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Pelagic tunicate grazing on marine microbes revealed by integrative approaches

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Abstract

Marine microorganisms comprise a large fraction of ocean carbon and are central players in global biogeochemical cycling. Significant gaps remain, however, in our understanding of processes that determine the fate, distribution, and community structure of microbial communities. Protists and viruses are accepted as being part of the microbial loop and a source of microbial mortality. However, pelagic tunicates (salps, doliolods, pyrosomes, and appendicularians), which are abundant in oceanic and coastal environments and consume microorganisms with higher individual grazing rates than other common grazers, remain underappreciated in their role controlling microbial communities, distributions, and flux through ecosystems. In spite of sampling challenges owing to their fragile nature and patchy distributions, recent developments in methodology have deepened understanding of grazing rates and selectivity of these ubiquitous grazers. Next-generation sequencing, quantitative polymerase chain reaction, high-resolution videography, improved microscopy, biomarkers, and in situ approaches are transforming our knowledge on the role of pelagic tunicates in determining the fate and function of microbial communities. Here, we review recent research on pelagic tunicate grazing with a focus on newer methodologies and their application across pelagic tunicate taxa. Synthesis of these studies points to a major role for pelagic tunicates in the control of marine microbial communities. Comparisons between pelagic tunicate taxa indicate important differences in prey selectivity, which will impact how these grazers are incorporated into global models. Application and integration of these methods will produce continued insights with the ultimate goal of illuminating the unique role of pelagic tunicates in the microbial loop and biological pump.

Top-down controls play a critical role in microbial ecology and contributions to global carbon and energy cycles (Fuhrman and Noble 1995; Chow et al. 2014). Protists, viruses, and small zooplankton are accepted as part of the microbial loop and a source of microbial mortality. However, pelagic tunicates (Fig. 1; salps, doliolids, pyrosomes, appendicularians) are one important group of grazers that is rarely considered in controlling marine microbial communities. These pelagic filter-feeders are distributed globally (Fig. 2) and all use a common strategy of passing large volumes of water over sheets of mucus to concentrate dilute food particles. Pelagic tunicates are abundant in the open and coastal ocean and consume microorganisms with higher individual grazing rates than other common grazers (e.g., copepods and protists). Owing to the challenges of studying fragile pelagic tunicates and their patchy distributions, their contribution to microbial mortality and the mediation of energy flow through marine ecosystems remains underappreciated.

New approaches to quantify grazing rates and selectivity including next-generation sequencing, quantitative polymerase chain reaction (PCR), high-resolution videography, improved microscopy techniques, biomarkers, and direct in situ sampling are improving our capacity to accurately estimate the abundance and ecological function of pelagic tunicates with respect to microbes. Gelatinous zooplankton, which includes the pelagic tunicates, are increasingly being integrated into food web models calling out a need to more accurately quantify grazing rates, particle selection patterns, and differences in microbial grazing behavior across grazer taxa. This review summarizes research to date on pelagic

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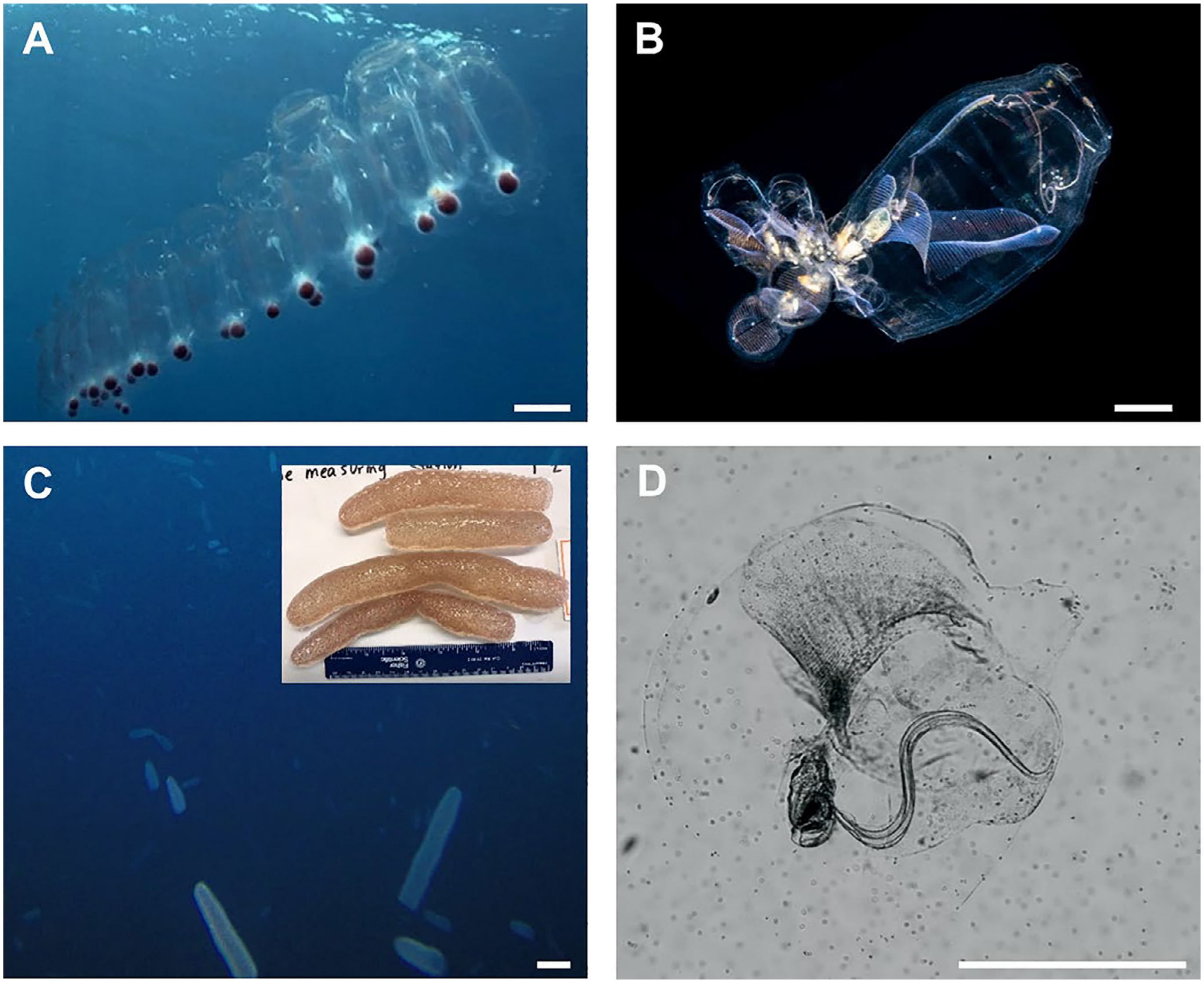

Fig. 1. Pelagic tunicates are chordates with large watery bodies that exhibit high grazing rates of microbial prey. The four major lineages shown: (**A**) salp (blastozooid stage, *Pegea confoederata*), (**B**) doliolid (phorozooid stage, *Dolioletta gegenbauri*), (**C**) pyrosome (*Pyrosoma atlanticum*), shown in situ and in the lab after capture (inset), and (**D**) appendicularian (*Oikopleura dioica*). Scale bars are approximate: (**A**, **C**) = 2 cm; (**B**, **D**) = 1 mm. (**B**) *Source*: Linda Ianniello.

tunicate grazing on microbial constituents with the goal of updating our view of trophic ecology, the microbial loop, and inspiring avenues for future research. We consider marine microbes broadly, including phytoplankton, nonpigmented eukaryotes, cyanobacteria, heterotrophic bacteria, and viruses. Although we cite studies from the past ~ 50 yr, here we highlight work from the past ~ 10 yr in this rapidly advancing area of research.

