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Prey taxonomy rather than size determines salp diets

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Abstract

Salps are gelatinous planktonic suspension feeders that filter large volumes of water in the food-dilute open ocean. Their life cycle allows periodic exponential growth and population blooms. Dense swarms of salps have a high grazing impact that can deplete the photic zone of phytoplankton and export huge quantities of organic matter to the deep sea. Previous studies described their feeding manner as mostly nonselective, with larger particles retained at higher efficiencies than small particles. To examine salp diets, we used direct in situ sampling (InEx method) of undisturbed solitary *Salpa maxima*. Aggregates ("chains") of *Salpa fusiformis* and *Thalia democratica* were studied using in situ incubations. Our findings suggest that in situ feeding rates are higher than previously reported and that cell removal is size independent with ~ 1 μm picoeukaryotes preferentially removed over both larger eukaryotes and smaller bacteria. The prey : predator size ratios we measured ($1 : 10^4$–$1 : 10^5$) are an order of magnitude smaller than previously reported values and to the best of our knowledge, are the smallest values reported so far for any planktonic suspension feeders. Despite differences among the three species studied, they had similar prey preferences with no correlation between salp body length and prey size. Our findings shed new light on prey : predator relationships in planktonic systems—in particular, that factors other than size influence filtration efficiency—and suggest that in situ techniques should be devised and applied for the study of suspension feeding in the ocean.

Salps are gelatinous, planktonic tunicates ranging in size from few millimeters to > 20 cm. Similar to other tunicates, these suspension feeders gather their food by pumping water through a mucus mesh that is continuously secreted by the endostyle and ingested with the trapped particles (Bone et al. 2000). Among the tunicates, salps are unique in using muscular peristaltic pumping to force water and associated food particles through the mucous mesh. This mode of pumping also propels the salp through the water, thus allowing it to exploit unfiltered water parcels and possibly orient toward patches of preferred food concentration (Madin and Deibel 1998). The powerful muscular pumping and the relatively loose mesh (reported mesh width 0.3×5.4 μm and up to 1.3×1.3 μm for *Salpa fusiformis* (Bone et al. 2003) allows salps to filter large volumes of water with reported values ranging from 1.5 to 55 L h^{-1} for individual zooids (Bone et al. 2003), a filtration rate higher than most holoplanktonic organisms. For example, a single solitary salp of $\sim$ 5 cm length has the same grazing impact as 450 large copepods (Harbison and Gilmer 1976).

Salps are abundant throughout the world ocean (Hereu et al. 2010) with some species found mainly on the continental shelf (e.g., *Thalia democratica*) and others more common in the open ocean (e.g., *Thetys vagina*, *Pegea confoederata*, and *Cyclosalpa affinis*). Some salp species also exhibit diel vertical migration (e.g., *Salpa aspera* and *S. fusiformis*; Wiebe et al. 1979; Madin et al. 1996). Salps are well adapted to the food-dilute open ocean environment and are able to adjust quickly to fluctuating food concentrations due to three characteristics: high filtration rates, ability to prey on a wide range of prey size, and their complex life cycle, which allows populations to grow exponentially under suitable conditions, which usually occur in response to increased prey concentrations (Alldredge and Madin 1982). Salps usually appear in low densities but under optimal conditions, the budding salp aggregates can form chains reaching a few meters in length and populations

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can reach densities of thousands of ind. per meter cubed (Henschke et al. 2016) that are typically distributed in the upper 100 m where phytoplankton are most abundant. Such "salp blooms" are facilitated by a complex life cycle that allows populations to grow exponentially when shifting from sexual (solitary) to asexual (aggregate) generations (Madin 1974; Hereu et al. 2010) using a high growth rate, short generation time, high fecundity, direct development, viviparity, maternal nutrition, alternation of generations, and hermaphroditism, which together allow for flexibility and adjustment to unpredictable patchiness of prey concentration (Alldredge and Madin 1982). Dense swarms have a high grazing impact, which may prevent phytoplankton blooms or end blooms before nutrients are depleted (Huskin et al. 2003). Other planktonic grazers can become rare, mainly because the rapid reproduction of salps limits competitors (Alldredge and Madin 1982). Salp swarms produce high concentrations of fast sinking (> 1000 m d^{-1}) fecal pellets and carcasses, enhancing the oceanic carbon pump by exporting large amounts of planktonic carbon to the deep sea (Smith et al. 2014; Henschke et al. 2016) and by transferring energy from ultra-plankton to higher trophic levels (Deibel 1985; Lavaniegos and Ohman 2003).

The relationships between prey and predator sizes are commonly considered as a master variable in marine food-web ecology, and prey : predator size ratio is considered a strong predictor of feeding efficiency for most planktonic suspension feeders (Hansen et al. 1994). As reviewed by Boyce et al. (2015), in most cases, the prey : predator size ratio is consistent within groups. These relationships range between 1 : 1 for raptorial-interception feeders such as dinoflagellates (Hansen et al. 1994) and up to 50 for cladocerans and meroplankton larvae (Hansen et al. 1994). Pelagic tunicates specialize on the filtration of relatively small particles with reported prey : predator size ratios of $\sim$ 1 : 200 for appendicularians (Lombard et al. 2011) and up to $1 : 5 \times 10^4$ for large salps (Harbison and McAlister 1979; Kremer and Madin 1992).

According to the literature, salps graze primarily on particles in the range between 1 μm and 1 mm (Harbison and Gilmer 1976; Deibel 1985; Kremer and Madin 1992) with larger particles retained at higher efficiencies than small particles (Harbison and Gilmer 1976; Harbison and McAlister 1979; Mullin 1983). For example, Harbison and McAlister (1979) showed that *Cyclosalpa* spp. retained bacteria much less effectively than particles > 4 μm in size. Using a Coulter counter, Harbison and Gilmer (1976) showed that *P. confoederata* can filter cells as small as 0.7 μm, but retention of 2.5 μm cells was higher and 4 μm particles were retained at the highest efficiency. Similarly, Mullin (1983) working with *T. democratica* also demonstrated higher retention of 5–7 μm phytoplankton in comparison to bacteria. In most cases, retention efficiency vs. cell size (measured with Coulter counters) showed a typical hyperbolic shape with all particles larger than 2–4 μm considered to be retained at 100% efficiency. In most cases, larger animals were less efficient in removing smaller particles than smaller animals (Harbison and Gilmer 1976; Harbison and McAlister 1979; Kremer and Madin 1992).

Active selection has historically not been considered possible for mucus net filter feeders and thus salps have been thought to be unselective (Conley et al. 2018). However, as recently demonstrated by Dadon-Pilosof et al. (2017), surface property interactions between prey cells and the tunicate mucous mesh may result in size-independent retention efficiency and differential retention of specific prey populations that are not equipped to evade filtration. Therefore, hereafter the use of the terms "selectivity" and "preference" is limited to their technical definition, i.e., removal of a food type in higher proportions than its proportion relative to other food types present in the environment (Chesson 1978, 1983).

Quantification of salp diets is a challenging task (Madin and Deibel 1998) due to the delicate makeup of the salp body and their patchy distribution. Invariably, existing sampling methods create stress that can affect feeding behavior, swimming, filtration, and ingestion. As reviewed by Madin and Deibel (1998) and Sutherland et al. (2010), experimental methods applied to date have mostly been indirect and/or involved capture and confinement. These included laboratory experiments with cultured diatoms as prey (Harbison and Gilmer 1976), in situ incubations with radioactively labeled bacteria and phytoplankton (Mullin 1983), and analysis of phytoplankton pigment in gut contents or fecal pellets (Madin and Cetta 1984; Madin and Purcell 1992). In most cases, the measured clearance rates have been considerably lower than reported pumping rates measured in situ with undisturbed animals (Sutherland et al. 2010; Sutherland and Madin 2010), suggesting either very low efficiency of filtration or more likely, experimental bias and disturbance of normal feeding behavior.

