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EFFECTS OF MULTIPLE SPECIES ON MOTILITY IN DIATOM ASSEMBLAGES

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EFFECTS OF MULTIPLE SPECIES ON MOTILITY IN DIATOM ASSEMBLAGES

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Table of contents

List of Tables and Figures

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ABSTRACT

      Diatoms are unicellular algae that, like other phototrophs, depend on light in order to survive. Many diatoms are known to have regulated motile responses to light, giving them a competitive advantage in their complex community, often containing many other species of algae. In order for similar diatom species to coexist in the same assemblage it is thought that each species will resource partition; a behavioral response that allows closely related species to be successful and cohabitate by using slightly different resources. Many experiments have demonstrated that other organisms exhibit this behavior, although the effect of co-existing diatom species on motility responses to irradiation have not been studied. Multiple species-specific motility experiments have been previously investigated (Cohn & Weitzell 1996, Cohn 2001) with three freshwater, pennate diatom species, *Craticula cuspidata* (Kützing) D.G. Mann, *Pinnularia viridis* (Nitzsch) Ehrenberg, and *Stauroneis phoenicenteron* (Nitzsch) Ehrenberg. This research, extending the work of previous experiments, explores the effects of the presence of multiple diatom species on motility for these three diatom species in order to determine whether they have behavioral differences that could potentially result in resource partitioning. Experiments were performed using an epi-illumination microscope to irradiate gliding diatoms in the leading or trailing end with blue (470 nm) or red (650 nm) light at high irradiation (ca. 10^5 µmol m^{-2}s^{-1}). When placed in the presence of other species, only one species, *S. phoenicenteron*, had statistically significant differences in the average direction change response times to blue irradiation at the leading end in the presence of other species. The presence of *C. cuspidata* alone resulted in a 2 fold increase in response times for *S. phoenicenteron*, while the addition of *P. viridis* alone caused no significant change in response times. However, such changes were particularly significant for *S. phoenicenteron* in the presence

of both *C. cuspidata* and *P. viridis*, which resulted in a 3 fold increase in response times. To determine if the altered response of *S. phoenicenteron* was dose-dependent, new motility experiments were preformed using samples with different percentages of *C. cuspidata* and *S. phoenicenteron* and, similar to the earlier motility experiments, diatoms were irradiated at the leading end with high intensity blue light. These experiments resulted in motility differences for *S. phoenicenteron* in the presence of *C. cuspidata*, where the average direction change response times increased as a function of increasing percentage of *C. cuspidata* cells in the sample. Specifically, the response times for single-species samples of *S. phoenicenteron* were significantly longer from response times of all samples containing percentages of *C. cuspidata* greater than 10%, and samples that contained 90% of *C. cuspidata* cells showed a 4 fold increase in response times for *S. phoenicenteron*. These results suggest that the behavior of some diatom species is altered in the presence of other species, and demonstrates how similar diatom species might differentially respond to resources. The observed effects of multiple species on motility suggest how some species might compete for slightly different resources in an algal community, such as directing cells into different areas of light wavelength or intensity. This research suggests potential areas for future studies, such as analysis of diatom stratification in different natural algal assemblages that could further determine the role of multi-species interactions in resource partitioning. Understanding such interspecies behavior and algal resource partitioning could lead to better management of healthy, stable aquatic ecosystems.

INTRODUCTION

Diatoms are a unique group of single-celled algae that have siliceous cell walls, which are known for their elaborate ornamentation and rigidness. These microscopic eukaryotes are photosynthetic and, like other photosynthetic algae, obtain most of their energy from light by absorption and conversion of solar energy to chemical energy. Diatoms are one of the most abundant primary producers, providing up to 30% of oxygen on Earth through photosynthesis (Round et al. 1990, Armbrust 2009). Diatoms arose during the early Triassic Period (250 mya) (Sorhannus 2007) and ever since then been ecologically successful, existing in all aquatic environments from lakes, rivers and ponds, to open oceans and estuaries.

Diatoms are often categorized into two groups, the Order Coscinodiscophyceae, known as centric diatoms, and the Order Bacillariophyceae, known as pennate diatoms. Centric diatoms are usually cylindrical in shape and have radial symmetry, whereas pennate diatoms are more elongated and have bilateral symmetry. Although all diatoms fall into these two categories, some centric and pennate diatoms deviate from the standard shape; for example some diatoms are triangular or square in shape (Round et al. 1990). Morphologically, diatoms have a wide variety of forms, where each species is thought to be adapted for their particular environment. Centric diatoms drift in the water column and are usually non-motile, whereas pennate diatoms sink into the sediment and are usually motile. This adaptation of motility, regulated by external factors such as light, temperature, pH, and salinity, allows pennate diatoms to regulate their location within the sediment, providing them with better access to nutrients and optimal light conditions, as well as the ability to avoid predation (Round et al. 1990).

Morphology

Diatoms are well known for their glass-like cell wall, also known as a frustule, which consist of two halves, each known as a valve or a theca. The two valves, the epivalve (larger) and the underlying hypovalve, have extended edges that overlap each other like a petri dish to form the cell wall (Round et al. 1990). Separate silicified bands, known as girdle bands, overlap and encircle the two valves. Pennate diatoms have three planes or axes of symmetry used to describe their morphology: the apical plane, which transverses the long axis of the cell wall, the transapical plane, which transverses the shorter side of the cell wall, and the valvar plane, which is parallel to the valves (Cox 1996). Because centric diatoms generally have radial symmetry, they often do not have a separate characteristic transapical plane. Instead, centrics have a valvar plane and an infinite number of radial planes that bisect and run parallel with the radius of the cell (Cox 1996).

There are three orientations of the frustule of pennate diatoms generally used when discussing their structure, valve view, girdle view, and the end view. The valve view, or face view, is when the valvar plane is perpendicular to one's vision, allowing the viewer to directly observe the valve surface. The girdle view is when the cell is observed from the valvar plane and is perpendicular to one's visions along the long axis of the cell, allowing one to observe the side of the cell (Round et al. 1990). The end view is when one observes the cell from the small end, perpendicular to both the valvar plane and the plane of bilateral symmetry (Round et al. 1990). Centric diatoms also have the valve and girdle view, however they often do not have a separate characteristic end view due to their radial symmetry. Detailed observations of valve structures as seen in each of these different orientations are used to help identify the species as well as determine species-specific structural characteristics.

The diatom frustule is often very rigid, yet highly ornamented with numerous pores, or areolae, that allow for passage of ions and nutrients. Most diatoms range from 5-200 μm in size, although some diatom species form stalk-like colonies that exceed this range (Round et al. 1990). While diatoms contain the same organelles as other eukaryotic algae (e.g. a nucleus, vacuoles, Golgi, and chloroplasts) they also have other specialized organelles. Most prominent among these is the silicalemma, used for the production of their silicified cell walls (Round et al. 1990) that are produced during somatic cell division (see Cell Division section below).

Diatoms have been around for millions of years and over time have evolved with various shapes and sizes. Due to the diversity in morphology, diatoms can be classified by the configuration and ornamentation of their frustule (Round et al. 1990, Cox 2012). Morphology also plays a crucial role in the survival of diatoms in their environment. For example, the radial symmetry and spines that exist in some centric diatoms allow them to remain suspended in the water column. Centric diatoms usually have silica thickenings, known as ribs, which extend out from a ring, reinforcing the stability of their radially symmetric valve (Round et al. 1990). Many centric diatoms also form long chains or circular colonies that are held together by the formation of additional silica bands (Round et al. 1990), further increasing their buoyancy.

In contrast to centrics, pennate diatoms have flat, more elongate bodies, which causes them to be more dense and allows them to settle in the sediment. This bipolar, elonglated shape is thought to make it easier for them to move through the sediment. Like centrics, many pennate diatoms have periodic silica thickenings (i.e. ribs) that extend from a central portion in the cell wall and are thought to provide additional structure to the cell (Round et al. 1990).

A major structural feature within the valve of motile diatoms, known as the raphe, consists of one or two slits in the cell wall that runs down the central axis of the valve. This

raphe is thought to be where extracellular mucilage strands are secreted through the hardened cell wall. These mucilage strands that originate within the cell, attach to the substratum and allow the diatom to move (Wetherbee et al. 1998, Cohn 2001). The raphe is located along the middle of the valve face or along specialized wings or ridges for certain species of diatoms (Round et al. 1990). The raphe is continuous along the length of the cell, allowing diatoms to glide forwards and backwards in a bi-directional fashion. Most pennate diatoms are biraphid, consisting of raphe slits on both valves, although there are some species of pennates that are monoraphid, having a raphe on only one of the cells' two valves.

Physiology

The physiological responses of diatoms are species-specific and strongly influenced by the environment. For instance, diatoms that inhabit rivers, streams, or tidal basins are faced with the challenge of water flow. In response to increased water flow, these diatoms often form mucilage pads or stalks, which is thought to aid in attachment to substrata and therefore, produces groups of cells that are less likely to be washed away by the current (Lamb & Lowe 1987, Celler et al. 2013). Diatom species that form mucilage pads usually have a less rigid cell wall, allowing the pads to adhere tightly to surfaces such as rock or vegetation. Stalk formation also aids in current resistance by increasing the weight of the diatom, as well as adding more surface area to the cell itself. This adaptation not only aids in their stability in the environment, but is also thought to increase nutrient uptake and gives diatoms the advantage to move higher up in their community and thus better competitors for sunlight (Aboal et al. 2012). As previously stated, buoyancy is an adaptation that open water centric diatoms depend on in order to stay

afloat in the water column, and this buoyancy can also be regulated by physiological changes to the amount or content of the lipids that are stored in the cell (Karleskint et al. 2013).

Diatoms contain photosynthetic pigments like other phototrophs, with some that differ from most other plants. Uniquely, diatoms lack chlorophyll b, which is a major pigment for most green plants. Chlorophyll a and c are the main photosynthetic pigments, although diatoms also contain accessory pigments such as beta-carotene and fucoxanthin, which are responsible for their golden color (Stauber & Jeffrey 1988). While higher green plants often store energy in the form of cellulose or other starches, diatoms can store energy in the form of chrysolaminarin (another glucose-based carbohydrate), which is often stored in specialized vacuoles (Hildebrand et al. 2012). Many biotechnology corporations are looking at this macromolecule, along with diatom lipids, as potentially important sources of biofuel (Hu et al. 2008). Algae, in general, are known to be renewable sources of energy, although such algal-based fuel has not been very successful for large-scale productions thus far. This process involves lipids and carbohydrates being extracted from diatoms that are then used for fuel, which is used in many industrial applications such as pharmaceutical and commercial applications (Hildebrand et al. 2012). Such work may allow algae to eventually be an important alternative to fossil fuels, known to be a major contributor of greenhouse gases such as carbon dioxide.

The success of diatoms is often indicative of the quality of the environmental conditions in their habitat. Diatoms are very sensitive to their surrounding environment (e.g. metals, ions, pH) and disturbances within their habitat can strongly influence their success. Therefore, the species and the abundance of diatoms are often used to determine the quality of water as well as changes within aquatic ecosystems (Smol & Stoermer 2010, Cibic et al. 2012, Bennion et al. 2014). One of the most essential environmental nutrients for diatoms is silicon, which is vital for

diatom reproduction and valve formation. Diatoms take up silicon from the environment, in the form of orthosilicic acid ($Si(OH)_4$), in order to precipitate it into a hardened form of silica for their cell wall (Round et al. 1990). Silicon is readily available in almost every aquatic environment, being one of the most abundant elements and is found in numerous inorganic materials, such as rocks and sand. The absence of silicates in a diatom's environment can lead to abnormal frustule production as well as inhibition of protein and pigment synthesis (Round et al. 1990, Debenest et al. 2008). Like the responses to other environmental conditions, the amount of silicon needed for frustule production is species-specific, although some silicon is required for frustule formation and cell division in virtually all diatoms.

Cell Division

Cell division in diatoms also has characteristic processes unique to these cells, including valve formation that begins soon after cytoplasmic division. Valve development starts with the accumulation of silicon in diatoms. Intracellular silica deposition vesicles (SDV), produced by each daughter cell, are located internally and fuse to form a larger specialized vesicle membrane bounded by a specialized membrane known as the silicalemma, although it is unknown exactly how silicon is transported via the SDV's to the interior of the silacalemma (Crawford 1980, Sumper & Kroger 2004). However, valve morphogenesis is known to require silica transporters (Smol & Stoermer 2010) as well as silaffins, which are proteins thought to be involved in precipitating the silica into spheres and plates that regulate the species-specific nanomorphology of the cell wall.