Biology and ecology of pelagic tunicates

Pelagic tunicates are invertebrates from the phylum Chordata (Fig. 1) with high potential for impacting microbial communities but poor representation in ecosystem models due to sampling challenges and lack of data on grazing rates, selectivity, and global distributions. Pelagic tunicates (salps, doliolids, pyrosomes, and appendicularians) use sheets of mucous to capture and concentrate food particles from seawater. This feeding strategy results in the highest individual filtration rates among filter-feeding plankton (Alldredge and Madin 1982), exceeding those of other important grazers, including copepods, flagellates, and ciliates (Conley et al. 2018c). Pelagic tunicates may directly compete with protists for microbial constituents. Although they have overlapping grazing preferences, an energetic model showed that pelagic tunicates and protists employ distinct—and equally viable—feeding strategies: protists have small carbon-dense bodies and use remote prey sensing, while pelagic tunicates have large,

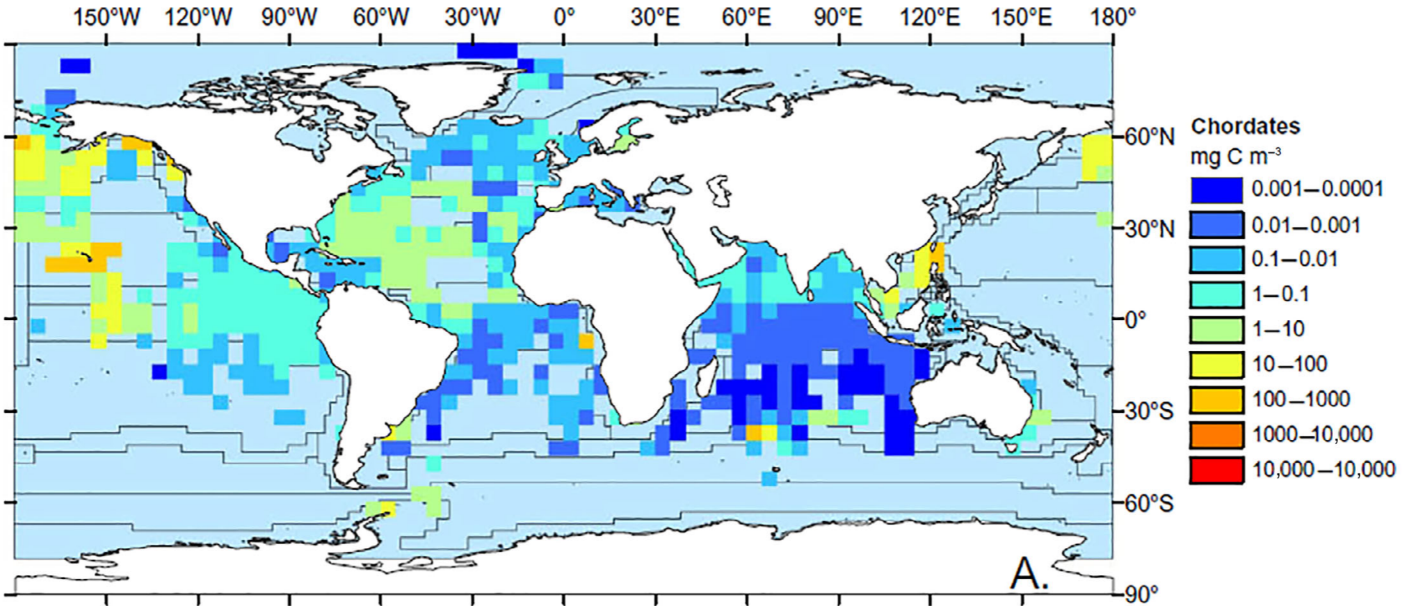

Fig. 2. Pelagic tunicates are broadly distributed and globally abundant. Geometric mean biomass (mg C m^{-3}) of 18 species of thaliaceans from the Jellyfish Database Initiative (JeDI) plotted as 5° grid cells over Longhurst provinces. Data primarily came from net sampling therefore biomass measurements are underestimates. *Source*: Lucas et al. (2014).

watery, carbon-dilute bodies with large capture surfaces for intercepting food in the open ocean (Dölger et al. 2019). More nuanced studies will be required to test the full suite of interactions between pelagic tunicates and protists, including the extent to which pelagic tunicates predate on protists while also competing with them for food, as well as the very different fates of fecal pellets and carcasses.

Although all pelagic tunicates pass water over a large mucous mesh during feeding, the details of feeding differ among pelagic tunicate lineages. Appendicularians secrete an external mucous house comprising an inlet filter and a food-concentrating filter. Particles must reach a second internal pharyngeal filter to be ingested (Morris and Deibel 1993). Sinusoidal tail beating drives a steady feeding current through the mucous house. The mucous house is discarded and replaced up to 40 times per day (Sato et al. 2003). In contrast, Thaliaceans—from the orders Salpida, Doliolida, and Pyrosomida—secrete an internal mucous mesh, resembling a plankton net, which is rolled into a mucous strand and then conveyed to the esophagus. To drive fluid over the mesh, salps use muscular pumping while doliolids and pyrosomes use ciliary beating. In all three Thaliacean taxa, water moves in through an incurrent siphon, passes over the mucous mesh, and then exits through an excurrent siphon after food particle removal.

While typical prey to predator size ratios range from 1:10 to 1:100 (Hansen et al. 1994), pelagic tunicates concentrate tiny organisms down to picoplankton (Flood et al. 1992; Sutherland et al. 2010), and even viruses (Lawrence et al. 2018). Prey particles can be smaller than mucous mesh-openings and several orders of magnitude smaller than the grazers. Mounting evidence demonstrates that not only do pelagic tunicates consume submicron-sized prey but they are selective in what they consume, potentially influencing the community structure of surrounding microbial communities and altering microbially driven biogeochemical cycling (Conley et al. 2018c). Furthermore, grazing on micron and sub-micron-sized prey could "short circuit" the microbial loop (Gorsky and Fenaux 1998), transferring carbon directly from pico-microplankton to higher trophic levels, impacting our understanding of the controls on microbial populations and carbon biogeochemical cycles.

Pelagic tunicates also have a role in carbon and energy export from the surface ocean. Undigested, or partially digested, prey cells are incorporated into fast-sinking fecal pellets (salps, doliolids, and appendicularians) or discarded mucous houses (appendicularians) and therefore have the potential to remove substantial amounts of organic material from surface waters (Silver and Bruland 1981; Robison et al. 2005). Intact phytoplankton cells have been observed in salp fecal pellets (Silver and Bruland 1981). Discarded mucous houses, fecal pellets, and pseudofeces have high sinking rates (Silver and Bruland 1981; Noji et al. 1997) and are also consumed by metazoan predators, including copepods, ostracods, and euphausiid larvae (Gorsky and Fenaux 1998; Lombard et al. 2010). Export of material can be accelerated by vertical migration (e.g., salps and pyrosomes) (Wiebe et al. 1979; Angel 1989; Andersen and Sardou 1992).

Though pelagic tunicates are frequently overlooked in ecosystem models, they are ubiquitous and abundant members of marine ecosystems (Fig. 2; Lucas et al. 2014). Pelagic tunicate life cycles are characterized by alternation of generations (with the exception of appendicularians) and short generation times (days to weeks), allowing rapid population growth when conditions are favorable (Alldredge and Madin 1982). Populations in temperate and cold waters along continental shelves periodically reach bloom proportions that extend for hundreds of kilometers (Madin et al. 2006). Appendicularians, for example, are among the two or three most abundant mesozooplankton in surface waters (Gorsky and Fenaux 1998). Pelagic tunicates are also important prey for planktonic carnivores including sea birds, larval and adult fish, chaetognaths, cnidarian medusae, ctenophores, copepods, and foraminifera (reviewed by Gorsky and Fenaux 1998; Purcell et al. 2005).

Thus, pelagic tunicates support several ecologically and economically important species (Fortier et al. 1994), occupy an important position in the marine food web that directly links lower and higher trophic levels (Sommer et al. 2002; Strom 2002), and contribute to export of microbially derived carbon to the deep ocean. Because of these properties, there was early recognition that pelagic tunicates have an important role to play in the microbial loop (Fortier et al. 1994; Pomeroy et al. 2007) but the details of that role are only recently emerging.

Integrative approaches provide new insight into particle selection and grazing impact

Methodological developments, especially integration across methods, have updated our understanding of the grazing impact and selectivity of pelagic tunicates (Fig. 3). Here, we summarize common and powerful approaches and provide examples of how they have been applied to study pelagic tunicate grazing. Considering that different methods can produce clearance rates that vary by more than an order of magnitude, even for a single species (Fig. 4), it is paramount to consider carefully the validity and relevance of each method when designing a research study. Combining multiple approaches that are informed by an understanding of the feeding ecology of the pelagic tunicate under study will yield the most rigorous results.

In situ study

The numerous challenges of studying pelagic tunicates explain why their importance has been overlooked in marine ecosystems. While globally abundant, pelagic tunicate distribution is patchy, and thus hard to sample predictably. Most biomass estimates of pelagic tunicates, such as the reports summarized in Fig. 2, are severe underestimates owing to the reliance on net tow sampling, which destroys these delicate animals beyond recognition. Their extreme fragility also inhibits most cultivation and experimentation using conventional methods (i.e., net tows). If captured intact, many species do not swim and feed normally in tanks, even in specialized plankton kreisels (Greve 1968). Updated measures of feeding impact and contributions to the carbon cycle require in situ-based quantification.