In most studies, measurements of prey size were conducted using standard Coulter counters, which are limited to particles > 1 μm, thus missing a large fraction of the marine picoplankton. Coulter counters also grouped different particle types together into a single "size" category, thus providing limited and potentially distorted information of the retained particles (Rosa et al. 2015). Deibel (1985) stated another disadvantage of Coulter counters: they discriminate between prey types based on volume, whereas planktonic grazers sometime discriminate between prey types on the basis of their linear dimensions (i.e., length, width, or diameter) as recently demonstrated by Conley and Sutherland (2017) and Conley et al. (2018). Estimation of prey size from gut content samples suffers from differential preservation of prey in the gut (Kremer and Madin 1992), and therefore, prey size quantification from gut content often miss the smallest and less armored cells. Moreover, barcoding of gut contents does not directly show that the prey item was captured as a single object as finding bacteria or cyanobacteria may be the result of aggregation or adhering to larger particles (Kremer and Madin 1992).

Flow cytometry and microscopic-based feeding studies (e.g., Scheinberg et al. 2005) provide significant benefits over

alternative methods (e.g., standard Coulter counters) by facilitating a combination of rough taxonomic discrimination and size ranking rather than size-based analyses that grouped together detrital and inorganic particles with different picoplanktonic and nanoplanktonic cell populations. Moreover, some flow cytometers can efficiently quantify and discriminate different submicron populations that are below the detection range of most other methods. The shortcomings and limitations of the use of flow cytometry-derived size estimates have been widely discussed during the last three decades (e.g., Spinrad and Brown 1986; Cunningham and Buonnacorsi 1992; Green et al. 2003; Laney and Sosik 2014; Agagliate 2017; Agagliate et al. 2018). These and other studies have demonstrated that the different refraction indices of different marine particles render absolute size estimates problematic. Nevertheless, it is also widely accepted that flow-cytometry–derived forward scatter (FSC) provides reliable size ranking to particles at the micron and submicron range, and when calibrated with polystyrene beads, reasonable approximation of cell sizes > 1 μm (Petersen et al. 2012). That is, particles with lower FSC are smaller than particles with higher FSC that are run under similar settings. An important point often missed when planktonic cell sizes are discussed is that these populations have a very wide size distribution that largely overlap as exemplified in, e.g., fig. 2 of Yahel et al. (2009) and fig. 2b of Jacobi et al. (2017)).

Our goal in this study was to test the hypothesis, raised by Sutherland et al. (2010) that feeding on submicrometer cells can provide a large proportion of salps' diets and the hypothesis of Dadon-Pilosof et al. (2017) that different prey taxa are retained at different efficiencies, regardless of their size. To obtain realistic measurements of salp feeding in the natural environment, we used a combination of in situ sampling that ensured minimum interference to the animals and sample analysis with flow cytometry. This approach allowed us to compare grazed particles to the natural background population and differentiate between prey populations based on pigments, nucleic acid content and relative size. Our findings suggest that in situ feeding rates are higher than previously reported and that cell removal was size independent with ~ 1 μm picoeukaryotes preferentially removed over both larger eukaryotes and smaller bacteria.

Methods

Salp feeding was studied in situ in the oligotrophic Mediterranean Sea, using blue-water SCUBA (Haddock and Heine 2005, see below). To allow efficient sampling, work was conducted in late spring during salp "blooms" when salp concentrations were high enough for divers to encounter at least several individuals (or chains) per minute. The unpredictable nature of the blooms and logistics involved with blue-water work posed a major challenge and restricted the number of samples collected and regularity of the sampling scheme. For example, during a 10-d expedition made in 2014 to Villefranche sur Mer (France), we were not able to sample even a single salp. Similarly, ad hoc expeditions made to the Red Sea, after major blooms were reported, either missed the bloom or arrived when the animals were at a final stage of decay. In fact during the 3 yr of the study, only once we were able to target a bloom of animals large enough and at sufficient numbers so that the direct sampling method could be applied, and to that end, A.D.-P. flew from Israel to France at a 24 h notice. As a result, our sample size was fairly limited (*Salpa maxima* $n = 13$, *S. fusiformis* $n = 12$, and *T. democratica* $n = 15$; Table 1).

Study sites

In situ sampling of *S. fusiformis* and *S. maxima* was conducted in the northwest Mediterranean Sea, outside the bay of Villefranche sur mer, France (43°42′N, 7°18′E, hereafter, NWMS) during April 2016 (water temperature ~ 14°C, chlorophyll *a* [Chl *a*] 0.1–0.4 μg L^{-1}). Sampling occurred at 8–20 m depth, and the bottom depth was greater than 100 m. In situ incubations of *T. democratica* were conducted in the eastern Mediterranean Sea, Michmoret, Israel (32°24′N, 34°52′E, hereafter, EMS) during May 2014 (water temperature ~ 23°C) and April 2015 (water temperature ~ 19°C, Chl $a < 0.1$ μg L^{-1}). Sampling occurred at 3–6 m depth, and the bottom depth was 5–20 m.

Direct in situ sampling (InEx)

Direct sampling of the water inhaled and exhaled by salps was possible only for the large zooids of *S. maxima* and was conducted during drift dives using blue-water SCUBA at 8–20 m. Pumping activity of each specimen was visualized before sample collection. Seawater collected next to the studied specimen was dyed with sodium fluorescein and gently released through a 0.2 μm filter at the entrance to the inhalant siphon. The speed and magnitude of the exhalant jet provided a clear indication of the animal's pumping activity.

To cleanly collect inhaled and exhaled water, we used a modification of the VacuSIP technique (Morganti et al. 2016) that allows simultaneous, clean, and controlled collection of the water inhaled and exhaled by the salps with minimal contact or interference with the studied animal (Supporting Information Movie S1). Water samples were collected by carefully positioning tubes (PEEK, external diameter 1.6 mm, internal diameter 0.27 mm, IDEX 1531) inside the exhalant siphon and next to the inhalant siphon of the sampled salp. Piercing the septum of an evacuated 10.5 mL sampling vessel (VACUETTE® Urine Tube, Round Bottom 10.5 mL, Greiner Bio-One, cat No. 455007) with a hypodermic syringe needle attached to the distal end of each tube allowed the external pressure to slowly force the sampled water into the vessel. The use of the PEEK tubing with its minute internal diameter (0.27 mm) allowed accurate point sampling, ensured controlled and slow suction, minimized the dead volume (< 25 μL, $< 0.23\%$ of the sampler volume), and served as a prefilter that prevented the suction of large and rare aggregates and marine snow particles. After collection, samples were kept on ice until preservation (within

Table 1. Ambient prey concentrations and predation rate for the three salp sampling expeditions in 2014, 2015, and 2016 in the EMS and NWMS. Note that *S. maxima* in 2016 was sampled using the direct InEx method, whereas the two other species were sampled using the indirect incubation method.