Immediately after cytoplasmic division, the two daughter cells have formed inside the confinement of the parental cell and each daughter cell produces a valve-forming SDV that

expands when silica is deposited into it, such that each daughter cell obtains a new valve. The SDV fuses with the cell membrane when the valve is complete, secreting the valve to the exterior of the cell, which will become the hypovalve of each daughter cell (Mann & Stickle 1991). Additional girdle bands that formed in other SDVs are brought to the cell surface in the same way, enhancing the structural integrity and stability of the newly formed theca (Sumper & Kroger 2004).

Upon completion of the new valves, the two daughter cells enlarge, separating themselves from each other, so that at the end of cell division each individual daughter cell contains one parental valve (epivalve) and one new valve produced from the SDV (hypovalve). This process leads to smaller cell sizes with each division because each hypovalve formation must occur inside the parental cell. Cell size is regained when diatoms go through meiosis and sexual reproduction (Drebes 1972, Werner 1977, Mann 1993).

Sexual reproduction is thought to take place once the diatom population reaches a size threshold that is small enough (Round et al. 1990). Sexual reproduction, and more specifically zygote formation, varies from species to species. For most centric diatoms, sexual reproduction is oogamous (Drebes 1972, Mann 1993). This means that the female gamete is significantly larger than the male gamete and is non-motile. A male gametangial cell divides to form microspores that undergo meiosis to form flagellated sperm (Drebes 1972, Mann 1993). The sperm migrates to, then fuses and fertilizes with the female gamete, which gives rise to subsequent cell division and cytokinesis.

For most pennate diatoms sexual reproduction is isogamous, meaning that the gametes are similar in size and shape (Werner 1977, Mann 1993). Pre-meiotic cells, presumably of two different mating types, often line up and pair with each other, which stimulates the cells to

undergo meiosis and secrete a protective layer of mucilage around the pair to protect the gametes (Mann & Stickle 1991). Each pre-meiotic cell then produces two meiotic gametes, which fuse together, one from each parental cell, to form two diploid zygotes (Werner 1977, Mann 1993). The zygotes quickly swell and elongate, forming specialized cells known as auxospores. These auxospores secrete specialized silica bands along their length as they elongate, protecting the cell inside. The auxospore then produces a primary vegetative cell that will eventually undergo regular cell division (Cohn et al.1989). This form of sexual reproduction for diatoms not only leads to growth in cell size, it also involves the process of genetic recombination, which results in considerable genetic diversity and aids in evolution over time.

Habitat

There are over 30,000 species of diatoms and each species has their own unique morphology that makes them suitable to the environment that they inhabit (Round et al. 1990). There are two natural environments for diatoms: the sediment, habitat for most of the benthic diatoms consisting of mostly pennates, and open water, habitat for most planktonic diatoms consisting of mostly centrics. Both planktonic and sediment dwelling diatoms exist in freshwater as well as salt-water environments. Each species of diatom has different physiological adaptations because of the diverse environments each species occupies.

Every adaptive behavior of diatoms to environmental conditions involves ecological trade-offs. Specific adaptive characteristic that help diatoms be successful for one particular environmental condition is also a constraint or limitation for other conditions. For example, most centric diatoms are planktonic and have been selected for the adaptation of buoyancy. Thus, most centric diatoms are non-motile and stay afloat in the water column where they are freely

accessible to nutrients, allowing them to access sunlight more easily while also providing the ecosystem with a major source of oxygen production. Although the buoyancy of centric diatoms gives them easy resource access, they lack the ability to quickly alter their position in response to environmental cues, which makes them more vulnerable to predation. Such diatoms are primary producer in many food chains, making them vital contributors to the success of the ecosystem, providing nutrition for many secondary consumers, from small snails to large filter feeders (Armbrust 2009).

Benthic diatoms have different ecological tradeoffs than centric diatoms because of their challenges with sediment. The majority of benthic diatoms are pennates and cannot float in their environment. Instead, many pennate diatoms have motility mechanisms that help them move through the substrata aided by the addition of their flat, more elongate morphology, which provides for more substratum-surface contact. This adaptation allows pennate diatoms to better regulate their access to light (in near shore environments that are often more shaded) as well as avoid predation by vertically migrating through the sediment, although the active control of motility requires a considerable expenditure of energy.

Another tradeoff is adhesion, which is especially critical for pennate diatoms. Some diatom species adhere tightly to surfaces, which makes motility more difficult. On the other hand, species that don't adhere as well are more prone to detaching from the substrata and loss from their assemblages due to water flow. Thus, each species is involved in a trade-off between the strength of adhesion and the flexibility of rapid motile responsiveness.

The many distinct species of diatoms thrive under different conditions. Major environmental factors that can influence diatom success are: temperature, pH, salinity and light (Cohn & McGuire 2000, Cohn et al. 2003, Cibic et al. 2012). Previous research has indicated

that each diatom species has a distinct range of environmental conditions they can survive in.

Although all diatoms need sunlight, each species has different light sensitivities for movement

(Cohn & Weitzell 1996, Cohn 2001, Cohn et al. 2003, Cohn et. al 2015), which suggest that each

species might occupy different light areas, or a particular niche of the benthic algal community.

For example, diatoms are known to stratify different layers of their community, where some

species are located closer to the top of the photic zone than others (Winder et al. 2009).

The changes in distribution of diatoms can be indicators for changes in aquatic

environments. The abundance and diversity of diatoms in virtually all aquatic environments

provides for a wide variety of species with specific ecological limitations. Historical records can

be used to determine how diatoms respond to their changing environment (Cibic et al. 2012). By

knowing what species of diatoms are in a body of water, one can often determine a number of

chemical and natural characteristics of the environment (Bennion et al. 2014). Diatoms can also

be indicators of toxins and polluted waters (Cohn & McGuire 2000, Smol & Stoermer 2010),

whereby abnormalities in the frustule can be an indicator of an environment that does not have

the proper nutrients or has toxic chemicals (Debenest et al. 2008).

Diatom Movement

The adaptation of motility for pennate diatoms is a unique mechanism that is different

from most microorganisms. Unlike other protists that have flagella for swimming or amoeboid

movement for crawling, the confinement of diatoms within their cell wall restricts their

movement to a type of gliding. Gliding, in general, is the movement of a cell over a surface

without the cell changing or distorting its shape. Many microbes move by gliding, although there

are different mechanisms in which this is done. For diatoms, this gliding is bidirectional, by

moving in the direction of the long axis of the cell body. Diatoms are thought to glide by the secretion of extracellular polysaccharide fibrils through the raphe, coupled with the movement of these fibrils along cellular cytoskeletal pathways (Drum & Hopkins 1966, Edgar & Picket-Heaps 1983). However, the underlying mechanism of how the mucilage is secreted and moved is still not known.

Most pennates are biraphid and have two raphe fissures, on opposite sides of the cell, where one slit, known as the "driving" raphe, is attached to substratum, while the other slit is not attached to the substratum (Higgins et al. 2003). Although the adhesion mechanism is not well understood on the molecular level, the proposal that diatoms use an actin-myosin mechanism is well supported (Wetherbee et al. 1998). Edgar and Picket-Heaps (1983) proposed that the actin cables that run parallel, near the raphe, connect to myosin molecules that are attached intracellularly to mucilage strands that extend outside the cell wall, generating the force to propel the cell forward (Cohn et al. 1996, Poulsen et al. 1999, Cohn 2001). The myosin heads can connect and disconnect from the two stationary actin cables, which are thought to be oriented in opposite directions, allowing the cell to be propelled in a bi-directional fashion. Mucilage strands that are connected to the internal myosin are secreted from the raphe, allowing the diatom to attach to the substratum and move. Higgins et al. (2003) found evidence of substantial tethers that are involved in cell adhesion and reorientation, reinforcing this mucilage secretion mechanism.

Diatom Behavior

Diatoms, like most organisms, regulate their motility in response to environmental stimuli. Many factors influence diatom motility and adhesion, where each species has

characteristic sensitivities to environmental conditions. The specific behavioral and motile responses to environmental conditions depend on the diatom species, where some species are more sensitive than others to particular stimuli. Previous research has determined that temperature plays a major role in cell speed, where cell movement was greatest in a distinct range of temperatures for each species (Cohn et al. 2003). Increasing temperature over these ranges lead to a rapid decrease in cell speed, which was most likely due to the denaturation of the proteins involved in motility (Cohn et al. 2003).

Another characteristic that affects motility is adhesion. The composition of mucilage, the temperature of the environment, as well as the sediment composition all affect how well diatoms can attach to the substratum. Changes in temperature can affect adhesion in some diatom species (Cohn et al. 2003), although it is unclear whether this is due to changes in mucilage composition or mucilage structure. The composition of mucilage (extracellular polymeric substances, EPS) for diatoms is often species-specific. EPS is mainly composed of polysaccharides, although the type and amount is often different among different species (Hoagland et al. 1993, Chiovitti et al. 2006, Poulsen et al. 2014). Differences in mucilage composition might potentially affect how species interact and may further relate to how species might segregate into different areas of their environment. Other abiotic factors, such as the physical characteristics of the substratum, for example, grain size of the sediment particles, can also affect the ability of diatoms to adhere and move properly (Du et al. 2010).

Although many abiotic factors influence diatom motility, light is likely the greatest regulator of short-term movement and direction change. Previous experiments have focused on the conditions that affected vertical migration and concluded that even though endogenous factors play a role, irradiance had the strongest influence (Coelho et al. 2011). The ability of cells

to demonstrate such a strong response is thought to be due to diatoms having intracellular photoreceptors that react to light stimuli (Cohn et al. 1999; Cohn 2001, Depauw et al. 2012, Costa et al. 2013). Red and blue photoreceptors have been found in algae, and diatoms are also thought to have at least these two types of photoreceptors (Falkowski & LaRoche 1991, Costa et al. 2013). Previous research has determined that diatoms react most strongly to light at their tips (i.e. the leading and trailing ends of the cell) (Cohn et al. 1999, Cohn et al. 2004). Irradiating the diatom at the center of the cell or the whole cell does not have a net effect on motility (Nultsch & Hader 1988, Cohn et al. 1999). This suggests that diatoms predominantly have photoreceptors at their tips, although photobehavior has only been studied by irradiating diatoms and observing their behavioral response, direct localization of photoreceptors has not been determined. The two ends of the diatom seem to have similar sensitivity when irradiated with the same wavelengths under the same conditions, therefore, the light receptor in the two ends are thought to be the same (Cohn 2001). Like many other factors that effect diatom motility, light sensitivity is also species-specific (Cohn 1999, Depauw et al. 2012, Cohn et al. 2015).

Diatoms are sensitive to both high and low light intensities and will regulate their movement accordingly. In general, at low to moderate intensities (<100 µmol $m^{-2}\,s^{-1}$), diatoms move into the light (Cohn et al. 1999). This intensity of light is similar to light levels found on a regular sunny day. The exposure to high intensity light (10^3-10^5 µmol $m^{-2}\,s^{-1}$) will cause diatoms to move away from the high irradiance (Cohn et al. 1999, Cohn et al. 2004). This response is known as behavioral photoprotection, allowing cells to avoid damage to their cellular components, particularly their photoreceptors (Serodio et al. 2006, Cartaxana et al. 2011).

Diatoms are also sensitive to changes in the tide and will synchronize their movement with the diurnal and tidal cycles. During the daytime when the tide is low, diatoms will migrate

towards the photic zone to maximize photosynthesis and before dark diatoms will vertically

migrate down to avoid high tide and predators (Cartaxana & Serodio 2008). Diatom behavior

during tidal changes may be connected to their light sensitivities as diatoms also migrate

vertically with diurnal changes in the light intensity, moving upwards or downwards with the

sediment, as appropriate. Regulation of their migratory behavior within their assemblage gives

diatoms an ecological advantage to survive their complex and ever-changing environment.