Successful measurements require direct in situ observations or careful collection by hand—ideally in situ—followed by experiments within hours of collection. Blue-water diving approaches developed in the 1970s (Hamner et al. 1975) are still relevant and allow careful collection and imaging. Modern updates for diver-based sampling (Dadon-Pilosof et al. 2017) enable real-time sampling from in situ incubation chambers and direct sampling from the incurrent and excurrent siphons of pelagic tunicates to produce more accurate grazing rates from natural assemblages (Dadon-Pilosof et al. 2019). Towed (e.g., In Situ Ichthyoplankton Imaging System; Guigand et al. 2005), autonomous (Zooglider; Ohman et al. 2019), and vertically dropped (Underwater Vision Profiler 5; Picheral et al. 2010) in situ imaging systems consistently reveal higher abundances of pelagic tunicates than other methods. For example, appendicularians frequently outnumber copepods and other hard-bodied plankton when measured using in situ imagery (Briseño-Avena et al. 2020; Hagemeyer et al. 2020). Advanced imaging and sample collection from remotely operated vehicle (ROVs) also yield new insights into undisturbed feeding behaviors at the organism scale (Katija et al. 2017). Depth-keeping Autonomous Underwater Vehicle Workshops (Yoerger et al. 2018) coupled to machine learning tracking (Katija et al. 2021) can follow individual animals as they move through the water column illuminating feeding behaviors and vertical transport mechanisms. Together these in situ techniques help update abundance estimates to more closely match reality (Luo et al. 2014) and enable high-resolution and quantitative comparisons between grazer abundance, behavior, and oceanographic parameters.

Pigment analysis

Pigment analysis of complex microbial populations differentiates coexisting phytoplankton and advances understanding of the selectivity of pelagic tunicate grazing on phytoplankton (van Heukelem and Hooker 2011; Falkowski 2014; Kramer and Siegel 2019). Pigment analysis has been applied to both grazer tissue (either whole or partial animals or dissected guts) and in situ prey fields or incubations toward detecting phytoplankton in grazer guts, grazing selectivity, grazing rates, and gut clearance rates (Nelson 1989; Pfannkuche and Lochte 1993; Dubischar and Bathmann 1997; Perissinotto et al. 2007; Ahmad Ishak et al. 2017; Décima et al. 2019).

While pigment analysis is useful for establishing the predation of pelagic tunicates on phytoplankton, numerous limitations exist. Although some phytoplankton pigments are chemotaxonomic markers for specific phytoplankton groups, many pigments are shared between phytoplankton groups, limiting the taxonomic resolution of the method. Also, pigment analysis applied alone misses nonpigmented microbial

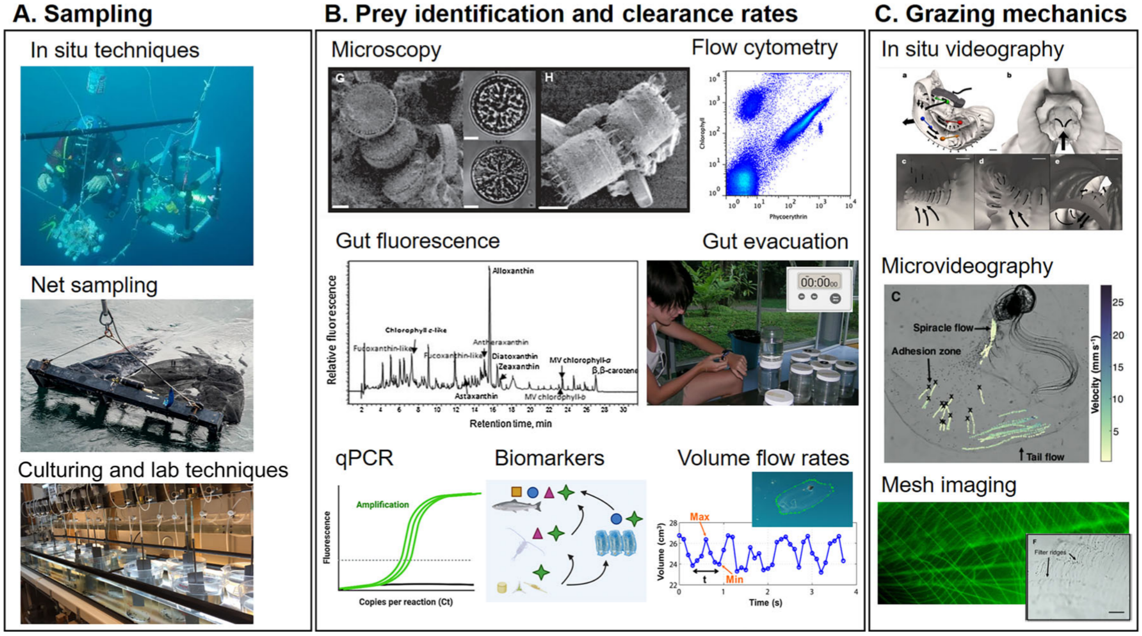

Fig. 3. Methods can be integrated to robustly measure grazing rates and selectivity of pelagic tunicates. (**A**) Sampling methods include in situ approaches (image credit: G. Yahel), net sampling (MOCNESS net shown), and laboratory culture (appendicularian culture facility at Sars International Center in Norway shown). (**B**) Grazing rates and prey identification can be determined using microscopy (Thompson et al. 2021), flow cytometry, gut fluorescence (Ahmad Ishak et al. 2017), gut evacuation time, quantitative qPCR, fatty acid biomarkers, and volume flow rates (Sutherland and Madin 2010). (**C**) Grazing mechanics are revealed from in situ videography (Katija et al. 2020), microvideography (2018b) and mesh imaging (Sutherland et al. 2010; Conley et al. 2018b). qPCR and biomarker images were created with BioRender.com.

prey. In addition, pigment breakdown during gut passage, which is especially problematic in detecting phytoplankton community composition in grazer digestive organs and fecal pellets, must be considered quantitatively (Conover et al. 1986; Durbin and Campbell 2007). Finally, as phytoplankton pigment concentrations vary with cell size, nutrient, and light availability, pigment analysis is not reliable for quantitative analyses of phytoplankton prey except in paired experiments on the same phytoplankton population.

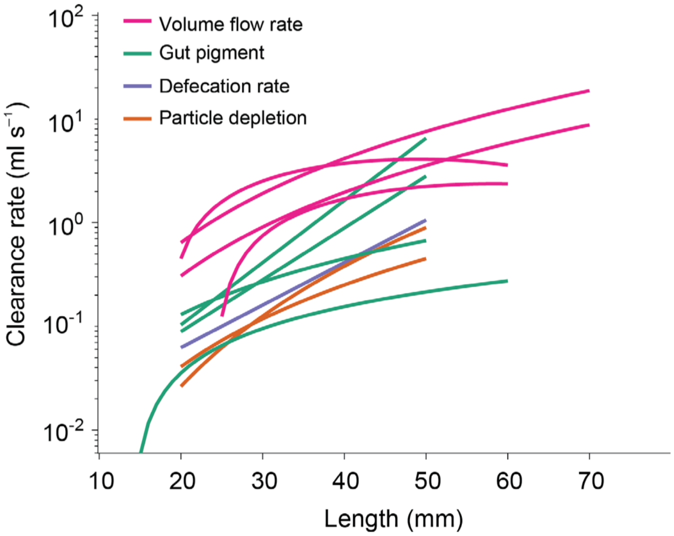

Fig. 4. Comparison of clearance rates by the salp *Pegea confoederata* obtained using various methods. Measurements vary by orders of magnitude with volume flow rates producing the maximum values. *Sources*: Harbison and Gilmer (1976), Madin and Cetta (1984), Madin and Kremer (1995), Hereu et al. (2010), Sutherland and Madin (2010).

