LIMNOLOGY and OCEANOGRAPHY

ASLO

Limnol. Oceanogr. 9999, 2020, 1–12

doi: 10.1002/lno.11658

Evasive plankton: Size-independent particle capture by ascidians

Yuval Jacobi,[1,2*] **Noa Shenkar**,[3,4] **J. Evan Ward**,[5] **Maria Rosa**,[6] **Guy Z. Ramon**,[1] **Uri Shavit**,[1] **Gitai Yahel**[2]

[1]Department of Civil and Environmental Engineering, Technion – Israel Institute of Technology, Haifa, Israel
[2]The Faculty of Marine Sciences, Ruppin Academic Center, Michmoret, Israel
[3]School of Zoology, George S. Wise Faculty of Life Sciences, Tel-Aviv University, Tel-Aviv, Israel
[4]Steinhardt Museum of Natural History, Israel National Center for Biodiversity Studies, Tel-Aviv University, Tel-Aviv, Israel
[5]Department of Marine Sciences, University of Connecticut, Groton, Connecticut
[6]Department of Biology, Connecticut College, New London, Connecticut

Abstract

Direct measurements of the capture efficiency of planktonic cells by seven solitary ascidians were made in situ and in the laboratory and compared with the capture efficiency of polystyrene microspheres. The capture efficiency of the microspheres was significantly higher than that of planktonic cells over the entire tested size range (0.3–15 μm). Submicron polystyrene spheres with a surface modification consisting of an adsorbed layer of a nonionic, long-chain surfactant were removed at lower efficiencies than uncoated particles whereas for larger microspheres (1–3 μm), the coating had no effect. Our findings strengthen the concept that some planktonic cells evade capture by mucus-based suspension feeders, and that evasion happens throughout the pico- and nanoplankton size range. Thus, the common assumption that particles larger than $\sim$ 1 μm are always captured at a 100% efficiency by ascidians should be reconsidered. Some large microalgae cells (> 3–12 μm) were captured at a lower efficiency than the largest microspheres used (3 μm) suggesting that other factors, such as surface interactions and particle shape, play an important role in capture throughout the tested size range. Furthermore, given the lack of a known active selection mechanism in ascidians, we propose that some plankton possess traits that allow them to evade predation by mucus-based suspension feeders.

Small, single-celled organisms constitute a major part of the marine biomass, far exceeding that of all multicellular organisms (Pomeroy et al. 2007). Many animals feed on these suspended food particles. This trophic strategy is called suspension feeding and it is unique to aquatic organisms (Gili and Coma 1998). Suspension feeders process water that contains a relatively dilute suspension of food particles (concentration in the order of ppm to ppb). The energetic demands of suspension feeders are met by efficient mechanisms that extract the available food resources from large volumes of water (Jørgensen 1975). Suspension feeding is widespread throughout the animal kingdom, and almost every animal phyla in the marine environment has some members that are suspension feeders (Riisgård and Larsen 2010), from microscopic zooplankton to macroscopic whales.

The particle capture systems of many suspension feeders is assumed to be mechanical by nature: It is dependent on particle size and the mechanical properties of the capture apparatuses(i.e., its dimensions, geometry, and the water flow through it, see, e.g., Hansen 1991; Silverman et al. 1996; Petersen 2007; Sumerel and Finelli 2014). Consequently, important quantities that describe biological filtration such as particle capture efficiency are often described only by size-dependent mechanisms that lead to particle encounter with the filtering elements (Rubenstein and Koehl 1977). While this approach has led to a better understanding of suspension feeding and its dependence on a range of factors, it does not account for other potentially important mechanisms such as surface interactions.

Ascidians (phylum: Chordata, subphylum: Tunicata, class: Ascidiacea) are a group of suspension feeders that colonize hard substrates in all marine habitats (Shenkar and Swalla 2011). These animals draw water through a mucous filter, which retains suspended food particles. The mucous filter is constantly replaced; being continuously secreted, used for retaining particles, and propelled into the digestive tract by ciliary action. The mucous filter is thought to be made of one layer of cylindrical fibers, 10–40 nm in diameter, that are organized into a mesh-like structure with rectangular pores of $\sim$ 0.5 μm width and $\sim$ 2.2 μm length (Flood and Fiala-Medioni 1981; Pennachetti 1984; Turon 1990).

The ascidian filter was described in the past as a simple sieve (Petersen 2007); however, evidence from recent years

*Correspondence: yuvaljacobi@technion.ac.il

Additional Supporting Information may be found in the online version of this article.

suggest that some bacterial clades are able to escape from being captured by ascidians as a result of their cell-surface properties (Dadon-Pilosof et al. 2017). This finding, as well as other data that have accumulated over the years, points to the importance of surface interactions in particle capture by suspension feeders. These results include a positive correlation between cell surface hydrophobicity and capture for ascidians and nanoflagellates (Monger et al. 1999; Dadon-Pilosof et al. 2017, 2019), a negative hydrophobicity-capture correlation for adult bivalves (Rosa et al. 2017), and surface-charge related enhanced capture in larval bivalves (Solow and Gallager 1990; Cole and Galloway 2015).

In this study, we measured, in situ, and under controlled laboratory conditions, the capture efficiencies of naturally occurring bacteria and microalgae by ascidians and compared them to the capture efficiencies of polystyrene artificial particles of known sizes. We also tested the influence of surface-attached polymers on capture efficiency by adsorbing a surfactant onto the surface of the artificial particles.

Methods

Study sites

In situ experiments were conducted at three different environments: the Eastern Mediterranean (EMT), the Northern Red Sea, and the Northwest Atlantic (Long Island Sound [LIS]). The Eastern Mediterranean study site was a ~ 10 m deep rocky ridge roughly 800 m west of Michmoret marina, Israel (32°24′8″N, 34°51′29″E). Sampling at the EMT was done during September and October 2014, and August and November 2015. The ascidians that were tested at the EMT were *Microcosmus exasperatus* (in laboratory and in situ experiments), *Phallusia nigra* (in situ experiments), and *Styela plicata* (laboratory experiments). Experiments at the Northern Red Sea (RS) were conducted at ~ 6 m depth on the coral reef next to the Interuniversity Institute for Marine Sciences, Israel (29°30′6″N, 34°55′04″E). The ascidians species that were tested at the RS were *Polycarpa mytiligera* (in laboratory and in situ experiments), and *Halocynthia spinosa* (in situ experiments). Work at the LIS study site was done during June 2016 at ~ 1 m depth on the submerged part of the floating docks of the Avery Point Campus of the University of Connecticut, U.S.A. (41°18′59″N, 72°3′38″W). The species studied at the LIS were *Ciona intestinalis* (laboratory and in situ experiments), and *Styela clava* (laboratory experiments).

Sampling scheme

To compare the efficiency by which ascidians capture artificial microsphere and similar sized planktonic cells, we collected paired samples of water before and after passage through the filtering system with and without the addition of microspheres to the inhaled water (see below). Inhaled and exhaled water were directly and simultaneously collected as described by Jacobi et al. (2017 and see fig. 1 and SI videos therein). This nonintrusive approach has previously been used to study feeding and metabolism of active suspension feeders in situ (Dadon-Pilosof et al. 2017; Jacobi et al. 2017; Morganti et al. 2017). Briefly, two small sampling tubes (PEEK, outer diameter 1.6 mm, inner diameter 0.23 mm, cat no. 1531B, IDEX) were gently placed a few millimeters into the siphons of individual ascidia. The distal end of each tube was fitted with a hypodermic needle that was used to pierce the septum of evacuated collecting vessels (Greiner Bio-One International, cat no. 455007). The pressure difference between the collection vessel and ambient water generated flow into the collection vessel. The rate of the sampling flow was adjusted to ~ 1 mL min^{-1} by changing the length of the sampling tubing as needed. To minimize disturbance of the animals during sampling, the collection tubes were kept at a minimum distance of 50 cm from the sampled animal. Manipulation of sampling tubes at this distance eliminated the stress that may be caused by diver activity. Before and after each collection of the paired inhaled and exhaled water samples, the proper position of the exhalant water collection tube was verified by injecting small amount of local seawater dyed with fluorescein into the inhalant siphon. Since the animals were continuously pumping, the dye left the ascidians after a few seconds through the exhalant siphon making the exhalant jet visible and allowing us to verify that the system sampled exhalent water rather than ambient water. Additionally, the relatively slow sampling flow rate (~ 1 mL min^{-1}) was at least one order of magnitude lower than the pumping rate of the ascidians (Fiala-Medioni 1978; Riisgård 1988; Petersen et al. 1999; Petersen and Svane 2002). This low sampling rate also reduced the potential risk of interference with the natural flow generated by the ascidians.

In situ measurements of microsphere capture by ascidians followed protocols in Jacobi et al. (2017). Briefly, after collecting a paired (inhaled and exhaled) water sample of untreated ambient water to measure the capture efficiency of planktonic cells, polystyrene microspheres (Fluoresbrite® YG Carboxylate Microspheres, Polysciences) of different sizes (3, 1, 0.5, and 0.3 μm in diameter) suspended in local seawater were delivered to the ascidians. This suspension was distributed using a loop of perforated tube that was placed around the inhalant siphon of each individual animal (SI video 2 in Jacobi et al. 2017). The microsphere suspension was injected through the perforated tube at a slow steady rate (~ 5 mL min^{-1}), while inhaled and exhaled water were collected. Water collection and microsphere distribution were stopped at the same time after approximately 4 min.

Animals for laboratory experiments were collected from the above-mentioned study sites, placed in individual 1-liter borosilicate beakers, and secured in place within a small pile of beach pebbles. Ascidians from LIS were held in the Rankin laboratory at the Avery Point Campus of the University of Connecticut. Ascidians from RS were held at the facilities of the Inter-University Institute for Marine Sciences. Ascidians from

EMT were kept at the aquaculture center of the School of Marine Science at Michmoret. All three facilities are equipped with a constant supply of flowing seawater. The flow of seawater through beakers containing individual ascidians was set to a rate of 20 L h^{-1}. The seawater at the facility near the EMT study site were sand-filtered as the water supply there tends to carry large amounts of sediments, which cause the ascidians to contract often, making sampling difficult. These EMT ascidians were therefore fed with fresh algae (*Nannochloropsis* sp., $\sim 10^6$ algal cells per beaker per day). The seawater supply at the LIS and RS facilities is unfiltered, and the ascidians there were kept with no additional food.