Mixed Species Behavior

Organisms use different behavioral strategies to survive in a complex community where

resources are limited. Research has shown that some organisms have adapted more successful

behaviors by resource partitioning. Previous research has indicated that specialist species (i.e.

those that exploit a specific resource) optimize more specific resources than generalist species

(Finke & Snyder 2008). For example, similar bumblebee species in the same community will

specialize on different types of flowers (Griffin & Silliman 2012). Even though these two bee

species were closely related and occupied the same general geographic area, some of the bee

species obtained nectar from flowers with longer length petals, while other species used flowers

with shorter length petals. This way the two species of bees can occupy the same habitat without

having to compete for the same resource. These two species could exploit slight differences in an

available resource, thus partitioning that resource between the two species, which allowed both

species to better succeed. Many closely related species that exist within confined environments

are thought to behave similarly by resource partitioning, allowing species to coexist in a

community with less competition for the same resources (Macarthur & Levins 1967, Schoener

1974, Finke & Snyder 2008). This situation, where closely related species are confined within a

limited area, is similar to the case of diatoms where many closely related species are found in the same assemblage.

Behavioral changes of closely related species based on intercellular communication regarding environmental conditions can also be seen in prokaryotes, for example in bacteria that use quorum sensing. Quorum sensing is when groups of bacteria communicate with each other to coordinate their behavior by secreting chemical signal molecules that usually results in altered motility of other cells around them. Bacteria use quorum sensing when they are in an environment with a lot of other bacteria (Bassler & Waters 2005) and often send out inhibitory or excitatory chemical signals that will affect the behavior of other cells. It is favorable under certain conditions for bacteria to be sensitive to the density of other species surrounding them. This means that individual species behavior can change when they are in a community of cells.

More often than not, diatoms are found within a complex assemblage of algae, sometimes in tightly confined aggregations referred to as microphytobenthos or biofilm. The build up of mucilage secretion and density of cells, including green algae and other microbes, can produce microbial mats. Diatoms often make up the majority of species within these algal communities, making them one of the most important primary producers of aquatic ecosystems (Johnson et al. 1997, Armbrust 2009). Within these algal assemblages there has been evidence for diatom stratification, where different species were located in various layers of the community during different times (Litchman et al. 2007, Winder et al. 2009, Cibic et al. 2012). In addition, previous research has determined that some diatom species have species-specific light sensitivities (Cohn 2001), which may potentially drive each species into a particular microniche. The question of how many different species can exist in close, confined areas such as ponds and lakes where intense competition is expected has been debated for many years (Hutchinson 1961) and it is

likely that active resource partitioning is one of the mechanisms in which this diversity remains possible. Specialization would allow similar diatoms species to co-exist in an algal community while minimizing direct competition and provide a symbiotic relationship between species. However, the mechanism for diatom stratification and whether this stratification relates to niche partitioning is unclear. Most research has focused on algal stratification as a whole (i.e. biofilms) or a specific species behavior. However, little research has been done on the effects of multiple diatom species and how their interactions affect motility.

Thesis Hypothesis and Rational

Previous research on diatoms has determined that adhesion is affected by the presence of other species (Cohn et al. 2003). The current research described below is designed to examine potential motile and behavioral adaptations of diatoms in the presence of other closely related species. Most research has analyzed diatom behavior by determining changes in overall diatom abundance within an assemblage (Baillie 1987, Coelho et al. 2011, Cartaxana et al. 2011). This study analyzes the motile responses of individual cells to irradiation in three species to determine if these responses are affected by the presence of other species. The three species used in our study are *Pinnularia viridis* (Nitzsch) Ehrenberg, *Craticula cuspidata* (Kützing) D.G. Mann, and *Stauroneis phoenicenteron* (Nitzsch) Ehrenberg (Figure 1). All of these species are benthic, freshwater, pennate diatoms that are motile, and were isolated from the same shallow pond, and mud samples were collected about 2-4 feet below the surface of the water.

The focus for this research is to determine changes in motility as measured by the functional response of diatoms to high irradiation. These three diatom species were used because they are easy to detect, isolate, and manipulate. For instance, they are large enough in size to

16

easily isolate and observe them under the microscope, and they move relatively quickly compared to other motile diatoms. Ecologically, all of these species are from the same small pond in Boulder, Colorado, and being from the same ecosystem suggests that they all had access to the same nutrients during development, making them ideal species for comparative motility experiments. Even though the species studied are motile, pennate diatoms, they vary slightly in size and basic structure and they all have a specific response to different types of light (e.g. blue and red). This suggests that they might occupy different areas of the sediment in their natural habitat. This research attempts to better understand these diatom species by analyzing changes in individual movement as a function of the presence or absence of other cells in their environment.

The objective, therefore, is to understand whether *Craticula cuspidata, Pinnularia viridis,* and *Stauroneis phoenicenteron* modify their photoresponsive behavior to high irradiance of either blue or red light in the presence of other species. Previous research has determined in single-species assays that *C. cuspidata* migrates into blue moderate light, *P. viridis* moves towards moderate blue-green light, and *S. phoenicenteron* moves towards moderate blue light and low to moderate red light (Cohn & Disparti 1994, Cohn 2001, Cohn et al. 2004, Cohn et al. 2015). Since each of these three species has characteristic sensitivities to different light wavelengths, we preformed experiments using both blue and red light. To test the effect of species being present, *S. phoenicenteron, P. viridis,* and *C. cuspidata* were irradiated in their own single-species population and then in mixed-species assemblages with the other species to determine if their motility responses changed. It was hypothesized that there would be a difference in diatom motility when cells were put into an environment containing multiple species as compared to their responses in single-species samples. Investigating the physiological

behavior of diatoms in multi-species samples can thereby help us to better understand whether diatoms are involved in species-dependent resource partitioning.
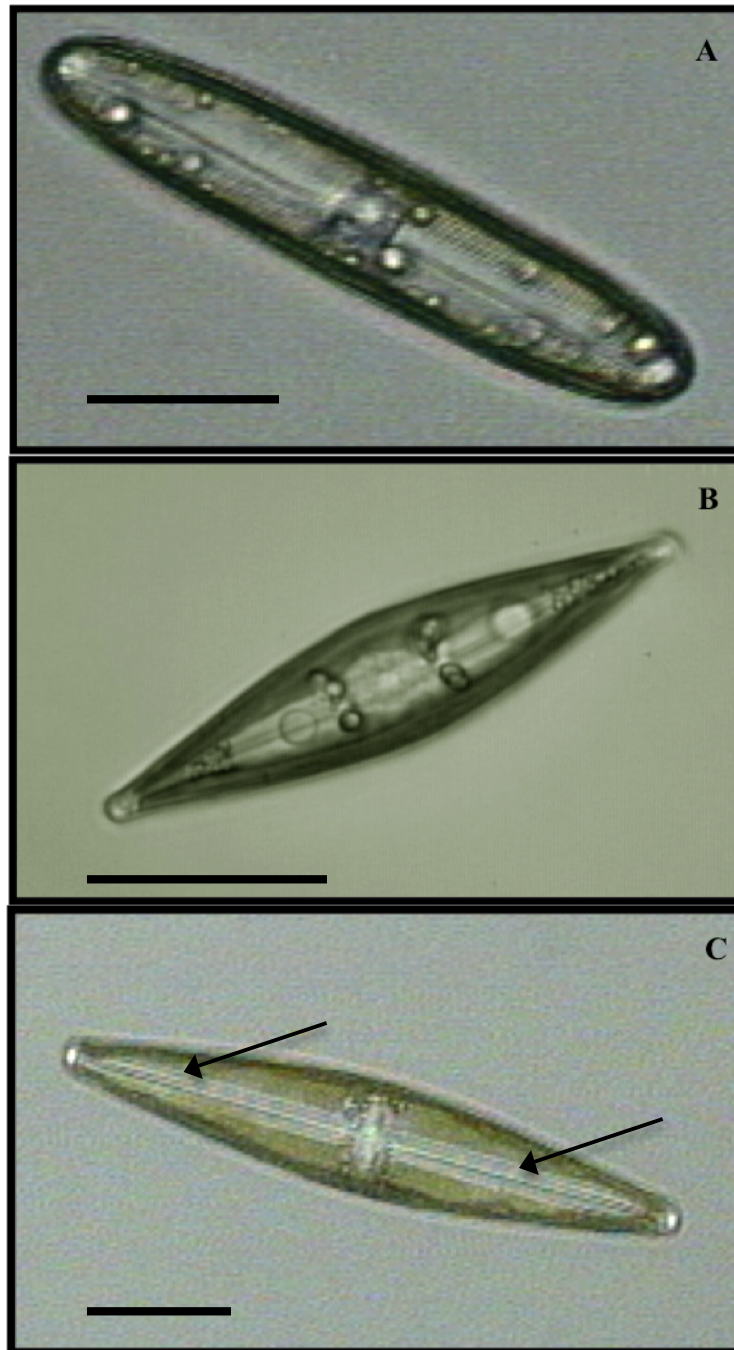
Figure 1: Three diatom species researched: ***Pinnularia viridis*, *Craticula cuspidata*, and**
Stauroneis phoenicenteron This figure illustrates the morphology and approximate cell length
of the three pennate diatom species used in the motility experiments. A) *Pinnularia viridis* cell
length ranges from 120-160 μm. B) *Craticula cuspidata* cell length ranges from 80-120 μm. C)
Stauroneis phoenicenteron cell length ranges from 150-250 μm. The arrow points to the raphe
(two slits in the cell wall) where mucilage is secreted to aid in motility and attachment. Scale
bars equal 50 μm.

MATERIALS AND METHODS

Diatom Sampling

The pennate diatoms used for experimentation were initially isolated from sediment samples taken from a small, freshwater pond in Boulder, Colorado during 2011-2015, and transferred to the laboratory. *Stauroneis phoenicenteron* (Nitzsch) Ehrenberg, *Craticula cuspidata* (Kützing) D.G. Mann, and *Pinnularia viridis* (Nitzsch) Ehrenberg were isolated from the sediment samples via drawn glass micropipette. All diatoms were washed in spot well plates three times with deionized water before being cultured separately in petri plates containing diatom medium (DM, recipe below). The diatoms were stored in an incubator set at 14°C with a 14:10 light:dark cycle and with a light irradiance of ca. 50 μmol/m^{2}sec. Diatoms were subcultured into petri plates with fresh diatom medium every 2-3 weeks. *Craticula cuspidata* cells isolated from these samples were approximately 90 μm long, *Stauroneis phoenicenteron* were approximately 200 μm long, and *Pinnularia viridis* were approximately 150 μm long (Figure 1).

Diatom Medium

Diatom medium (DM) was prepared as shown in Table 1, by combining 10 mL of 30mM Ca(NO$_3$)$_2$·7H$_2$0, 10 mL of 40 mM KH$_2$PO$_4$, 10 mL of 10 mM MgSO$_4$·7H$_2$0, 5 mL of 0.02% v/v saturated Na$_2$SiO$_3$ (20 mL/L, pH 8.5), 100 μL of 1 M Na$_2$HCO$_3$, 1 mL of 0.1 μM FeSO$_4$·7H$_2$0, 1 mL of 0.1 μM MnCl$_2$·4H$_2$0, and 50 mL soil extract (5% v/v, boiled and triple filtered with a final filter size of 0.2 microns) into a 1 L Erlenmeyer flask (Andersen 2005). Deionized water was added to the flask to equal 1 L. The solution was boiled while mixing on a hot plate (400°C). After the solution was cooled, vitamins were added: 1 mL of 1 g/L

niacinamide, 1 mL of 0.1 g/L biotin, 1 mL of 1 g/L thiamine, and 1 mL of 1 mg/L B_{12} (Andersen

2005). After the addition of the vitamins, the pH of the solution was adjusted to 6.8. Fresh

diatom medium was placed in the culture petri plates every week.

Substance	Stock Solution Concentration	Amount of Stock Solution Used (mL/L)	Final Concentration
$Ca(NO_3)_2 \cdot 4H_2O$	30 mM	10	0.3 mM
KH_2PO_4	40 mM	10	0.4 mM
$MgSO_4 \cdot 7H_2O$	10 mM	10	0.1 mM
Na_2SiO_3 (~1M) saturated solution	20 mL/L 2% v/v (pH~8)	5	0.02% v/v
$FeSO_4 \cdot 7H_2O$	1 µM	1	0.01 µM
$MnCl_2 \cdot 4H_2O$	0.1 µM	1	0.001 µM
Soil Extract boiled and filtered	saturated	50	5% v/v
Niacinamide	1 g/L	1	1mg/L
Biotin	0.1 g/L	1	0.1 mg/L
Thiamine	1 g/L	1	1 mg/L
B_{12}	1 mg/L	1	1µg/L
$NaHCO_3$	1 M	100 µL/L	100 µM

Table 1:Composition of Diatom Medium This table displays the composition of diatom medium and final mixture concentration, with a final pH around 6.8.

