MINIREVIEW

Connecting alveolate cell biology with trophic ecology in the marine plankton using the ciliate Favella as a model

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Abstract

Planktonic alveolates (ciliates and dinoflagellates), key trophic links in marine planktonic communities, exhibit complex behaviors that are underappreciated by microbiologists and ecologists. Furthermore, the physiological mechanisms underlying these behaviors are still poorly understood except in a few freshwater model ciliates, which are significantly different in cell structure and behavior than marine planktonic species. Here, we argue for an interdisciplinary research approach to connect physiological mechanisms with population-level outcomes of behaviors. Presenting the tintinnid ciliate Favella as a model alveolate, we review its population ecology, behavior, and cellular/molecular biology in the context of sensory biology and synthesize past research and current findings to construct a conceptual model describing the sensory biology of Favella. We discuss how emerging genomic information and new technical methods for integrating research across different levels of biological organization are paving the way for rapid advance. These research approaches will yield a deeper understanding of the role that planktonic alveolates may play in biogeochemical cycles, and how they may respond to future ocean conditions.

Introduction

Planktonic alveolates (ciliates and dinoflagellates) are key members of marine planktonic communities (Sherr & Sherr, 2002; Landry & Calbet, 2004). Although often overlooked by physiologists, ecologists, and microbiologists, these protists are highly evolved unicellular alternatives to metazoan zooplankton with whom they successfully compete (Fig. 1a). As members of the microzooplankton (Box 1), these unicellular predators consume c. 60% of global marine primary production (Schmoker et al., 2013). Excretion of wastes is correspondingly high, regenerating nutrients (nitrogen, phosphorous, trace elements) that support primary production and the microbial loop (Dolan, 1997; L’Helguen et al., 2005). Planktonic alveolates also transfer substantial amounts of material energy from microbial food webs to larger metazoan consumers, such as the copepods and krill that support early life stages of some marine fish and mammals.

The importance of alveolates in marine ecosystems is due in part to life cycle adaptations: resting stages and other metabolic strategies that allow persistence during times of scarcity, coupled with high population growth rates that support rapid responses to blooms of diverse prey. The latter can include harmful algal species (Jeong et al., 2003); toxin-resistant alveolates have been implicated in the demise of harmful raphidiophyte and dinoflagellate blooms (Calbet et al., 2003; Waite & Lindahl, 2006). The ecological success of marine planktonic alveolates is also undoubtedly linked to the range and sophistication of their prey capture and predator–evasion strategies. Some species can consume the smallest planktonic organisms (including bacteria) at high rates (Strom, 1991; Christaki et al., 1999). At the other extreme, some dinoflagellates can prey on diatoms many times larger than themselves, using extracellular digestion or direct engulfment (Hansen & Calado, 1999). Another widespread alveolate nutritional strategy is mixotrophy. Photosynthetic dinoflagellates generally also consume phytoplankton and other prey (Jeong et al., 2010), while numerous dinoflagellates and ciliates harbor endosymbiont algae (Stoecker et al., 2009) or sequester prey chloroplasts (kleptoplasty) and obtain fixed carbon from these organelles (Johnson, 2011). Predator defense strategies are...
equally varied. For example, dinoflagellates may produce chemical compounds that deter grazing (Kubanek et al., 2007), and both ciliates and dinoflagellates can modify their swimming behavior and exhibit defensive morphologies to evade predation (Stoecker & Sanders, 1985; Jakobsen, 2001; Jakobsen et al., 2006).

These diverse behavioral strategies and responses clearly underpin planktonic alveolate ecological success. Nonetheless, we know little about their sensory biology, in particular, how environmental cues are sensed and transduced into behavioral responses. Exciting recent work has shown that planktonic alveolates respond to microscale heterogeneity in environmental cues (Mitchell et al., 2008; Seymour et al., 2010; Harvey & Menden-Deuer, 2011). Clearly, the utilization of cell signaling pathways is critical in allowing microzooplankton to successfully navigate such patchiness in their biotic and abiotic environment. In addition the aggregate of these sensory interactions between microzooplankton and prey- or predator-associated cues results in population and community-level responses. However, research on planktonic alveolates (and other groups of marine microorganism) is largely conducted in a discipline-specific manner, that is, either at a cellular/mechanistic level, or with a population ecology emphasis. Moreover, what we do know of alveolate cell signaling and its links to behavior comes primarily from study of model organisms such as the ciliates Paramecium and Tetrahymena. These organisms have served as useful models providing insight into alveolate sensory biology, specifically with regard to conserved sensory mechanisms (as discussed below). However, they differ in critical ways from marine planktonic alveolates: being freshwater, benthic, largely bacterivorous, and adapted to nutrient- and detritus-rich habitats. Further, they exhibit little of the morphological specialization that typifies the planktonic ciliates (Fig. 1b). These limitations – marginally relevant model organisms and lack of an interdisciplinary approach – hamper our ability to understand how sensory biology regulates marine alveolate behaviors and to predict the population and community-level outcomes of behavioral traits. An interdisciplinary focus on representative organisms is needed to address these questions and ultimately to develop broadly applicable ecosystem models that incorporate the behavioral traits of marine planktonic alveolates.

**Purpose of this review**

There is a need for several model organisms to adequately represent the diversity of planktonic alveolates. It is therefore important that both dinoflagellates and ciliates are developed as models for studies that link cell biology to
Microzooplankton are historically defined as heterotrophic and mixotrophic members of the plankton that are 20–200 μm in size (although this size classification may vary based on context) and inhabit fresh and marine waters. Most are unicellular eukaryotes (protists) that consume a variety of living prey or detrital particles (phagotrophy) and in some cases may supplement nutrient acquisition with uptake of dissolved nutrients (osmotrophy) by parasitism or use a combination of strategies. Smaller microzooplankton eat bacteria (bacterivores) or the smallest eukaryotes (< 2 μm, termed picoplankton), while others consume a variety of nanoplanckton (2–20 μm) or microplankton (20–200 μm) prey. Some of these particle feeders harbor endosymbiotic algae; others retain chloroplasts of ingested algal prey and use these for photosynthesis (chlorokleptoplasty). Still others have their own plastsids, but also consume prey cells. Mixotrophy describes these diverse trophic combinations.

Alveolates are a eukaryotic phylum consisting of ciliates, dinoflagellates, and apicomplexa. All are derived from a photosynthetic ancestor, but only some dinoflagellate species retain functional chloroplasts. Some of these photosynthetic dinoflagellates make neurotoxins and are important contributors to harmful algal blooms (HABs); most are mixotrophic. Alveolates are characterized by sac-like alveoli that underlie the cell surface, which store Ca²⁺ for rapid release, representing an evolutionary alternative to sarco-endoplasmic reticulum of animal muscle cells.