Stable isotopes

The transitory nature of gut-content samples can be addressed through stable isotope analysis, which provides a time-integrated view of grazed and assimilated prey (Frost et al. 2012). Nitrogen isotope ratios ($\delta^{15}N$) estimate trophic position (Pitt et al. 2009), while carbon isotope ratios ($\delta^{13}C$) indicate carbon sources in food webs. Seasonal and interannual variability in stable isotope signatures are driven by changes in primary productivity and the environment (El-Sabaawi et al. 2012), verifying the value of this method in quantifying variability in bottom-up resource availability. Combining stable isotope analysis with microscopy and molecular analysis of gut contents and fecal pellets can identify both transitory and longer-term grazing preferences (D'Ambra et al. 2015). For example, in a recent study from the eastern tropical pacific, stable isotope analysis of grazers and background particulate organic matter (POM) collected from distinct depth layers revealed pyrosome grazing below the surface mixed layer (Décima et al. 2019). The same study showed that appendicularians, doliolids, and pyrosomes were primarily grazing on phytoplankton, whereas salp isotopic signatures indicated omnivory and carnivory. In some cases where POM samples were not available, pelagic tunicates have been substituted as baseline consumers (trophic position 2) under the assumption that they feed indiscriminately on POM. A recent review, however, points to heterogeneous trophic enrichment in ^{13}C and ^{15}N compared to POM indicating that heterotrophic microzooplankton are an important dietary source for tunicates (Pakhomov et al. 2019). Therefore, pelagic tunicates are not reliable baseline consumers. In cases when baseline isotopic values are unavailable, compound-specific stable isotope analysis of individual amino acids or fatty acids (FAs) can untangle trophic relationships (Popp et al. 2007). For example, variations in δ^{15}N values of amino acids from leatherback turtles and gelatinous zooplankton were recently used together with Bayesian models to establish trophic structure (Hetherington et al. 2019).

FA profiles

FAs are particularly valuable for lower trophic level inference because primary producers have distinctive lipid signatures (Dalsgaard et al. 2003; Galloway and Winder 2015), which can be traced into primary consumers (Galloway et al. 2015). Essential FAs (EFAs) are components of cell membranes in heterotrophs, yet they are only synthesized by some autotrophs, making them useful indicators. For example, diatoms, dinoflagellates and prymnesiophytes each have unique FA compositions. Ratios between certain FA indicate dominance of particular phytoplankton, for example dinoflagellates over diatoms (Parrish et al. 2000; Dalsgaard et al. 2003). Appendicularian FA composition was shown to directly reflect nutritional sources, suggesting the utility of FA in determining the diet of pelagic tunicates (Troedsson et al. 2005). Similarly, pyrosome FA profiles revealed diatoms, dinoflagellates, prymnesiophytes, coccolithophores, chlorophytes, cyanobacteria, and bacteria as dietary components (Perissinotto et al. 2007; Richoux 2011; Schram et al. 2020). By their FA composition, doliolids from the northeast Atlantic grazed on diatoms and dinoflagellates (Pond and Sargent 1998). Antarctic salp FAs were dominated by flagellates year-round with only minor contributions of diatoms (von Harbou et al. 2011). As with other biomarker analyses, FA data interpretation can be bolstered using lab experiments with known prey sources to account for differences in prey assimilation and modification (Galloway and Budge 2020).

Flow cytometry

Flow cytometry is a proven tool for enumerating distinct microorganisms in aquatic samples that adapts easily to study the impact of pelagic tunicates on autotrophic (i.e., pigmented) and heterotrophic (i.e., nonpigmented) cells. Data from flow cytometry yield insight into grazing selectivity based on taxa, DNA content, and size properties as well as grazing rates when used in culture, mesocosm, or in situ experiments. This technique replaces the Coulter counter, which was limited by its ability to distinguish cells on properties other than size and unable to detect submicron particles (Deibel 1982; Harbison et al. 1986; Cucci et al. 1989).

Flow cytometry can be applied to both grazer tissue (i.e., guts) or to suspended microbial prey. Recent work on the grazing preferences of pyrosomes used flow cytometry to distinguish macerated grazer tissue and nonpigmented cells from phytoplankton present within pyrosome zooids (Thompson et al. 2021). Ratios of *Synechococcus* to small (pico-nano) eukaryotic phytoplankton in guts were compared to the relative abundance of these taxa in the prey field. Estimates of grazing rates and retention efficiency of delicate pelagic tunicates have also been facilitated by the application of flow cytometry to in situ sampling and incubations. Using a modification of the VacuSIP technique (Morganti et al. 2016), Dadon-Pilosof et al. (2019) compared the size distribution and taxonomy of grazed cells at the exhalant and inhalant siphons of free-swimming and incubated salps. Quantification of particle cell size distributions at the exhalant and inhalant siphons closely matched size estimates from microscopy analysis, and enabled estimates of the role of particle size in driving selectivity patterns (Dadon-Pilosof et al. 2019).

The full advantages of flow cytometric analysis of pelagic tunicate grazing on marine microbes have yet to be thoroughly explored. For example, the use of multiple excitation lasers simultaneously distinguishes at least eight different groups of co-existing eukaryotic phytoplankton in seawater samples (Thompson and van den Engh 2016). If applied to grazing studies, this approach could provide more information on grazer selectivity. In addition, flow cytometry can distinguish the light history of photosynthetic cells (Falkowski 1983; Falkowski and LaRoche 1991), thus depth of origin. Quantifying the relative chlorophyll concentration of individually grazed phytoplankton could reveal the depth where the grazer was feeding, providing insight into how pelagic tunicates impact phytoplankton populations in different euphotic zone layers.

Sequencing

High throughput sequencing approaches are beginning to yield numerous insights into the impact of pelagic tunicate feeding on complex natural communities. Sequencing approaches hold several advantages over more traditional approaches of pigment analysis, stable isotope analysis, microscopy, and flow cytometry. Sequencing allows the detection of small and morphologically unremarkable, partially degraded, and rare prey across diverse taxonomic lineages. In addition, high taxonomic resolution allows prey identification down to the genus, species, and subspecies levels. Metagenomic analysis offers a method to infer grazing impact on potential metabolic functions and ecological interactions in a microbial community.

Sequencing-based approaches can be applied to grazing questions in two ways. First, direct sequencing of dissected tissue from feeding organs of pelagic tunicates can reveal the taxonomic range of grazed prey and any feeding selectivity (Walters et al. 2019; Frischer et al. 2021; Thompson et al. 2021). Second, coupling of sequencing to in situ incubation experiments can reveal diversity in retained prey, clearance rates, and enable comparative analysis between coexisting tunicate taxa (Dadon-Pilosof et al. 2017).

While powerful, sequenced-based approaches face numerous pitfalls that must be carefully considered toward understanding pelagic tunicate grazing. Datasets created by high throughput sequencing are inherently compositional because the number of returned sequence reads is limited by the sequencing platform (Gloor et al. 2017). Thus, sequence counts are not a method for counting the number of genes associated with cells in the sampled population. For example, two samples (i.e., a seawater prey field vs. a tunicate gut sample) can have the same counts of a microbial taxa but different sequence proportions of the same taxa (Gloor et al. 2017). This effect could result in misleading grazing rates and feeding efficiency calculations. As reviewed in Gloor et al. (2017), many compositionally appropriate analysis tools exist, which must be incorporated into sequence-based approaches to microbe-tunicate grazing interactions. Another challenge is in managing environmental contamination, particularly where sequencing is applied to grazer tissue rather than seawater. Incorporating appropriate negative controls can help tease apart sequences derived from grazed cells, the seawater environment, and molecular reagents (Kim et al. 2017). Evidence suggests that the surfaces of some gelatinous animals are low in microbial biomass (Hammer et al. 2019), leaving these samples vulnerable to the amplification of contaminating DNA from the environment and reagents. Another major challenge is determining the ecological nature of the relationship between a grazer and its associated phytoplankton sequences. Microbes recovered from grazer guts or fecal pellets could be prey, symbionts, pathogens, and/or contaminants. Existing knowledge of the ecology of potential microbial prey can aid with interpretation of their association with grazer guts and fecal pellets.

Quantitative PCR

Quantitative PCR (qPCR) approaches offer a robustly quantitative view of prey identity, prey concentration, and feeding rates for pelagic tunicates. This contrasts with the compositional data provided by sequence-based approaches, but is limited to a few select prey taxa.

Comparison between guts or whole organisms and surrounding seawater using qPCR can provide quantitative data on the degree to which the grazer concentrates prey, feeding rates, and prey preferences. For example, qPCR applied to appendicularian feeding revealed the highest ingestion efficiencies for the smallest phytoplankton species and showed how small prey species inhibit ingestion of larger algae when both prey are present (Troedsson et al. 2007). Most recently, qPCR assays for Bacillariophyta (diatoms), Haptophyta, and Cryptophyta were developed and applied to doliolids across their life stages (Frischer et al. 2021), which revealed grazing preference for diatoms but the ingestion of diverse microbial prey.