Laboratory experiments were conducted using the same protocols as those used for the in situ experiments. To sample the inhaled and exhaled water, a Polytetrafluoroethylene tube (75 cm long, ID 400 μm, OD 800 μm) was positioned a few millimeters into the siphons of each ascidian. The distal ends of the sampling tubes were placed outside the water table and below water level, and the resulting gravitational flow was adjusted to an average rate of ~ 1 mL min^{-1}. For the microsphere experiments, water supply to the beaker was temporarily stopped, a suspension of microspheres added, the water gently mixed, and then inhaled and exhaled samples taken.

To test if surface properties affect capture efficiency, polystyrene microspheres in a range of sizes (3, 1, 0.5, 0.3 μm in diameter) were coated with a tri-block copolymer (Poloxamer 188, cat. no. P5556, Sigma-Aldrich; P188) and fed to the ascidian *P. mytiligera*. P188 is a nonionic surfactant made of two hydrophilic blocks of poly(ethylene oxide) and a hydrophobic core made of poly(propylene oxide). Coating was done by adding 0.01% of P188 to a suspension of microspheres in filtered seawater and incubating for at least 1 h at room temperature.

Analytical methods

Flow cytometry

Particle concentration and composition of inhaled and exhaled water samples was measured by means of flow cytometry. The instrument used for analyses (Attune® Acoustic Focusing flow cytometer, Applied Biosystems) is equipped with acoustic focusing and a syringe-based fluidic system that ensure high precision measurement of particle concentrations (± 5%). The most significant advantage of performing measurements with flow cytometer rather than other types of particle counters (e.g., Coulter counter or LISST) is the ability to categorize particles into different types based on their fluorescence and light scatter properties. Particle types were identified and quantified using the protocol presented by Dadon-Pilosof et al. (2017) and Jacobi et al. (2017).

Briefly, samples for planktonic cells were incubated for 15 min at room temperature with Glutaraldehyde (at a final concentration of 0.1%) and were either kept at 4°C and analyzed within 2 d, or frozen in liquid nitrogen and stored at −80°C until analyzed (within 20 d). The planktonic cells (0.3–15 μm) in each sample were categorized into high- and low-scatter eukaryotic algae (NanoEuk and PicoEuk, respectively), *Synechococcus* sp. (Syn), *Prochlorococcus* sp. (Pro), and nonphotosynthetic microbes (Bact). In order to count nonphotosynthetic microbes, a subsample was stained with the nucleic acid stain SYBR Green I (20–120 min dark incubation at room temperature, 1 : 10^4 of SYBR Green commercial stock). Discrimination between the different autotrophic groups was done by plotting the forward scatter of the cells (FSC, a proxy of cell size) against the orange fluorescence of phycoerythrin, and the red fluorescence of chlorophyll. In some cases, the *Prochlorococcus* group could not be separated from the noise signal and was not counted. Each run on the flow cytometer was done with the addition of reference beads (Polysciences™, cat no. 23517, Flow Check High Intensity Green Alignment 1 μm) as an internal standard.

To estimate the size of planktonic cells that are larger than the instrument's detection limit (~ 0.3 μm), the forward scatter signal (FSC) from the flow cytometer was calibrated using two complementary size calibration kits (Molecular probes, cat no. F13838, F13839). Calibration results were in agreement with the predictions of the Mie scattering theory (Koch et al. 1996). Since the particles used to calibrate the FSC signal to cell size probably have a larger refractive index than that of planktonic cells this calibration process may cause an overestimation of plankton size. We refer to the measured diameter of plankton as "FSC-diameter" to remind the reader of this limitation. Cell counts from the flow cytometer were converted to biomass concentration by multiplying the size estimation from the forward scatter signal and using conversion factors from Houlbrèque et al. (2006) and references therein.

The samples from experiments with microspheres were also preserved with Glutaraldehyde and kept at 4°C until they were analyzed (within 2 weeks). Samples were not frozen so as to prevent breaking and loss of the polystyrene particles. In order to verify that this method was an appropriate procedure for storing samples with microspheres, several control samples were kept for 90 d and repeatedly analyzed by the flow cytometer. Results showed no variability in concentration that exceeds the precision level of the flow cytometer used (± 5%).

Microspheres surface properties

Surface properties of the microspheres before and after coating with Poloxamer 188 were characterized using five methods. Zeta potential was determined using a Zetasizer Nano ZS© (Malvern Instruments, UK). Measurements were performed on 0.3-μm Fluoresbrite® YG Carboxylate Microspheres, (Polysciences) in double distilled water containing 15 g L^{-1} NaCl and a pH of 8. Surface wettability was measured using the "captive bubble" method and a drop shape analyzer (DSA25E, KRÜSS). A "cake" of 0.5 μm microspheres was created by filtering a suspension of microspheres with a 0.2 μm polycarbonate membrane filter (Whatman, no. 110606). This

filter was then placed in a cuvette filled with water with the microsphere coated side facing down. An air bubble was then allowed to deposit on the microsphere covered surface and was immediately photographed. The contact angle between the air bubble and the surface was measured by fitting an ellipsoid and a tangent line on to the images. The contact angle of the microsphere surface and water was calculated as the difference between 180° and the measured air-microsphere angle. Following these measurements, the microsphere-covered membrane was incubated for 30 min at room temperature in a bath of 0.01% Poloxamer 188, after which contact angle measurements were repeated. Contact angle was measured on three different "bead cakes." On each of these surfaces, we have laid multiple independent air bubbles and for each bubble the contact angle was measured independently. These data points were lumped together and presented as box plots in Fig. 2a. For the statistical analysis, we conservatively used the number of "cakes" as sample size ($n = 3$).

To examine the effect of coating particles with Poloxamer 188 on their surface morphology, we imaged both coated and uncoated particles with an environmental scanning electron microscope (Quanta 200, FEG, FEI) at the Center for Nanoscience, Tel-Aviv University.

Finally, to measure the surface hydrophobicity of the microspheres, a chromatographic method, previously described by Monger et al. (1999), was used following the procedures of Dadon-Pilosof et al. (2017). Triplicates of microspheres suspended in filtered seawater were passed through pairs of hydrophobic (Sep-pak C18, Waters, cat no. WAT036810) and hydrophilic (Waters, WAT020530) solid phase columns. Using the concentration of the microspheres after passage through either the hydrophobic or hydrophilic columns, the Hydrophobic Interaction Chromatography (HIC) index was calculated for each particle type (Poloxamer 188 coated or uncoated particles) as: $\mathrm{HIC} = (C_{\mathrm{hydrophilic}} - C_{\mathrm{hydrophobic}})/C_{\mathrm{hydrophilic}}$, where $C_{\mathrm{hydrophilic}}$ and $C_{\mathrm{hydrophobic}}$ are the microsphere concentrations after passage through the hydrophilic and hydrophobic columns, respectively. A HIC index value close to 1 indicates relatively hydrophobic particles and low HIC values indicate a hydrophilic particle surface.

Data analysis

Data analysis was carried out with "RStudio" (Version 1.2.5033, © 2009–2019 RStudio). To test whether the capture efficiency of large eukaryotes is lower than that of similar sized microspheres, we did a multiple linear regression of the logit transformed capture efficiencies over the particle diameter and type as explanatory variables. Particles were categorized into two types: microspheres (3 and 10 μm in diameter) and microalgae. We permutated the capture efficiency data 1000 times and compared the observed "type" coefficient from the multiple linear regression to the random coefficients that were calculated for each permutation. In order to avoid the problems of logit transforming capture efficiencies that are equal to 1, we set these data to be 0.99. We verified that the data meets the Gauss–Markov assumptions prior to any linear regression analysis (for details, see Boldina and Beninger 2016). For power analysis, we used the G*Power software (version 3.1.9.2, written by Franz Faul, University of Kiel, Germany). For ANOVA statistical tests, the assumptions of homogeneity of variances, normality (using the Anderson-Darling test) and (for repeated measures) sphericity were checked (with Mauchly's test of sphericity) and where violated, data were transformed (percent data were usually logit transformed) or an alternative permutation test was used. Data are reported as average ± 95% confidence interval of the mean unless stated otherwise.

Encounter model

The problem of predicting the probability of encounter between particles smaller than the openings of a filter and the fibers of a rectangular mesh was previously addressed by Silvester (1983). This model considers three mechanisms that lead to encounter: direct interception, diffusional deposition, and inertial impaction. For the capture of polystyrene particles by the ascidian filter in the size range, we studied here it is possible to neglect inertial impaction (Sutherland et al. 2010; Jacobi et al. 2017). The results from the model were compared to measured data about the efficiency of particle capture by the ascidian *P. mytiligera*. Since the exact dimensions of the mesh of this species are unknown, we used the capture efficiency data of untreated 0.5 μm polystyrene microspheres (Jacobi et al. 2017) to derive the mesh size of its mucous filter. For additional details, see the Supporting Information.