Ciliates are obligate predators, and are classified by the arrangement of their cilia around the cell. Some of the more highly evolved groups display specialization of feeding (oral) and locomotory cilia in specific areas of the cell (Fig. 1b). Tintinnids, an important group of choricroth (dancing hair!) ciliates in the pelagic realm (upper water column), have a protective lorica (Latin for body armor) that is known to be composed of protein in some genera, but whose chemical composition remains uncharacterized in others. They can retract into the lorica via a contractile stalk (spasmoneme) to avoid danger. Like most planktonic ciliates, their cilia are fused into bundles of adoral membranelles. These are used in complex sensory and behavioral processes such as detecting prey or modifying swimming behaviors, to move or orient the cell in response to environmental cues, such as those presented in prey patches. The adoral membranelles form a ring around the peristomial cavity where food particles are processed. Tentaculoids (flap like cytoplasmic extensions) may be interspersed among adoral membranelles and aid in particle capture. Particles are ingested at the cytostome, located at the edge of the peristomial cavity, pass through the cytopharynx, and are encapsulated in digestive vacuoles. Hypotrich ciliates are even more highly specialized, using clusters of cilia (cirri) to ‘walk’ on the sediment surface (benthos).

Phagocytic ciliates and dinoflagellates feed by several distinct strategies: ambush feeding, filter feeding, and feeding by direct interception. Feeding capabilities can be quantified by clearance rate: the equivalent volume of water processed to remove particles per unit time. Some alveolates also have secretory proteinaceous organelles (trichocysts) that can be rapidly released for offense or defense.

Box 1. Small but complex cells: a microzooplankton primer for cell biologists

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Trophic ecology. The dinoflagellate Oxyrrhis has many advantages for use as a model planktonic dinoflagellate, and interested readers are directed to a recent collection of articles covering this topic (The Journal of Plankton Research, Volume 3). To connect alveolate cell biology to population-level ecology, we have focused on marine ciliates, specifically Favella (Claparede and Lachmann, 1858), a tintinnid ciliate (Fig. 1c and d, Box 1) recently proposed as a model for studying cellular mechanisms that regulate ecological interactions (Montagnes, 2013).

Favella have a long history of study, with the first biological study probably by Campbell (1927). A variety of aspects of Favella biology have subsequently been studied; these studies represent the most complete account of lorica formation and variability (Laval-Peuto, 1981, 1983) and factors affecting encystment and excystment (Kamiyama & Aizawa, 1987; Kamiyama, 1994; Kamiyama et al., 1995). For a more general overview of Favella biology, the reader is directed to excellent recent texts that thoroughly review the characterization and classification of ciliates (Lynn, 2010) and the use of tintinnids as models in biological and ecological studies (Dolan et al., 2013). Due to their protective shell (lorica), tintinnids are more readily sampled and cultured, and thus their ecology is better understood than that of naked choricroth. Moreover, unlike other potential models for examining selective feeding behavior such as the planktonic alveolate dinoflagellate Oxyrrhis marina (Davidson et al., 2011; Roberts et al., 2011) Favella have a relatively broad geographic distribution and are found in neritic temperate–tropical plankton communities (Dolan & Pierce, 2013). In addition, Favella is a large cell (c. 150 μm) amenable to a range of biophysical and imaging techniques that are critical for understanding the cell signaling mechanisms that regulate trophic behaviors. They are also more tractable for genomic studies that are important for identifying cellular machinery involved in signal transduction. Favella sp. fulfill a number of requirements that have been proposed as prerequisite for a model species including its general biological relevance, the fact that it is relatively easy to culture, and its usefulness for interdisciplinary study (Montagnes et al., 2011).
The remainder of this review focuses on planktonic ciliates, using Favella as a model organism. We review research on the population ecology, cell behavior and cellular/molecular biology of Favella and other ciliates in the context of their sensory biology. Importantly, planktonic ciliates have a shared evolutionary history so that conserved signal transduction machinery between ciliates and dinoflagellates may be similar despite behavioral and ecological differences between them. Our goal is to stimulate interdisciplinary research examining the mechanistic basis of ecological interactions in marine ciliates (and planktonic ciliates in general) and to suggest how this research can be used to inform predictions of planktonic ciliate population and community dynamics.

**Trophic behaviors of ciliates**

Marine planktonic ciliates fill a variety of trophic niches (Box 1). Collectively, they feed on a wide range of prey including phytoplankton, bacteria, detritus and microzooplankton such as flagellates and other ciliates; some species also acquire nutrition through kleptoplasty (Stoecker et al., 1981, 1987; Stoecker & Evans, 1985; Christaki et al., 1999; Schoener & McManus, 2012). Ciliates have a repertoire of feeding behaviors that suit their different nutritional strategies. Larger prey items may be captured by direct interception (M.L. Echevarria and A.R. Taylor, unpublished) or by ambush predation (Jakobsen et al., 2006). In contrast, bacterivores apparently filter pico-plankton from the water (Christaki et al., 1998). Ciliates can sense and respond to mechanical (touch), hydrodynamic (shear, pressure) and chemical cues from prey to increase consumption rates and efficiencies (Buskey & Stoecker, 1988; Lueken et al., 1996; Jakobsen et al., 2006). The range of predators on ciliates and the corresponding ciliate defense strategies are similarly diverse. They may be consumed by dinoflagellates, by other ciliates, and by larger metazoan predators such as copepods and jellies (Stoecker & Evans, 1985; Stoecker & Sanders, 1985; Uchida et al., 1997). Dinoflagellate parasites may also compete with predators for tintinnids as a food source and promote retention of nutrients in the microbial loop (Coats et al., 1994, 2010; Coats & Bachvaroff, 2013). Tintinnid ciliates are surrounded by a protective lorica (Fig. 1) that likely serves a variety of functions that may include regulation of buoyancy, regulation of swimming by modifying fluid flow, increase of feeding rate efficiency by either acting as a tether to detrital particles or increasing fluid flow in the region of cilia, and protection from ultraviolet radiation (Dolan, 2013). Here, we focus on the defensive capabilities the lorica may confer against predation (Stoecker, 2013). In contrast, naked ciliates such as Strobilidium spiralis, Mesodinium pulex, and Balanion co-

**Feeding behavior of Favella: From detection to consumption**

Planktonic ciliates subsist on a dilute suspension of food particles that vary in size, motility, nutritional value and toxicity. Therefore, they have evolved sophisticated behaviors for detecting and consuming preferred prey and avoiding others (Fig. 2a–c, Box 1). Selectivity can vary even among closely related species: within the genus Favella, F. erehenbergii feed preferentially on dinoflagellates over other groups of phytoplankton, whereas other species (e.g. F. campenula and F. serrata) appear to be less selective (Gold, 1969; Blackbourn, 1974; Stoecker et al., 1981). Such variation can have profound ecological consequences; for example, predators that reject toxic cells in favor of benign prey in mixed communities might enhance harmful algal bloom formation and persistence, while less selective predation would contribute to bloom reduction.