Salp species (number of samples)	Sampling date and site	Size of individuals (mm)	Prey type	Ambient concentration (cells mL^{-1})		Retention efficiency (%) or clearance rate (mL $ind.^{-1}$ h^{-1})	
				Mean ± CI95%	Median	Mean ± CI95%	Median
S. maxima (13)	April 2016, NWMS	135 ± 0	NanoEuk	$9.44 \times 10^2 \pm 1.32 \times 10^2$	8.75×10^2	30.6 ± 11.9%	39.40%
			PicoEuk	$5.88 \times 10^3 \pm 9.25 \times 10^2$	6.12×10^3	66.1 ± 13.7%	75.30%
			Syn	$1.40 \times 10^5 \pm 2.05 \times 10^4$	1.43×10^5	1.66 ± 2.3%	0.00%
			PLP	$2.17 \times 10^4 \pm 2.63 \times 10^3$	2.21×10^4	2.85 ± 2.9%	0.00%
			HNA-Ls	$4.16 \times 10^5 \pm 7.36 \times 10^4$	4.40×10^5	12.4 ± 7.99%	11.50%
			HNA-Hs	$6.46 \times 10^3 \pm 1.54 \times 10^3$	6.86×10^3	14.8 ± 10.8%	5.21%
			LNA	$4.29 \times 10^5 \pm 7.26 \times 10^4$	4.41×10^5	8.99 ± 5.96%	5.23%
S. fusiformis (12)	April 2016, NWMS	38.7 ± 5.7	NanoEuk	$8.39 \times 10^2 \pm 2.83 \times 10^2$	7.10×10^2	754 ± 949	120
			PicoEuk	$4.38 \times 10^3 \pm 1.28 \times 10^3$	4.96×10^3	1084 ± 596	962
			Syn	$1.56 \times 10^5 \pm 1.60 \times 10^4$	1.60×10^5	115 ± 158	43
			PLP	$2.24 \times 10^4 \pm 2.19 \times 10^3$	2.17×10^4	276 ± 384	0
			HNA-Ls	$4.83 \times 10^5 \pm 5.09 \times 10^4$	5.09×10^5	90 ± 136	18
			HNA-Hs	$4.00 \times 10^3 \pm 7.23 \times 10^2$	4.09×10^3	560 ± 622	169
			LNA	$4.70 \times 10^5 \pm 5.32 \times 10^4$	5.03×10^5	92 ± 160	0
T. democratica (5)	April 2015, EMS	14.3 ± 3.8	NanoEuk	$6.72 \times 10^2 \pm 1.87 \times 10^2$	6.61×10^2	256 ± 304	206
			PicoEuk	$2.57 \times 10^3 \pm 2.87 \times 10^3$	1.62×10^3	1135 ± 1487	782
			Syn	$5.01 \times 10^3 \pm 5.44 \times 10^3$	4.18×10^3	262 ± 439	53
			PLP	$1.27 \times 10^4 \pm 6.09 \times 10^3$	1.38×10^4	1108 ± 2281	329
			HNA-Ls	$2.95 \times 10^5 \pm 6.92 \times 10^4$	2.98×10^5	8 ± 25	0
			HNA-Hs	$2.62 \times 10^4 \pm 5.14 \times 10^4$	1.15×10^4	138 ± 398	19
			LNA	$3.47 \times 10^5 \pm 1.06 \times 10^5$	3.43×10^5	21 ± 40	16
T. democratica (10)	May 2014, EMS	15 ± 0	NanoEuk	$7.23 \times 10^2 \pm 1.90 \times 10^2$	6.61×10^2	207 ± 192	59
			PicoEuk	$2.88 \times 10^3 \pm 9.50 \times 10^2$	2.89×10^3	1068 ± 1063	102
			Syn	$1.41 \times 10^4 \pm 7.28 \times 10^3$	1.19×10^4	216 ± 243	0
			PLP	$2.00 \times 10^4 \pm 4.91 \times 10^3$	1.82×10^4	719 ± 1168	55
			HNA-Ls	$2.38 \times 10^5 \pm 4.56 \times 10^4$	2.44×10^5	337 ± 579	0
			HNA-Hs	$1.32 \times 10^4 \pm 1.76 \times 10^4$	4.08×10^3	14 ± 34	0
			LNA	$3.59 \times 10^5 \pm 3.57 \times 10^4$	3.67×10^5	469 ± 501	176

2 h) for cell counts with a flow cytometer. The retention efficiency (RE) of planktonic cells was calculated from the difference in concentration between the inhaled (In) and exhaled (Ex) water using the formula: RE (%) = 100 × (In − Ex)/In.

In situ incubations

For small salps and chains (*T. democratica* and *S. fusiformis*), which were too small for direct sampling as described above, we modified the indirect clearance rate method techniques described by Riisgård (2001). Organisms were collected into open-ended incubators (2 liter) during drift dives using blue-water SCUBA. A control sample was immediately collected with 15 mL tube. Both containers were incubated at the collection site (3–6 m depth) for 15–30 min. Water was suctioned from the incubator after 15 min using the VacuSIP technique as described above.

At the end of the incubations, the incubators were pulled onto the boat and a second water sample was collected from each incubator and preserved for further analysis. Number and length of zooids were measured for each incubator.

Prey concentration and relative size

Flow cytometry

Flow cytometry was used to quantify the concentrations and the cell characteristics of nonphotosynthetic microbes (hereafter referred to as nonphotosynthetic bacteria), and the four dominant autotrophic groups (*Prochlorococcus* [Pro], *Synechococcus* [Syn], picoeukaryotic algae [PicoEuk], and nanoeukaryotic algae [NanoEuk]). We used an Attune® Acoustic Focusing Flow Cytometer (Applied Biosystems) equipped with a syringe-based fluidic system that allows precise adjustment of the injected sample volume and hence high precision of the

measurements of cell concentrations (± 5%). The optics system contained violet and blue lasers (405 and 488 nm, respectively) and was further adapted for the analysis of marine ultraplankton samples as described below.

Aliquots of 1.8 mL were collected from each water sample and transferred into 2 mL cryovials (Corning cat No. 430659). Samples were first incubated for 15 min at room temperature with glutaraldehyde 50% (electron microscopy grade, Sigma-Aldrich, cat No. 340855) at 0.1% (final concentration) for the oligotrophic EMS and 0.2% (final concentration) for the more productive NWMS water. Samples were frozen in liquid nitrogen (at least 60 min) and then stored at −80°C until analysis (within a few weeks).

Each sample was analyzed twice. First, 600 μL of the sample water was analyzed at a high flow rate (100 μL min^{-1}) for the determination of ultra-phytoplankton with a dual threshold (trigger) on the red fluorescence channels of the violet and blue lasers. A second run was used to analyze cells with no autofluorescence, i.e., nonphotosynthetic microbes. To visualize these cells, a 300 μL aliquot of the sample water was incubated with the nucleic acid stain SYBR Green I (20–120 min dark incubation at room temperature, 1 : 10^4 of SYBR Green commercial stock). For this run, we used a low flow rate of 25 μL min^{-1} and the instrument was set to high sensitivity mode. Seventy-five microliters of the sample water was analyzed with a dual threshold (trigger) on green fluorescence channels of the violet and blue lasers. Taxonomic discrimination was made based on orange fluorescence (Bl2, 574 ± 13 nm) of phycoerythrin and red fluorescence (Bl3, 690 ± 20 nm and VL3, 685 ± 20 nm) of chlorophyll (Tarao et al. 2009); side scatter provided a proxy of cell surface complexity and cell volume (Marie et al. 1999), and FSC was a proxy of cell size (Cunningham and Buonnacorsi 1992; Simon et al. 1994).

Where possible, the nonphotosynthetic bacteria were further divided based on their green fluorescence (proxy for nucleic acid content) and FSC (proxy for size) into three groups: LNA, low-nucleic acid nonphotosynthetic bacteria; HNA-Ls, high-nucleic acid low-scatter nonphotosynthetic bacteria; and HNA-Hs, high-nucleic acid high-scatter nonphotosynthetic bacteria (Zubkov et al. 2004). Similarly, the eukaryotic algae were separated into pico and nano-categories (Simon et al. 1994). The literature regarding *Synechococcus* size is vague but the reported size range is 0.3 to 1.2 μm (e.g., Uysal 2001; Garcia et al. 2016). For picoeukaryotic algae, we followed Worden and Not (2008), which suggested a size range of up to 3.0 μm, whereas larger eukaryotes were designated to nanoeukaryotic algae (2.0–20 μm). As a rough proxy of cell size, we used the ratio of the median FSC of each cell population to that of the median FSC of reference beads (Polysciences™, cat# 23517, Flow Check High Intensity Green Alignment 1.0 μm) that were used as an internal standard in each sample.

Cell size estimates: Test and validation

To validate our flow cytometry results, we compared cell size estimates from six samples analyzed with an epifluorescence microscope (Olympus BX43) to those from the same samples analyzed with the flow cytometer. For cell quantification with Epifluorescence microscopy, we followed the protocol of Sherr and Sherr (1993) and Lindelll and Post (1995). Briefly, seawater samples were preserved with 0.75% formaldehyde immediately upon collection and stored in the dark at 4°C. Within a day from collection samples were filtered on a ø25 mm, 0.2 μm polycarbonate membrane using low vacuum, mounted on a microscope slide with ultra-low fluorescence immersion oil (Sigma-Aldrich 56822), and stored frozen (−20°C) until analysis. For photosynthetic cell counts, we filtered 100 mL of unstained seawater, whereas for nonphotosynthetic prokaryotes (bacteria), 14 mL of the 0.75% formaldehyde sample was first incubated with the nucleic acid stain 6-diamidino-2-phenylindole dilactate (DAPI; Molecular Probes D-3571).