Results

Picoplanktonic cells (0.3–2 μm) dominated the water at the study sites in the EMT and the Red Sea (RS) where they accounted for > 97% of the total cell number and most of the planktonic biomass (Jacobi et al. 2017). Submicron, nonphotosynthetic microbes (Bact) accounted for > 50% of the pico- and nanoplanktonic biomass in these oligotrophic sites (for more details regarding the planktonic populations at the RS and EMT study sites, see Jacobi et al. 2017). In contrast, at the Northwest Atlantic (LIS) study site, >94% of the planktonic biomass was attributed to nanoeukaryotic microalgae. These cells were relatively large and abundant with a median FSC-diameter of 11.8 ± 6.24 μm (median ± interquartile range) and a concentration $4.7 \times 10^3 \pm 1.8 \times 10^3$ cells mL^{-1}. The concentration of nonphotosynthetic microbes (Bact) at the Northwest Atlantic ($5.64 \times 10^5 \pm 1.28 \times 10^5$ cells mL^{-1}) resembled those of the oligotrophic sites ($3.61 \times 10^5 \pm 0.66 \times 10^5$ and $6.79 \times 10^5 \pm 1.01 \times 10^5$ cells mL^{-1} for the RS and EMT, respectively), but the cells were roughly three times larger than those of the oligotrophic study sites, with a median FSC-diameter of 1.04 ± 0.25 μm (median ± interquartile range). While these picoplanktonic cells accounted for more than 95% of the total cell numbers in the

LIS water, their contribution to the planktonic biomass was small. Cyanobacterial particles were rare at the Northwest Atlantic site and accounted for less than 1% of the planktonic biomass. *Synechococcus* sp. particles (with a median FSC-diameter of 1.05 μm) were found at low concentrations ($6.2 \times 10^3 \pm 5.5 \times 10^3$ cells mL^{-1}) and *Prochlorococcus* sp. particles could not be detected at the LIS study site.

Overall, the results indicated that planktonic cells were captured less efficiently than similar-sized microspheres by all ascidian species studied. This was most evident with the capture of submicron heterotrophic bacteria (Fig. 1) but, surprisingly, the capture efficiency of the larger eukaryotic microalgae was also different than that of similar-sized microspheres ($p < 0.001$), which were captured, by most species, at between 75% and 100% efficiency (see also Jacobi et al. 2017). Exceptions to these trends were some of the capture efficiencies measured in laboratory experiments, specifically for the ascidians *M. exasperatus* and *P. mytiligera* that capture the planktonic cells at high efficiency.

The species tested in situ (*C. intestinalis*, *H. spinosa*, *M. exasperatus*, *P. mytiligera*, and *P. nigra*) captured nanoeukaryotic algae (NanoEuk) with an average efficiency of 68% ± 8%, *Synechococcus* sp. at 64% ± 8%, picoeukaryotes (PicoEuk) at 70% ± 9% and *Prochlorococcus* with 60% ± 11% (PicoEuk and Pro data are only from experiments in the EMT and RS), and heterotrophic bacteria at 13% ± 1%. See Supporting Information Table S1 for mean capture efficiencies for each species studied in situ.

The difference between the inhaled and exhaled concentrations of nano- and picoeukaryotic algae exhibits a monotonic dependence on inhaled concentration throughout the measured concentration range (Supporting Information Fig. S1). This means that the capture efficiency of these algae by ascidians is roughly constant and is independent of the inhaled concentration over the range of ambient concentrations encountered during this study. In contrast, nonphotosynthetic bacteria are captured by ascidians from the RS and EMT with low efficiency regardless of inhaled concentration. For the LIS ascidian *C. intestinalis*, the number of nonphotosynthetic bacteria captured is weakly dependent on inhaled concentration.

Two methods were used to assess the hydrophobicity of the microspheres: contact angle measurements and chromatography (HIC index). Both methods revealed a similar trend. As expected, the polystyrene microspheres were hydrophobic; however, surprisingly, coating them with P188 led to a slight increase in hydrophobicity. The contact angle for 0.5 μm uncoated spheres

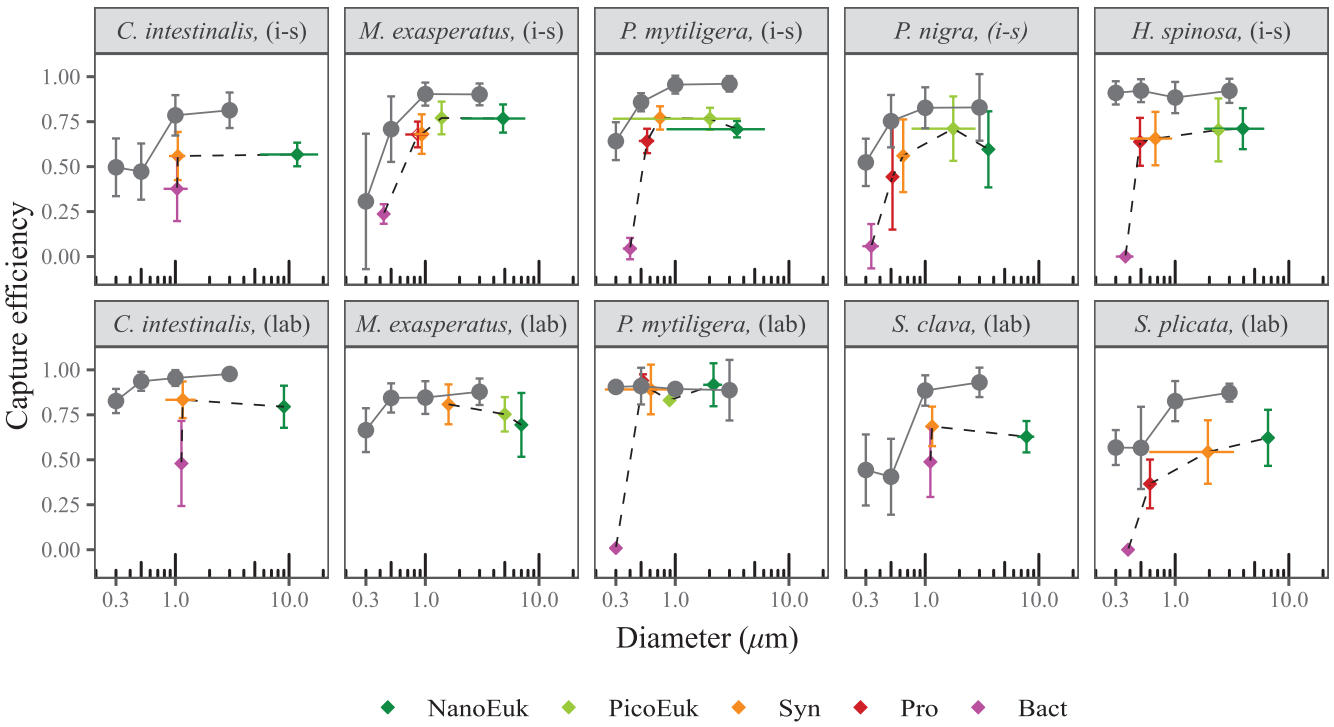

Fig 1. A comparison of the capture efficiency of planktonic cells (diamonds) and uncoated microspheres (gray circles) by different ascidian species as measured in situ (i-s, upper panels) and at the laboratory (lab, lower panels) plotted vs. particle diameter. Planktonic cells are categorized into five groups: Bact—nonphotosynthetic microbes (pink), Pro—*Prochlorococcus* sp. (red), Syn—*Synechococcus* sp. (orange), PicoEuk—low-scatter eukaryotic microalgae (light green), and NanoEuk—high-scatter eukaryotic microalgae (dark green). Vertical error bars are 95% CI. Plankton diameters are the median diameters that were calculated based on the forward scatter signal as measured using a flow cytometer (FSC-diameter). The horizontal error bars are the interquartile range of the sizes of each cell population (in some cases, the bars are smaller than the symbol width). See Supporting Information Table S3 for sample size in each experiment.

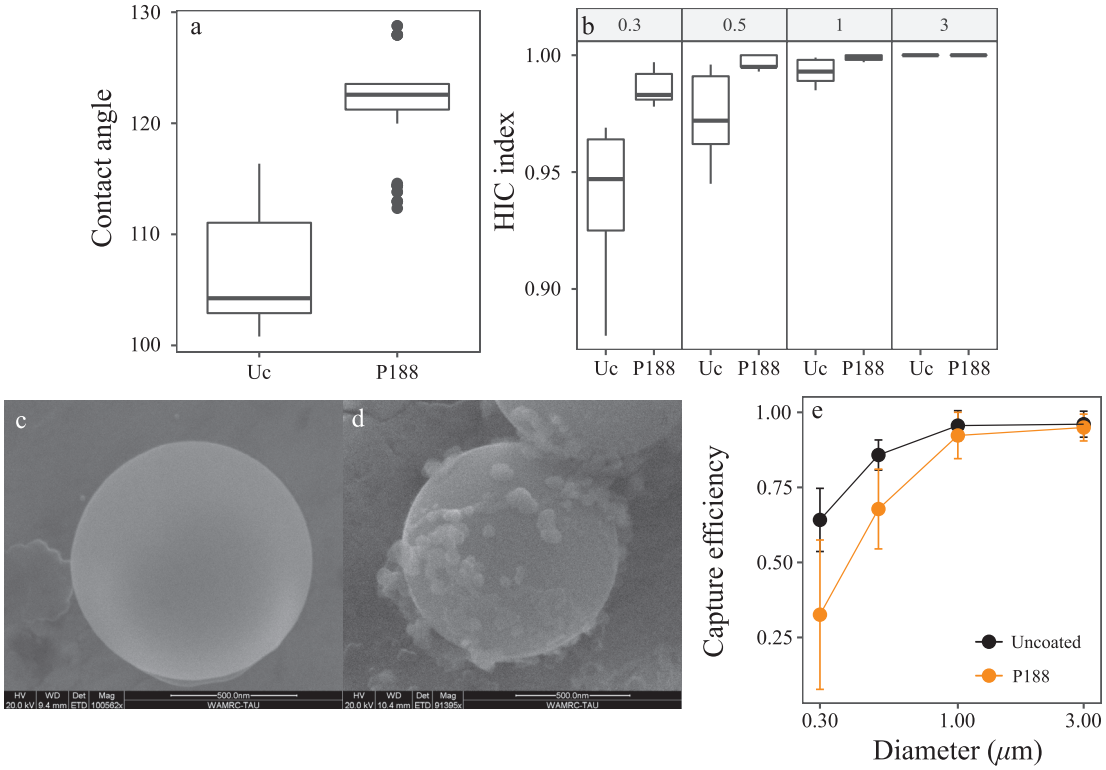

Fig 2. Effects of coating microspheres with Poloxamer 188. Uc—uncoated microspheres, P188—the same microspheres coated with Poloxamer 188. (**a**) Box plots presenting the contact angle of 0.5 μm spheres. Coating increased the contact angle, indicating an increase in hydrophobicity (n = 32 and 25 for Uc and P188, respectively). (**b**) The HIC index of experimental microspheres. Coating increased the HIC index of small particles (0.3 and 0.5 μm), indicating an increase in hydrophobicity. The HIC index of larger particles (1 and 3 μm) is not affected by coating (n = 5). For the boxplots of panels (**a**) and (**b**), the median is presented as horizontal line, the box encompass the second and third quartiles, and whiskers extend to the nearest point that is < 1.5 times the interquartile range. Dots represent outliers. (**c**, **d**) Scanning electron microscopy images of an uncoated and coated 1 μm sphere, respectively. Coating resulted in visible structures on the sphere's surface. (**e**) Capture efficiency of uncoated (black circles) and Poloxamer 188 coated (orange circles) microspheres by the ascidian *P. mytiligera* (error bars are 95% CI, n = 9).

