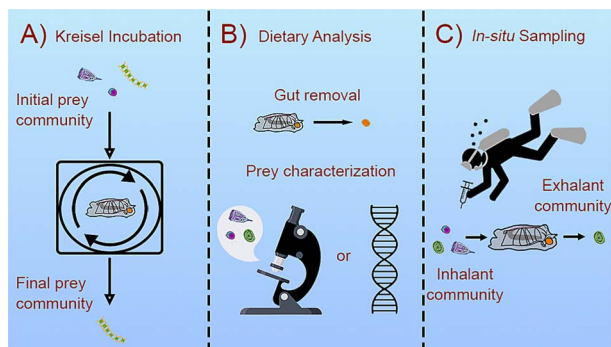
As an alternative to sieving, direct interception whereby particles strike and adhere to the mucous fibers themselves as a function of the prey's "stickiness" relative to the salp's feeding filter may also occur (Sutherland *et al.*, 2010). Dadon-Pilosof *et al.* (2017) found that both appendicularians and benthic ascidians cleared members of the SAR11 clade with notably lower efficiency than picocyanobacteria of the same size due to their less hydrophobic cell surfaces. Differences in cell surface hydrophobicity among natural populations of heterotrophic bacteria, *Synechococcus*, and picoeukaryote natural populations have been shown to affect clearance rates by heterotrophic nanoflagellates (Monger *et al.*, 1999), further supporting the importance of such molecular forces in governing prey selectivity. At least some species of salps possess chemoreceptors (Fedele, 1933) such that selection on this basis is not out of the question and may be related to the consistently positive selection for dinoflagellates we see. These results agree with several other similar reports of dinoflagellates being favored (Tanimura *et al.*, 2008; Metfies *et al.*, 2014; Ahmad Ishak *et al.*, 2017; Pauli *et al.*, 2021; Sutherland and Thompson, 2022) and may relate to salp distributions at both regional and local scales. Whether the dinoflagellates themselves are favored for nutritional reasons like higher assimilation efficiencies (Andersen, 1986) or if the nutrient landscapes that produce particle loadings and characteristics advantageous to salps also happen to favor dinoflagellates remains to be seen.

Although much less frequently considered, there is also an upper limit to prey size dictated by the size of the salp's esophageal opening. A clogging phenomenon has been observed in which particularly large particle loads lead to the formation of a bolus blocking the esophagus and subsequent ejection of the filter (Harbison *et al.*, 1986; Pakhomov *et al.*, 2003). This has previously been proposed as a mechanism to explain surprisingly low retention efficiencies for large particles in salps (Vargas and Madin, 2004), as particularly large phytoplankton such as *Chaetoceros* sp., *Proboscia* sp. and *Rhizosolenia* sp. that were found to dominate larger centric diatoms in the water column here may cause similar blockages that force salps to reject them (Fender *et al.*, 2023). For *Chaetoceros* sp. in particular, the spines of its frustules likely exacerbate this rejection by inflating its

effective size such that it has also been observed to clog the inlet filters of appendicularians far more than similarly sized but non-spiny cryptophytes (Troedsson *et al.*, 2007). The rejection of these species, of which none were found in any of the 58 guts examined, is reflected in their selectivity and likely explains why five of the seven salp species negatively selected large centric diatoms. Indeed, independent 18S rDNA metabarcoding of additional salp guts and fecal pellets also found significantly lower representation of bacillariophyte sequences compared to the water column, mirroring our results. Metabarcoding also revealed substantially lower species richness within the salps compared to the water column regardless of cycle, further supporting some degree of selection. If this rejection of large diatoms is mediated by filter clogging, it suggests the phenomenon may not be solely dependent on particle abundance (Harbison *et al.*, 1986; Perissinotto and Pakhomov, 1998) but is rather a complex function of bulk prey community characteristics correlated with conditions that typically favor high phytoplankton abundance.