Diatom Cleaning and Preparation of Slides

　　Diatoms from growing cultures were cleaned prior to motility experiments by using a multi-spot well plate where two wells were filled with deionized water and the third spot well was filled with fresh diatom medium. Diatoms isolated from culture were transferred sequentially via glass micropipette from their petri plate culture into the two deionized spot wells and then into a spot well with diatom medium. After this cleaning, cells were transferred onto a glass slide pre-prepared with VALAP (vaseline:lanolin:parafin 1:1:1 w:w:w) spacers along the top and bottom edges of the slide to protect the cells from being crushed. A glass cover slip was added over the cells and enough diatom medium was added to fill the slide chamber. The cover slip was then sealed in place with VALAP. Every slide prepared contained ca. 100-150 diatoms, low enough to prevent overcrowding, but high enough to make numerous measurements in one experiment. The slide was placed in a dark room for ten minutes before experimentation, allowing the cells to equilibrate to the same light environment. For each multi-species experiment, two slides were prepared: one slide with a single species and one slide with the desired multiple species in a defined approximate ratio. Different ratios consisted of a 1:1 mixture of two different species, or a 1:1:1 mixture of three different species. For the dose-dependent experiments on *Stauroneis phoenicenteron* and *Craticula cuspidata*, samples containing different percentages of both species were prepared (i.e. 10%, 20 %, 50%, 80%, and 90%). For the multiple species slides, cells from each of the species were washed one time in their own species spot well containing deionized water. For the second wash all species were washed together in the same well with deionized water. Then all species were transferred together for the last wash in diatom medium. The glass slide with multiple species was prepared the same way the single species slide was prepared.

Irradiation of Diatoms

Diatoms were irradiated as described previously (Cohn et al. 1999, Cohn 2001, Cohn et al. 2015). Prepared slides were placed on the stage of a Zeiss Axioskop microscope fitted with epi-illumination optics. This epi-illumination microscope, fitted with a 100 W mercury arc lamp, was used to irradiate diatoms through the objective. Precise bandwidth fluorescent irradiation filters were placed along the light path so that desired specific wavelengths of light (blue: 470 nm, or red: 650 nm) would irradiate the diatoms through the objective. A shutter box (Uniblitz Model VMM-T1, Vincent Associates, Rochester, New York) was also connected to the microscope to control the duration of irradiations (red: 2700 ms, or blue: 1000 ms). The duration times for red and blue light were made so that at each wavelength the cells were irradiated with equal total energy of irradiance (Cohn 2004, Cohn et al. 2015). There was also a filter slider on the microscope that allowed the blocking of the light path during shutter irradiations for un-irradiated controls (closed: no irradiance, or open: full irradiance).

A DAGE-MTI 68 video camera connected to a video monitor was attached to the microscope, which allowed for viewing the diatoms on the video monitor. Background illumination used to observe cell movement was of low irradiance (<5 µmol m^{-2}s^{-1}) in order to minimize any light effects on cell movement. The image projected on the screen passed through a time generator, in order to determine the amount of time for each cell to make direction changes and thereby the response times for cells to make direction changes. The location of the epi-illumination spot (set at a radius of ca. 130 µm) was outlined on the video monitor. Diatoms were irradiated at their leading or trailing tip by observing cells moving into this marked spot on the video monitor. When a diatom's tip (ca. 25 µm) entered the irradiation circle on the video monitor, the shutter trigger was pressed, allowing the epi-irradiation path to open and irradiate

the diatom (Figure 2). Movement of diatoms was observed from the time of the irradiation to the time of their direction change to determine the amount of time for the cell to respond to the exposure of high irradiance.

Cells were observed prior to irradiation to ensure cells were healthy and had adequate motility for testing. For every experiment, both the single-species slide and the multiple-species slide was prepared. Previous experiments determined the average response times of these three species when irradiated at the leading end with blue light (Cohn 2001, Cohn et al. 2015). Therefore, leading end irradiations with blue light was the positive control to ensure the apparatus was set up properly. Experimental diatoms were irradiated with a single pulse of high intensity light (ca. 10^5 μmol photons m^{-2}s^{-1}) with either blue or red light. The diatoms were irradiated at the leading or trailing ends (Figure 2) only once, and direction change response times were recorded. Un-irradiated diatoms were the negative control to compare response times of irradiated diatoms with the direction change times for untreated diatoms that had no additional light stimulus.

Experimental Design

For the irradiation experiment, two to three assays were prepared for each day's observations, a single species slide and one or two mixed-species slides. For each slide about 15 measurements were taken (i.e. direction change response time), making a total that ranged from 30-45 measurements for each experiment. These measurements consisted of leading end irradiations (e.g. red or blue light), trailing end irradiations, and un-irradiated cells. Such sets of observations were run at least 2-5 times, on separate days, for each experiment, so that each final data point contained at least 10 measurements. Measurements were made on diatoms that were

selected randomly, and the order of test (e.g. leading, trailing, un-irradiated) was changed

between replicates to ensure time under the microscope was not a determining factor. The total

number of diatoms measured for this research was over 2000.

Statistical Analysis

The statistics was performed using the computer software Statview (previously purchased

through SAS Inc., Cary, North Carolina). ANOVA was used to make pairwise comparisons

between and within multiple groups, for example, comparing front-end responses with un-

irradiated responses (control) for *C. cuspidata, S. phoenicenteron,* and *P. viridis* as well as all

three together in an assemblage. Scheffe's post-hoc test was used to determine significance

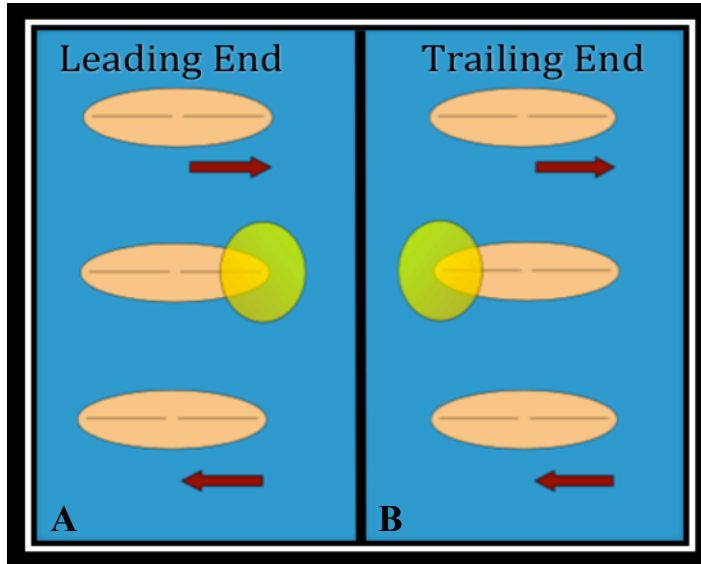levels for specific comparisons.

Figure 2: **Approximate Position of Diatom Irradiation** This diagram shows the approximate location of where the diatoms were irradiated with light. The slit on the diatom is the raphe. (A) Left: diatom was irradiated with light in the leading end; (B) Right: diatom was irradiated in the trailing end.

RESULTS

Single Species Response to Irradiation

Each of the three species used in this study, *P. viridis*, *C. cuspidata*, and *S. phoenicenteron*, were irradiated with blue (470 nm) or red (650 nm) high intensity light in their own single-species samples and direction change response times were recorded as described in materials and methods (Figures 4-9). For each sample prepared, direction change response times were also recorded for un-irradiated cells (Figure 3).

When each of the individual diatom species were irradiated at their leading end with blue light, response times were significantly different compared to un-irradiated cells. The average direction change response times for un-irradiated *C. cuspidata*, *S. phoenicenteron,* and *P. viridis* cells were 58 ± 4 s, 140 ± 10 s, and 150 ± 19 s, respectively (Figure 3). This can be compared to the average direction change response times for leading end irradiations with blue light for *C. cuspidata, S. phoenicenteron,* and *P. viridis* cells, which were 15 ± 3 s, 43 ± 4 s, and 13 ± 1 s, respectively (Figures 4a, 6a, 8a). All of these leading end irradiations with blue light resulted in a significantly faster response time (*P*<0.0001 for all three species).

Red light irradiations at the leading end did not result in a significantly different response times compared to un-irradiated cells for two of the species. The average direction change response times for leading end red light irradiations for *C. cuspidata, S. phoenicenteron,* and *P. viridis* cells were 51 ± 8 s, 96 ± 10 s, and 162 ± 20 s, respectively (Figures 5a, 7a, 9a). Unlike blue light irradiations, leading end irradiations with red light were not significantly different for *C. cuspidata* and *P. viridis* compared to un-irradiated cells (*C. cuspidata P*=0.71*, P. viridis P*=0.95). Red light irradiations of *S. phoenicenteron* cells resulted in significantly faster response times compared to un-irradiated cells (*P*=0.01).

In contrast to leading end irradiations, each diatom species had longer response times when irradiated at the trailing end. The average direction change response times for trailing end irradiations with blue light for *C. cuspidata, S. phoenicenteron*, and *P. viridis* cells were 139 ± 10 s, 266 ± 25 s, and 198 ± 27 s, respectively (Figures 4b, 6b, 8b). For each species, blue trailing end irradiations had significantly longer response times than the corresponding leading end irradiations ($P<0.0001$ for all three species). For *S. phoenicenteron* and *C. cuspidata*, blue trailing end irradiations had significantly longer response times than response times for un-irradiated cells ($P<0.001$). Blue irradiations at the trailing ends for *P. viridis* were not significantly different than response times for un-irradiated cells ($P=0.25$).

The average direction change response times to red light irradiations at the trailing end for *C. cuspidata, S. phoenicenteron*, and *P. viridis* cells were 104 ± 10 s, 147 ± 17 s, and 125 ± 28 s, respectively (Figures 5b, 7b, 9b). The response times for red trailing irradiations were significantly longer than corresponding leading end irradiations for both *C. cuspidata* ($P<0.0001$) and *S. phoenicenteron* ($P<0.04$). Red irradiations at the trailing ends for *P. viridis* were not significantly different from the corresponding leading end irradiations ($P<0.57$). For *C. cuspidata*, red trailing end irradiations had significantly longer response times than response times than response times for un-irradiated cells ($P=0.001$). Red irradiations at the trailing ends for *S. phoenicenteron* were not significantly different than response times for un-irradiated cells ($P=0.94$). For *P. viridis*, response times were not significantly different between red irradiations at the leading end, trailing end, and un-irradiated cells ($P=0.54$).

Multiple Species Response to Irradiation

To determine the effects of the presence of multiple species on motility, mixed-species samples were prepared and cells were irradiated with blue or red light and direction change response times were recorded as described in materials and methods (Figures 4-9). Similar to single-species samples, response times for un-irradiated cells for samples with multiple species were also recorded for every assay (Figure 3).

Un-irradiated Cells

To determine if the presence of other species had an effect on diatom motility in general, we investigated the direction change response times for un-irradiated cells of each species in mixed-species samples. The presence of multiple diatom species in the samples had little to no effect on the response time of un-irradiated cells compared to single-species samples (Figure 3). The average direction change response time for un-irradiated *C. cuspidata* cells in single-species samples was 58 ± 4 s, compared to response times of 74 ± 6 s, 59 ± 16 s, and 77 ± 19 s, respectively, for samples containing *C. cuspidata* along with equal numbers of *S. phoenicenteron, P. viridis*, or both species (Figure 3a). The average direction change response times for un-irradiated *C. cuspidata* cells were not significantly different between these four treatment groups (*P*=0.57).

The average direction change response time for un-irradiated *P. viridis* cells in single-species samples was 150 ± 19 s, compared to response times of 178 ± 34 s, 176 ± 29 s, and 216 ± 36 s, respectively, for samples containing *P. viridis* along with equal numbers of

C. cuspidata, *S. phoenicenteron,* or both species (Figure 3b). Similar to *C. cuspidata*, response times for un-irradiated *P. viridis* cells were not significantly different between the four treatment groups (P=0.44).