was 107° ± 2° and for coated spheres it was 122° ± 1° (two sample t-test $p < 0.001$, Fig. 2a). The HIC index of uncoated 0.3 and 0.5 μm spheres was 0.94 ± 0.03 and 0.97 ± 0.02, respectively (Fig. 2b). These indices were significantly different for P188-coated spheres (two-way ANOVA with permutations $p < 0.001$). The HIC index of P188-coated spheres was measured as 0.98 ± 0.01 and 0.99 ± 0.00 for 0.3 μm and 0.5 μm spheres, respectively. Both the hydrophilic and hydrophobic columns removed > 95% of the larger (1 and 3 μm) particles; thus, coating particles larger than 0.5 μm with P188 had no measurable effect on the HIC index (Fig. 2d). Coating 0.3 μm spheres with P188 reduced the magnitude of the (negative) zeta-potential from −10 ± 1.2 to −2 ± 1 mV (mean ± SD), indicating a weaker surface charge for coated microspheres. Environmental scanning electron microscopy images indicated that coating microspheres with Poloxamer 188 produced structures that covered the microsphere surface (Fig. 2c,d).

The mean capture efficiencies of coated and uncoated submicron spheres by ascidians were statistically different (Repeated measures ANOVA, $F_{1,8}$ = 7.15, p = 0.04; post hoc pairwise t-test with Bonferroni corrections p = 0.013 and 0.034 for 0.5 μm and 0.3 μm spheres, respectively; Fig. 2e). The coated spheres were captured at a lower efficiency than uncoated ones (18% and 32% lower for 0.5 and 0.3 spheres, respectively; Fig. 2e). However, for particles larger than one-micron, coating had no effect on capture efficiency (repeated measures ANOVA, $F_{1,8}$ = 1.13, p = 0.32, Power > 0.8, assuming an intermediate effect size; Fig. 2e).

Discussion

The lower capture efficiencies of pico- and nanoplankton relative to similar-sized microspheres (Fig. 1) indicate that particle size is not the only factor that governs capture. In addition to particle size and the pore size of the filter, particle shape (Conley et al. 2018*a*) and surface properties (Labarbera 1984; Dadon-Pilosof et al. 2017; Rosa et al. 2017) are thought to control particle capture by suspension feeders. Our results support the idea that some particle attributes, other than size, influence the capture of particles by ascidians. Coating particles with Poloxamer 188 (P188) has no measurable effect on the capture efficiency of microspheres larger

than 1.0 μm (Fig. 2e). This finding is consistent with the description of the ascidian filter as a thin mesh with rectangular pores of uniform, submicron size (Flood and Fiala-Medioni 1981), at least in one dimension of the rectangular pores. This description implies that particles larger than the mesh size should always be captured at 100% efficiency by simple sieving, regardless of their surface properties. Submicron planktonic cells were also captured with a lower efficiency than submicron polystyrene particles (Fig. 1). To be captured, particles smaller than the mesh size must first encounter the fibers of the mesh and then be held by some retaining force. Thus, surface interactions may influence the capture of smaller particles by altering the efficiency of this retention. Supporting this idea is the effect of coating polystyrene particles with P188 had on the capture efficiency of submicron artificial particles. However, the lower than expected capture efficiency (< 100%) of pico- and nanoplankton cells that are most likely larger than the mesh size (Fig. 1) does not seem to fit this conceptual model.

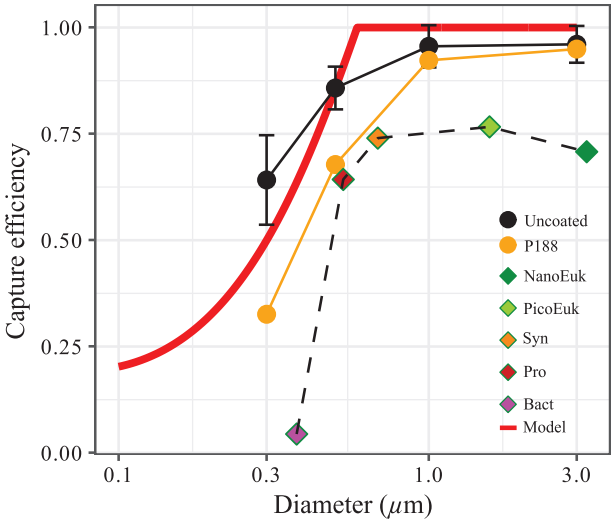

Fig 3. Observed and calculated capture efficiencies of cells and microspheres by the ascidian *P. mytiligera*. The model suggested by Silvester (1983) (red line) was fitted to the observed capture efficiency for uncoated microspheres (black circles) by adjusting the pore size of the modeled mesh. The capture of P188 coated particles (orange circles) indicates that the probability of encounter of those particles with the mesh does not represent their capture (i.e., retention efficiency < 1). Capture efficiencies of various sized plankton (green line and diamonds) also do not fit the model. NanoEuk—nanoplanktonic microalgae (dark green), PicoEuk—picoplanktonic microalgae (light green), Syn—*Synechococcus* sp. (orange), Pro—*Prochlorococcus* sp. (red), and Bact—heterotrophic bacteria (pink). Error bars (95% CI of the mean) are presented for the capture efficiencies of uncoated microspheres and are omitted for other particles for clarity (*see* Figs. 1, 2e).

The rectangular mesh-filter model

The discrepancy between the lower than expected capture efficiency (< 100%) of nanoplankton and large picoplankton relative to the dimensions of the filter is highlighted by calculating the probability of particle encounter with fibers of the mesh using the model suggested by Silvester (1983). This model is frequently used to predict different quantitative aspects of particle capture by rectangular meshes (Riisgård 1988; Loudon and Alstad 1990; Sutherland et al. 2010; Jacobi et al. 2017). As seen in Fig. 3, when tested at the field, the capture efficiency of naturally occurring pico- and nanoplankton by the ascidian *P. mytiligera* is significantly lower than the 100% efficiency predicted for a sieve with such pore size.

A possible explanation for the low capture efficiency of plankton in the 1–10 μm size range may be that, in contrast to what has previously been suggested (Flood and Fiala-Medioni 1981; Pennachetti 1984; Turon 1990), the pores in the mesh are nonuniform in size and so a fraction is large enough to allow some plankton to escape. If this is the case, however, then similar capture patterns for large (1 and 3 μm) microspheres should have been measured. In contrast, 1 and 3 μm spheres were captured by ascidians, on average, at > 95% efficiency (Fig. 1 and Jacobi et al. 2017).

The role of surface properties on particle capture

The capture of ellipsoid particles by ascidians is controlled by the size of their minimum axis, providing a mechanistic and quantitative explanation for the effect of particle shape in suspension-feeding (Conley and Sutherland 2017; Conley et al. 2018*b*). In contrast, basic questions regarding the way in which surface properties of particles affect their capture by suspension feeders are still unanswered. For example, an ambiguous "stickiness" trait is often mentioned in this context by some authors (Shimeta and Koehl 1997; Bone et al. 2003; Riisgård and Larsen 2010), yet, it is unclear whether adhesion is an important factor in particle capture. Another frequently mentioned assumption is that because of the high ionic strength of seawater, electrostatic forces are unimportant in marine suspension feeding (Rubenstein and Koehl 1977). It is true that the decay of the electric potential with distance from the surface is enhanced by the presence of ions, but when a particle is brought close to the vicinity of a filtering structure, distance-dependent forces may still alter its trajectory and potentially change the probability of capture. As demonstrated by these examples, to gain a better understanding of the role of surface interactions in suspension feeding, a formal quantitative framework and coherent definitions need to be established. The results presented in this study, involving the capture of particles coated with the surfactant P188, as well as the work of other authors in the field of suspension feeding (Cucci et al. 1985; Pales Espinosa et al. 2010, 2016; Rosa et al. 2017) are initial attempts to establish such a framework.

Attractive and repulsive forces

The deposition of small particles onto biological surfaces is usually described by the balance of attractive and repulsive forces that can be categorized into DLVO and non-DLVO

forces. The DLVO theory, described by Derjaguin and Landau and independently by Verwey and Overbeek (reviwed by Hermansson 1999), considers attractive forces that arise from Van der Waals interactions and repulsive forces from overlapping electric double layers, when the surface and the particles carry the same charge (Smart 2014). Among the non-DLVO forces are steric effects and osmotic pressure which may induce repulsion. Additionally, if the surfaces are hydrophobic, the deposition of particles may be thermodynamically favorable. Therefore, hydrophobic interaction may be considered as an additional attractive force (Smart 2005). The mucous filter of ascidians is thought to carry a negative charge (Flood and Fiala-Medioni 1981). The particles used in this study were carboxylate-modified polystyrene spheres that carried a negative surface charge as well. Since the zeta-potential measurements of the microspheres showed a decrease after coating with P188 (from −10 ± 1.2 to −2 ± 1 mV, mean ± SD) and concomitantly capture efficiency dropped, it can be concluded that the repulsion associated with the electric double layer is not an important surface interaction in particle capture by ascidians.