Prey selection may occur during any of four successive feeding stages: searching, prey contact, capture and processing, and ingestion (Montagnes et al., 2008).

**Searching**

Favella exhibits a helical swimming pattern that comprises a circular swimming path and rotation of the cell along the longitudinal axis. Aggregation or dispersion in response to environmental cues (Fig. 2) is modified by altering the rate of longitudinal rotation relative to the radius of the swimming path (Montagnes, 2013) and by periodic bouts of backward swimming and turning. These swimming modifications represent a kinesis (nondirected changes in behavior in response to a locally perceived signal) rather than a true taxis (directed swimming along a perceived signal gradient). Observation of swimming behavior in Favella reveals both chemokinetic and mechanokinetic responses, although the underlying sensory reception and transduction pathways are unknown. Cues leading to increased aggregation include preferred dinoflagellate prey cells, equivalently sized beads and filtrate from cultures of preferred prey (Buskey & Stoecker, 1988). Similarly, other tintinnids such as Tintinnopsis sp.
and *Strombidium* sp. exhibit apparent chemotactic (but likely chemokinetic) responses to phytoplankton prey, with the resulting direction of travel dependent on prey physiological condition (Verity, 1988). Thus, *Favella* are selectively sensitive to both chemical and physical prey cues (Fig. 2).

Chemical cues such as cyclic nucleotides, amino acids, vitamins, dimethylsulfonio propionate (DMSP), and
related compounds are attractants for some freshwater and marine planktonic alveolates (Yang et al., 1997; Bell et al., 1998; Van Houten, 1998; Seymour et al., 2010). In apparent contrast, DMSP and dissolved amino acids caused decreased feeding rates in Favella and other ciliates, although whether these decreases were related to changes in swimming behavior is unclear (Strom et al., 2007). Dispersive swimming behavior by Favella was not seen in a study with a toxic alga, either, although the salinity gradients used to generate prey patches were a covariate that could have masked the effect of the alga (Harvey & Menden-Deuer, 2011). Aggregative swimming in the presence of mechanical (contact) cues is attributed to increased frequencies of ciliary reversals (Fig. 2b) that result in periods of backward swimming, decreased net displacement, and increased rates of directional change (Buskey & Stoecker, 1988; Stoecker et al., 1995). This is supported by high-speed video microscopy of tethered Favella in which dinoflagellate prey cells (and microspheres of similar size) consistently elicited ciliary reversals upon contact with adoral membranelles, while smaller cells and microspheres rarely did so (Stoecker et al., 1995). The ability of Favella to sense and respond to a diverse range of prey cues with modifications in swimming behavior suggests they possess complex sensory mechanisms that regulate these behaviors.

Contact

Together with optimized searching behaviors, the contact probability, governed by relative size, and swimming speed of predator and prey, will influence feeding rates (Gerritsen & Strickler, 1977). In addition to physical size of predators and prey, the extent of the perception field of the predator is influenced by its sensory capabilities. Despite considerable research on Favella, relatively little empirical evidence is available to determine their overall feeding strategy, that is, how do they remove particles from the water? Recent theoretical estimates indicate that microzooplankton in general cannot achieve sufficiently high clearance (and consequent prey encounter) rates to sustain observed growth unless they function as relatively passive filter feeders (Kjørbøe, 2011). In addition hydrodynamic constraints are believed to restrict microzooplankton such as Favella to filter feeding strategies (Kjørbøe, 2011). These contentions are at odds with experimentally observed searching and prey selection behaviors. These behaviors, which remain unaccounted for in such theoretical approaches, almost certainly enhance alveolate feeding rates and the nutritive value of their diet and support the contention that alveolate sensory capabilities play a critical role in their ecological success.

The specialized morphology of planktonic ciliates also contributes to prey capture success (see Fig. 1b). Favella captures small (4 μm) cells less efficiently than larger (20 μm) cells because the former pass through the spaces between adoral membranelles (Stoecker et al., 1995; see Fig. 1d), but more sophisticated behaviors appear to be at play, as well. High-speed video microscopy shows that preferred prey frequently contact the adoral membranelles, while toxic prey of similar size are rarely contacted (Taniguchi & Takeda, 1988). Apparently Favella may modify feeding and swimming currents, and thus encounter rates, based on dissolved chemical cues from prey. However, because feeding and swimming currents in Favella are interrelated, it is not clear how these observations would apply to free-swimming cells. Understanding how Favella make contact with prey particles is an important area of future research that may be aided by recently developed techniques as discussed below in ‘Future research and challenges’.

Capture

Following contact, particles are captured; this may be aided by tentaculoids that are cytoplasmic extensions among the ring of adoral membranelles (Montagnes, 2013). Particles are then processed and may be selectively ingested or rejected based on receptor–ligand and electrostatic interactions (Montagnes et al., 2008). Taniguchi & Takeda (1988) observed that toxic cells of the raphidophyte Heterosigma akashiwo were ejected from the peristomal cavity upon contact. The mechanistic basis for this processing behavior is unknown, although it most likely involves rapid biochemical recognition of H. akashiwo at the peristome or membranellar band by Favella (Fig. 2c). Such particle recognition could lead to selective feeding behavior in prey mixtures, as was demonstrated in feeding experiments: Favella consumed low levels of H. akashiwo when it was presented alone, but selectively avoided ingesting it when other nontoxic species were also present (Graham & Strom, 2010). Particle recognition and handling behaviors are clearly important components of the selective feeding for Favella and most other planktonic alveolates. The range of plasticity of such behaviors and their modification in response to environmental cues are almost completely unknown.

Ingestion

The final steps in the feeding sequence of Favella involve phagocytosis via the cytopharynx, formation and maturation of the food vacuole, recycling of vacuolar membrane, and expulsion of residual waste, although this process has not been studied in detail at the cellular level (see
Fig. 1d). The rate of particle ingestion by Favella and other ciliates may be ultimately limited by the recycling of vacuolar membrane material (Verity, 1991; Radek & Hausmann, 1996; Hausmann, 2002). On shorter time scales, Favella will reject prey while a newly formed digestive vacuole remains at the cytopyharynx (Taniguchi & Takeda, 1988). These observations indicate that the ciliate must sense satiety but the intracellular mechanisms involved are unknown. It is possible that the metabolite DMSP (Strom et al., 2003) and perhaps amino acids (Strom et al., 2007) produced by phytoplankton prey act as chemical cues to inhibit feeding in Favella by activating a satiety response. Such responses have the potential to substantially alter individual and population predation rates, and shape the composition of the prey community.