For *S. phoenicenteron*, the average direction change response time for un-irradiated cells in single-species samples was 140 ± 10 s, compared to response times of 190 ± 16 s, 138 ± 21 s, and 194 ± 34 s, respectively, for samples containing *S. phoenicenteron* along with equal numbers of *C. cuspidata, P. viridis*, or both species (Figure 3c). The response times for un-irradiated *S. phoenicenteron* cells were not significantly different between the four treatment groups (P=0.09).

Since the average direction change response times for un-irradiated cells were not significantly different between treatment groups for each species (single species, 1:1 ratio of two species (2), and 1:1:1 ratio of all three species samples), the average response time for all four treatment groups combined was used for comparisons between response times of irradiated cells and un-irradiated cells. The combined treatment groups average direction change response times for un-irradiated cells of *S. phoenicenteron, C. cuspidata,* and *P. viridis* were 178 ± 10 s, 65 ± 4 s, and 176 ± 14 s, respectively.
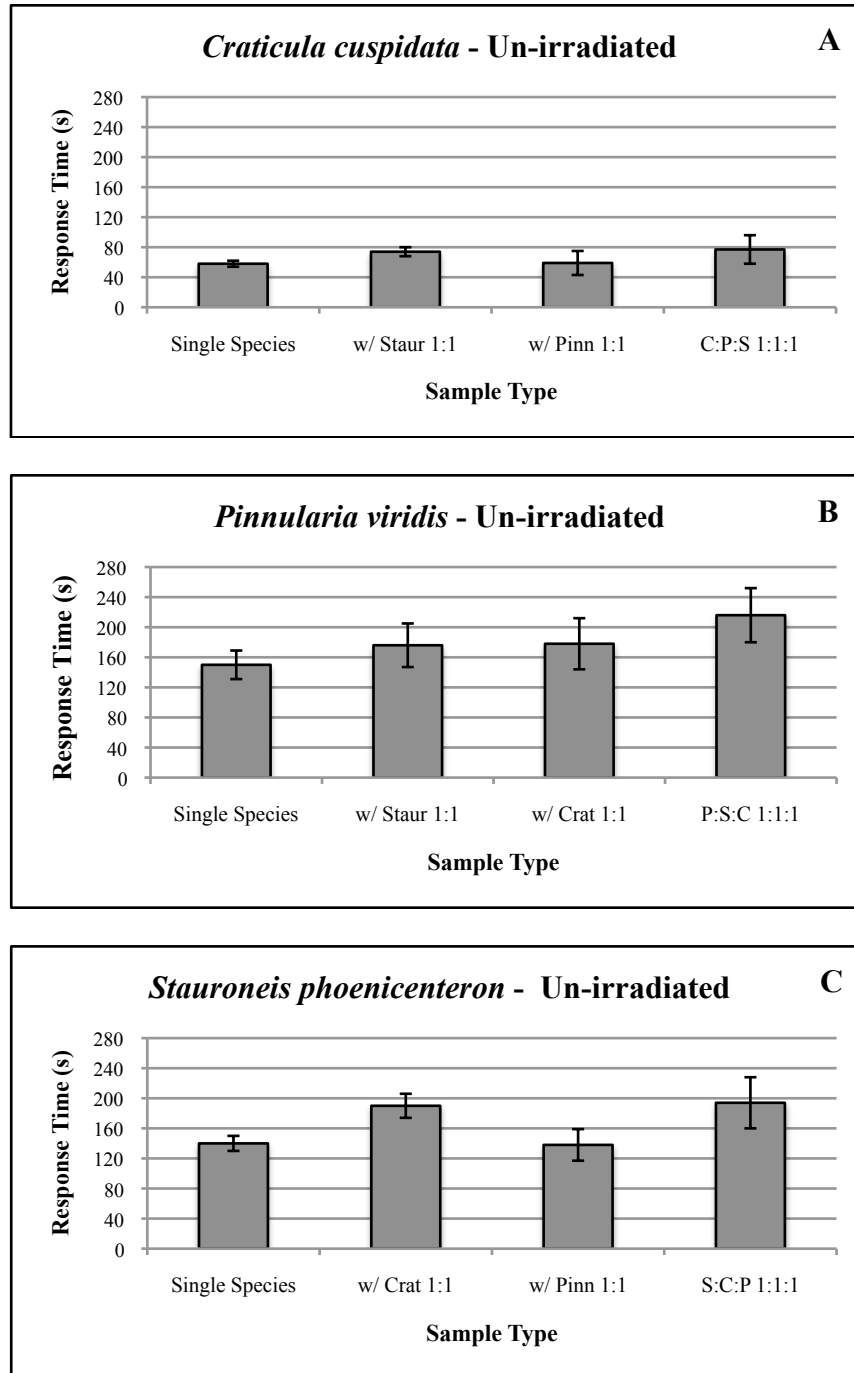
Figure 3: Average Direction Change Response Times for Un-irradiated Cells This figure displays the average direction change response times for un-irradiated cells in single species and multiple species assays. (A) *Craticula cuspidata* response times for un-irradiated cells in single species, 1:1 ratio with *S. phoenicenteron* (Staur), 1:1 ratio with *P. viridis* (Pinn), and 1:1:1 ratio with all three species (C:P:S). (B) *Pinnularia viridis* response times for un-irradiated cells in single species, 1:1 ratio with *S. phoenicenteron* (Staur), 1:1 ratio with *C. cuspidata* (Crat), and 1:1:1 ratio with all three species (P:S:C). (C) *Stauroneis phoenicenteron* response times for un-irradiated cells in single species, 1:1 ratio with *C. cuspidata* (Crat), 1:1 ratio with *P. viridis* (Pinn), and 1:1:1 ratio with all three species (S:C:P). Error Bars represent ± 1SE.

32

Pinnularia viridis Irradiations

In the presence of multiple species, *P. viridis* response times to leading end irradiations with blue light were not significantly different from response times observed in single-species samples (Figures 4 and 5). Specifically, the average direction change response time for blue light irradiations at the leading end for *P. viridis* cells alone was 13 ± 1 s, compared to response times of 14 ± 2 s, 14 ± 2 s, and 33 ± 11 s, respectively, for samples containing *P. viridis* along with equal numbers of *C. cuspidata, S. phoenicenteron*, or both species (Figure 4a). These response times were not significantly different from response times for single-species samples (P=0.08).

The average direction change response time for leading end red light irradiations for *P. viridis* alone was 162 ± 20 s, compared to response times of 143 ± 20 s, 141 ± 19 s, and 168 ± 24 s, respectively, for samples containing *P. viridis* along with equal numbers of *C. cuspidata, S. phoenicenteron*, or both species (Figure 5a). These response times were also not significantly different compared to the single-species samples (P=0.79).

Similarly, the presence multiple species had no effect on response times for trailing end irradiations with blue or red light for *P. viridis* compared to response times for single-species samples. The average direction change response times for trailing end blue light irradiations for *P. viridis* alone was 198 ± 27 s, compared to response times of 244 ± 33 s, 207 ± 23 s, and 300 ± 45 s, respectively, for samples containing *P. viridis* along with equal numbers of *C. cuspidata, S. phoenicenteron*, or both species (Figure 4b). These values were not significantly different from the average response time observed in single-species samples (P=0.10).

The average direction change response times for trailing end red light irradiations for *P. viridis* alone was 125 ± 28 s, compared to response times of 121 ± 40 s, 108 ± 24 s, and 144 ± 25 s, respectively, containing samples of *P. viridis* along with equal numbers of

C. cuspidata, S. phoenicenteron, or both species (Figure 5b). These response times were not

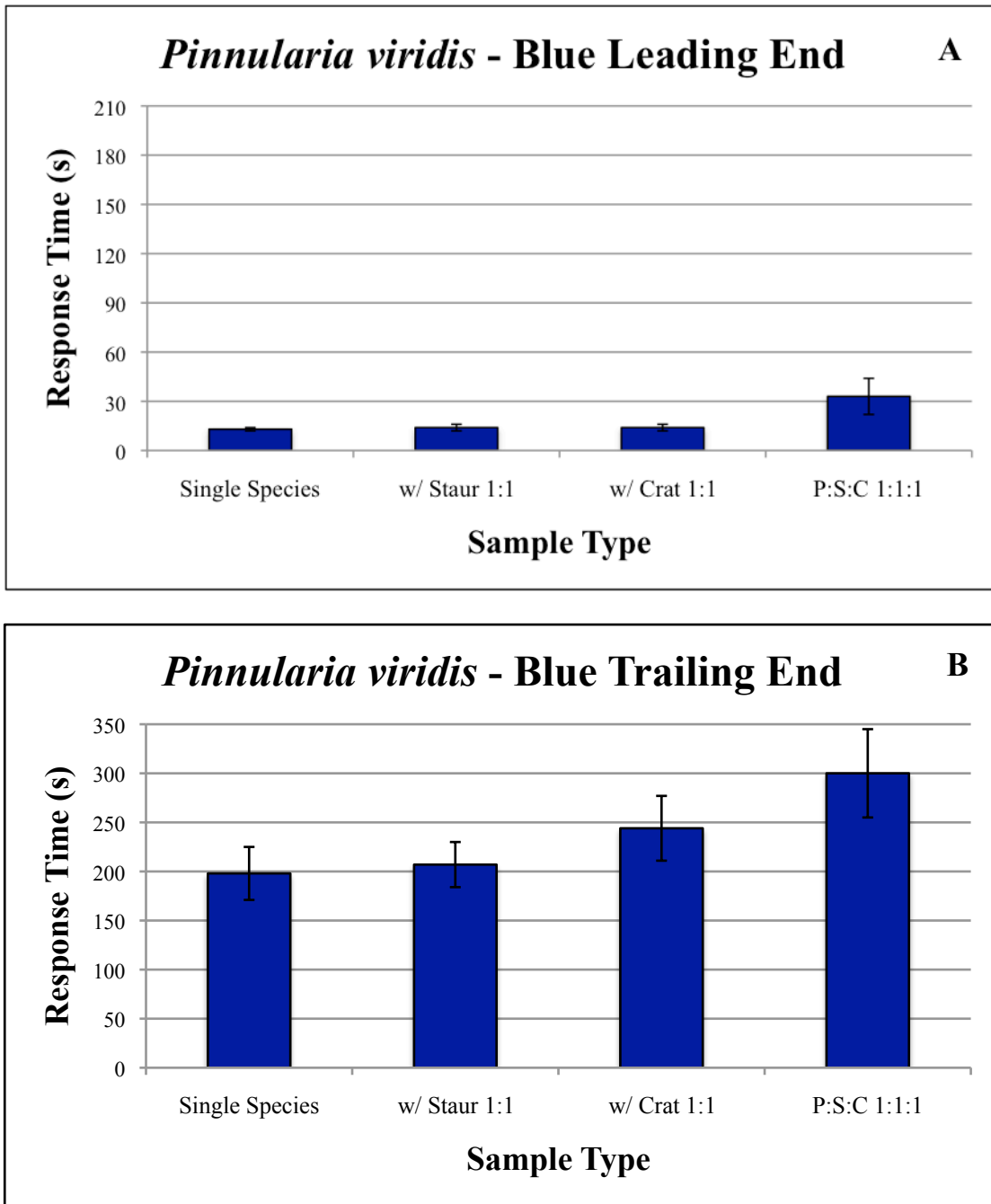significantly different from single-species samples (*P*=0.94).

Figure 4: *Pinnularia viridis* **Average Direction Change Response Times to Blue Light Irradiations** This figure displays the average direction change response times of *P. viridis* to irradiations with high intensity blue light (470 nm) in single-species assays and mixed-species assays containing *P. viridis* along with equal numbers of *S. phoenicenteron* (Staur), *C. cuspidata* (Crat), or both (P:S:C) (ratios of 1:1 or 1:1:1). A) Average response times of *Pinnularia viridis* leading end irradiations. B) Average response times of *Pinnularia viridis* trailing end irradiations. Error Bars represent ± 1SE.