While powerful, qPCR faces several challenges, which must be navigated for studies on pelagic tunicate feeding (discussed in Frischer et al. 2014). As with any qPCR assay, attention to reaction specificity and efficiency are central to obtaining biologically relevant results (Bustin et al. 2013). Attention to the relationship between gene copies (e.g., 18S rRNA gene) and cell number is needed as phytoplankton can harbor multiple genome copies per cell and these copy numbers vary with the phytoplankton physiological state (Worden and Allen 2010). Specific to feeding studies, work on copepods (Nejstgaard et al. 2008; Durbin et al. 2012) and nonmarine consumers (Weber and Lundgren 2009; Pompanon et al. 2012; Bowen and Iverson 2013; Traugott et al. 2013) revealed different degrees of prey DNA degradation across grazer taxa, which affects amplification during qPCR. Degraded DNA can lead to underestimates of prey abundances (Nejstgaard et al. 2008), thus should be quantified. For example, Frischer et al. (2014) tested the degree to which doliolids degrade diatom DNA using a differential length amplification qPCR (dla-qPCR) assay and found little DNA degradation, which supported additional studies that compared the doliolid diatom concentration factor across many life stages (Walters et al. 2019) and numerous other phytoplankton taxa (Frischer et al. 2021). Such analysis of DNA degradation levels would be a precursor to studies in other pelagic tunicate taxa and microorganisms. In addition, our recent work and that of others shows that some pelagic tunicate guts can inhibit PCR (Metfies et al. 2014), although the inhibitory compound is unknown. Use of internal positive controls (Kavlick 2018) can quantify the level to which the DNA has inhibited qPCR, and should be tested for each sample analyzed.

Microscopy

Microscopy was pivotal in the earliest studies examining feeding mechanics of pelagic tunicates and still has an important role in quantitative and qualitative study of impacts on microbial communities. Microscopy has revealed mesh properties including arrangement patterns, shape, and pore size. Bone et al. (2000) used microscopy to reveal the rectangular shape, configuration, and genesis of filter filaments of salp mesh from several species (Bone et al. 2000). However, measurements of mesh fiber and pore dimensions made by scanning electron microscopy and transmission electron microscopy are subject to experimental artifacts from shrinking and drying (Bone et al. 2003; Conley et al. 2018c). Freshly collected (i.e., wet) salp mesh imaged by epifluorescence microscopy indicated a fiber dimension of 0.1 μm, which coupled with slow filtration rates, suggests feeding as a low Reynolds number process leading to high encounter rates with submicron-sized particles such as picocyanobacteria and other small abundant microbes (e.g., *Pelagibacter*) (Sutherland et al. 2010). Likewise, microscopy revealed very small openings of appendicularian mesh (0.1×0.8 μm) and an elongated rectangular shape, similar to insect larva (Wallace and Malas 1977). However, few such measurements of wet or in vivo meshes have been made relative to the number and diversity of pelagic tunicates (Conley and Sutherland 2017). Microscopy is also key to understanding the identity, size, and shape of phytoplankton ingested by pelagic tunicates as flow cytometry can be problematic for robust size determination even when calibrated to spherical beads (Shapiro 2005) and pigment- or sequence-based approaches only infer size from taxonomy.

While powerful in positively identifying prey, quantifying prey size, and revealing mesh properties, limitations of microscopy make it more powerful in combination with other approaches. Microscopy is not capable of resolving taxonomy of partially digested, unrecognizable, or morphologically unremarkable prey species that can be discovered with molecular approaches. Similarly, for quantification of prey abundance in guts, mesh, or fecal pellets, microscopy is low throughput and would be powerful in strategic combination with high throughput methods of sequence and flow cytometry analysis.

Biomechanics and fluid mechanics

Pelagic tunicates use muscular pumping, or ciliary beating, in combination with a large mucous particle capture surface to process particles at high rates. Flow speeds of water passing through the animal and morphology determine the source and volume of fluid that is processed, therefore setting an upper limit on filtration capacity (Bochdansky et al. 1999; Sutherland and Madin 2010). In situ images of swimming salps have shown how body volume changes over time to yield time-varying filtration rates (Madin and Kremer 1995; Sutherland and Madin 2010) and high speed video of particle trajectories revealed flow rates in doliolids (Deibel and Paffenhofer 1988). Flow properties at the scale of the filtering mesh determined how and whether particles stick to the mucous mesh and are ultimately ingested (Deibel and Paffenhofer 1988; Conley and Sutherland 2017). Particle image velocimetry near the filter of a salp—while preliminary—suggested that particles are captured by tangential flow filtration (Sutherland et al. 2010), similar to solitary benthic tunicates (Conley et al. 2018a). While flow rates indicate potential clearance rates, mesh morphology determines the upper and lower limits of particle retention, which vary appreciably among taxa. Mesh dimensions are powerful for determining the lower limits of particle retention, which can be accurately predicted from mathematical models (Silvester 1983; Sutherland et al. 2010). Advancing understanding of flow dynamics and mesh morphology during grazing by different gelatinous taxa will provide a mechanistic understanding of the traits governing prey capture that can be broadly applied across marine environments where these grazers are found (Litchman et al. 2013).

Lab-based approaches (culture and feeding experiments)

In spite of the importance of in situ work for accurately revealing pelagic tunicate grazing rates and trophic ecology, laboratory studies have provided a strong foundation for feeding in controlled settings. Experiments in large volumes of water minimize the disruption to the filtering process and bolster data from quantitative in situ feeding measurements. The appendicularian *Oikopleura dioica* can be reared and maintained over numerous generations in the laboratory (Paffenhöfer 1973) and therefore has been developed as a model for cross-disciplinary research, including studies of feeding ecology with active cultures (Bouquet et al. 2009). Lab experiments revealed appendicularian grazing on viruses (Lawrence et al. 2018). Numerous studies have combined appendicularian culture with the use of artificial prey (i.e., polystyrene or glass particles). Tracking of colored particles provided measurement of salp gut passage times (Madin and Cetta 1984). Studies with cultured microalgae and fluorescent polystyrene beads of known sizes revealed size-based retention efficiencies (Kremer and Madin 1992). Feeding with polystyrene beads indicated that gelatinous grazers select for certain particle shapes and sizes (e.g., appendicularians: Fernández et al. 2004; salps: Sutherland et al. 2010). Feeding appendicularians ellipsoidal microbeads showed that minimum particle diameter determined prey interaction with the feeding-filters, which meant that ellipsoidal particles were not retained as well as spherical particles with the same maximum diameter (Conley and Sutherland 2017). The finding showed that particle shape allows more streamlined cells to escape grazing by globally abundant pelagic tunicates and may help explain the prevalence of ellipsoidal cells in the ocean.

What are pelagic tunicates grazing?

The integrative approaches discussed above reveal that pelagic tunicates can strongly mediate microbial populations through high filtration of large volumes of seawater combined with fine mesh filters. Existing evidence shows that pelagic tunicates as a whole preferentially select larger eukaryotic phytoplankton over picocyanobacteria (Fig. 5) and heterotrophic bacteria, thus grazing by these animals shapes microbial community structure and can impact biogeochemical cycles. Incorporation of microbes into fast sinking fecal pellets alters carbon cycles by removing primary production from the surface ocean (Fig. 6). In spite of the common strategy among pelagic tunicates to filter large volumes over mucous meshes, differences between grazer taxa in the details of morphology, kinematics, and fluid mechanics of feeding result in taxon-