Besides physical and chemical selection mechanisms, predator behaviors may also allow for mucous mesh grazers to exhibit preference (Conley *et al.*, 2018). Phytoplankton community compositions often change with depth due to varying light and nutrient availabilities, leading to microscale heterogeneity in the form of thin layers that motile filter feeders may utilize. Even within a DCM, phytoplankton communities can exhibit fine-scale differences in vertical distribution (Latasa *et al.*, 2017). Doliolids are able to decouple their locomotion from their feeding such that they can take advantage of these microlayers if they are rich in preferred prey (Walters *et al.*, 2019). This behavior represents a mechanism to exhibit preference even for filter-feeders that lack discrete physiological means, potentially shifting prey community structure. Certain larger, more motile species of salps are also well known for their ability to migrate on a diel cycle (Purcell and Madin, 1991; Nishikawa and Tsuda, 2001; Nogueira *et al.*, 2015), which day/night paired MOCNESS casts confirm occurred in *S. thompsoni* and *S. fusiformis* in the present study (Fig. 2). Since feeding and locomotion in salps are coupled, migration may yield differences in gut content relative to the surface if the prey community changes with depth. While FlowCam and flow cytometry indicated little difference in the composition of prey types within the mixed layer and at the DCM, these deeper samples were sometimes still within the mixed layer such that little difference is to be expected. Although the FlowCam analyses that provide the bulk of our water column taxonomic identifications are broadly representative of the euphotic zone where salps do the majority of their feeding, they were generally limited to the upper 40–70 m and would average over any such microscale differences which salps may be able to exploit. Furthermore, *S. thompsoni* were often found well <200–300 m where much of the potential planktonic prey will have aggregated onto sinking particulates and may, therefore, look quite different from the surface. For at least this strongly swimming species, and potentially others for which our sample size was too low to detect diel patterns, this may help explain the stark differences we see in these deeper salps' gut contents compared to the results from the mixed layer.

While the various examples of selection in filter-feeders provided above establish precedence for it in salps, it is important to consider their methodological differences and associated



**Fig. 9.** Conceptual diagram of the primary methods currently available to sample salp diets. (a) *Ex-situ* incubations in specially designed circular flow kreisels where removal is determined based on the difference in community composition between an initial water sample and at the end of the experiment. (b) Removal of the gut and characterization of its contents via microscopy, genetics, or metabolomics. (c) *In-situ* sampling of the prey community within the salp's inhalant current compared to that of its exhalant current.

limitations. Many of the prior studies rely on incubations to establish controlled prey fields that can be measured at the start and end of an experiment (Fig. 9A). Such incubations allow for robust determinations of selective removal but are suspect with respect to the representativeness of the prey community provided. Disruptions in natural salp feeding behavior have also been observed in confinement (Madin and Deibel, 1998; Sutherland *et al.*, 2010). Alternatively, blue-water diving and direct collection of water samples before and after passing through the feeding filter is much less disruptive and allows characterization at the exact feeding depth (Fig. 9C; Dadon-Piloso *et al.*, 2019). However, blue-water methods are labor intensive such that few samples can be collected over a limited depth range. Net collection and subsequent diet characterization via microscopy as utilized here, genetics or metabolomics allows for broader sampling and higher taxonomic resolution of prey (Fig. 9B), yet the net's vertical resolution is still limited such that it is unclear to what degree the trends we see are influenced by motility as opposed to different sampling depths. Clearly, there is no one-size-fits-all solution, although we feel the technique employed here is the most appropriate to the question at hand, which is currently available.

We also note that, as with any gut content analyses, our results may be subject to digestion biases that impact prey types in different ways (Köster *et al.*, 2011). For example, the surprising scarcity of coccolithophores could have been influenced by preferential digestion dissolving their characteristic calcium carbonate coccoliths (White *et al.*, 2018), a phenomenon we expect also explains the absence of the athecate dinoflagellates *Polykrikos* sp. and *Gyrodinium* sp. as well as aloricate ciliates. One of the most common objects observed in the guts of all salps were nondescript spherical particles of typically  $\sim 10 \mu\text{m}$ , although they ranged in size from 1 to  $20 \mu\text{m}$ . Given their abundance, broad range in size and importance to assembling complete size-spectra (Fender *et al.*, 2023), we believe these particles include the above groups as well as other various nanoplankton. We, therefore, grouped these particles as well as their corollaries in the FlowCam images, such as the aloricate ciliates, in the generic

size-structured prey classes such that this bias should be largely mitigated. Complete digestion such that certain prey types were not counted at all is possible and may still have biased selections low. However, selection was largely negative for silica-frustule-bearing diatoms, which are typically some of the most digestion-resistant prey types. Dinoflagellates were still positively selected in most salps despite the removal of athecate species, suggesting the true selection may have been even higher. We therefore consider both of these results robust.