Figure 5: *Pinnularia viridis* **Average Direction Change Response Times to Red Light Irradiations** This figure displays the average direction change response times of *P. viridis* to irradiations with high intensity red light (650 nm) in single-species assays and mixed-species assays containing *P. viridis* along with equal numbers of *S. phoenicenteron* (Staur), *C. cuspidata* (Crat), or both (P:S:C) (ratios of 1:1 or 1:1:1). A) *Pinnularia viridis* leading end irradiations. B) *Pinnularia viridis* trailing end irradiations. Error Bars represent ± 1SE.

36

Craticula cuspidata Irradiations
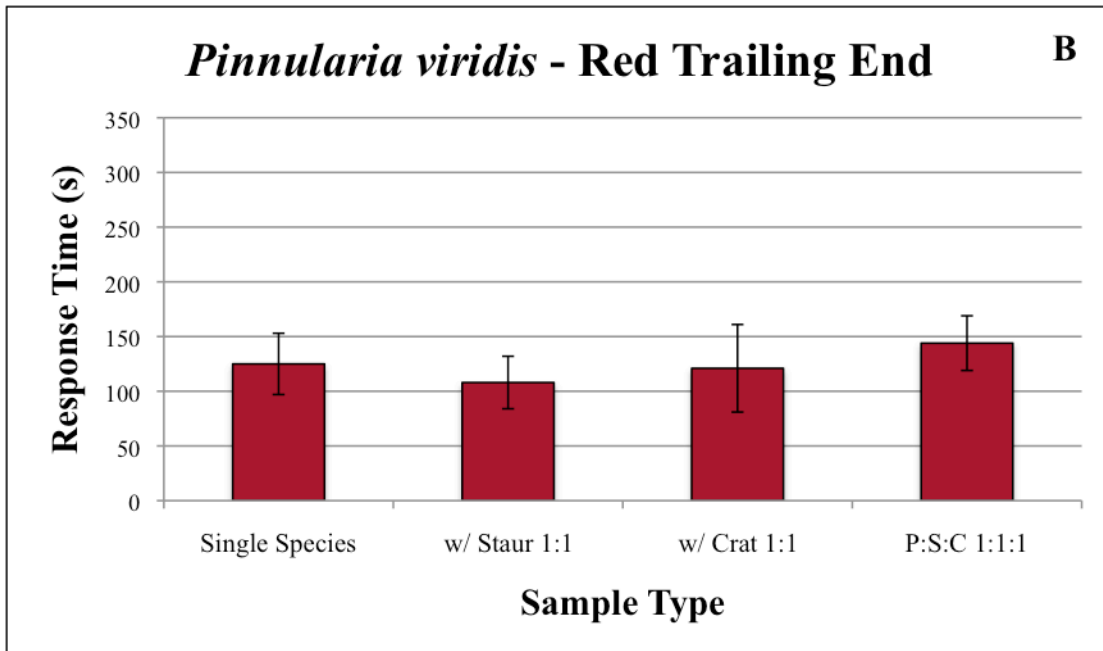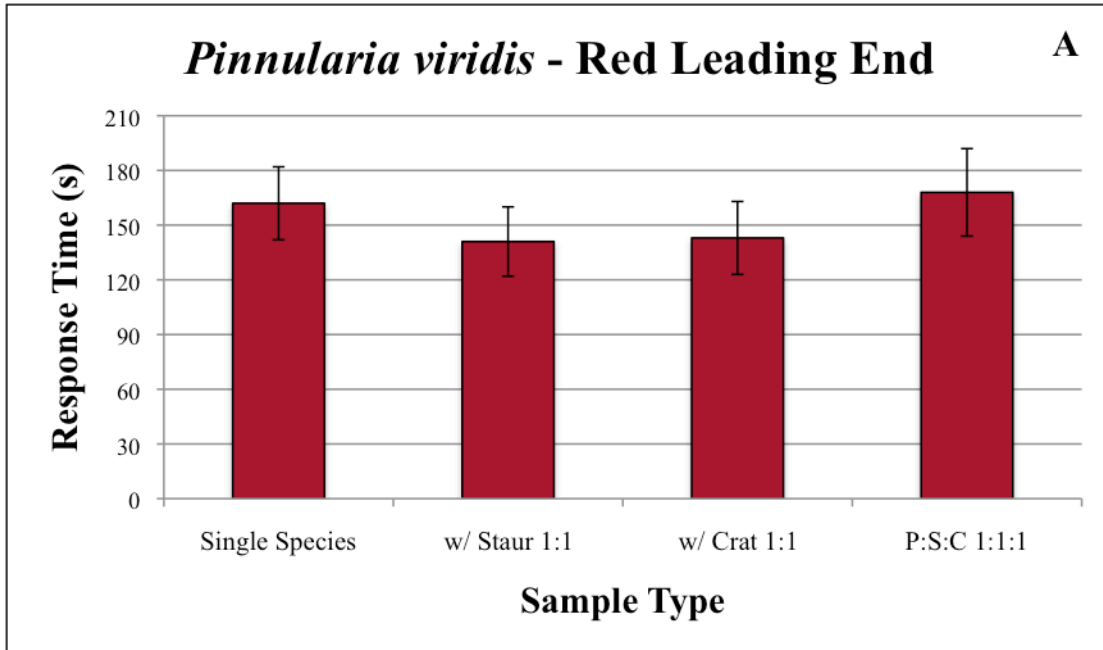
In the presence of multiple species, *C. cuspidata* response times to leading end irradiations were not significantly different from response times for single-species samples (Figures 6 and 7). Specifically, the average direction change response time for leading end blue light irradiations for single-species samples of *C. cuspidata* cells was 15 ± 3 s, compared to response times of 13 ± 2 s, 17 ± 2 s, and 12 ± 2 s, respectively, for samples containing *C. cuspidata* along with equal numbers of *P. viridis, S. phoenicenteron*, or both species (Figure 6a). The average direction change response time for leading end red light irradiations for *C. cuspidata* alone was 51 ± 8 s, compared to response times of 58 ± 13 s, 43 ± 6 s, and 53 ± 19 s, respectively, for samples containing *C. cuspidata* along with equal numbers of *P. viridis, S. phoenicenteron*, or both species (Figure 7a). Response times for leading end irradiations with either red or blue light in mixed-species samples for *C. cuspidata* were not significantly different from single-species samples (blue light: P=0.52; red light: P=0.77).

Similarly, *C. cuspidata* response times to trailing end irradiations in the presence of other species were not significantly different from response times for single-species samples. The average direction change response time for trailing end blue light irradiations for single-species samples of *C. cuspidata* cells was 139 ± 10 s, compared to response times of 148 ± 16 s, 138 ± 22 s, and 187 ± 28 s, respectively, for samples containing *C. cuspidata* along with equal numbers of *P. viridis*, *S. phoenicenteron*, or both species (Figure 6b). The average direction change response time for trailing end red light irradiations for single-species samples of *C. cuspidata* cells was 104 ± 10 s, compared to response times of 118 ± 20 s, 113 ± 14 s, and 106 ± 20 s, respectively, for samples containing *C. cuspidata* along with equal numbers of

P. viridis, *S. phoenicenteron*, or both species (Figure 7b). Response times for trailing end irradiations with either red or blue light in mixed-species samples for *C. cuspidata* were not significantly different from single-species samples (blue light: P=0.49; red light: P=0.43).

Figure 6: *Craticula cuspidata* **Average Direction Change Response Times to Blue Light Irradiations** This figure displays the average direction change response times of *C. cuspidata* to irradiations with high intensity blue light (470 nm) in single-species assays and mixed-species assays containing *C. cuspidata* along with equal numbers of *S. phoenicenteron* (Staur), *P. viridis* (Pinn), or both (C:P:S) (ratios of 1:1 or 1:1:1). A) *Craticula cuspidata* leading end irradiations. B) *Craticula cuspidata* trailing end irradiations. Error Bars represent ± 1SE.
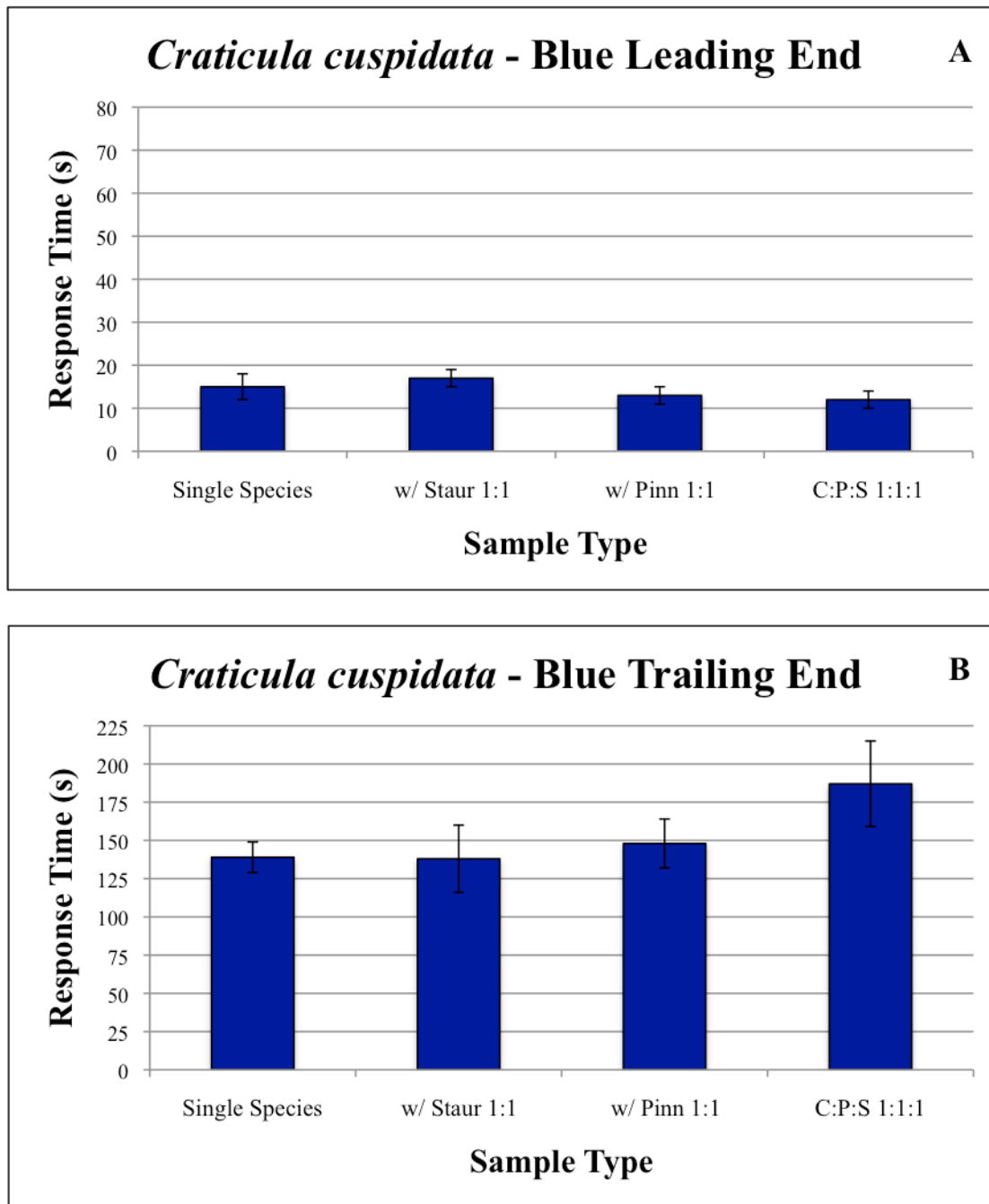
Figure 7: *Craticula cuspidata* **Average Direction Change Response Times to Red Light Irradiations** This figure displays the average direction change response times of *C. cuspidata* to irradiations with high intensity red light (650 nm) in single species-assays and mixed-species assays containing *C. cuspidata* along with equal numbers of *S. phoenicenteron* (Staur), *P. viridis* (Pinn), or both (C:P:S) (ratios of 1:1 or 1:1:1). A) *Craticula cuspidata* leading end irradiations. B) *Craticula cuspidata* trailing end irradiations. Error Bars represent ± 1SE.
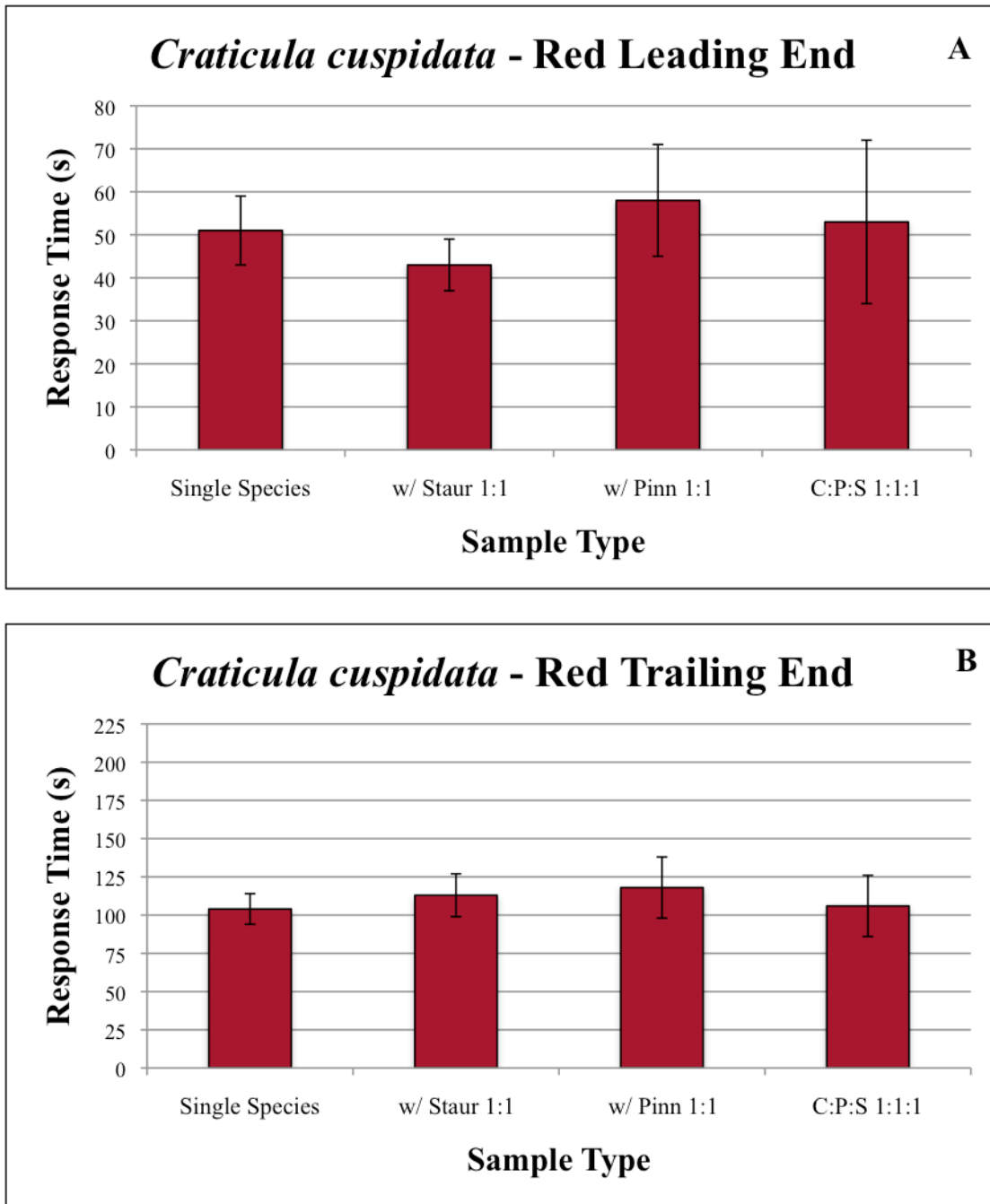
Stauroneis phoenicenteron Irradiations