**Predator avoidance: evasion and escape**

Studies of Favella and other ciliates have demonstrated their capability of sensing and responding to chemical, mechanical, and hydrodynamic stimuli from predators (Fig. 2d). Although a response to predator chemical signals has not yet been shown in Favella, these signals can be important in other ciliates. The marine ciliate Euplotes octocarinatus increased motility and decreased foraging behaviors when exposed to chemical cues from a flatworm predator (Hammill et al., 2009), which led to decreased ciliate mortality. The same ciliate exhibited avoidance reactions in response to chemical cues from an ameboid predator, but only if had been previously exposed to predator chemical cues, suggesting inducible defense reactions (Kusch, 1993a, b). Ciliates can respond to hydrodynamic stimuli from copepods by performing ‘escape jumps’: periods of high-velocity, directed swimming away from copepod feeding currents (Broglio et al., 2001; Wu et al., 2010). The fluid deformation produced by predator feeding currents, rather than velocity or acceleration, is thought to trigger this response (Jakobsen, 2001). Although Favella do not perform escape jumps, anecdotal observations (M.L. Echevarria and A.R. Taylor, unpublished) suggest that Favella exhibit periods of backward swimming to escape from suction produced by a pipette.

Perhaps the most obvious form of defense/avoidance in the tintinnid ciliates is withdrawal into the proteinaceous lorica by means of a contractile cytoplasmic stalk (see Fig. 1d). A copepod predator was able to remove Favella cells from their loricas, leaving empty loricas at the end of the experiments; however, crushed loricas with live intact cells were also observed, supporting a protective role for this structure (Stoecker & Sanders, 1985). The lorica probably provides less protection against predatory dinoflagellates, which were able to extract and consume the cell bodies of tintinnid Eutintinnus tubulosus even though the ciliates were fully contracted into their loricas (Uchida et al., 1997). The cell body of Favella contracts into the lorica in response to mechanical stimulation of the membranelles (see below), which may aid in survival against at least some predators (Fig. 2d). The range of cues triggering this behavior is unknown.

**Cellular mechanisms governing planktonic ciliate behaviors**

**Sensory mechanisms**

The complex behaviors outlined above begin with sensation. However, the sensory mechanisms and signal transduction machinery utilized by planktonic ciliates, that ultimately give rise to population-level responses, are virtually unknown. The cell biology and genetics of several model ciliates has been extensively described, although rarely in the context of their trophic interactions – not surprising given this type of interdisciplinary research is still in its infancy – even among well-studied metazoans (Bargmann, 2006). Because most of the work in this area has focused on Tetrahymena and Paramecium, they are a starting point from which to begin exploration. For example, ionotropic signaling mechanisms – those governed by voltage-, chemo-, or mechano-sensitive ion channels (Box 2) – are well studied in model ciliates. These species possess numerous ion channel variants localized to discrete regions of the cell, resulting in finely tuned responses to stimuli (Connolly & Kerkut, 1983; Machemer, 1989; Machemer & Sugino, 1989; Machemer et al., 1998). Calcium is a particularly important signaling molecule in regulating ecologically significant ciliate behaviors (reviewed by Plattner (2014) for Paramecium tetraurelia, Box 2). For example, ciliary beating in P. tetraurelia is regulated by localized oscillating waves of Ca2+ in the cilia, the patterns of which are shaped by Ca2+ release from and sequestera in alveoli (Plattner et al., 2006) combined with infra-ciliary Ca2+ buffering (Sahling et al., 2009). Such exquisite localized control of Ca2+ dynamics allows regulation of a multitude of processes. For instance during Ca2+-mediated exocytosis Ca2+ release from alveoli is also sufficient to trigger cilia reversals and backward swimming, but Ca2+-mediated ciliary reversals do not result in exocytosis due to the infraciliary Ca2+ buffering (Huser et al., 2004). The repertoire of spatial and temporal Ca2+ patterns that underlie behavioral responses in ciliates is extended by different isoforms of the Ca2+ binding protein calcineurin that have variable roles in regulating exocytosis, calcium dynamics, and backward swimming (Fraga et al., 2010). Ciliates also possess metabotropic – enzyme-linked or second messenger-linked – signaling machinery (Box 2) similar to that in metazoans. G-protein-coupled receptor
Box 2. Converting signals to behavior: a primer for ecologists

Like all eukaryotes, alveolates employ several basic signal transduction mechanisms. Ionotropic signaling mechanisms involve ion channels. The cell’s membrane potential ($V_{mem}$, net negative inside) is maintained by electrogenic pumps (e.g. Na$^+$/K$^+$ pumps) that, together with ion channels, set the steady state $V_{mem}$. Steady-state $V_{mem}$ can be depolarized (made less negative) or hyperpolarized (made more negative) by changes in either ion concentrations or ion channel activity, which in turn activates voltage-gated ion channels to cause changes in $V_{mem}$. Other chemosensitive ion channels are activated by ligand binding (ion/cyclic nucleotides e.g. cAMP or cGMP, or organic solutes) while mechanosensitive ion channels respond to physical deformation of the cell membrane. Ionotropic components can be studied with microelectrodes to monitor changes in $V_{mem}$ or ion currents.

Calcium signaling plays a critical role in alveolates. For example, transient depolarizations may activate voltage-activated Ca$^{2+}$ channels to trigger release of Ca$^{2+}$ from alveolar sac stores, leading to rapid increase in cytoplasmic Ca$^{2+}$ that results in changes in ciliary beating, causing reversals or other cell movements. Action potentials (APs) are rapid all-or-nothing depolarizations that propagate spatially around the cell and result in a large influx of Ca$^{2+}$ that causes contraction of the cell into the lorica. Most commonly associated with neuromuscular systems of multicellular animals, they are in fact common in many groups of unicellular eukaryotes (Taylor, 2009), and utilized in coupling environmental sensing and behavior in alveolates. As with the sarcoplasmic reticulum of muscle cells, the alveolar Ca$^{2+}$ stores of alveolates are replenished by Ca$^{2+}$ pumps. In addition calcium induced calcium release channels (CICRs), such as the ryanodine receptors (RyR) are common in metazoan muscle and neuronal cells as well as in alveolates. They mediate the release of Ca$^{2+}$ from the sarcoplasmic reticulum in metazoans and alveoli in alveolates. Calcium release channels can in turn stimulate membrane trafficking and fusion by SNAREs.

Metabotropic signaling machinery involves enzyme-linked mechanisms similar to those that are well studied in metazoans. Membrane-spanning GPCR bind external solutes and trigger signal cascades inside the cell via GTP hydrolysis via second messenger-linked cascades. Common downstream pathways involve adenyl cyclase, which catalyzes the conversion of ATP to cyclic AMP (cAMP), often leading to phosphorylation of targets by protein kinase A. This can ultimately activate ion channels, linking metabotropic and ionotropic pathways.

Finally, lectins (carbohydrate-binding proteins) on the cell’s surface can identify specific carbohydrate moieties and trigger signal cascades; this is a highly conserved signaling.