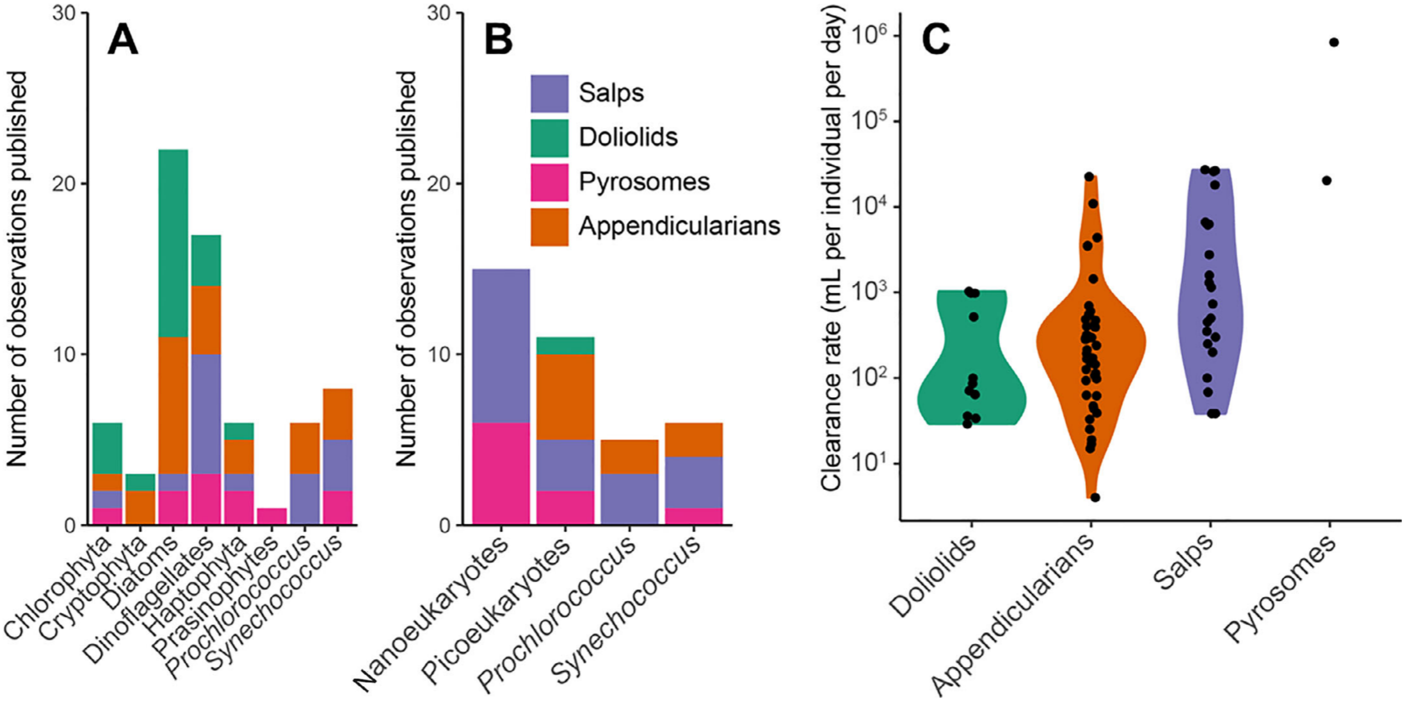

Fig. 5. Published observations of pelagic tunicates (color coded by lineage) prey selection and clearance rates. (**A**) Observations of feeding on specific phytoplankton taxa. (**B**) Observations of feeding on defined microbial size classes. (**C**) Mean published clearance rates by pelagic tunicate group. Each unique observation is shown with a black dot (Supporting Information Table S1).

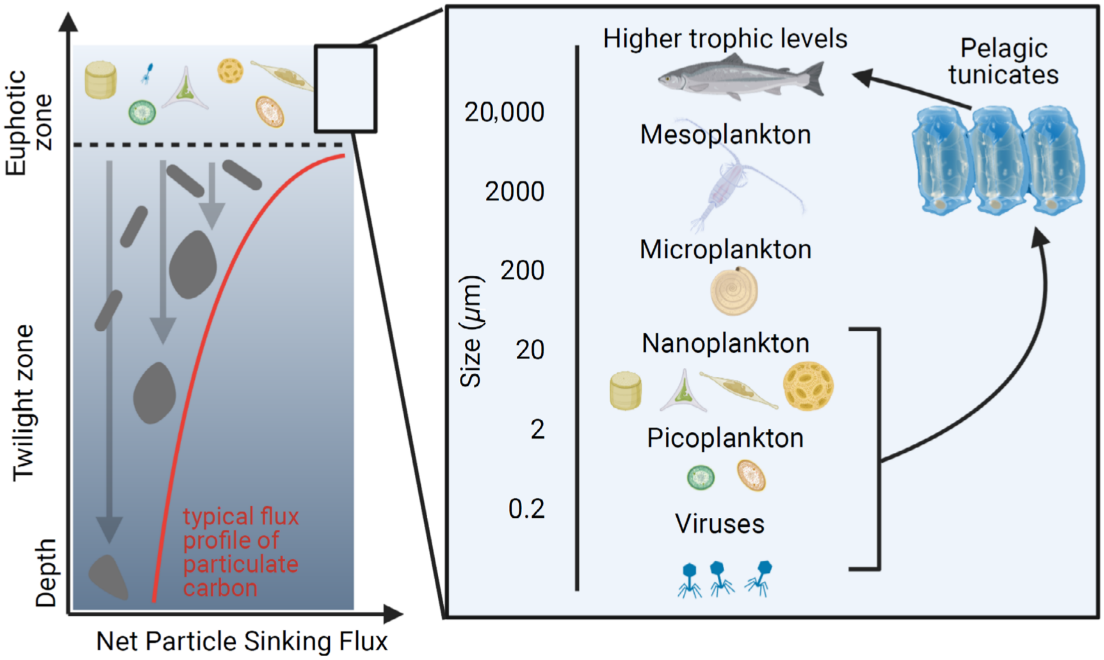

Fig. 6. (Right panel) Pelagic tunicates have a considerable but overlooked influence on microbial mortality and the microbial loop. Increasing evidence suggests that pelagic tunicate grazing can bypass carbon and energy transfers of the microbial food web, making primary production more directly available to the highest trophic levels. (Left panel) Pelagic tunicates strongly mediate the flux of carbon to the sea floor. Future studies will expand our knowledge of grazing rates and selectivity and other interactions with the microbial community including symbiosis and the microbial modification of sinking mucus aggregates, fecal pellets, and carcasses.

specific grazing rates and selection for particular microbes (Fig. 5).

Salps

Salps are barrel-shaped animals that pump water across a mucous mesh using muscular pumping that also propels the animal through the water (Fig. 1A). This feeding strategy allows the salp to process large volumes of seawater and move constantly to fresh prey fields. Most taxa are adapted to the open ocean although some inhabit coastal ecosystems; widespread distributions of salps have earned them incorporation into global carbon models (Luo et al. 2020). The complex life history of salps comprises an alternation of generations between asexual solitary animals and sexually reproducing clonal chains. Together, these characteristics enable salps to respond quickly to new oceanographic conditions, reaching extremely high densities where conditions are favorable (Alldredge and Madin 1982). Starting with early microscopy studies on salp fecal pellets (Silver and Bruland 1981), salp feeding on phytoplankton is well established. Coupled with their high filtration rates (Bone et al. 2003), and small mesh sizes (Sutherland et al. 2010), salps are expected to dramatically impact microbial communities down to submicron cells.

Recent application of integrative approaches demonstrates salp selective feeding on different microbial taxa, which contrasts with previous understanding of nonselective feeding (Vargas and Madin 2004; von Harbou et al. 2011). Metfies et al. (2014) applied high-throughput sequencing to the guts of several salp species from Antarctic waters to resolve conflict between previous studies of FA signatures and microscopy to identify salp feeding preferences. Their results were consistent with FA analysis, revealing preferential feeding by three salp species on dinoflagellates. Furthermore, high taxonomic resolution of microbial taxa (sub-Class level) offered by sequencing 18S rRNA gene sequences indicated differences between coexisting salp species in their prey preference. These data suggest that salps can shift the balance of microbial communities, selectively reducing dinoflagellate population sizes. However, with the sequencing approach alone, insight into the size classes of preferred dinoflagellates remained unresolved.

Using a novel direct in situ sampling technique (InEx) in combination with flow cytometry, Dadon-Pilosof et al. (2019) measured size classes of salp-selected prey and further tested whether salps selectively feed on different prey types. Three salp species preferred prey about 1 μm (i.e., picoeukaryotes), with preference against both smaller (cyanobacteria and bacteria) and larger prey (nanoeukaryotes). This evidence from in situ studies coupled to flow cytometry contrasts with previous understanding that salps preferentially retain larger eukaryotic phytoplankton and explains the conflicting FA and microscopy results that inspired Metfies et al. (2014), as smaller cells would be difficult to detect, thus undercounted, with microscopy. In addition, the in situ approach applied by Dadon-Pilosof et al. (2019) measured higher salp feeding rates and smaller predator-to-prey ratios than previously determined from non in situ approaches. These data emphasize the importance of studying salps in situ for accurate feeding behavior on diverse microbial prey.

Doliolids

Doliolids, similar to salps, are barrel-shaped animals that move water from an incurrent to an excurrent siphon over a mucous mesh filter (Fig. 1B). In contrast to the muscular pumping by salps, mucociliary action transports a slow, steady current across the mucous mesh filter to capture prey particles. Numerous laboratory and field-based experiments have shown the potential for doliolids to impact microbial communities through their high water clearing rates (Paffenhöfer et al. 1995; Deibel 1998a), efficient particle capture (Tebeau and Madin 1994), and high phytoplankton production needed for doliolid blooms (Deibel 1985; Paffenhöfer et al. 1995). However, how doliolids impact microbial community structure through selective feeding is less well-studied.