Unlike the response times of *P. viridis* and *C. cuspidata* cells, response times of

S. phoenicenteron cells in the presence of other species were often significantly different from

response times for single-species samples (Figures 8 and 9). Specifically, the average direction

change response time for leading end blue light irradiations for *S. phoenicenteron* alone was

43 ± 4 s, compared to response times of 58 ± 7 s, 88 ± 9 s, and 127 ± 21 s, respectively, for

samples with equal numbers of *P. viridis, C. cuspidata*, or both species (Figure 8a). Response

times of *S. phoenicenteron* with equal numbers of *P. viridis* cells present were not significantly

different from response times for single-species samples of *S. phoenicenteron* (P=0.99).

However, the presence of *P. viridis* and *C. cuspidata*, significantly increased response times for

S. phoenicenteron compared to response times for single-species samples of *S. phoenicenteron*

(P=0.01). Moreover, the presence of *C. cuspidata* significantly increased response times for

S. phoenicenteron compared to response times for single-species samples of *S. phoenicenteron*

(P=0.05).

The response times for *S. phoenicenteron*, irradiated at the leading end with red light in

the presence of multiple species, were not significantly different compared to response times of

single-species samples. The average direction change response time for leading end red light

irradiations for single-species samples of *S. phoenicenteron* was 96 ± 10 s, compared to response

times of 159 ± 29 s, 148 ± 13 s, and 139 ± 33 s, respectively, for samples containing

S. phoenicenteron along with equal numbers of *P. viridis, C. cuspidata*, or both species (Figure

9a). These response times for *S. phoenicenteron* in the presence of other species were not

significantly different from response times for single-species samples of *S. phoenicenteron*

(*P. viridis* P=0.39, *C. cuspidata* P=0.07, both species present P=0.75).

Similar to response times for leading end red light irradiations, trailing end irradiations with either red or blue light for *S. phoenicenteron* in the presence of multiple species resulted in response times that were not significantly different from response times for single-species samples of *S. phoenicenteron*. Specifically, the average direction change response time for trailing end blue light irradiations for *S. phoenicenteron* alone was 266 ± 25 s, compared to response times of 177 ± 20 s, 188 ± 17 s, and 282 ± 39 s, respectively, for samples containing *S. phoenicenteron* along with equal numbers of *P. viridis, C. cuspidata*, or both species (Figure 8b). These response times for *S. phoenicenteron* in the presence of multiple species were not significantly different from single-species samples of *S. phoenicenteron* (P=0.30).

The average direction change response time trailing end red light irradiations for single-species samples of *S. phoenicenteron* was 147 ± 17 s, compared to response times of 115 ± 16 s, 162 ± 19 s, and 192 ± 43 s, respectively, for samples containing *S. phoenicenteron* along with equal numbers of *P. viridis, C. cuspidata*, or both species (Figure 9b). Response times for *S. phoenicenteron* irradiated at the trailing end with red light in presence of other species were not significantly different from response times for single-species samples of *S. phoenicenteron* (P=0.24).

Figure 8: *Stauroneis phoenicenteron* **Average Direction Change Response Times to Blue Light Irradiations** This figure displays the average direction change response times of *S. phoenicenteron* to irradiations with high intensity blue light (470 nm) in single-species assays and mixed-species assays containing *S. phoenicenteron* along with equal numbers of *C. cuspidata* (Crat), *P. viridis* (Pinn), or both (S:C:P) (ratios of 1:1 or 1:1:1). A) *Stauroneis phoenicenteron* leading end irradiations. B) *Stauroneis phoenicenteron* trailing end irradiations. Error Bars represent ± 1SE.
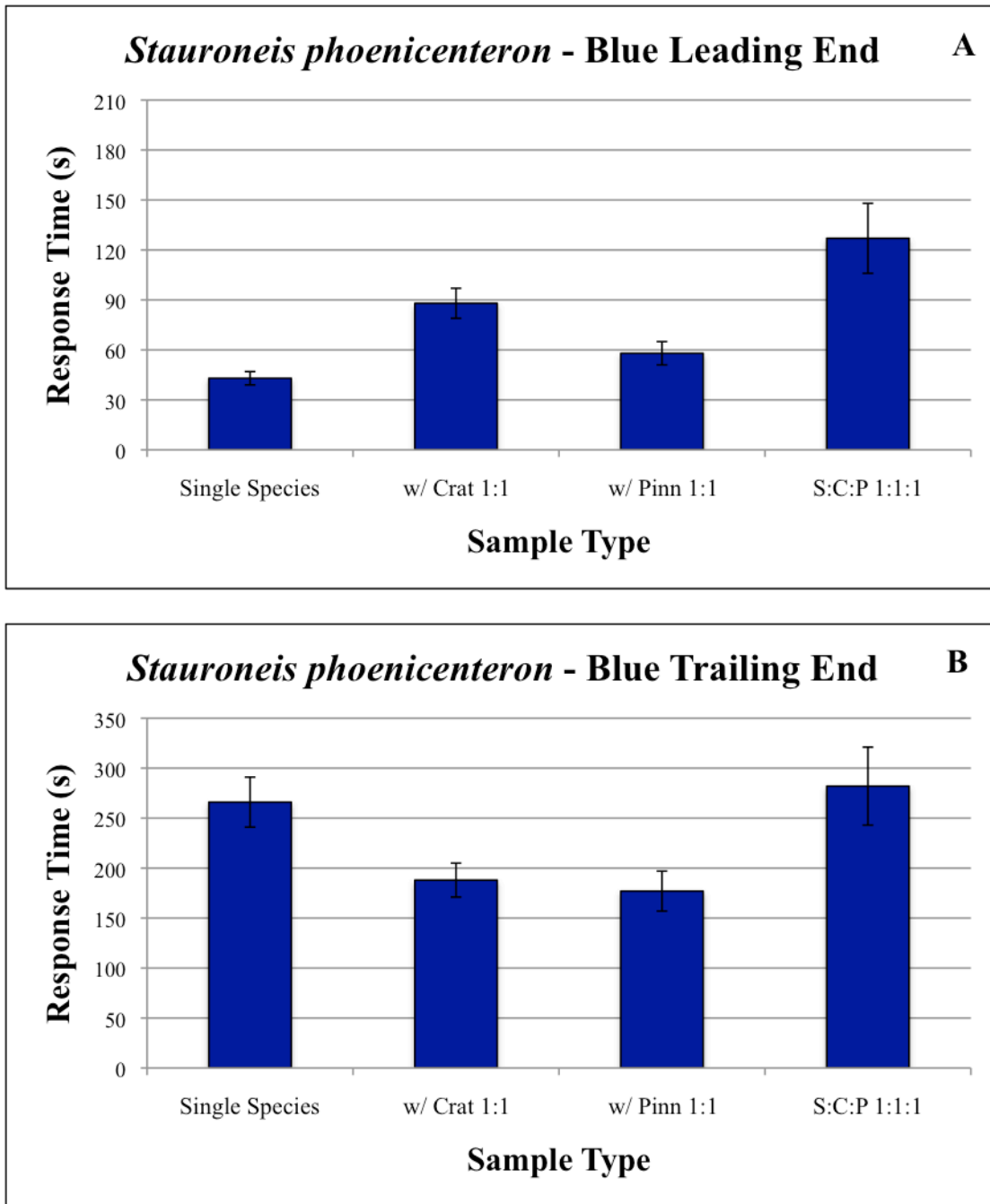
Figure 9: *Stauroneis phoenicenteron* **Average Direction Change Response Times to Red Light Irradiations** This figure displays the average direction change response times of *S. phoenicenteron* to irradiations with high intensity red light (650 nm) in single-species assays and mixed-species assays containing *S. phoenicenteron* along with equal numbers of *C. cuspidata* (Crat), *P. viridis* (Pinn), or both (S:C:P) (ratios of 1:1 or 1:1:1). A) *Stauroneis phoenicenteron* leading end irradiations. B) *Stauroneis phoenicenteron* trailing end irradiations. Error Bars represent ± 1SE.
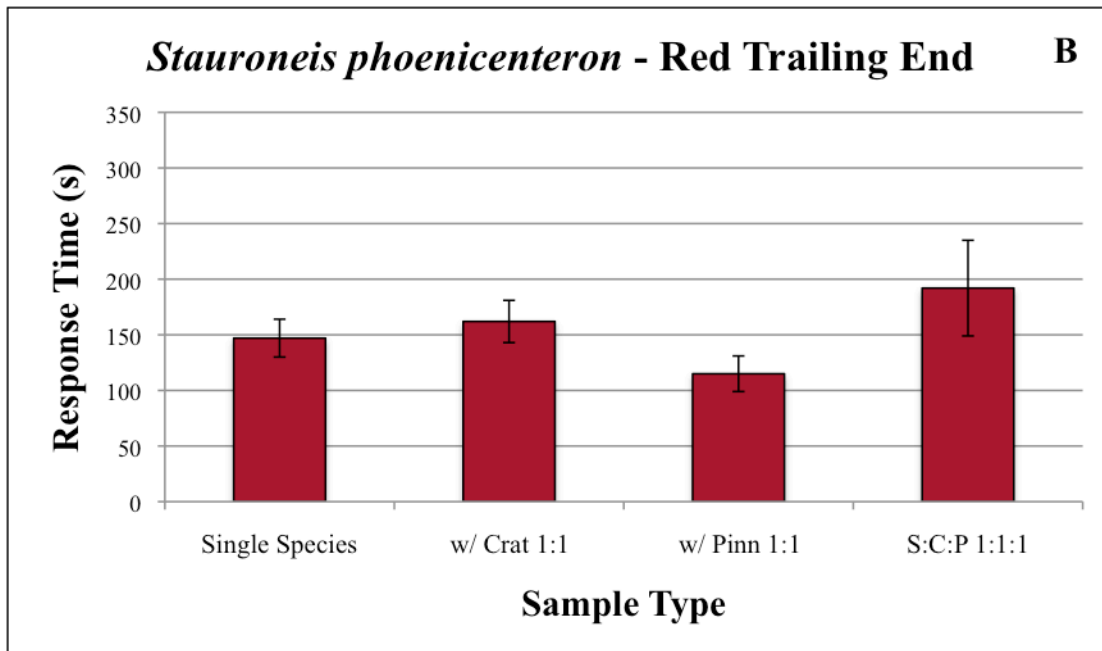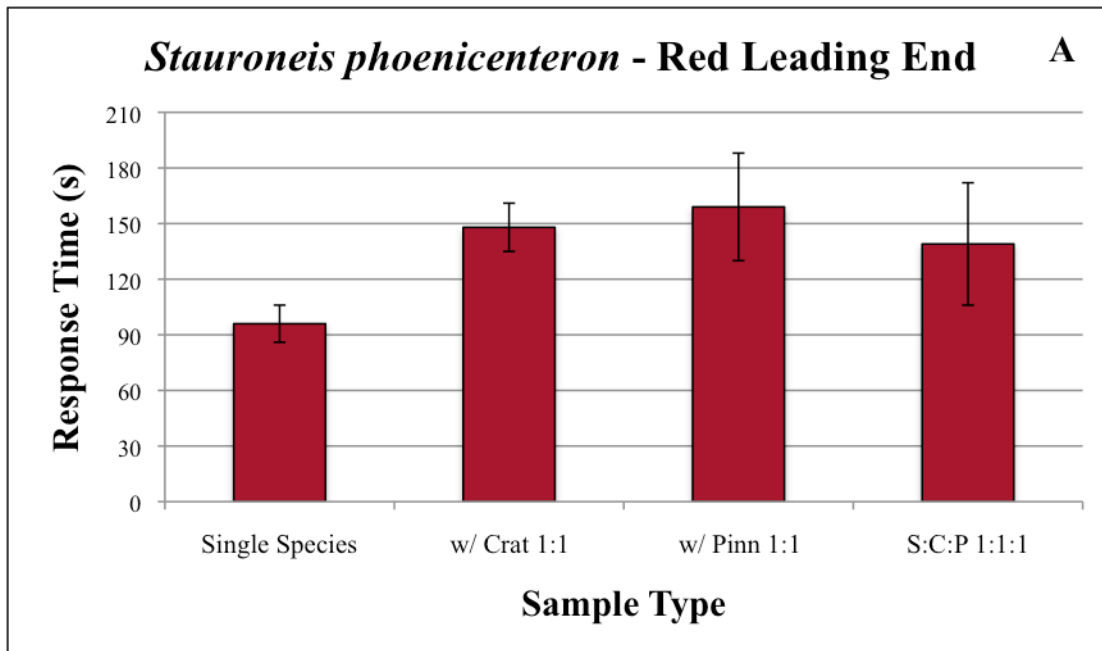
Dose-Dependent Response of Stauroneis phoenicenteron and Craticula cuspidata in Mixed Species Samples