Filters were examined and imaged under an epifluorescence BX43 Olympus microscope using 100X magnification. For photosynthetic cells, we used a wide blue optical filter set with excitation range of 420–490 nm and a barrier filter at 515 nm. For DAPI counts, we used standard cell counts, and area measurements were carried out using the "Analyze particles" procedure of ImageJ (scientific image analysis, NIH). Measurement parameters were adjusted according to cell fluorescence and published estimated size of each group: nonphotosynthetic microbes 0.01–4 μm^2, DAPI fluorescence; *Prochlorococcus* (Pro) 0.01–0.64 μm^2, red fluorescence; *Synechococcus* (Syn) 0.04–3 μm^2, orange fluorescence; and eukaryotic algae (Euk) 0.64–20 μm^2, red fluorescence.

A cluster of *Prochlorococcus*-like particles (PLP) with low FSC (very small size), significant red fluorescence and low or null orange fluorescence were present in all water types and seasons. The ratio of the median FSC of this cell population to the median FSC of 1 μm yellow–green reference beads that were run with each sample ranged from 0.01 to 0.3. The best estimates for *Prochlorococcus* cell size were made on cultures and provide a range of 0.5–0.8 μm for length and 0.4–0.6 μm for width (Partensky et al. 1999). Therefore, these very small particles are hereafter designated as "*Prochlorococcus*-like particles" or "PLP."

Data analysis

Our sampling design was especially robust due to the paired sampling design (InEx and before/after incubations) applied throughout sample collection and analysis. That is, the same prey populations were compared in the same water prior to and after the passage through the salps' filtration apparatus using identical analytical methods. Normalization to calibration beads provided protection against instrumental drift.

To allow a comparison between the filtration efficiency of the three salp species and across the two sampling modes used (direct and incubation), we calculated an apparent retention efficiency for each prey population in each incubation experiment by normalizing its measured clearance rate to those of the prey population with highest clearance rate measurement using the equation $RE'_{i,j} = CR_{i,j}/CR_{i_{max},j}$, where RE′ is the apparent

retention efficiency of prey population *i* in incubation *j*, CR is the measured clearance rate, and i_{max} is the prey population that was cleared at the highest rate in the specific incubation (mostly picoeukaryotic algae).

A mathematical encounter model based on hydrosol mechanisms (Silvester 1983) was applied to test the prediction that salp particle retention efficiencies are determined solely on the basis of size. The model has previously been applied with the salp *P. confoederata* (Sutherland et al. 2010) and was expanded here to include *T. democratica* and *S. fusiformis*. Briefly, capture efficiency by a rectangular mesh was calculated for a range of particle sizes (0.01–10 μm) using Silvester's (1983) model. Mesh fiber diameter, mesh dimensions, realistic particle concentrations, and flow speeds were taken from the literature (Bone et al. 2003; Sutherland et al. 2010; Supporting Information Table S1). Particles were assumed to be spherical and encounter was modeled based on direct interception and diffusive deposition of particles on the mesh. Encounter by *S. maxima* was not modeled because there are no published mesh dimensions (length and width of mesh opening and fiber diameter) for this species.

To obtain a rough estimate of the contribution of each prey type to the salp's diet, we applied published conversion factors (Houlbrèque et al. 2006; Buitenhuis et al. 2012) to the Mediterranean Sea and applied them to the main cytometric populations in our samples: nanoeukaryotic and picoeukaryotic algae, *Synechococcus*, *Prochlorococcus*, and three nonphotosynthetic bacterial populations as summarized in Table 2. As with any biovolume-based conversion factors, these should be considered as the best available first-order approximations (Li et al. 1993; Buitenhuis et al. 2012).

A "within subject" design (i.e., paired *t* test, repeated measure analysis of variance [ANOVA], and their nonparametric alternative: Wilcoxon Matched Pairs Test and Friedman Repeated Measures ANOVA on Ranks, respectively) was used throughout the analysis to test the null hypothesis of nonselective retention. Post hoc analysis was done with Holm-Sidak or Tukey post hoc Pairwise Multiple Comparison. Where needed to meet ANOVA requirements of homogeneity of variance and normality, clearance rate was square root transformed and filtration efficiency was square root and arcsine transformed. Due to the low number of samples collected from *T. democratica* in 2015, these samples were pooled with the 2014 data for statistical analyses. Data are reported as mean ± 95% confidence interval of the mean (CI95%) unless specified otherwise. Analysis was done using SigmaPlot (Systat Software, ver. 13.0) and Statistica (Statsoft, Ver 13.2).

Results

Direct in situ sampling

Sampling of the water inhaled and exhaled by otherwise undisturbed, freely swimming *S. maxima* (solitary zooids,

Table 2. Estimated contribution of the dominant microbial populations to the planktonic biomass in the salp diets as sampled at EMS for *T. democratica* and NWMS for *S. fusiformis* and *S. maxima*. Calculations are based on measured concentration of each prey population and published conversion factors (Houlbrèque et al. 2006; Buitenhuis et al. 2012). Carbon content in the diet of *T. democratica* and *S. fusiformis* is based on average clearance rate and for *S. maxima* is based on average retention efficiency. This is a summary table, detailed statistics for each parameter are reported in Table 1.

				Feeding measurement			Carbon content in diet			
		Average ambient concentration (cells mL^{-1})		Average clearance rate (L ind.$^{-1}$ h^{-1})		Average retention efficiency (%)	(μg ind.$^{-1}$ h^{-1})		(μg L of pumped water)	
Group	Carbon content (fg C cell^{-1})	EMS	NWMS	*T. democratica*	*S. fusiformis*	*S. maxima*	*T. Democratica*	*S. Fusiformis*	*S. Maxima*	Reference
NanoEuk	7628	70.2×10^2	8.9×10^2	0.238	0.269	30.59%	1.311	1.835	2.09	Houlbrèque et al. (2006)
PicoEuk	1319	2.8×10^3	5.2×10^3	1.161	1.452	66.09%	4.406	9.880	4.50	Buitenhuis et al. (2012)
Syn	154	1.4×10^4	1.4×10^5	0.246	0.354	1.66%	0.535	8.067	0.38	Buitenhuis et al. (2012)
PLP	60	2.0×10^4	2.2×10^4	0.896	1.763	2.85%	1.074	2.330	0.04	Buitenhuis et al. (2012)
Bact	14	5.3×10^5	8.7×10^5	0.185	0.410	10.20%	1.387	5.029	1.25	Houlbrèque et al. (2006)

length = 11–15 cm) provided a measure of their in situ diet composition under minimal interference conditions. Flow cytometry counts of the main groups of nanoplanktonic and picoplanktonic prey revealed that *S. maxima* consumed mainly eukaryotic algae despite their lower concentrations in ambient water ($3.4 \times 10^3 \pm 2.7 \times 10^3$ mean ± CI95%, cells mL^{-1}; Table 1). Interestingly, the smaller picoeukaryotes were retained at the highest retention efficiency (66% ± 14%; Fig. 1), whereas larger nanoeukaryotes were retained at significantly lower retention (30.6% ± 12%; Fig. 1; repeated measures ANOVA [RM-ANOVA], $F_{[12,6]} = 26$, $p < 0.001$). Surprisingly, the retention of picocyanobacteria (*Synechococcus* and *Prochlorococcus*) with intermediate abundance in the ambient water ($8.1 \times 10^4 \pm 6.5 \times 10^4$ cells mL^{-1}) was negligible (1.6% ± 2.3% and 2.8% ± 2.9%, for *Synechococcus* and PLP, respectively; Supporting Information Fig. S1). Non-photosynthetic bacteria that numerically dominated the planktonic community ($8.3 \times 10^5 \pm 2.1 \times 10^5$ cells mL^{-1}) were also retained at relatively low efficiency (15% ± 11%, 12% ± 8%, 9% ± 6% for HNA-Hs, HNA-Ls, and LNA, respectively), but this retention was significantly different from zero (Fig. 1; Table 1; Supporting Information Fig. S1).