Surface modification of experimental microspheres

In the current study, the surfactant used to modify surface properties of polystyrene microspheres was Poloxamer 188 which is a tri-block copolymer composed of a poly(propylene glycol) core attached to two poly(ethylene glycol) chains (PPG and PEG, respectively). Poloxamers adsorb to polystyrene particles via the hydrophobic PPG core leaving the PEG chains in the aqueous phase, presumably resulting in a polymer brush (Alexandridis and Hatton 1995). This surface brush structure fits the theory of chain molecules at an interface as well (Alexander 1977) and likely formed on the coated microspheres. If so, the brush layer would have induced repulsion between the microspheres and elements in the mucous filter, resulting in increased mobility of the particles within the mucus. Such increased mobility was previously observed for particles covered with a PEG brush in human cervical mucus (Lai et al. 2007; Wang et al. 2008). Increased mobility in the mucous filter as a result of a steric repulsive effect would cause a larger proportion of coated microspheres to cross over to the downstream side of the filter resulting in a lower capture efficiency.

Steric repulsion is potentially of importance in particle capture by mucus-based suspension feeders. In other biological processes, steric repulsive effects due to surface bound polymer brush layers were shown to have an important role. Among these are reduced cell adhesion onto brush covered surfaces (Bridgett et al. 1992; Nejadnik et al. 2008), altered distribution of small particles in living mice (Schipper et al. 2009), and possibly the increased migration of cancer cells throughout the body (Iyer et al. 2009). The hydrophobicity of particles also might be important in suspension feeding, as addressed by several studies (e.g., Monger et al. 1999; Rosa et al. 2013, 2017; Dadon-Pilosof et al. 2017). Results from the contact angle and HIC index measurements of P188 coated and uncoated microspheres demonstrate that hydrophobicity alone cannot explain the efficiency at which particles are captured. Additionally, data from the current study suggest that the HIC index, as we measured it, is sensitive to particle size since the hydrophilic diol column retains large particles more efficiently. For example, 99% of uncoated 3 μm particles were retained by the diol column while similar 0.3 μm particles were retained at 88%. This means that HIC index may not be a reliable method to characterize hydrophobicity of different size particles.

The idea that interactions between the surface of food particles and biological filters may have a dramatic effect on the efficiency by which particles are captured was recognized decades ago (Jørgensen and Goldberg 1953; Labarbera 1984; Shimeta and Jumars 1991). Observations of the effect of surface properties on particle capture have been reported for *Daphnia magna*, which captures neutral particles more efficiently than negatively charged ones (Gerritsen and Porter 1982). Rotifers were shown to be able to select between phytoplankton and clay particles (Kirk 1991). Particle capture by bivalves was found to be not strictly size-dependent (Yahel et al. 2009) and to be affected by the presence of surface bound neoglycoproteins (Rosa et al. 2017). Some bacteria are able to escape from being captured by suspension feeding, most likely because of their surface properties (Dadon-Pilosof et al. 2017). However, the mechanism underlying these phenomena remains elusive. Furthermore, to date, the properties involved in this process are not well understood. Some authors were able to positively correlate measures of particle hydrophobicity to particle capture (Monger et al. 1999; Dadon-Pilosof et al. 2017); however, no such correlation was found in this study, suggesting that hydrophobic interactions are not necessarily involved in particle capture. Similarly, while theory predicts that surface charge cannot be important in marine suspension-feeding due to the high ionic content of seawater, some evidence suggests that surface charge is an important property in particle capture (Labarbara 1978; Gerritsen and Porter 1982; Rosa et al. 2017). The nature of the surface interactions that underpin the evasiveness of some planktonic cells from capture by mucous filters remains unclear. Further progress in this field will require a quantitative mechanistic description of this phenomenon.

Addressing potential pitfalls

Our results suggest that polystyrene microspheres are captured by ascidians at higher efficiency than their natural planktonic prey across the entire tested size range (0.3–15 μm). The concentrations of large cells (> 2 μm) in samples from the inhalant siphons were relatively low (mean of 1.95×10^3 cells mL^{-1}). As such, capture efficiency of these size groups could be possibly underestimated because of background noise in the flow cytometer analysis. If background noise was in fact significant, sample pairs with low inhalant

particle concentrations would be most sensitive and effected. Plotting the number of captured cells per volume of water pumped against inhaled concentration reveals that a linear relationship was maintained even at the lowest concentrations (Supporting Information Fig. S1). Therefore, the effect of background noise on calculated capture efficiencies was minimal.

Sampling coupled pairs of inhalant and exhalant water has an inherent advantage because sample pairs are subjected to identical handling procedures making the analysis more robust and final calculations less susceptible to errors. The method we used for sampling in situ, however, is sensitive to a potential artifact that may result in trends in the data, similar to the ones presented here. If the collection tube of the exhalant water is misplaced in such a way that the exhalant sample becomes "contaminated" by ambient seawater, the resulting calculated capture efficiency of plankton will be underestimated. When microspheres are delivered to the animal inhalant siphon, the same error will lead to an overestimation of microsphere capture efficiency. To avoid this potential artifact, only relatively large animals with siphons that enabled careful positioning of the exhalant collection tube a few millimeters into the exhalant siphon were examined. The correct location of the collection tube was repeatedly tested before and during each trial by injecting fluorescein dye into the inhalant siphon and verifying that the tube is positioned amidst the exhalant jet visualized by the dye. Ambient water "contamination" may also occur if the flow rate of the exhalant water sampling system is higher than that of the animal. To avoid this problem, the collection flow rate was adjusted to ~ 1 mL min^{-1}, at least one order of magnitude lower than the flow rate of ascidians (Fiala-Medioni 1978; Riisgård 1988; Petersen et al. 1999; Petersen and Svane 2002; Yahel et al. 2005). Given these precautions, the relatively low capture efficiency of plankton that was repeatedly observed is most likely a true phenomenon which can be explained by the shape of plankton, their surface properties, their mechanical traits, or a combination of those.

The large plankton groups (PicoEuk and NanoEuk) are most likely larger than the mesh size reported for ascidians ($\sim 0.5 \times 2.2$ μm; Flood and Fiala-Medioni 1981); however, their lower than expected capture efficiency disagrees with the current description of the ascidian mucous filter. The means used to estimate the sizes of plankton are prone to error, since the FSC signal from the flow cytometer was calibrated to a set of microspheres of known sizes, but with a different refractive index than that of planktonic cells. Nevertheless, as scattering theory predicts, particles presenting a larger FSC signal are larger than lower forward scattering particles (Koch et al. 1996). In addition, under the frequently used assumption that the refractive index of different pico- and nanoplankton groups is similar (Ackleson and Spinrad 1988), the sizes estimated for different planktonic groups represent true size differences relative to each other. Thus, cell sizes estimated in this study are likely to be close to the actual sizes.

Grazing evasion

Grazing by suspension feeders exert top-down control on pico- and nanoplankton populations. Grazing evasion mechanisms therefore provide an adaptive benefit for microbial plankton. While small size provides partial grazing refugia (Boyce et al. 2015), a diminished cell size carries costs (Giovannoni et al. 2005; Partensky and Garczarek 2010). Larger cells have therefore developed alternative mechanisms such as grazing deterrence (Kirchman 2008) and the thick biomineralized armor of diatoms, dinoflagellates, and coccolithophores (Reynolds 2006). Many pico- and nanoeukaryote cells are known to be motile (Visser and Kiørboe 2006). Flagella- and cilia-based mechanisms may allow eukaryotic microalgae to swim out of the feeding currents of some suspension feeders and escape capture. For a large and motile cell that encounters the ascidian mucous filter, escaping is only possible if it is able to swim through the mucous structure and cross the brachial sack to get into the excurrent jet. As we focused on the effect of surface properties, motility was not investigated as a grazing evasion mechanism in this study.

Our results suggest yet another grazing avoidance mechanism where some pico- and nanoplankton developed surface features that increase their mobility through mucus and lower their capture efficiency by mucus-based filters. The results presented here suggest that particle capture by suspension feeders is greatly influenced by physical–chemical surface interactions. We postulate that in order to gain a better understanding of suspension feeding and its role in marine microbial ecology the role of surface interactions in particle capture needs to be thoroughly explored.

References

Ackleson, S. G., and R. W. Spinrad. 1988. Size and refractive index of individual marine particulates: A flow cytometric approach. Appl. Opt. **27**: 1270–1277. doi:10.1364/ao.27.001270

Alexander, S. 1977. Adsorption of chain molecules with a polar head a scaling description. J. Phys. **38**: 983–987. doi:10.1051/jphys:01977003808098300

Alexandridis, P., and T. A. Hatton. 1995. Poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) block copolymer surfactants in aqueous solutions and at interfaces: Thermodynamics, structure, dynamics, and modeling. Colloids Surf. A Physicochem. Eng. Asp. **96**: 1–46. doi:10.1016/0927-7757(94)03028-X

Boldina, I., and P. G. Beninger. 2016. Strengthening statistical usage in marine ecology: Linear regression. J. Exp. Mar. Biol. Ecol. **474**: 81–91. doi:10.1016/j.jembe.2015.09.010

Bone, Q., C. Carré, and P. Chang. 2003. Tunicate feeding filters. J. Mar. Biol. Assoc. U. K. **83**: 907–919. doi:10.1017/S002531540300804Xh

Boyce, D. G., K. T. Frank, and W. C. Leggett. 2015. From mice to elephants: Overturning the "one size fits all" paradigm in marine plankton food chains. Ecol. Lett. **18**: 504–515. doi:10.1111/ele.12434

Bridgett, M. J., M. C. Davies, and S. P. Denyer. 1992. Control of staphylococcal adhesion to polystyrene surfaces by polymer surface modification with surfactants. Biomaterials **13**: 411–416. doi:10.1016/0142-9612(92)90159-L