Strong evidence of doliolid selective feeding comes from Walters et al. (2019) through an integrative approach that combined qPCR with qualitative sequence analysis of gut samples. This integrative approach demonstrated that doliolids capture a range of eukaryotic phytoplankton across size classes of pico- to nanoplankton, including diatoms and dinoflagellates. Diatom-specific qPCR assays revealed concentration of diatoms 10 million times above background seawater. This combined approach also enabled study of differences in feeding selectivity across doliolid life stages. Small actively growing gonozooids concentrated diatoms most, suggesting that diatoms fuel the growth of doliolid blooms. A follow-up study addressed selective feeding across doliolid life stages during bloom and non-bloom conditions through multiple techniques (Frischer et al. 2021). Molecular sequencing and qPCR from wild-caught doliolids showed a range of prey types and sizes, but larger prey (including large diatoms, ciliates, and metazoans) comprised most of the recovered gut sequences. However, the metazoan prey were not digested and assimilated. Stable isotope analysis of starved doliolids revealed isotopic signatures similar to background POM, suggesting detrital feeding in maintaining growth and reproduction, and a very complex interaction with the microbial food web (Frischer et al. 2021).

The doliolid's mechanism for selecting larger eukaryotic phytoplankton, specifically diatoms, remains unclear. Walters et al. (2019) suggest two possibilities. The ability of doliolids to position themselves in micro-layers of high diatom concentrations could enable selective feeding on diatoms. This behavior contrasts with salps, as doliolids filter without propulsion (Mouritsen and Richardson 2003; Durham et al. 2013). Another possibility is that doliolids reject undesired prey by altering their feeding currents (Walters et al. 2019). Collectively, a picture is emerging of doliolids as selective grazers on a diverse prey assemblage that includes detritus, picoplankton, and zooplankton in addition to eukaryotic phytoplankton.

Pyrosomes

Pyrosomes capture planktonic microorganisms by filtering seawater across the individual mucous meshes of hundreds of individual colonial zooids that form a hollow tube (Alldredge and Madin 1982; Godeaux et al. 1998) (Fig. 1C). These colonial animals graze just below the mixed layer at the chlorophyll maximum (Décima et al. 2019; Lyle 2020). Thus, chlorophyll *a* standing stocks can be reduced during pyrosome blooms (17.5% reduction; Décima et al. 2019).

Combinations of techniques are starting to provide insight into the selectivity and efficiency of prey capture by pyrosomes. Given their 0.6 μm mesh pore size (Bone et al. 2003), the retention of phytoplankton prey ranging from picocyanobacteria and small heterotrophic bacteria to large eukaryotic phytoplankton is possible. Stable isotope analysis on pyrosome species in different oceans showed near pure feeding on phytoplankton (Décima et al. 2019; Schram et al. 2020). FA analysis suggested selective feeding on dinoflagellates (Schram et al. 2020) and possibly ciliates, although ciliates could be symbionts rather than grazed prey (Perissinotto et al. 2007). Pigment analysis showed high concentrations of chlorophyll within individual pyrosome zooids and selection of larger eukaryotic phytoplankton prey rather than *Synechococcus* (Décima et al. 2019). 16S rRNA gene sequencing revealed the capture of a wide range of microbial prey from the picocyanobacterium *Synechococcus* to plastid sequences from eukaryotic phytoplankton lineages including centric diatoms, pennate diatoms, prymnesiophytes, cryptophytes, dinoflagellates, and prasinophytes (Thompson et al. 2021). Microscopy confirmed the presence of some lineages discovered in sequence analysis, revealed undigested phytoplankton in pyrosome guts, and quantified phytoplankton prey sizes (1–120 μm with the majority of the cells greater than 10 μm). Application of flow cytometry to pyrosome tissue and surrounding seawater showed preference for larger eukaryotic phytoplankton over smaller *Synechococcus* (confirming the pigment-based results of Décima et al. 2019). Flow cytometry also demonstrated heterogeneity between individual pyrosomes in their selective grazing. Future experiments, especially utilizing in situ approaches, could test how differences in pyrosome colony size, age, or vertical migration history influence selective feeding preferences. While pyrosomes are one of the most understudied pelagic tunicate taxa (Fig. 5C), the existing studies show pyrosome feeding on a range of phytoplankton types and sizes with preference for larger eukaryotic phytoplankton. Combined with high seawater filtration rates (Perissinotto et al. 2007; O'Loughlin et al. 2020) and massive abundances during blooms (Lebrato and Jones 2009; Archer et al. 2018; Brodeur et al. 2018; Sutherland et al. 2018), these observations signal a major role for pyrosomes in restructuring marine microbial communities and microbe-driven biogeochemical cycling.

Appendicularians

Appendicularians are unique among pelagic tunicates as they build an external mucous structure, or "house," that is renewed from 2 to 40 times per day (Sato et al. 2003; Fig. 1D). The sinusoidal beating of the appendicularian's tail sets up a feeding current where water and food particles enter through two inlet filters, are passed over a food concentrating filter, conveyed to the internal pharyngeal filter and finally to the esophagus. Particle-depleted water then leaves the house through an exit spout. The challenges of imaging such an ephemeral, three-dimensional structure, and complex flows in a free-swimming organism have made description of the feeding process challenging (Alldredge 1977; Flood 1991; Morris and Deibel 1993; Conley et al. 2018b). The different parts of the filter comprise mucus that has different mesh opening sizes and fiber sizes (Morris and Deibel 1993). Furthermore, the meshes are not uniform in their physiochemical properties; mesh fibers have varying roles that relate to pore size but likely also to stiffness, elasticity, and stickiness (Acuña et al. 1996; Conley et al. 2018b). Unlike other tunicates that consume mucus together with attached food particles, appendicularians do not consume the mucous house and therefore must unstick accumulated particles from the food-concentrating filter before consumption. High-speed micro-videography revealed how particles attach to the mucus and then are ultimately detached via pulsatile flow and filter fiber elasticity in order to be conveyed to the internal pharyngeal filter for consumption (Conley et al. 2018b). In situ three-dimensional imaging from submersibles recently showcased the complex details of structure and flow through the chambers of midwater appendicularians (Katija et al. 2020).

Appendicularians are widespread and numerous in the plankton, sometimes rivaling or even exceeding copepod densities (Landry et al. 1994; Briseño-Avena et al. 2020b). Furthermore, through rapid growth and frequent shedding of mucous houses, appendicularians have production rates that can be 10 times higher than copepods (Hopcroft and Roff 1995). The coarse inlet filters (13–54 μm) exclude large or spiny prey items, although some species lack inlet filters (Alldredge 1977). A number of grazing rates and metabolic measurements of appendicularians have been conducted in the laboratory, frequently with cultured phytoplankton (Deibel 1998b; Fig. 5D). Field studies have focused on appendicularian growth and production, which exceeded that of copepods (Hopcroft et al. 1998). Qualitative studies based on microscopy of fecal pellets indicated bacteria, cyanobacteria, pennate and centric diatoms, dinoflagellates, choanoflagellates, ciliates, and coccolithophores as important dietary constituents (Deibel and Turner 1985; Urban et al. 1992; Acuña et al. 2002). More recently, in situ sampling combined with flow cytometry and sequencing revealed that appendicularians are capable of grazing picocyanobacteria at high rates (*Synechococcus* and *Prochlorococcus*) (Scheinberg et al. 2005; Dadon-Pilosof et al. 2017) but other abundant microbes, notably *Pelagibacter ubique*, evade capture (Dadon-Pilosof et al. 2017). Prey similar in size to *P. ubique*, and 0.3 μm polystyrene beads, were captured at higher rates than *P. ubique*. This enigma could be explained by the observation that *P. ubique* has a less hydrophobic surface than other particles and therefore is less likely to stick to mucous filters, as shown with reversed-phase chromatography. In laboratory incubations, appendicularians efficiently grazed *Emiliania huxleyi* viruses (160–180 nm diameter) (Lawrence et al. 2018). The finding that appendicularians can graze on viruses extends the lower limit of possible prey resource use. Furthermore, viruses were present in discarded mucus houses and fecal pellets, suggesting a role for appendicularians in transfer of viruses to depth. Further studies could elucidate if other pelagic tunicates graze viruses and the extent to which prey surface properties, rather than size, determine susceptibility to grazing. In spite of grazing on some prokaryotic prey and viruses, the emerging consensus is that appendicularians consistently graze autotrophic eukaryotes at higher rates than prokaryotic prey (Dadon-Pilosof et al. 2017).