In situ incubations

In situ incubations of the smaller *S. fusiformis* at the NWMS (aggregates zooids, length = 2.3–5.5 cm) showed similar size-independent preferential removal of the picoeukaryotic algae (1080 ± 600 mL ind.$^{-1}$ h^{-1}; Fig. 2a; RM-ANOVA, $F_{[11,6]} = 10$, $p < 0.001$) over both the larger nanoeukaryotes and smaller picocyanobacteria (750 ± 950 and 195 ± 195 mL ind.$^{-1}$ h^{-1}, respectively; Table 1; Fig. 2a). The abundant nonphotosynthetic bacteria groups that numerically dominated the planktonic community were removed at a significantly lower clearance rates (90 ± 130 and 90 ± 160 mL ind.$^{-1}$ h^{-1}, for HNA-Ls and LNA, respectively; Fig. 2a), whereas the rarer and larger HNA-Hs bacteria were removed at intermediate and more variable rates (560 ± 620 mL ind.$^{-1}$ h^{-1}). In contrast to *S. maxima*, the filtration of picocyanobacteria with intermediate abundance in the ambient water was similar to that of HNA-Ls and LNA bacteria (115 ± 160 and 280 ± 390 mL ind.$^{-1}$ h^{-1}, for *Synechococcus* and PLP, respectively; Fig. 2c; Supporting Information Fig. S2).

In the oligotrophic EMS, in situ incubations of the smallest salp studied, *T. democratica* (aggregates, 1–1.5 cm), showed similar size-independent preferential removal of the picoeukaryotic algae (1160 ± 800 mL ind.$^{-1}$ h^{-1}; Fig. 2b,c) over both the larger nanoeukaryotes (230 ± 150 mL ind.$^{-1}$ h^{-1}, RM-ANOVA, $F_{[15,6]} = 5.7$, $p < 0.001$; Fig. 2) and smaller picocyanobacteria (Table 1; Fig. 2b,c) as observed for both *S. maxima* and *S. fusiformis* in the NWMS (Fig. 2; Supporting Information Fig. S1, S2). Nonphotosynthetic bacteria that numerically dominated the planktonic community were removed at lower clearance rates (60 ± 100, 217 ± 340, and 306 ± 320 mL ind.$^{-1}$ h^{-1}, for HNA-Hs, HNA-Ls, and LNA, respectively; Fig. 2b,c). On average, clearance rates of picocyanobacteria with intermediate abundance in the ambient water ($8.9 \times 10^4 \pm 7.1 \times 10^4$ cells mL^{-1}; Supporting Information Fig. S2) were somewhat higher than clearance rate on non-photosynthetic bacteria (346 ± 196 and 896 ± 976 mL ind.$^{-1}$ h^{-1}, for *Synechococcus* and PLP, respectively; Fig. 2; Supporting Information Fig. S2).

Over the 3 yr of sampling, the diet composition of the three salp species studied showed surprising similarity despite the

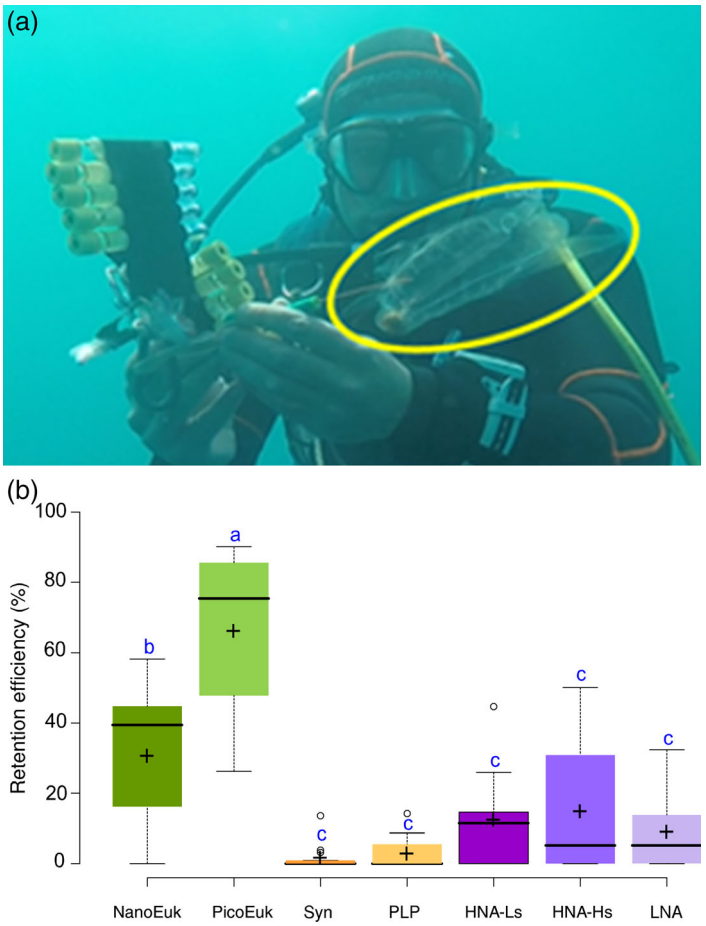

Fig. 1. Direct in situ sampling of *S. maxima*. (**a**) Sampling of the water inhaled and exhaled from a freely swimming *S. maxima* using the VacuSip method. (**b**) Retention efficiency (%) of different prey types by *S. maxima* measured by direct in situ sampling of the water inhaled and exhaled by each specimen in the NWMS (10–20 m depth) in April 2016 ($n = 13$). Eukaryotic cells: NanoEuk, nanoeukaryotic algae; PicoEuk, picoeukaryotic algae; Syn, *Synechococcus* and PLP, *Prochlorococcus*-like particles are two picocyanobacterial taxa. The nonphotosynthetic bacteria were subdivded into: HNA-Ls, high-nucleic acid low-scatter cells; HNA-Hs, high-nucleic acid high-scatter cells; LNA, low-nucleic acid cells. In each box, center lines show medians; +s show means; box limits indicate the 25th and 75th percentiles; whiskers represent local minima and maxima, i.e., they extend to data points that are less than 1.5 × IQR away from the 25th and 75th percentiles (IQR is the interquartile range), outliers are represented by open circles. Letters represent significantly different groups ($p < 0.05$, SNK post hoc multiple comparison pairewise tests).