(GPCR) signal transduction pathways, in particular, are critical for chemo- and mechano-sensation of predators and prey (Yang et al., 1997; Marino et al., 2001; Ramoino et al., 2003; Rosner et al., 2003; Kissmehl et al., 2006; Walerczyk et al., 2006; Bartholomew et al., 2008). Additionally, freshwater ciliates have been proposed as model systems for studying the relationship between sensory mechanisms and cell structure (Hufnagel, 1992). Although these conserved signaling mechanisms are likely to be common to freshwater and marine ciliates, freshwater ciliates have less complex morphologies and different ecological functions. Therefore, the ways that these two groups utilize these molecular mechanisms are expected to be very different.

Mechanistic basis of movement

Ciliate movement is tightly regulated by the bioelectrical activity of the cell. Mechanical and chemical stimuli from predators and prey interact with cell surface receptors and downstream signaling pathways to modulate this bioelectrical activity and associated behavioral responses. Marine planktonic ciliates generally have an oral cavity surrounded by a ring of adoral cilia (see Fig. 1b) that generate swimming and feeding currents. These ciliary bands also serve a sensory function during prey and predator interactions, leading to more complex behavioral adaption. Most of the work investigating cellular mechanisms that regulate movement has utilized the bacteriovorous freshwater models Tetrahymena and Paramecium. We are unable to review the extensive literature on freshwater ciliates, but refer readers to reviews of pioneering work by Van Houten, Preston, and others (Naith & Sugino, 1984; Preston & Saimi, 1990; Stock et al., 1997; Van Houten, 1998, 2000; Hennessey & Kuruvilla, 1999; Van Houten et al., 2000). Here, we focus on the marine hypotrich Euplotes, to develop hypotheses regarding the bioelectrical machinery that might underlie signaling processes in Favella. Although Euplotes is a benthic bacteriovore that creeps along the substrate, it has cilia arranged in complex subsets for specialized function (like Favella). The movement patterns of Euplotes sp. have been well described in a detailed ethogram that contains three different types of side stepping reactions that create a characteristic movement pattern (Ricci et al., 1988). The beat patterns of different subsets of cirri and the mechanisms by which they function to propel Euplotes crassus were subsequently described by Erra et al. (2001). Euplotes also inhabit an environment with an ionic composition similar to that of Favella. Therefore, the signaling capabilities of Euplotes — specifically those related to bioelectrical responses (reviewed in (Machemer, 1996; Machemer &
Teunis, 1996; Machemer, 2001) – may more closely resemble those of Favella.

**Interactions with prey**

Mechanical cues from prey are known to cause aggregative searching behaviors in Favella, as described above. Our recent work (M.L. Echevarria and A.R. Taylor, unpublished) shows these searching behaviors are under direct bioelectrical control. For example, contact of prey with adoral membranelles initiates transient depolarizations that result in ciliary reversals and backward swimming that cause aggregation of Favella in patches of prey (Fig. 3), translating prey cues to changes in Favella cell distributions. While the bioelectrical machinery that regulates this behavioral response in Favella has yet to be fully elucidated, the marine hypotrich Euplotes vannus serves as a useful proxy. (Krüppel et al., 1993; Lueken et al., 1996; Stock et al., 1997). When E. vannus are mechanically stimulated at the cell’s anterior, stretch-activated ion channels allow Ca$^{2+}$ influx, triggering depolarization via voltage-activated Ca$^{2+}$ and Na$^{+}$ channels that leads to backward creeping responses (Krüppel et al., 1993, 1995). Normal forward walking resumes once voltage-activated K$^{+}$ channels return $V_{\text{mem}}$ to resting levels (Krüppel et al., 1995). Similar bioelectrical mechanisms appear to regulate chemosensory processes, causing E. vannus to aggregate in patches containing dissolved chemical cues from prey (Lueken et al., 1996). Interestingly, these chemical cue-induced sidestepping reactions of E. vannus are regulated by transient depolarizations, much as we observe for Favella (Fig. 3). How such dissolved chemical cues are initially perceived by planktonic ciliates is poorly understood, although GPCR signal transduction pathways are likely important in regulating chemosensory searching behaviors. The GPCR gene family is well described in model freshwater ciliates (see below) and conserved among marine planktonic organisms (Port et al., 2013). Moreover, in behavioral experiments with the marine ciliate Uronema sp. and the dinoflagellate O. marina, pharmacological inhibitors of GPCR pathways influenced chemosensory and predation responses (Hartz et al., 2008).

There is good evidence that the final step of prey capture and selection by planktonic alveolates involves receptor–ligand interactions between the cell surfaces of both predator and prey. Lectins (carbohydrate-binding proteins) are widely used as surface-recognition systems in eukaryotes (Lis & Sharon, 1998) and have been detected in a range of marine alveolates, including ciliates (Roberts et al., 2006). In the dinoflagellate O. marina, cell surface lectins function in prey selection and capture by binding specifically to mannose residues on the surface of prey particles. Blocking these lectins with mannose decreased ingestion of preferred prey by up to 60% (Wootton et al., 2007). Similarly surface proteins anchored to the...
extracellular surface by glycosylphosphatidylinositol play an important role in predator–prey recognition processes in freshwater ciliates (Simon & Kusch, 2013). These studies suggest fruitful avenues for investigation of the unknown particle recognition processes employed by marine planktonic ciliates.

**Predator evasion**

Few studies have explicitly addressed the cellular mechanisms that regulate predator evasion in microzooplankton. We recently demonstrated that contraction of the *Favella* cell into the lorica is caused by action potentials (APs), rapid all-or-nothing depolarizations (M.L. Echevarria and A.R. Taylor, unpublished). These events bear superficial similarity to excitation–contraction in the sessile freshwater ciliate *Vorticella* sp., whose cell body is attached to its substrate by a long stalk comprising an ordered array of Ca²⁺-dependent contractile proteins and filaments (the spasmoneine) that rapidly coils in response to mechanostimulation in an intensity-dependent manner (Katoh & Naitoh, 1992). The underlying mechanism involves a mechanically induced depolarization leading to rise in intracellular Ca²⁺ that activates the contractile proteins of the spasmoneine (Katoh & Kikuyama, 1997). The increase in stalk Ca²⁺ concentration is mediated by Ca²⁺-induced Ca²⁺ release from membranous tubules adjacent to the stalk (Katoh & Naitoh, 1994). These membranous tubules therefore function analogously to sarcoplasmic reticulum of animal muscle cells. Predator evasion in tintinnid ciliates such as *Favella* may well be mediated by a similar excitation–contraction mechanism.

The best-studied predator–evasion model in ciliates is the *Didinium–Paramecium* bi-ciliate trophic interaction (Wesenberg & Antipa, 1970), for which the cellular mechanisms are known (Hara & Asai, 1980; Hara et al., 1985; Miyake & Harumoto, 1996; Iwadate et al., 1997, 1999, 2004). When predatory *Didinium nasutum* contact *Paramecium* spp., membrane depolarization triggers a rise in intracellular Ca²⁺ and a discharge of toxicysts, toxin bearing extrusive organelles that immobilize and capture the *Paramecium* spp. (Iwadate et al., 1999). In defense, *Paramecium* may discharge nontoxic trichocysts in a Ca²⁺-dependent manner to propel themselves away from predators (Wesenberg & Antipa, 1970; Hara & Asai, 1980; Hara et al., 1985; Iwadate & Kikuyama, 2001). Extrusive organelles are a common feature among alveolates (Kugrens et al., 1994; Rosati & Modeo, 2003), and some oolocytic ciliates are known to possess them (Modeo et al., 2001). This is supported by observations of the predatory ciliate *Litonotus lamella* that discharge toxicysts into its prey, *Explotes*. The toxicysts depolarize the prey and thus prey presumably affect their movement and ability to escape (Morelli et al., 2002).