Pelagic tunicates link the ocean's sunlit surface and interior on local and global scales

Pelagic tunicate grazing on surface production links the sunlit surface waters and deep ocean via fast-sinking fecal pellets and detritus laden with surface-derived carbon (Fig. 6). Quantification of carbon exports mediated by pelagic tunicates demonstrates a major role in the biological pump. Observations with high-quality videography in the field and laboratory show that among gelatinous zooplankton, salps and pyrosomes have some of the highest sinking rates per day (Lebrato et al. 2013). In the North Atlantic, modeled salp contributions to vertical carbon flux revealed fecal pellets as the largest source of carbon flux, which totaled 11% of the particulate organic carbon (POC) flux in sediment traps (Stone and Steinberg 2016). A data-driven modeling-based approach revealed that global pelagic tunicate fluxes (2.1–2.6 Pg C yr^{-1}) contributed 72% of overall POC export by gelatinous zooplankton (cnidarians, ctenophores, and pelagic tunicates). Pelagic tunicate fluxes were largest primarily due to salp fecal pellets (Luo et al. 2020). The size and properties of the pelagic tunicate fluxes indicate an underappreciated role of pelagic tunicates in the global biological pump and balance of carbon between the air and sea (Luo et al. 2020). The addition of pelagic tunicates (and other gelatinous zooplankton) to ecosystem models could increase estimates of carbon deposited on the seafloor by 8–35% (Luo et al. 2020). However, data also indicate differences between pelagic tunicate taxa in the efficiency of carbon export from the surface. For example, detritus from appendicularians (i.e., discarded mucous houses) disaggregates above 200 m, thus is not a major source of carbon to the deep sea (Lombard et al. 2010). In contrast, salp carbon export is high at 200 m (Stone and Steinberg 2016). Thus, continued study of the mechanisms by which all taxa of pelagic tunicates export microbially derived carbon to the deep sea—including the relative size and density of fecal pellets and discarded mucus as well as degree of vertical migration—will further improve global predictive models.

Observations also suggest regional and seasonal variability in how pelagic tunicate feeding links the surface ocean to the deep ocean. On local or regional scales, pelagic tunicates may play an especially important role in mediating flux at the shelf break where several species bloom during optimal conditions (Madin et al. 2006; Deibel and Paffenhöfer 2009). At shelf breaks, sufficient food is available from the productive coastal waters to trigger and sustain a bloom but not clog filtration apparatus. Intrusions of cold, nutrient-rich water from cold-core eddies can spur salp and doliolid blooms well in excess of 1000 ind. m^{-3} (Deibel and Paffenhöfer 2009; Everett et al. 2011). There are also latitudinal differences in pelagic tunicate carbon fluxes. At high latitudes, sinking occurs quickly with little remineralization, while at tropical and temperate latitudes, pyrosome and salp derived-carbon decomposes above 1500 m (Lebrato et al. 2013). Furthermore, significant differences in salp contributions to carbon fluxes to the deep ocean occurred across seasons and in bloom vs. nonbloom conditions, indicating pelagic tunicate grazing as a dynamic element of the biological pump (Stone and Steinberg 2016). Further studies of bloom initiation and termination will deepen our understanding of the frequency, intensity, and impacts of blooms. More broadly, comparisons of pelagic tunicates with other key grazers in planktonic systems will help tease out their relative contributions. One of the few studies measuring pelagic tunicate and protist grazing in the same system showed that protist grazing exceeded that of salps (Stukel et al. 2021), although the authors discussed the very different contributions of the two grazer types to the biological pump.

In addition to their horizontal patchiness, pelagic tunicates exhibit nonuniform distributions in the vertical dimension. They have been observed in dense layers (~ 1 m thick) coinciding with environmental gradients in density and chlorophyll (Paffenhöfer et al. 1991; Lyle 2020). Species of salps and pyrosomes are also strong vertical migrators. *Salpa aspera* (Madin et al. 2006), *Salpa thompsoni* (Henschke et al. 2021), and *Pyrosoma atlanticum* (Angel 1989; Henschke et al. 2019) migrate to depths of 800–1000 m. Further studies of vertical distribution will be required to better understand the potential for pelagic tunicates to accelerate the biological pump.

Finally, phytoplankton can remain intact within fecal pellets (Silver and Bruland 1981; Caron et al. 1989; Paffenhöfer and Köster 2005). These cells could remain viable as they sink to depth either as prey or in diapause state that will seed future phytoplankton blooms. Thus, sinking fecal pellets may serve as a yet-to-be studied mechanism of linking surface phytoplankton to the deep ocean.

Pelagic tunicates will play a unique role under global change scenarios

Global change is predicted to influence the ecology and ecosystem function of both pelagic tunicates (Brotz et al. 2012; Lucas et al. 2014) and microbial communities (Legendre et al. 2015; Moran et al. 2016; Hutchins and Fu 2017). Long term studies (20+ yr) of pelagic tunicate abundance indicate that populations can fluctuate dramatically, by several orders of magnitude, from year to year due to changes in temperature and stratification (Ménard et al. 1994; Lavaniegos and Ohman 2003; Licandro et al. 2006).

Some large bloom events are linked to climate change. For example, salps flourish in regions of the southern ocean, sometimes outnumbering and outcompeting the krill that underlie Antarctic food webs (Perissinotto and Pakhomov 1998; Atkinson et al. 2004) and pyrosomes have expanded their range into higher latitudes off the west coast of North America (Brodeur et al. 2018; Sutherland et al. 2018). A number of variables, many of them interrelated, are influenced by changes in climate and may explain these bloom events. Changes in ocean temperature, density gradients (i.e., stratification), pH and nutrient distributions can directly influence grazers (Atkinson et al. 2004; Bouquet et al. 2018) or may indirectly influence grazing through changes in microbial cell abundance, size, shape, and community structure (Montes-Hugo et al. 2009). Even in productive regions, increases in surface temperatures drive stronger density gradients thereby limiting nutrient delivery to surface waters and can result in oligotrophic conditions favoring smaller cell sizes. Pelagic tunicates, with their propensity to form blooms and efficiently filter small particles, may be uniquely poised to capitalize on these emerging conditions and could influence biogeochemical cycling in these areas in future climate scenarios. For instance, a multi-year pyrosome bloom in the northern California Current, a biologically rich eastern boundary current, coincided with a marine heat wave (2014–2016) followed by a strong El Niño (Brodeur et al. 2018). The bloom grazed up to 22% of the phytoplankton standing stock (O'Loughlin et al. 2020), which may have shifted carbon from other basal consumers including copepods and krill with ramifications for higher trophic levels. The same heat wave in the eastern North Pacific stimulated a doliolid bloom with densities reaching 3847 ind. m^{-3} in the Gulf of Alaska (Pinchuck et al. 2021). A more detailed understanding of the particle preferences of the different pelagic tunicates and the mechanistic underpinnings of feeding selection (e.g., size limits as dictated by mesh morphology) will improve predictions of the unique role of these grazers as community dynamics shift.

Future work and conclusions

In this review, we synthesize the growing body of work on pelagic tunicate feeding pointing to a global role for pelagic tunicates in controlling the abundance, community structure, and fate of microbial carbon. Pelagic tunicates modify the pathway that microbes take through the carbon cycle; grazed microbes are exported via mucous aggregates, fecal pellets, and jelly falls (Fig. 6). These impacts are especially acute during grazer blooms, which are part of normal life history cycles (Condon et al. 2013). Against a backdrop of global change, blooms are increasing in some regions of the world (Atkinson et al. 2004; Brodeur et al. 2018). Future work should bring more quantitative studies of grazing rates and selectivity under in situ conditions, expand the number of pelagic tunicate species that have been studied, and link grazing impact on microbial communities to more dimensions of the physical, chemical, and biological ocean environment.

Future studies could integrate laboratory and in situ techniques in novel and multiscale ways to achieve unprecedented resolution of predator–prey relationships at the base of pelagic food webs. Knowledge of the mesh dimensions, fluid flow rates across the mesh, mesh stickiness, and how these parameters vary in response to environmental conditions can help define grazing rates and the particle selection across a range of oceanographic conditions. Beyond understanding grazing impact, work to resolve how pelagic tunicates mediate the biological pump will inform global carbon cycling models. In situ imagery deployed with high temporal resolution can provide detailed vertical distribution data over diel cycles (Maas et al. 2021). High-resolution imagery may also provide new insights into the settling behavior of discarded mucus, fecal pellets, and carcasses after release (e.g., Briseño-Avena et al. 2020a). Direct in situ sampling of prey-laden mucus and fecal pellets as they sink could reveal the dynamics of settlement, fragmentation, and restructuring by the microbial community as this material descends to depth. Quantifying rates of grazing, selectivity, and feeding mechanisms on microbial prey by pelagic tunicates is once again transforming our understanding of microbial ecology, the microbial loop, and of the transfer of carbon and energy through ocean food webs.

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Conflict of Interest

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