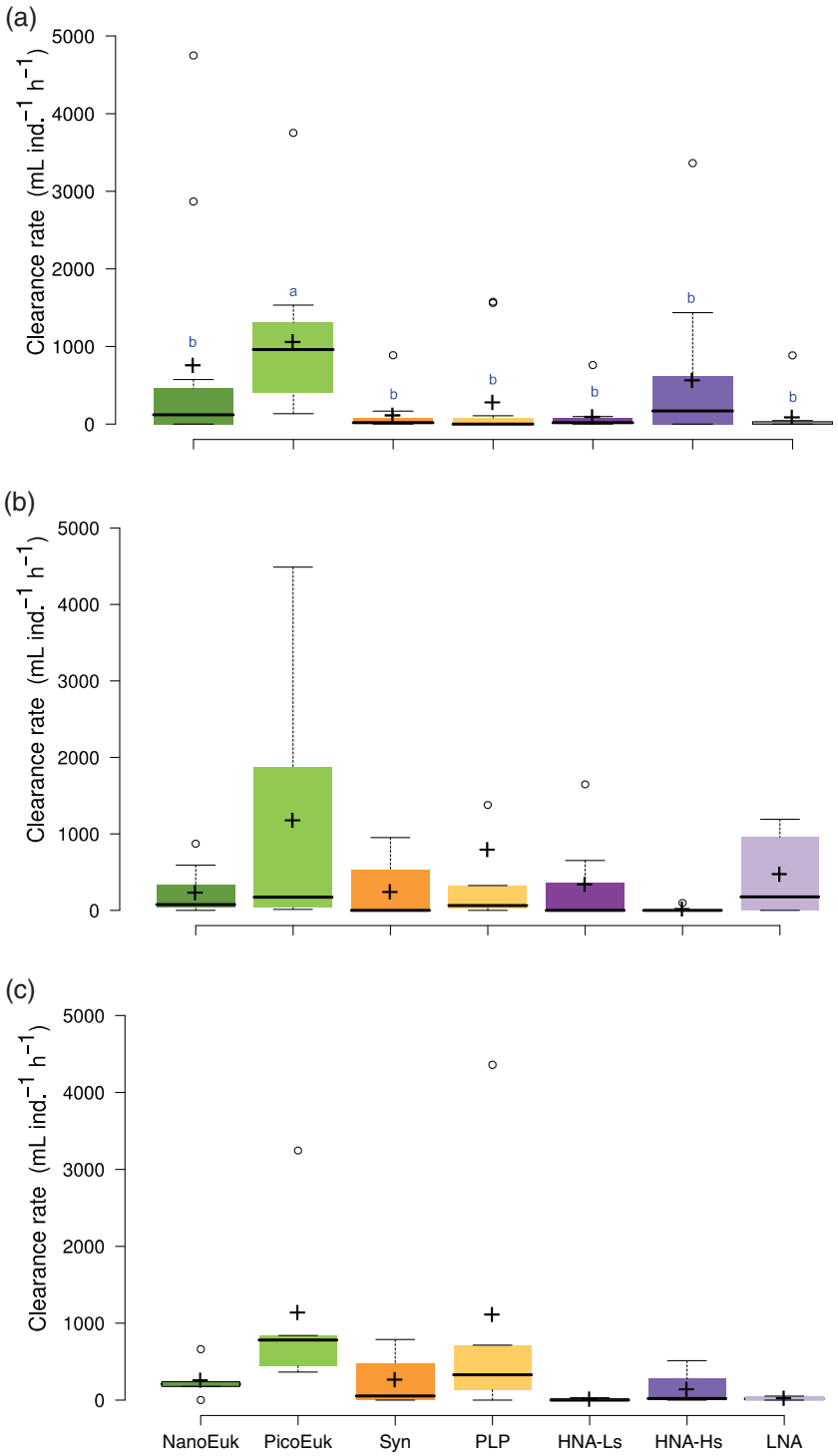

Fig. 2. Clearance rates of marine microorganisms by salps from in situ incubations. (**a**) *S. fusiformis*, NWMS, $n = 12$, April 2016. (**b**) *T. democratica*, East Mediterranean Sea (EMS), $n = 10$, May 2014. (**c**) *T. democratica*, EMS, $n = 5$, April 2015. Abbreviations and box plots markers are as in Fig. 1. Due to the low N, no statistics are provided for panels b and c (see text).

large differences in size and life stages. Generally, all three species showed size-independent removal of small picoeukaryotes over both larger nanoeukaryotic algae and smaller prokaryotic cells. For example, filtration of picoeukaryotes differed significantly from both *Synechococcus* and HNA-Hs despite their similar size ($\sim 1\ \mu$m; Fig. 3a–d).

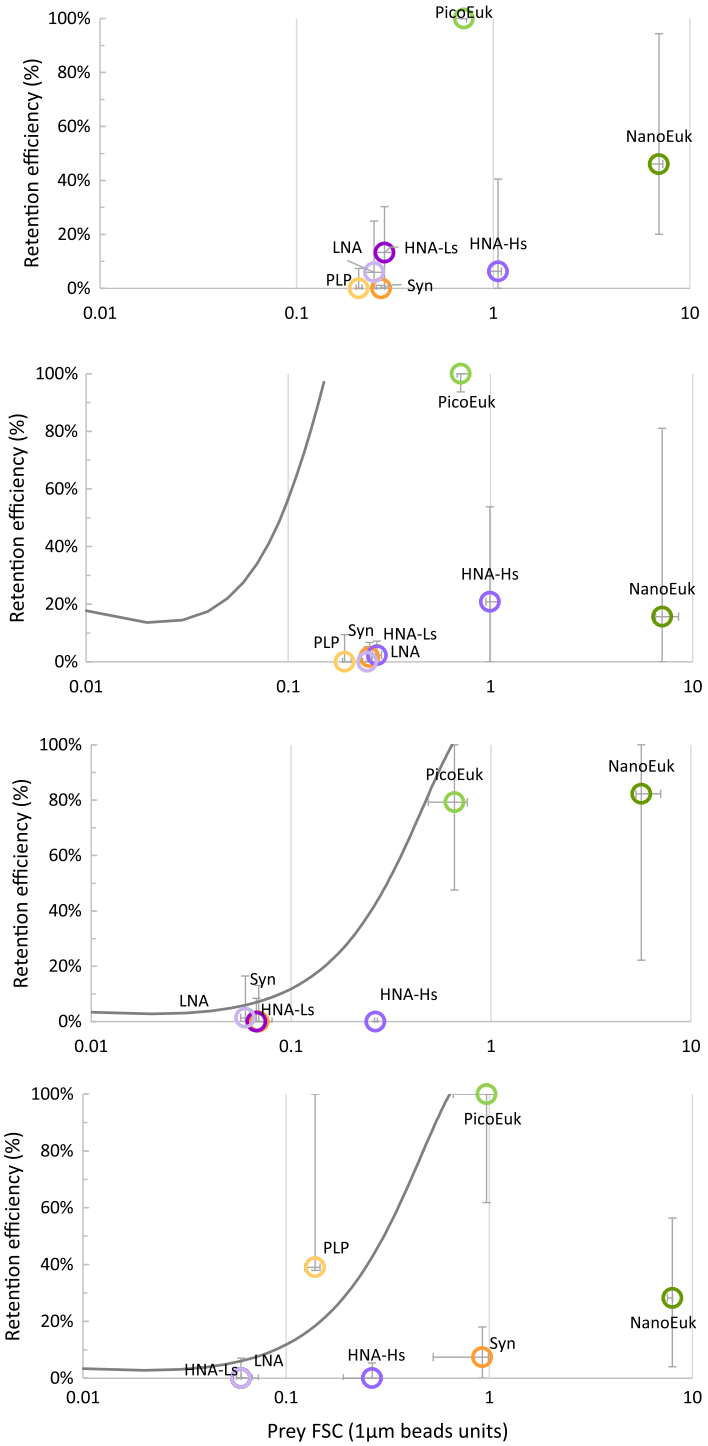

Fig. 3. Relationship between the retention efficiency (%) of different prey types and their relative size conservatively calculated as the ratio of cells FSC to the FSC of 1 μm beads (microscopy-based size estimates were higher). The size-based retention efficiencies predicted by the encounter model of Sutherland et al. (2010) are plotted as grey lines in **b**–**d**. Vertical error bars are lower and upper quartiles of retention efficiency (%), and horizontal error bars are lower and upper quartiles of the FSC normalized to the FSC of 1 μm beads. While the size estimates of particles above 0.8 μm are quite accurate, size estimates of smaller particles are questionable. (**a**) Direct in situ sampling of *S. maxima*, NWMS (10–20 m depth), April 2016, $n = 13$. (**b**) In situ incubations of *S. fusiformis*, NWMS, $n = 12$, April 2016. (**c**) In situ incubations of *T. democratica*, EMS, $n = 10$, May 2014. (**d**) In situ incubations of *T. democratica*, EMS, $n = 5$, April 2015. Abbreviations as in Fig. 1. *PLP is excluded from **c** due to its questionable size estimate.

Discussion

Direct in situ sampling of undisturbed salps feeding in their natural environment (*S. maxima*) revealed a significant size-independent preference for picoeukaryotic algae (~ 1 μm) over both larger nanoeukaryotic algae (~ 6–8 μm) and smaller prokaryotic cells (picocyanobacteria and nonphotosynthetic bacteria ≤ 1 μm; Figs. 1, 3a). Similar results were obtained from in situ incubations of two smaller salp species (*S. fusiformis* and *T. democratica*) during spring blooms in the oligotrophic Mediterranean Sea.

The differential retention of different planktonic cells is clearly revealed when the contribution of cell populations to the salp diet is compared to its contribution to the ambient ultraplankton biomass. Calculations based on cell carbon content derived from published conversion factors suggest that *S. maxima* gained ~ 55% of their carbon from picoeukaryotic algae that represented only 14% of the ultra-planktonic carbon in the ambient water of the NWMS (Fig. 4; Table 2). Similarly, picoeukaryotic algae contributed 36% of the diet of *S. fusiformis* in the NWMS and 51% of the diet of *T. democratica* in the EMS where they represent only 19% of ultra-planktonic carbon in the ambient water.