**Genomic basis of sensation and behavior**

The examples above clearly illustrate that similar cellular mechanisms may regulate behaviors in diverse groups of ciliates. Are similar commonalities present at the genomic level? Over the past decade genomic research has yielded a great deal of information on the sensory mechanisms that ciliates possess. Ciliates have evolved through multiple rounds of endosymbiosis (Keeling, 2010), and their genetics are dauntingly complex, so these studies have generally been limited to a few model taxa. Ciliates have both somatic micronuclei that govern gene expression during growth, development and asexual reproduction, and germine micronuclei for gene exchange, and recombination during sexual reproduction (Orias et al., 2011). The genomes of ciliates are very large, repetitive and have unusual codon usage. However, over the past decade pioneering genomic studies on *Paramecium* and *Tetrahymena* (Aury et al., 2006; Eisen et al., 2006) have revealed these organisms possess molecular machinery similar to that used by multicellular organisms, with a high potential for functional specificity due to multiple paralogs arising from repeated gene duplication and divergence. For example, *Paramecium tetraurelia* has more K⁺ channel genes than humans (Haynes et al., 2003), and the 165 ATP-binding cassette transporter genes found in *Tetrahymena thermophila*, important in regulating influx and efflux of a wide variety of ions and biomolecules, dwarf the 48 known in humans (Xiong et al., 2012). The impressive size and diversity of these gene families indicate that ciliates possess a sophisticated sensory toolkit that underlies their sensory biology.

Genetic approaches have also given insight into the molecular mechanisms ciliates utilize at each step of the feeding process, from searching for prey through digestion. Searching behaviors that are dependent on chemoreception rely on metabotropic signaling processes such as the GPCR signal transduction pathway (Box 2). This was evidenced by the creation of a knockout mutant of *T. thermophila* deficient in GPCRs; this mutant lost the ability to respond to chemical attractants (Lampert et al., 2011). Genes for adenylyl cyclase, important in the GPCR signal transduction pathway, were cloned from *P. tetraurelia* and found to localize to cilia, where they potentially regulate the K⁺ channels that control ciliary movements, thus linking metabotropic signaling to behavior (Weber et al., 2004). Based on these genomic studies in model ciliates, it is reasonable to propose that in marine
planktonic ciliates, chemical cues from prey or predators may act to alter swimming behavior via a GPCR-cAMP-ion channel mechanism.

Once food particles have been successfully contacted, phagocytosis occurs through the cooperation of several different molecular components. GPCRs are also important in regulating phagocytosis, as knockdown of a putative GPCR in Tetrahymena greatly reduced phagocytosis of Salmonella (Agbedanu et al., 2013). Cytoskeletal regulation of phagosome formation is critical to successful ingestion of particles. This has been demonstrated using a knockdown of a homolog to the actin-modulating protein ADF/cofilin in T. thermophila; this mutation decreased the rate of food vacuole formation (Shiozaki et al., 2013).

Food particle digestion also requires tightly regulated membrane dynamics during phagosome formation, vesicle fusion, exocytosis, and membrane recycling. These processes use components of the highly conserved eukaryote membrane trafficking system (Dacks & Doolittle, 2004; Dacks et al., 2009), including a superfamily of proteins known as SNAREs (soluble N-ethylmaleimide sensitive factor attachment protein receptors) that, together with calcium release channels, mediate the membrane fusion events critical to many cell signaling processes. Genes coding for a group of SNARE proteins known as syntaxins have been identified in P. tetraurelia and are associated with vesicles in the region of the cytosol and cytopharynx (Schilde et al., 2010). In terms of calcium release channels, 34 candidate genes including those for inositol 1,4,5-triphosphate receptors and ryanodine receptors were identified in P. tetraurelia, where different paralogs localized to different areas of the cell, including the phagosome, endosome, and oral cavity (Ladenburger & Plattner, 2011). After food particles have been successfully ingested and compartmentalized, phagosomes mature into digestive vacuoles (see Fig. 1d). This process occurs through the action of vacuolar-type H+-ATPases that acidify the phagosome, facilitating prey digestion. In P. tetraurelia, RNA interference targeted to vacuolar-ATPase subunits inhibited phagosome formation, indicating that acidification of phagosomes might be necessary to signal the transformation of phagosomes into food vacuoles (Wassmer et al., 2009).

Genetic approaches have also demonstrated molecular mechanisms involved in defense against predators. GPCR knockout mutants of the ciliate Stentor coerulescens had increased mechanosensitivity, indicating that G proteins may influence the detection of predators by mechanical or hydrodynamic stimuli (Marino et al., 2001). Recently, homologs for genes that code for the proteins Piezo1 and Piezo2, responsible for mechanosensitive ion currents in mammals, were also found in the genome of T. thermophila and P. tetraurelia (Coste et al., 2010, 2012). This suggests a highly conserved mechanism for sensing mechanical cues across all eukaryotes, although their functional role in ciliates has yet to be determined. Finally, the important role that Ca2+ channels play in downstream signal transduction of environmental cues is illustrated by their role in defense mechanisms. Gene silencing of calcium release channels associated with the intracellular Ca2+ stores in P. tetraurelia impaired their ability to discharge defensive trichocysts (Ladenburger et al., 2009).

Although molecular insights from freshwater bacterivorous ciliate genomes provide a useful template from which to work, newly available Moore foundation marine microbial eukaryotes transcriptomic information (http://marinemicroeukaryotes.org) will greatly enhance our ability to probe the sensory biology of marine planktonic algae. There are 21 ciliate and five heterotrophic dinoflagellate species in the pipeline at present, as well as 31 species of ‘autotrophic’ (almost certainly mixotrophic) dinoflagellates. These emerging transcriptomes will provide a wealth of information on signal transduction machinery and will help identify molecular targets that might be important for trophic biology. Comparative transcriptomics has the potential to yield information that can address specific targeted hypothesis related to trophic behaviors. For instance, transcriptomic data are now available for Favella taraikaensis fed nontoxic Heterocapsa sp. and toxic H. akashiwo which may provide insights into the signal transduction machinery specifically involved in selective feeding on nontoxic vs. toxic prey.