Cole, M., and T. S. Galloway. 2015. Ingestion of nanoplastics and microplastics by Pacific oyster larvae. Environ. Sci. Technol. **49**: 14625–14632. doi:10.1021/acs.est.5b04099

Conley, K. R., and K. R. Sutherland. 2017. Particle shape impacts export and fate in the ocean through interactions with the globally abundant appendicularian *Oikopleura dioica*. PLoS One **12**: e0183105. doi:10.1371/journal.pone.0183105

Conley, K. R., A. Ben-Tal, Y. Jacobi, G. Yahel, and K. R. Sutherland. 2018*a*. Not-so-simple sieving by ascidians: Re-examining particle capture at the mesh and organismal scales. Mar. Biol. **165**. doi:10.1007/s00227-018-3300-8

Conley, K. R., F. Lombard, and K. R. Sutherland. 2018*b*. Mammoth grazers on the ocean's minuteness: A review of selective feeding using mucous meshes. Proc. R. Soc. B Biol. Sci. **285**: 20180056. doi:10.1098/rspb.2018.0056

Cucci, T., S. Shumway, R. Newell, R. Selvin, R. Guillard, and C. Yentsch. 1985. Flow cytometry: A new method for characterization of differential ingestion, digestion and egestion by suspension feeders. Mar. Ecol. Prog. Ser. **24**: 201–204. doi:10.3354/meps024201

Dadon-Pilosof, A., and others. 2017. Surface properties of SAR11 bacteria facilitate grazing avoidance. Nat. Microbiol. doi:10.1038/s41564-017-0030-5

Dadon-Pilosof, A., F. Lombard, A. Genin, K. R. Sutherland, and G. Yahel. 2019. Prey taxonomy rather than size determines salp diets. Limnol. Oceanogr. **64**: 1996–2010. doi:10.1002/lno.11165

Fiala-Medioni, A. 1978. Filter-feeding ethology of benthic invertebrates (Ascidians). III. Recording of water current in situ - rate and rhythm of pumping. Mar. Biol. **45**: 185–190. doi:10.1007/BF00390554

Flood, P. R., and A. Fiala-Medioni. 1981. Ultrastructure and histochemistry of the food trapping mucous film in benthic filter-feeders (ascidians). Acta Zool. **62**: 53–65. doi:10.1111/j.1463-6395.1981.tb00616.x

Gerritsen, J., and K. G. Porter. 1982. The role of surface chemistry in filter feeding by zooplankton. Science **216**: 1225–1227. doi:10.1126/science.216.4551.1225

Gili, J. M., and R. Coma. 1998. Benthic suspension feeders: Their paramount role in littoral marine food webs. Trends Ecol. Evol. **13**: 316–321. doi:10.1016/S0169-5347(98)01365-2

Giovannoni, S. J., and others. 2005. Genome streamlining in a cosmopolitan oceanic bacterium. Science **309**: 1242–1245. doi:10.1126/science.1114057

Hansen, B. 1991. Feeding behaviour in larvae of the opisthobranch *Philine aperta* - II. Food size spectra and particle selectivity in relation to larval behaviour and morphology of the velar structures. Mar. Biol. **111**: 263–270. doi:10.1007/BF01319708

Hermansson, M. 1999. The DLVO theory in microbial adhesion. Colloids Surf. B Biointerfaces **14**: 105–119. doi:10.1016/S0927-7765(99)00029-6

Houlbrèque, F., B. Delesalle, J. Blanchot, Y. Montel, and C. Ferrier-Pagès. 2006. Picoplankton removal by the coral reef community of La Prévoyante, Mayotte Island. Aquat. Microb. Ecol. **44**: 59–70. doi:10.3354/ame044059

Iyer, S., R. M. Gaikwad, V. Subba-Rao, C. D. Woodworth, and I. Sokolov. 2009. Atomic force microscopy detects differences in the surface brush of normal and cancerous cells. Nat. Nanotechnol. **4**: 389–393. doi:10.1038/nnano.2009.77

Jacobi, Y., G. Yahel, and N. Shenkar. 2017. Efficient filtration of micron and submicron particles by ascidians from oligotrophic waters. Limnol. Oceanogr. **63**: S267–S279. doi:10.1002/lno.10736

Jørgensen, C. B. 1975. Comparative physiology of suspension feeding. Annu. Rev. Physiol. **37**: 57–79. doi:10.1146/annurev.ph.37.030175.000421

Jørgensen, C. B., and E. D. Goldberg. 1953. Particle filtration in some ascidians and lamellibranchs. Biol. Bull. **105**: 477–489. doi:10.2307/1538464

Kirchman, D. L. 2008. Microbial ecology of the oceans, 2nd ed. John Wiley & Sons.

Kirk, K. L. 1991. Inorganic particles alter competition in grazing plankton: The role of selective feeding. Ecology **72**: 915–923. doi:10.2307/1940593

Koch, L. A., B. R. Robertson, and D. K. Button. 1996. Deduction of the cell volume and mass from forward scatter intensity of bacteria analyzed by flow cytometry. J. Microbiol. Methods **27**: 49–61. doi:10.1016/0167-7012(96)00928-1

Labarbara, M. 1978. Particle capture by a Pacific brittle star: Experimental test of the aerosol suspension feeding model. Science **201**: 1147–1149. doi:10.1126/science.201.4361.1147

Labarbera, M. 1984. Feeding currents and particle capture mechanisms in suspension feeding animals. Integr. Comp. Biol. **24**: 71–84. doi:10.1093/icb/24.1.71

Lai, S. K., D. E. O'Hanlon, S. Harrold, S. T. Man, Y.-Y. Wang, R. A. Cone, and J. Hanes. 2007. Rapid transport of large polymeric nanoparticles in fresh undiluted human mucus. Proc. Natl. Acad. Sci. USA **104**: 1482–1487. doi:10.1073/pnas.0608611104

Loudon, C., and D. N. Alstad. 1990. Theoretical mechanics of particle capture: Predictions for hydropsychid caddisfly distributional ecology. Am. Nat. **135**: 360–381. doi:10.1086/285051

Monger, B. C., M. R. Landry, and S. L. Brown. 1999. Feeding selection of heterotrophic marine nanoflagellates based on the surface hydrophobicity of their picoplankton prey. Limnol. Oceanogr. **44**: 1917–1927. doi:10.4319/lo.1999.44.8.1917

Morganti, T., R. Coma, G. Yahel, and M. Ribes. 2017. Trophic niche separation that facilitates co-existence of high and low microbial abundance sponges is revealed by in situ study of carbon and nitrogen fluxes. Limnol. Oceanogr. **62**: 1963–1983. doi:10.1002/lno.10546

Nejadnik, M. R., H. C. van der Mei, W. Norde, and H. J. Busscher. 2008. Bacterial adhesion and growth on a polymer brush-coating. Biomaterials **29**: 4117–4121. doi:10.1016/j.biomaterials.2008.07.014

Pales Espinosa, E., D. Hassan, J. E. Ward, S. E. Shumway, and B. Allam. 2010. Role of epicellular molecules in the selection of particles by the blue mussel, *Mytilus edulis*. Biol. Bull. **219**: 50–60. doi:10.1086/bblv219n1p50

Pales Espinosa, E., R. M. Cerrato, G. H. Wikfors, and B. Allam. 2016. Modeling food choice in the two suspension-feeding bivalves, Crassostrea virginica and *Mytilus edulis*. Mar. Biol. **163**: 1–13. doi:10.1007/s00227-016-2815-0

Partensky, F., and L. Garczarek. 2010. Prochlorococcus: Advantages and limits of minimalism. Ann. Rev. Mar. Sci. **2**: 305–331. doi:10.1146/annurev-marine-120308-081034

Pennachetti, C. A. 1984. Functional morphology of the branchial basket of *Ascidia paratropa* (Tunicata, Ascidiacea). Zoomorphology **104**: 216–222. doi:10.1007/BF00312033

Petersen, J., and I. Svane. 2002. Filtration rate in seven Scandinavian ascidians: Implications of the morphology of the gill sac. Mar. Biol. **140**: 397–402. doi:10.1007/s002270100706

Petersen, J. K. 2007. Ascidian suspension feeding. J. Exp. Mar. Biol. Ecol. **342**: 127–137. doi:10.1016/j.jembe.2006.10.023

Petersen, J. K., S. Mayer, and M. Å. Knudsen. 1999. Beat frequency of cilia in the brancial basket of the ascidian *Ciona intestinalis* in relation to temperature and algal cell concentration. Mar. Biol. **133**: 185–192. doi:10.1007/s002270050457

Pomeroy, L., P. l B. Williams, F. Azam, and J. Hobbie. 2007. The microbial loop. Oceanography **20**: 28–33. doi:10.5670/oceanog.2007.45

Reynolds, C. S. 2006. The ecology of phytoplankton. Cambridge Univ. Press.

Riisgård, H. U. 1988. The ascidian pump: Properties and energy cost. Mar. Ecol. Prog. Ser. **47**: 129–134. doi:10.3354/meps047129

Riisgård, H. U., and P. S. Larsen. 2010. Particle capture mechanisms in suspension-feeding invertebrates. Mar. Ecol. Prog. Ser. **418**: 255–293. doi:10.3354/meps08755

Rosa, M., J. E. Ward, S. E. Shumway, G. H. Wikfors, E. Pales-Espinosa, and B. Allam. 2013. Effects of particle surface properties on feeding selectivity in the eastern oyster Crassostrea virginica and the blue mussel *Mytilus edulis*. J. Exp. Mar. Biol. Ecol. **446**: 320–327. doi:10.1016/j.jembe.2013.05.011

Rosa, M., J. E. Ward, A. Frink, and S. E. Shumway. 2017. Effects of surface properties on particle capture by two species of suspension-feeding bivalve molluscs. Am. Malacol. Bull. **35**: 181–188. doi:10.4003/006.035.0212

Rubenstein, D. I., and M. a. R. Koehl. 1977. The mechanisms of filter feeding: Some theoretical considerations. Am. Nat. **111**: 981–994. doi:10.1086/283227

Schipper, M. L., and others. 2009. Particle size, surface coating, and PEGylation influence the biodistribution of quantum dots in living mice. Small **5**: 126–134. doi:10.1002/smll.200800003

Shenkar, N., and B. J. Swalla. 2011. Global diversity of Ascidiacea. PLoS One **6**: e20657. doi:10.1371/journal.pone.0020657

Shimeta, J., and P. A. Jumars. 1991. Physical-mechanisms and rates of particle capture by suspension-feeders. Oceanogr. Mar. Biol. **29**: 191–257.