While the contribution of picoeukaryotic algae to the salps' diet was significantly higher than their ambient concentration, the contribution of nanoeukaryotic algae to the diet of both *S. fusiformis* and *T. democratica* was in reduced proportion (7% and 15%, respectively) in comparison to their contribution to the ambient planktonic biomass (14% and 27%, respectively). In contrast, in *S. maxima,* the contribution of nanoeukaryotic algae to the salps' diet was larger than their contribution to the ambient biomass (25% in the diet vs. 14% in the ambient water).

Previous studies showed that salps are capable of grazing micron-sized particles (Madin 1974; Harbison and Gilmer 1976), although at relatively low rates (Kremer and Madin 1992), even when grazing on a natural assemblage (Vargas and Madin 2004). Our findings show that small nonphotosynthetic bacteria (most < 1 μm) were removed at relatively low retention (~ 10%). Nevertheless, as predicted by Sutherland et al. (2010) and hydrosol filtration theory (Rubenstein and Koehl 1977), due to the relative dominance of nonphotosynthetic bacteria in the oligotrophic Mediterranean (> 5×10^5 cells mL^{-1} EMS, > 8×10^5 cells mL^{-1} NWMS), the contribution of these cells to salp diets was significant, amounting to 15–20% of the salps' planktonic carbon intake (Fig. 4).

Picocyanobacteria abundance in the ambient water was intermediate (5×10^3–2×10^5 cells mL^{-1} EMS, 2×10^3–1×10^5 cells mL^{-1} NWMS) and these cells accounted for a high proportion (17% and 48%%) of the ambient planktonic biomass in the EMS and NWMS, respectively. Their contribution to salp carbon intake was variable with minimal contribution to the diet of *S. maxima* (3%) and 38% and 18% of the diet of *S. fusiformis* and *T. democratica*, respectively (Fig. 4). Taken together, despite the low retention efficiency of prokaryotic cells (cyanobacteria and nonphotosynthetic bacteria), they accounted for a considerable fraction of the salps' diet. This contribution was low (20%) for the large *S. maxima*, high (57%) for *S. fusiformis,* and intermediate (34%) for the small *T. democratica* in the oligotrophic EMS water.

These results stand in sharp contrast to the prediction of an encounter-based mathematical model (Sutherland et al. 2010) that assumed 100% retention of any particle larger than the mesh size and any particle that directly encountered the mesh elements. Model predictions based on published mesh dimensions suggested that *S. fusiformis* should retain all prey larger than 0.2 μm at 100% and that *T. democratica* should retain all cells larger than 0.8 μm at 100% (Fig. 3b–d; no published mesh dimensions were available for *S. maxima*). Our in situ measurements suggest that most of the smaller (submicrometer) and highly abundant prokaryotes (i.e., picocyanobacteria and nonphotosynthetic bacteria) were retained at much lower efficiencies than the model predictions (Figs. 2, 3b–d, excluding PLP in 2015). Surprisingly, larger nanoeukaryotic algae (~ 6–8 μm) were also retained at reduced efficiency in comparison to the picoeukaryotic algae (Fig. 3). As no postcapture selection mechanisms are known to exist in salps, our findings suggest that factors other than cell size control the probability of capture of planktonic microbes by salps.

The proxy we used for cell size was based on the median FSC of each cytometric population normalized to the median FSC of 1 μm beads that were run with each sample. Scatter-based size estimates are limited by the wavelength used (488 nm in our case) and the different refraction index of different cells types and size calibration beads (e.g., Green et al. 2003). Therefore, while the bead-normalized FSC proxy is likely a good representation of reality for particles > 0.6–1 μm, the absolute size estimates of smaller particles are unrealistic but suggest very small cells. Nevertheless, Mie Theory and empirical data suggest that FSC provides at the very least a reliable ranking of cell population sizes (e.g., Robertson and Button 1989; Cunningham and Buonnacorsi 1992; Green et al. 2003). This assertion was also corroborated in our small validation experiments, where we compared median cell size estimates of ultraplanktonic populations in six samples analyzed with an epifluorescence microscope to those from the same samples analyzed with the flow cytometer. This comparison revealed, as expected, a strong positive but nonlinear correlation of the FSC and microscopical-derived size estimates (Spearman $r = 0.74$), and extremely small (< 0.2 μm) PLP were also observed in the microscope (Supporting Information Fig. S4). Identification of aggregates and/or cells attached to particles as single cells could also disrupt and bias our data. Fortunately, the use of extremely small sampling tube diameter (270 μm) served as a prefilter the prevented the sampling of most aggregates. Moreover, particle attached cells will have very low fluorescence to scatter ratio and hence will fall in the right lower quadrant of our fluorescence vs. scatter cytograms (see Supporting Information Fig. S3), classified as detritus, and gated out of the analysis.

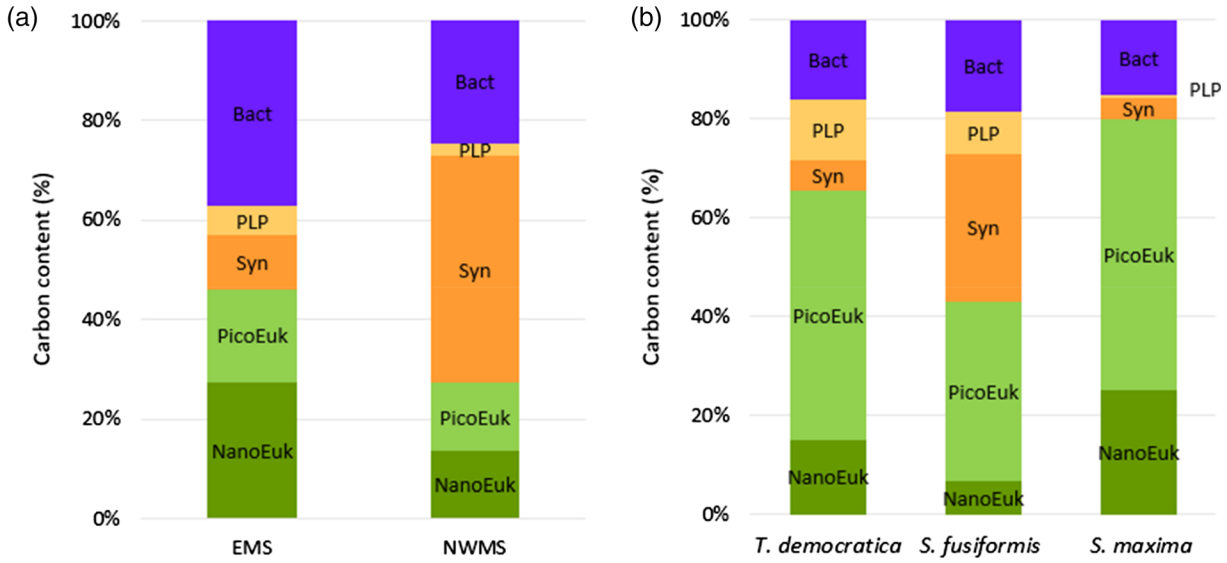

Fig. 4. Relative contribution (%) of the dominant microbial populations to: (**a**) the planktonic carbon in the ambient water during sampling, i.e., available food, and (**b**) to the salps' diet based on in situ clearance rates measurements for *T. democratica* (n = 15) sampled at EMS, and *S. fusiformis* (n = 12) sampled at NWMS and on retention efficiency for *S. maxima* (n = 13) sampled at NWMS. Calculations are based on measured concentrations of each prey population and estimated carbon content per cell for each population from Houlbrèque et al. (2006) and Buitenhuis et al. (2012), as detailed in Table 2.