**Developing a working model of sensory mechanisms of Favella**

To stimulate interdisciplinary research into the linked sensory behavior and ecology of Favella, we present a working model describing known and putative sensory mechanisms (Fig. 4). To test this model, we have developed techniques combining microelectrode recordings of membrane potential ($V_{mem}$) with high-speed (250 FPS) video microscopy that were used to collect the data presented below (see Fig. 3, M.L. Echevarria and A.R. Taylor, unpublished). Resting membrane potential of Favella is about 62 mV, and cells exhibit spontaneous rhythmic depolarizations (RDs) that result in ciliary reversals and contraction of the peristomal cavity. Although the function of peristomal contractions is unknown, they may be involved in prey capture (Taniuchi & Takeda, 1988; Stoecker et al., 1995). RDs are c. 0.5 s long with peak amplitudes of $+20$ mV relative to resting membrane potential. The superficial pattern of RDs in Favella resembles cardiac pacemaker cell activity, in which a calcium clock along with nonspecific cation leak channels regulate
oscillating changes in membrane potential that coordinate cardiac contractions (Lakatta et al., 2008). A similar mechanism may function in *Favella* (Fig. 4a). Transient depolarizations, similar in appearance to RDs, are also stimulated when live or artificial prey particles contact the adoral membranelles or peristomal cavity (Fig. 4b); these also result in ciliary reversal and peristomial contraction. Such mechanically induced depolarizations supply a mechanism whereby contact with prey cells alters individual swimming behavior and leads to population-level aggregation (see Fig. 3).

*Favella* exhibit spontaneous APs that are rapid (10 ms) and result in complete reversal of membrane voltage (from 60 to +20 mV) (Fig. 4c). APs result in contraction of the cell into the lorica and cessation of ciliary beating, suggesting that fast APs are required for excitation–contraction responses during predator avoidance. The ionic components of the currents responsible for generating the APs have not yet been identified, although voltage-gated Na+/Ca²⁺ channels are the most likely mediators (Taylor, 2009). In the working model (Fig. 4), spatial variability in mechanosensitivty is critical, as is the ability to exhibit cue-specific and graded response to environmental cues. As described above, weaker mechanical stimuli result in swimming behaviors that lead to prey capture and ciliate aggregation, while stronger stimuli result in contraction into the lorica for predator evasion.

Metabotropic signaling mechanisms likely allow *Favella* to respond to chemical stimuli from predators and prey with appropriate behavioral responses (Fig. 4d). In such a
scenario, prey metabolites bind to GPCRs, activating adenylyl cyclase and downstream targets, including voltage-gated $K^+$ and $Ca^{2+}$ channels, which result in increased frequency of periods of backward swimming and aggregation of *Favella* in prey patches.

**Integrating approaches: from cellular mechanisms to population dynamics**

Marine planktonic ciliates inhabit highly heterogeneous environments rich in chemical, mechanical, electrostatic, and hydrodynamic stimuli. To survive, they must integrate these stimuli over relevant spatial and temporal scales and utilize the resulting outputs to make behavioral decisions that will maximize fitness. Although behavioral responses to isolated stimuli by *Favella* and other alveolates have been studied, understanding the chain of connection from stimulus detection and transduction through individual and population behavioral response is a major challenge. The working model described for *Favella* (Fig. 4) provides a conceptual starting point to design studies addressing that chain of connection.

A range of technical approaches is needed in such an interdisciplinary effort, including genomics and application of genetic tools, cell physiological and behavioral studies, multi-trophic population studies, field observations, and modeling. Electrophysiology and high-speed video techniques described above may be combined with downstream signal transduction assays (e.g. $Ca^{2+}$-sensitive fluorescent dyes) to determine the link between sensation, bioelectrical activity, and behavior (Fig. 5). Combining these techniques to connect cell physiology with responses to stimuli from predators and prey has been performed for only a few taxa (Lueken *et al.*, 1996). These techniques could also be coupled to high-resolution analysis of fluid flow landscapes generated by *Favella* using particle image or tracking velocimetry (PIV/PTV) to examine the connection between signal transduction, associated behaviors and changes in the fluid landscape surrounding the cell (Stamhuis, 2006; Wereley & Meinhart, 2010). PIV has been used to characterize flow fields produced by copepods and to determine clearance rates, the energetic cost of feeding, escape responses, and the hydrodynamic effects of the flow field on mechanosensitive predators and prey (Catton *et al.*, 2007; Kierboe *et al.*, 2010; Kierboe, 2011; Murphy *et al.*, 2012), but to our knowledge has not been applied to microzooplankton. PIV would allow detailed examination of the hydrodynamics involved in approach, contact,
capture, and ingestion or rejection of food particles, as well as the sensitivity of *Favella* to hydromechanical signals from predators. Importantly, PIV will enable resolution of the feeding mode (e.g. filter feeding vs. direct interception, which require very different feeding strategies).

Cellular and microscale studies of behavior must also be linked with experiments that place them in a broader ecological landscape (Fig. 5). Newly developed 3D imaging techniques allow tracking of individuals and distributions of cells simultaneously so that behavioral changes may be linked to changes in population distribution. These techniques have revealed that predatory dinoflagellates exhibit behavioral changes in the presence of prey that increase capture efficiency of prey (Sheng et al., 2007, 2010; Harvey et al., 2013). A major challenge for population-level studies is to simulate the microscale patchiness of prey, predators, and related chemical cues at the microscale (Mitchell et al., 2008; Stocker, 2012). Structured columns are a powerful way to address this problem; they are chambers that contain heterogeneous and structured mixtures of particles (e.g. prey cells) or chemical stimuli that more closely resemble the patchy distributions of these substances in nature. Recent studies have combined this technique with 3D imaging to demonstrate that *Favella* did not avoid a layer of toxic *H. akashiwo* (Harvey & Menden-Deuer, 2011), while a population of predatory dinoflagellates aggregated rapidly to layers of prey cells or prey culture filtrate (Menden-Deuer & Grunbaum, 2006). This technology is ripe for exploitation by those seeking to integrate study of population and individual sensory biology.

By combining these levels of investigation (Fig. 5), it should be possible to define the aspects of tintinnid biology that are necessary to inform trait-based population and community models. These types of models allow for a greater understanding of the mechanisms underlying population dynamics of planktonic communities (Litchman & Klausmeier, 2008; Litchman et al., 2013) because they allow for reductions in model complexity by grouping organisms according to traits that determine functional (ecological) outcomes, allowing for ecological modeling at the level of whole communities. Trait-based models have been applied to a range of spatial scales, from regional blooms to global biogeographical distributions (Follows et al., 2007; Litchman & Klausmeier, 2008), but have mainly been used to predict phytoplankton distributions as the relationship between common phytoplankton taxa and important ecological traits (e.g. photophysiology, nutrient acquisition, N-fixation, and biochemical ratios) is relatively well characterized (Barton et al., 2013). In contrast, planktonic ciliates – indeed, all zooplankton – remain poorly represented in trait-based models because of the lack of cross-disciplinary studies in ecologically relevant model species (Litchman et al., 2013). An important step in integrating them into these models is characterizing the relationship between their traits and ecological functions. This includes investigating the sensory mechanisms that mediate interactions such as prey detection, selectivity, ingestion, and predator avoidance that characterized the relationship between ciliate traits and ecological functions. (see Table 1).