Shimeta, J., and M. A. R. Koehl. 1997. Mechanisms of particle selection by tentaculate suspension feeders during encounter, retention, and handling. J. Exp. Mar. Biol. Ecol. **209**: 47–73. doi:10.1016/S0022-0981(96)02684-6

Silverman, H., J. W. Lynn, E. C. Achberger, and T. H. Dietz. 1996. Gill structure in zebra mussels: Bacterial-sized particle filtration. Integr. Comp. Biol. **36**: 373–384. doi:10.1093/icb/36.3.373

Silvester, N. R. 1983. Some hydrodynamic aspects of fitter feeding with rectangular-mesh nets. J. Theor. Biol. **103**: 265–286. doi:10.1016/0022-5193(83)90028-0

Smart, J. D. 2005. The basics and underlying mechanisms of mucoadhesion. Adv. Drug Deliv. Rev. **57**: 1556–1568. doi:10.1016/j.addr.2005.07.001

Smart, J. D. 2014. Theories of mucoadhesion, p. 159–174. *In* Mucoadhesive materials and drug delivery systems. John Wiley & Sons.

Solow, A. R., and S. M. Gallager. 1990. Analysis of capture efficiency in suspension feeding: Application of nonparametric binary regression. Mar. Biol. **107**: 341–344. doi:10.1007/BF01319834

Sumerel, A., and C. Finelli. 2014. Particle size, flow speed, and body size interactions determine feeding rates of a solitary ascidian *Styela plicata*: A flume experiment. Mar. Ecol. Prog. Ser. **495**: 193–204. doi:10.3354/meps10571

Sutherland, K. R., L. P. Madin, and R. Stocker. 2010. Filtration of submicrometer particles by pelagic tunicates. Proc. Natl. Acad. Sci. USA **107**: 15129–15134. doi:10.1073/pnas.1003599107

Turon, X. 1990. Estructura de los filtros branquiales de ascidias (tunicados). Misc. Zool. **14**: 125–133.

Visser, A. W., and T. Kiørboe. 2006. Plankton motility patterns and encounter rates. Oecologia **148**: 538–546. doi:10.1007/s00442-006-0385-4

Wang, Y. Y., S. K. Lai, J. S. Suk, A. Pace, R. Cone, and J. Hanes. 2008. Addressing the PEG mucoadhesivity paradox to engineer nanoparticles that "slip" through the human mucus barrier. Angew. Chem. Int. Ed. **47**: 9726–9729. doi:10.1002/anie.200803526

Yahel, G., D. Marie, and A. Genin. 2005. InEx—a direct in situ method to measure filtration rates, nutrition, and metabolism of active suspension feeders. Limnol. Oceanogr.: Methods **3**: 46–58. doi:10.4319/lom.2005.3.46

Yahel, G., D. Marie, P. G. Beninger, S. Eckstein, and A. Genin. 2009. In situ evidence for pre-capture qualitative selection in the tropical bivalve Lithophaga simplex. Aquat. Biol. **6**: 235–246. doi:10.3354/ab00131

Acknowledgments

We thank the staff of the faculty for Marine Science in Michmoret (Ruppin Academic Center), the Inter-University Institute in Eilat (IUI), and the Department of Marine Sciences (UConn, U.S.A.) for support and use of the scientific diving centers and running seawater facilities. This research was supported by ISF Grant 1280/13 and BSF Grant 2012089 to GY; ISF Grant 2182/15 to NS; BSF Grant 2017622 to GY, US, and JEW; NSF-IOS Grant 1147122 to JEW; and NSF-IOS Grant 1755409 to GY, US, and JEW.

Conflict of Interest

YJ, GY, and US are collaborating with Professor Thomas Kiørboe who is an associate editor for *Limnology & Oceanography*. The other authors declare no conflict of interest.

Submitted 13 May 2020
Revised 02 November 2020
Accepted 08 November 2020

Associate editor: Kelly Benoit-Bird

Supplementary information for

Evasive plankton: Size-independent particle capture by ascidians

Yuval Jacobi[1,2], Noa Shenkar[3,4], J. Evan Ward[5],
Maria Rosa[6], Guy Z. Ramon[1], Uri Shavit[1], and Gitai Yahel[1]

1. Department of Civil and Environmental Engineering, Technion – Israel Institute of Technology, Haifa 32000, Israel
2. The Faculty of Marine Sciences, Ruppin Academic Center, Michmoret 4029700, Israel
3. School of Zoology, George S. Wise Faculty of Life Sciences, Tel-Aviv University, Tel-Aviv 6997801, Israel
4. Steinhardt Museum of Natural History, Israel National Center for Biodiversity Studies, Tel-Aviv University, Tel-Aviv 6997801, Israel
5. Department of Marine Sciences, University of Connecticut, Groton, Connecticut 06340, United States
6. Department of Biology, Connecticut College, New London, Connecticut 06320, United States

Yuval Jacobi - yuvaljacobi@technion.ac.il
Noa Shenkar - shenkarn@tauex.tau.ac.il
J. Evan Ward - evan.ward@uconn.edu
Maria Rosa - mrosa@conncoll.edu
Guy Z. Ramon - ramong@technion.ac.il
Uri Shavit - aguri@cv.technion.ac.il
Gitai Yahel - yahel@Ruppin.ac.il

Probability of encounter model of a rectangular mesh

The encounter of microspheres and microbial plankton with the ascidian filter is likely to be limited to non-inertial mechanisms: direct interception and diffusional deposition (Rubenstein and Koehl 1977; Jacobi et al. 2017). The probability of an encounter between particles and the mucous fibers of the ascidian filter was calculated by adapting the model presented by Silvester (1983). This model was used several times in the past to predict particle capture by the rectangular mesh filters of several suspension feeders (Loudon and Alstad 1990; Loudon 1990; Sutherland et al. 2010; Jacobi et al. 2017).

When considering only direct interception the probability of encounter, E_R, between a rectangular mesh made of cylindrical fibers with a diameter d_f and a spherical particle with of diameter d_p is (Silvester 1983; Sutherland et al. 2010):

$$E_R = (2R\ ln(R) - R + R^{-1})\ /\Lambda \tag{1}$$

where

$$R = 1 + \frac{d_p}{d_f} \tag{1a}$$

$$\Lambda = 1 - 2\ ln(\tau) + \frac{\tau^2}{6} - \frac{\tau^4}{144} + \frac{\tau^6}{1080} \tag{1b}$$

$$\tau = (\pi d_f \sqrt{W^2 + L^2})/(WL) \tag{1c}$$

and L and W are the inner length and width of the rectangular mesh.

The efficiency of a single fiber due to Diffusional deposition, E_D, is:

$$E_D = 3.7\Lambda^{-\frac{1}{3}} Pe^{-\frac{2}{3}} + 0.62Pe^{-1} \quad (2)$$

where $Pe = d_f u/D$ is the *Peclet* number with $u = 3 \times 10^{-4}$ $(m\ s^{-1})$ being the characteristic water flux through the mesh of ascidians (Petersen, 2007; Riisgård, 1988). The diffusion coefficient $D = kT/3\pi\rho_{sw}\nu d_p$ $(m^2\ s^{-1})$ was calculated with $k = 1.38 \times 10^{-23}$ $(m^2\ kg\ K^{-1}\ s^{-2})$ being the Boltzman constant, $T = 298$ $(K)$is temperature in Kelvin, $\rho_{sw} = 1030$ $(kg\ m^{-3})$ is the density of seawater, and $\nu = 9.3 \times 10^{-7} (m^2\ s^{-1})$ the kinematic viscosity of seawater.

Considering direct interception and diffusional deposition, the total probability of encounter of particles with the rectangular mesh filter is:

$$E_{tot} = \frac{W + L}{WL}(E_R + E_D)d_f \left[1 - \frac{(E_R + E_D)d_f}{W + L}\right] \quad (3)$$

The results from the model were compared to measured data about the efficiency of particle capture by the ascidian *Polycarpa mytiligera* (Fig. 3). Since the exact dimensions of the mesh of this species' are unknown, we used the capture efficiency data of untreated 0.5 µm polystyrene microspheres (Jacobi et al. 2017) to estimate the mesh size of its mucous filter. To do so we assumed a constant ratio between the length and width of the mash, $r = L/W$, as supported by average values of mesh size of other species obtained from the literature (Table S1). We estimated the mesh size, represented by the value of W (and $L = rW$), by minimizing the error, $|E_{obs} - E_{tot}|/E_{obs}$ where E_{obs} is the observed capture efficiency from Jacobi et al. (2017) and E_{tot} is the result of the model. The

812 calculations show that the estimated pore size of *P. mytiligera* is a rectangle 0.677 x

813 1.716 µm.