### Implications of niche breadth

While the various metrics used to calculate niche breadth make direct comparisons across different studies problematic, one generally expects higher values for unspecialized filter feeders than for ambush or raptorial feeders, which have evolved physiologies or behaviors to maximize their capture of preferred prey. Katechakis *et al.* (2004) investigated the differences in niche space between the copepod *Acartia clausi*, the cladoceran *Penilia avirostris* and the doliolid *Doliolum denticulata* and found significantly higher niche breadths in the filter-feeding doliolids than the raptorial copepod, with the variably selective filter-feeding cladoceran falling somewhere in between. Food niche breadth can also play an important role in the population dynamics of a species. The body size, abundance and/or reproductive success of consumers that are specialized to feed on relatively few prey types are often dependent on the availability of those preys, as is the case with many larval planktivorous fish (Gophen, 1980; Mayer and Wahl, 1996). For generalists, on the other hand, coupling to any one specific prey type is weaker, such that their success is dependent on total prey abundance.

Overall, our observed  $B_{PSI}$  are lower than would be expected for nonselective filter feeders (Fig. 6). While a portion of this is certainly due to the robust selectivities for many of the larger prey discussed above, much of this trend is due to our inclusion of  $< 1 \mu\text{m}$  particles as a discrete prey category which salps generally do not efficiently feed on (Harbison and McAlister, 1979; Fender *et al.*, 2023). Excluding this prey category leads to higher niche breadths for all salp species, particularly those of Cycles 2, 3 and 4, which rise to a more expected 0.61–0.75 (0.49, 0.77) while also reducing the differences between species (Supplementary Fig. 3). While these niche breadths still fall short of true generalism, they agree with evidence that blooms are dictated by total rates of primary production and/or temperature (Deibel and Paffenhöfer, 2009; Pascual *et al.*, 2016) rather than by the population of any one specific prey item.

### Niche overlap and potential competition

Overlaps in gut contents were moderately high, as expected, with both  $O_{PSI}$  and the PCA suggesting that collection location is not the sole determinant of diet. Contrary to this, the 18S salp gut and fecal pellet metabarcoding suggest collection location was the primary factor, although they also depict substantial differences between the diets of *S. thompsoni* and *T. democratica* of Cycle 4 that mirror our results of species-specific preference. Taken together, we interpret this to mean that while ambient conditions are of utmost importance in shaping salp diets, other factors, such as the physicochemical and behavioral considerations mentioned previously, may still play a role.

One of the most apparent explanations for these differences in diet is spatial partitioning. In the same way that selectivity of the more motile salp species may be driven by feeding at different depths, two populations doing so could minimize competition with one another while feeding at the same depth could increase potential competition. For example, *T. democratica* is constrained to feeding at the surface due to its smaller size and weaker motility (Fig. 2). Despite the evidence of diel migration by *S. fusiformis* in Cycle 3, both of these species in Cycle 4 were found exclusively in the upper 50 m such that their very high dietary overlap is likely the product of feeding within the same mixed layer. This may also relate to the lower overlaps and high diet variability seen for the highly motile *S. thompsoni* as well as the differences in diet compared to *T. democratica* via 18S DNA. As with selectivity, the degree to which this explanation may be applied to the other species is limited by our knowledge of particle compositions at depth and small sample sizes for the nondominant species.

## CONCLUSION

Our findings show that despite traditionally being labeled as nonselective filter feeders, salps do exhibit positive and negative selections for certain prey types, showing apparent preference that extends beyond the expected exclusion of small particles due to poor retention and large particles due to clogging. Dinoflagellates were consistently positively selected across salp species and sampling locations while large, spinous particles that may clog the feeding filter and cause it to be rejected were strongly negatively selected. These selectivities also differ slightly between species, potentially due to variable motility, subsequent partitioning of the water column, or mucous mesh spacing. This led to feeding niche breadths that were generally large yet well below that of true generalists, suggesting that despite being largely nonselective across the full range of possible prey, salps still exhibit meaningful preferences that may impact their distribution as well as phytoplankton community composition. The potential for competition between salps is, therefore, unsurprisingly high, possibly more so between species relegated to the same portion of the water column. Further investigation into the mechanisms behind these differences in prey utilization, be they chemical, behavioral or physical, is necessary to improve our understanding of the impact salp feeding has on the plankton community.

## SUPPLEMENTARY DATA

Supplementary data can be found at *Journal of Plankton Research* online.

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## DATA AVAILABILITY

FlowCam and salp gut data are accessible on the Biological and Chemical Oceanography Data Management Office website (<https://www.bco-dmo.org/project/754878>).

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