**Future prospects and challenges: responses of microzooplankton to a changing ocean**

Planktonic ciliates play critical roles in marine food webs and biogeochemical cycles. As such, it is crucial to understand how they will be affected by environmental perturbations, particularly anthropogenic changes in the coastal and surface oceans. Here again, the vast majority of experimental work has focused on primary producers; effects on microzooplankton, the most important link between primary producers and metazoans, are largely unknown (Caron & Hutchins, 2013). An integrated research approach is needed to address how anthropogenic change could affect planktonic ciliates and associated ecosystem...
Table 1. Traits of planktonic ciliates: a conceptual approach for linking subcellular and organism-level research to ecological modeling. ‘Ecological functions’, arising from multiple organism characteristics, are the currencies of fitness formalized mathematically in ecosystem models.

<table>
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<tr>
<th>Trait</th>
<th>Resource acquisition</th>
<th>Persistence &amp; growth</th>
<th>Predation mortality</th>
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<td>Prey size</td>
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<td>Clearance rate</td>
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<td>Prey selectivity</td>
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<td>Swimming behavior</td>
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<td>Nutritional strategy</td>
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<td>Life history</td>
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We consider three such functions: research acquisition, persistence and growth, and predation mortality, whose rates are governed by organismal phenotype traits. Here, we have grouped trait functions by color (yellow = morphological; red = behavioral; blue = metabolic) according to the biological property underlying individual or species-level variation. Such grouping recognizes that natural selection acts on trait phenotype and thus simultaneously on multiple ecological functions, as those functions may stem from the same trait (e.g. cell size) or are intimately related within a trait class (e.g. metabolism). Because many of behavioral functions (red) represent rate processes linked to environmental conditions through ciliate sensory pathways, knowledge of sensation, and signal transduction in a model taxon can be extended to diverse species with similar traits, and parameterized into more realistic models of community-level processes.

processes. Of the many important avenues of study, we focus on two: elevated CO₂ (ocean acidification) and anthropogenic particles.

Ocean absorption of atmospheric CO₂ resulting from fossil fuel combustion is well documented (IPCC, 2013) and has led to measurable decreases in surface ocean pH (ocean acidification) (Feely et al., 2009). A scientific imperative arising from these changes is to predict effects on marine food webs, so that political and societal responses can be proceed in an informed manner. A burgeoning literature has addressed effects on phytoplankton physiology, biochemistry, and genetic change, but we have almost no data on how microzooplankton will respond. Indirect evidence, mainly from mesocosm experiments, suggests that microzooplankton themselves are both resistant and resilient to changes in seawater pH and CO₂ content on the order of those predicted for the next century (Rose et al., 2009; Nielsen et al., 2010; Aberle et al., 2013). The more probable effects of ocean acidification on microzooplankton will arise from changes in their prey assemblage, including aspects such as species composition, cell size, and the biochemical composition of prey cells. For example, increased CO₂ levels altered the fatty acid content and composition of a diatom (Rossel et al., 2012) and increased the C: N and C: P ratios of a cryptophyte (Schoo et al., 2013). These changes in phytoplankton composition were detrimental to the reproduction and growth of copepod predators in these single-prey experiments. Expanding such findings to microzooplankton in mixed communities, and incorporating the potential effects of other climate stressors, is a task well beyond the reach of univariate experiments on individual species. To develop the needed predictive capability, it will be necessary to understand how prey attributes are perceived and how they relate to the various individual and population behaviors that ultimately dictate microzooplankton ingestion and growth.

As discussed, planktonic ciliates can account for high clearance rates of prey particles with varying degrees of selectivity. Historical studies have used artificial prey (e.g. plastic microbeads) to examine trophic behavior in planktonic ciliates (see above: Feeding behavior: From detection to consumption). Our work using *Favella* demonstrates that mechanical cues from plastic beads elicit the same electrical responses and behaviors as contact with natural prey cells of a similar size. This suggests that ingestion of anthropogenic particles can readily occur under natural conditions. Of the various anthropogenic particles of concern, marine microplastics are emerging as a significant ocean problem. About 300 million tonnes of plastic are produced annually with a small but significant fraction (estimated to be between 1% and 5%) ending up in aquatic ecosystems (Reisser et al., 2013). Although accurate estimates of microplastic abundance in the surface ocean do not currently exist (Cole et al., 2013; Lee et al., 2013), a dramatic increase is predicted in the coming decades due to weathering and breakdown of the plastics that continue to accumulate in the coastal and open oceans. Moreover, both the particles themselves and the toxic chemicals they release can harm aquatic organisms (Wright et al., 2013a, b). How will planktonic alveolates be affected by ingestion of non-nutritive and potentially toxic microplastic particles? How will this ingestion affect alveolate-dependent food webs and microplastic sedimentation? These questions can only be addressed using an integrated and interdisciplinary approach.

Planktonic ciliates comprise a key but greatly understudied component of planktonic food webs. In the world’s oceans they encompass trophic strategies from primary producers through predatory zooplankton. Their ubiquity, behavioral sophistication, and genetic diversity make these unicellular organisms central to the consumption of primary production and the regulation of nutrient cycles in both coastal and open ocean waters. To better predict how these fascinating organisms engender and respond to ecosystem change will require a much deeper...
understanding of how they perceive, process, and respond to environmental cues at all levels of biological organization. This review argues that such an understanding can only be achieved through multi-level investigation that ranges across fields now often logistically and conceptually distinct. The most successful studies are likely to share several characteristics. First, studies should use relevant model species and should explicitly address the range of responses possible for those species so that findings may be extrapolated to different time and space scenarios. Second, research should be trait-based (e.g. Table 1). That is, hypotheses, experiments (e.g. choice of cues to test), and responses investigated should relate to ‘major axes’ thought to represent the ecological niche of planktonic ciliates. This will facilitate extrapolation from individual species to functional groups of organisms in the plankton and will allow incorporation of findings into trait-based models (Kiorboe, 2011; Litchman et al., 2013). Finally, research should be integrative, both in the range of biological levels addressed and in study design. Only by adopting such interdisciplinary investigations to connect alveolate cell biology to trophic ecology can we hope to improve predictions of plankton community responses under current and future ocean scenarios.

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Statement

The genus Favella has been recently redescribed, and some former Favella spp. placed in the new genus Schmidingerella Agatha & Struder-Kypke (2012). Reconciling cladistic and genetic analysis in choreotrichid ciliates (Giliphora, Spirotricha and Oligotrichia). J Eukaryot Microbiol 59: 325–350. For consistency with the ecological literature and due to uncertainties in species-level identification in previous studies, we retain the name Favella throughout this review.

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