Table S1. Mesh size data from the literature. L and W are the mesh pore length and width respectively. r is the ratio L/W. *Pore dimensions of *P. mytiligera* were calculated based on capture efficiency data of microspheres from Jacobi et al. (2017)

Taxa	Pore size (nm)			Fiber thickness (nm)		
	L	***W***	***r***	Transverse	Longitudinal	reference
Ciona intestinalis	699	410	1.70	15	25	Flood and Fiala-Medioni (1981)
Ciona intestinalis	582	420	1.39	15	20	Flood and Fiala-Medioni (1981)
Phallusia mammillata	594	309	1.92	15	25	Flood and Fiala-Medioni (1981)
Ascidiella aspersa	792	297	2.67	10	20	Flood and Fiala-Medioni (1981)
Styela plicata	2167	497	4.36	10	20	Flood and Fiala-Medioni (1981)
Styela plicata	1751	514	3.41	10	20	Flood and Fiala-Medioni (1981)
Halocynthia papillosa	648	169	3.83	15	40	Flood and Fiala-Medioni (1981)
Microcosmus sabatieri	784	316	2.48	10	15	Flood and Fiala-Medioni (1981)
Cystodytes dellechiajei	197.8	143.7	1.38			Turon (1990)
Diplosoma spongiforme	498.4	285.1	1.75			Turon (1990)
Polysyncraton lacazei	548.2	352.9	1.55			Turon (1990)
Aplidium conicum	200.5	159.8	1.25			Turon (1990)
Sidnyum turbinatum	119.2	79.3	1.50			Turon (1990)
Ecteinascidia herdmanni	198.8	134	1.48			Turon (1990)
Ascidiella scabra	601.3	411	1.46			Turon (1990)
Halocynthia papillosa	1158	361.1	3.21			Turon (1990)
Microcosmus polymorphus	1209.6	205.9	5.87			Turon (1990)
Ascidia paratropa	500					Pennachetti (1984)
in-vivo estimate	2200	500	4.40	10	40	Flood and Fiala-Medioni (1981)
average	813	309	2.5	12.5	23.125	
*Polycarpa mytiligera**	1716	677	2.5			This work

814

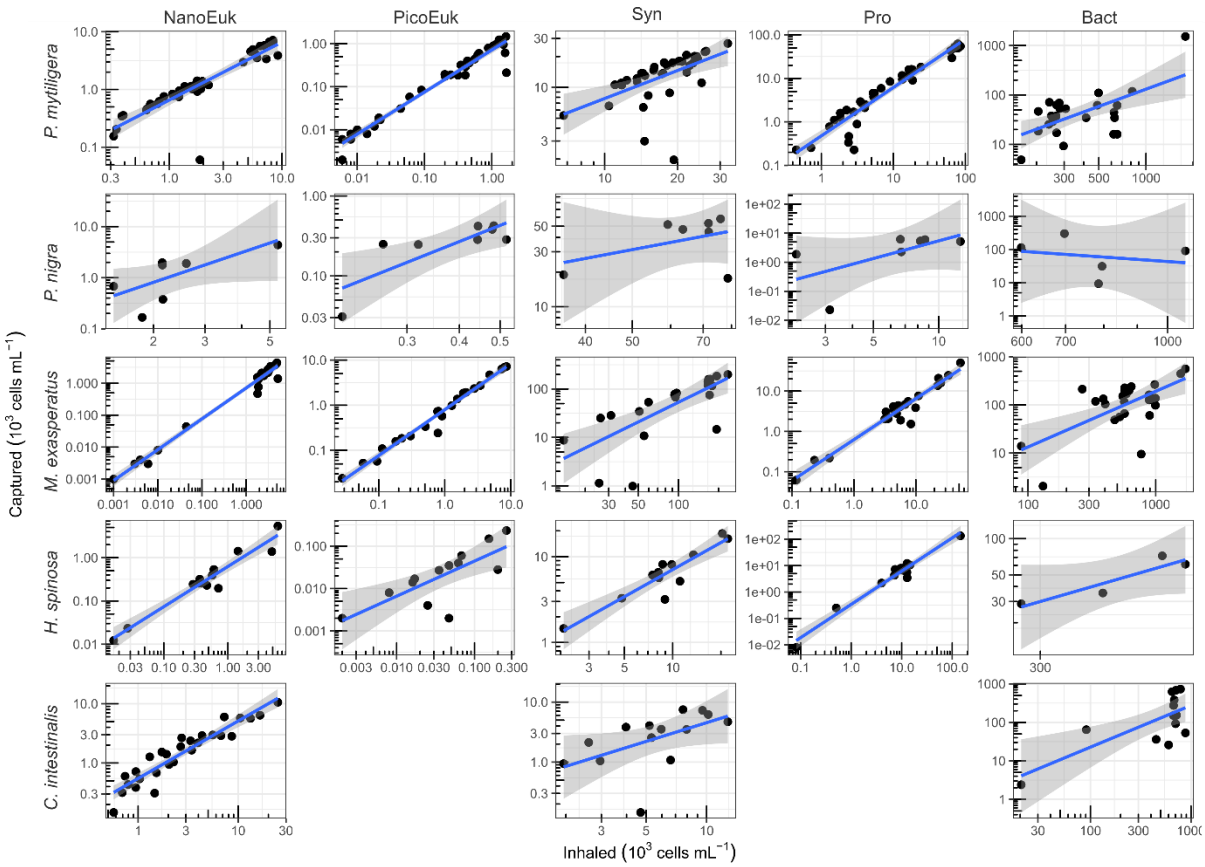

Figure S1. The amount of captured pico- and nano planktonic cells as a function of inhaled concentration. Each plot in this panel represents the capture of a certain prey type (columns) by one of the ascidian species tested (rows) in-situ. The blue line is a linear regression curve and the shaded area is the 95% confidence interval for the linear model. The slope, intercept, and R^2 values for each regression are presented in Table S4.

Table S2. Mean values (±95% confidence interval for the mean) of capture efficiency (%) of naturally occurring planktonic cells as measured in-situ for each of the studied ascidians. EMT is the eastern Mediterranean study site, RS the Red Sea study site, and LIS the Long Island Sound site in the Northeast Atlantic. Bolded numbers indicate cases where the capture efficiency was significantly different from zero. N is the number of animals tested in-situ. NanoEuk – high scatter eukaryotic microalgae, PicoEuk - low scatter eukaryotic microalgae, Syn – *Synechococcus*, Pro – *Prochlorococcus*, and Bact – heterotrophic bacteria.

			Capture efficiency (Mean±95% CI)				
Species	Basin	N	NanoEuk	PicoEuk	Syn	Pro	Bact
M. exasperatus	EMT	5	**76± 8**	**77±9**	**68±11**	**68±7**	**24±5**
P. nigra	EMT	7	**60±21**	**71±18**	**56±20**	**44±29**	6±12
P. mytiligera	RS	23	**71±5**	**77±6**	**74±9**	**64±7**	0±6
H. spinosa	RS	4	**71±11**	**70±18**	**66±15**	**64±13**	0±18
C. intestinalis	LIS	10	**57±7**	---	**56±13**	---	**38±18**

Table S3. Sample sizes (individual ascidians tested) for laboratory and in situ trials

		N	
Species	Basin	lab	in situ
M. exasperatus	EMT	10	5
P. nigra	EMT		7
S. plicata	EMT	27	
P. mytiligera,	RS	3	23
H. spinosa	RS	4	
C. intestinalis	LIS	10	7
S. clava	LIS	9	

Table S4. Slope (a), intercept (b), and R^2 values for the regression curves shown in Fig. S1. Bolded numbers indicate cases where the coefficient was significantly different from zero.

	NanoEuk			PicoEuk			Syn			Pro			Bact		
	a	b	R^2	a	b	R^2	a	b	R^2	a	b	R^2	a	b	R^2
P. mytiligera	**0.73**	-0.03	0.89	**0.67**	0.03	0.76	**0.80**	0.72	0.60	**0.67**	0.47	0.97	**0.76**	**-228.07**	0.72
P. nigra	**1.02**	-0.94	0.82	**0.89**	-0.06	0.63	0.42	13.95	0.14	0.50	0.41	0.52	-0.20	264.36	0.09
M. exasperatus	**0.71**	0.04	0.78	**0.87**	-0.03	0.99	**0.76**	-4.62	0.68	**0.87**	-1.47	0.95	**0.24**	-7.77	0.51
H. spinosa	**0.67**	-0.01	0.78	**0.70**	-0.01	0.67	**0.83**	-0.95	0.88	**0.94**	**-2.28**	0.99	0.45	-105.56	0.79
C. intestinalis	**0.41**	**0.45**	0.91	---	---	---	**0.51**	0.28	0.46	---	---	---	0.48	-44.9	0.18

References

Flood, P. R., and A. Fiala-Medioni. 1981. Ultrastructure and histochemistry of the food trapping mucous film in benthic filter-feeders (ascidians). Acta Zool. **62**: 53–65. doi:10.1111/j.1463-6395.1981.tb00616.x

Jacobi, Y., G. Yahel, and N. Shenkar. 2017. Efficient filtration of micron and submicron particles by ascidians from oligotrophic waters. Limnol. Oceanogr. doi:10.1002/lno.10736

Loudon, C. 1990. Empirical test of filtering theory: Particle capture by rectangular-mesh nets. Limnol. Oceanogr. **35**: 143–148. doi:10.4319/lo.1990.35.1.0143

Loudon, C., and D. N. Alstad. 1990. Theoretical mechanics of particle capture: predictions for hydropsychid caddisfly distributional ecology. Am. Nat. **135**: 360–381. doi:10.1086/285051

Pennachetti, C. A. 1984. Functional morphology of the branchial basket of ascidia paratropa (Tunicata, Ascidiacea). Zoomorphology **104**: 216–222. doi:10.1007/BF00312033

Rubenstein, D. I., and M. a. R. Koehl. 1977. The Mechanisms of Filter Feeding: Some Theoretical Considerations. Am. Nat. **111**: 981–994. doi:10.1086/283227

Silvester, N. R. 1983. Some hydrodynamic aspects of fitter feeding with rectangular-mesh nets. J. Theor. Biol. **103**: 265–286. doi:10.1016/0022-5193(83)90028-0

Sutherland, K. R., L. P. Madin, and R. Stocker. 2010. Filtration of submicrometer particles by pelagic tunicates. Proc. Natl. Acad. Sci. U. S. A. **107**: 15129–15134. doi:10.1073/pnas.1003599107

Turon, X. 1990. Estructura de los filtros branquiales de ascidias (tunicados). Misc. Zool. **14**: 125–133.