A recent publication demonstrated that the filtration of planktonic organisms by pelagic (appendicularians) and benthic tunicates is not size specific but is rather species (phylotype) specific and suggested that other properties such as cell shape and surface properties are more important in determining the probability of capture of planktonic organisms (Dadon-Pilosof et al. 2017). Monger et al. (1999) suggested that cell-surface hydrophobicity affects grazing rates of zooplankton and more recently it was shown that most members of the SAR11 clade, the most abundant clade in the ocean, had a significantly lower hydrophobicity index than other abundant bacterial groups (Dadon-Pilosof et al. 2017). These low hydrophobicity strains commonly avoided filtration by both benthic and pelagic tunicates (Dadon-Pilosof et al. 2017). Study of filtration by the appendicularian *Oikopleura dioica* using microvideography and microparticles, with identical surface chemistry but different shapes, demonstrated that the minimum diameter of ellipsoidal particles, rather than their length or equivalent sphere diameter, determined the capture efficiency of the particles (Conley and Sutherland 2017). The differential "preference" of salps' filtration as measured in this study suggests similar mechanisms—surface properties and/or shape— are likely to be involved here as well.

While the combination of hydrosol theory and "slippery" cell surfaces can explain the low removal of particles smaller than the mesh pores, the low retention of larger eukaryotic cells is more enigmatic. Variability in nanoeukaryotic cell shape may be responsible for their lower retention efficiency (Conley and Sutherland 2017; Conley et al. 2018) if slender cells with a minimum diameter smaller than the mesh pores were prevalent in the population. A better understanding these removal patterns should be gained from a detailed phylotype specific comparison (e.g., using 18s rDNA) of the cells retained by the salps and those that evade filtration.

Clearance rates measured by the incubation method in this study (Fig. 2) were higher than published values for these species (e.g., Madin and Deibel 1998). Previous workers (Madin 1974; Harbison and Gilmer 1976; Licandro et al. 2006; Sutherland et al. 2010) collected salps either by plankton nets or by SCUBA diving and transferred them to incubation containers onboard or in the laboratory. In some cases, salp incubations relied on artificial prey. In this study, we tried to minimize interference using in situ incubations with natural prey populations and minimal contact with the studied salp by fitting the incubators' size to the sampled organism. Nevertheless, clearance rates measured here are lower than the maximum pumping rate estimates for several similarly sized salp species using nonintrusive kinematic methods (Sutherland and Madin 2010). Considering the high retention efficiencies, we measured by the direct InEx technique (for *S. maxima*; Fig. 1), and assuming these reflect typical retention efficiencies for salps, it is suggested that clearance rates should have been similarly high. This discrepancy suggests that even careful in situ incubations may underestimate the actual clearance rates of these organisms. Future studies should therefore combine direct in situ measurements with kinematic analysis to accurately measure salp feeding rates.

Salps are adapted to oligotrophic water where they are found in low numbers throughout the year (Licandro et al. 2006). Due to logistical constraints, most salp feeding studies are conducted during dense salp blooms when their high filtration rates are reported to consume 35–70% of primary production

(Andersen 1998; Huskin et al. 2003; Madin et al. 2006). Therefore, salps in the center of dense swarms (where they are frequently sampled) may have high refiltration rates and the prey concentration and composition available to these salps is likely to be very different from that available to salps living outside the swarms or at the swarm edges. Nevertheless, salp blooms produce a significant ecological effect; hence, studying salps during this conspicuous phase is fully justified. In fact, due to the constraints of in situ work, which depended on encountering patchily distributed organisms, 3 yr of sampling were required to collect sufficient numbers of direct InEx samples. Still, an effort to sample in the early stages of a bloom should be part of future sampling as well as monitoring for potential shifts in prey composition before and during a bloom.

In the Mediterranean, summer-adapted species like *T. democratica* may consume cyanobacteria with some efficiency (Fig. 2b,c), whereas spring-adapted species like *S. fusiformis* filter mostly larger eukaryotes (Fig. 2a). Those differences may explain their seasonal succession or even biogeographic distribution within salp species (Kremer and Madin 1992) and among other planktonic groups such as copepods (Acuña 2001). Despite the fact that *T. democratica* has been characterized as a coastal species (Deibel 1985), able to survive in more eutrophic conditions than other salp species, in our sampling expeditions (2014–2016), we observed and collected small *T. democratica* in aggregates only in the oligotrophic EMS. This observation hints at niche differentiation between salp species, potentially based on prey type availability, but different patterns may occur in other areas. Replication of this study in other oceanic regions and seasons, as well sampling under bloom and nonbloom conditions should provide more information regarding this issue.

Salps in our study fed mostly on extremely small particles and the ratio of the salps' size to their optimal prey size in the Mediterranean Sea was considerably smaller than published values and is far beyond the predicted values based on a meta-analysis of many planktonic suspension feeders and their prey (Fig. 5; Boyce et al. 2015). This surprisingly high ratio cannot be explained by prey availability, as we sampled in two different environments and three years.

Further investigation is required to understand the mesh structure, particle retention mechanisms, differential removal mechanism, and which prey and predator factors are involved. It has been suggested that smaller salps can retain a greater fraction of small particles than larger ones (Harbison and McAlister 1979; Kremer and Madin 1992); our results do not support this assertion. Despite the large size difference (ranging from an average of 1.5 cm for *T. democratica* to 13.5 cm for *S. maxima*; Table 1), the three salps species studied had similar prey preferences (Figs. 3, 5) with no correlation between salp body length and prey size (Figs. 1–3). Surprisingly, the removal pattern of the large *S. maxima* was more similar to that of the small *T. democratica* than to the intermediate-sized *S. fusiformis,* suggesting a role of niche partitioning even among similarly sized salps.

Our findings shed new light on prey: predator relationships in planktonic systems and suggest that in situ techniques

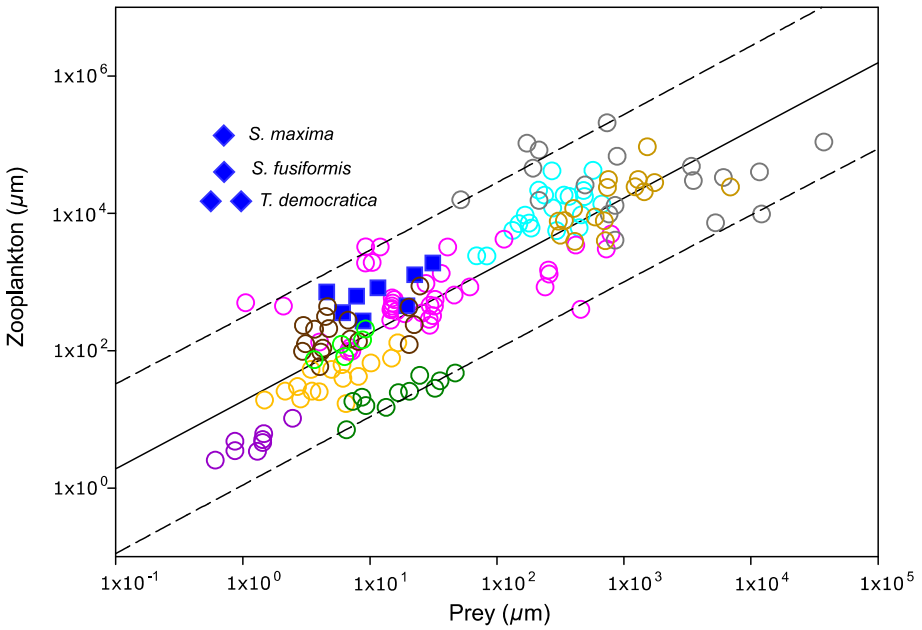

Fig. 5. Size relationships of marine plankton prey and their consumers. Blue diamonds represent measurements of salps feeding on their main food source (picoeukaryotic algae, ~ 1 μm) derived from in situ experiments (this study). All other data were adapted (with permission) from Boyce et al. (2015). Solid line; linear regression fit. Dashed lines, 95% prediction interval (for Boyce data); Blue squares represent measurements of doliolids and salps feeding as in Boyce et al. (2015). Other planktonic predators are represented by circles: Chaetognatha, cyan; Ciliate, orange; Copepod, pink; Ctenophore and Scyphomedusa, grey; Dinoflagellate, dark green; Flagellate, purple; various invertebrate, dark brown; Rotifer, light green; Siphonophore, light brown.

should be devised and applied for the study of suspension feeding in the ocean. Furthermore, factors other than size influence filtration efficiency and these should be further investigated.

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Conflict of Interest

None declared.

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