Since the earlier experiments of *S. phoenicenteron* resulted in significant differences in response times for multiple-species samples compared to single-species samples, additional motility experiments were conducted to determine if there was a dose-dependent pattern of increase. *Stauroneis phoenicenteron* was the only species that had significant increases in direction change response times in the presence of other species. In particular, blue leading end irradiations of *S. phoenicenteron* in the presence of *C. cuspidata* cells resulted in significantly longer response times compared to single-species samples. To determine the dose-dependent nature of this effect, samples were prepared with different ratios of *C. cuspidata* and *S. phoenicenteron* cells and the average direction change response times were recorded as described in materials and methods.

Interestingly, *S. phoenicenteron* response times continued to increase as the percentages of *C. cuspidata* present in the sample increased (Figure 10). Specifically, the average direction change response time for single-species samples of *S. phoenicenteron* cells irradiated at the leading end with blue light was 42 ± 4 s, compared to response times of 64 ± 8 s, 82 ± 12 s, 88 ± 9 s, 119 ± 22 s, and 172 ± 39 s, respectively, for samples containing *C. cuspidata* cells at 10%, 20 %, 50%, 80%, and 90% of the cells present (Figure 10). Single-species samples of *S. phoenicenteron* were significantly different from all samples containing percentages of *C. cuspidata* greater than 10% (for 10% *C. cuspidata*, $P=0.99$; 20-80% *C. cuspidata*, $P \leq 0.05$; 90% *C. cuspidata*, $P=0.04$).

In contrast to *S. phoenicenteron* response times, the percentage of *S. phoenicenteron* cells present had no effect on the response times for *C. cuspidata* cells. The average direction change response time for single-species samples of *C. cuspidata* cells irradiated with blue light was 15 ± 3 s, compared to response times of 7 ± 1 s, 9 ± 1 s, 17 ± 2 s, 12 ± 2 s, and 11 ± 5 s, respectively, for samples containing *S. phoenicenteron* cells at 10%, 20 %, 50%, 80%, and 90% of the cells present (Figure 10). *Craticula cuspidata* in the presence of different percentages of *S. phoenicenteron* cells resulted in response times that were not significantly different from response times of single-species samples of *C. cuspidata* ($P=0.98$).
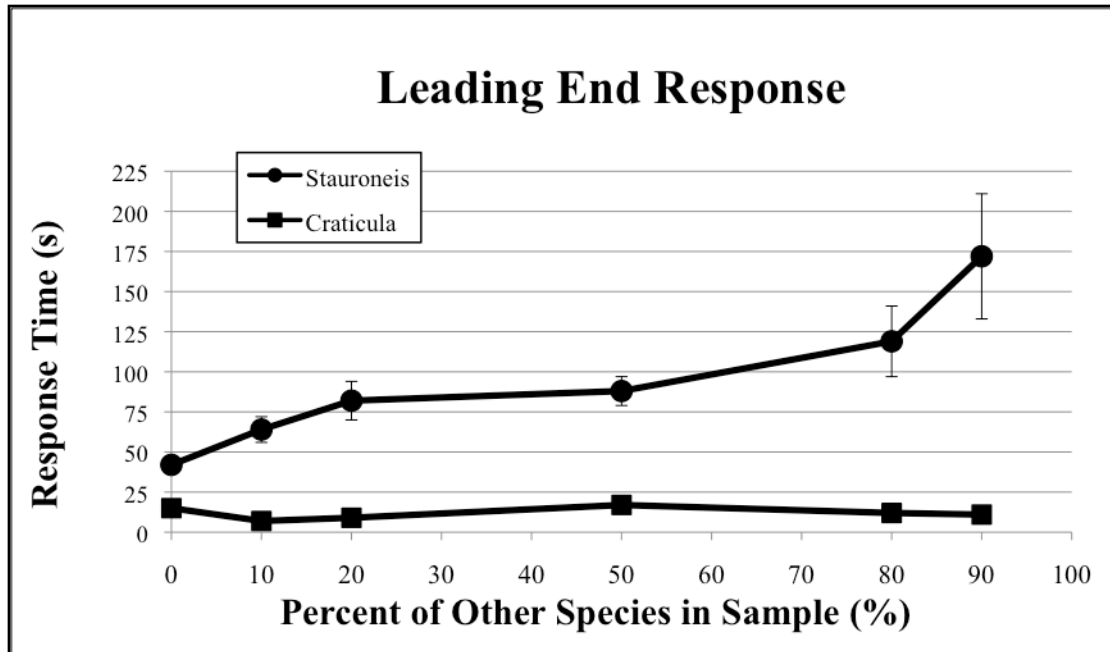
Figure 10: The Response Times of *Craticula cuspidata* and *Stauroneis phoenicenteron* to Leading End Blue Irradiations as a Function of Species Composition in Sample This figure displays the average direction change response times of *S. phoenicenteron* (Stauroneis) and *C. cuspidata* (Craticula) cells irradiated at the leading end with high intensity blue light (470 nm). Samples were prepared with different amounts of *S. phoenicenteron* and *C. cuspidata* cells. Error Bars represent ± 1SE.

DISCUSSION

Diatoms, like most algae, are often found in complex assemblages consisting of many different species (Stevenson et al. 1996, Armbrust 2009), which can create the possibility of active competition for limited resources. Therefore, diatoms that have the adaptation of motility can better respond to environmental stimuli such as light and temperature and thus can be more successful competitors in these diverse communities. Most of these algal assemblages contain an abundance of diatoms, which can potentially lead to closely related diatom species competing for the same resources. In nature, if two similar species in the same community are competing for the same limiting resource in a confined space, sometimes one of those species will have a slightly more efficient ability to use that resource. Therefore, over time that species would be able to reproduce more successfully and be more prolific than the other species, ultimately outcompeting the other species.

In order for multiple species in the same community to effectively succeed, they must use resources efficiently, where each species is likely to occupy their own localized area or niche, using slightly different resources. This process, known as niche or resource partitioning, allows individual species to optimize access to resources (e.g. light) while expending less energy competing with other species (Schoener 1974, Finke & Snyder 2008, Griffin & Silliman 2012). This is also thought to occur in algal communities, where closely related species of diatoms in the same assemblage will differentiate spatially into slightly different niches. It has been observed that diatom species can stratify into different microhabitats (Litchman et al. 2007, Winder et al. 2009, Cibic et al. 2012). Our results confirm previous research that each of the three diatom species has species-specific light sensitivity (Cohn & Disparti 1994, Cohn et al. 2004, Cohn et al. 2015), suggesting their differences in sensitivity to light could allow these

species to spatially partition into slightly different light areas. The experimental data provided here suggest that some diatom species have additional modifications to their motile and behavioral responses to light stimulus when in the presence of other species, which could provide a further mechanism for some species to carry out this partitioning.

Light is a strong environmental cue that is thought to affect physiological responses such as motile behavior and biofilm formation (Coelho et al. 2011). For motile photosynthetic organisms, such as diatoms, the ability to actively manage their access to light is crucial due to light generating most of their energy production. Therefore, it is not surprising that diatoms would be responsive to light conditions due to the importance of light to their success in obtaining energy. Motility allows these organisms to move into or out of specific light, allowing them to regulate their energy collection and provide greater efficiency of resource use.

Motile diatoms will move towards certain light stimuli when conditions are favorable, allowing them to maximize their position to the most optimal light conditions. In general, diatoms tend to move into light that is relatively low to moderate in intensity. However, when light conditions are unfavorable, for example when light intensity is too high, diatoms will respond by changing their direction and moving away from the light (Cohn et al. 1999, Cohn et al. 2004, Cartaxana et al. 2011). This response keeps the cells moving away from the high intensity light by inducing direction change at the leading end and repressing direction change at the trailing end.

Our results showed that even though each species showed its own characteristic sensitivity to light, as a whole, all species had shorter direction change response times to high intensity irradiations of their leading end compared to similar irradiations of their trailing end, resulting in a net movement of the cells out of bright light. Specifically, *C. cuspidata* and

S. phoenicenteron response times to irradiations of their leading end ranged from 15-51 s and 43-96 s, respectively, while the trailing end response times ranged from 104-139 s and 147-266 s, respectively (Figures 6-9). This behavior of cells to remove themselves from very high intensity light is often referred to as photoprotection and is thought to be a physiological response to avoid photodamage, especially for the photopigments that are responsible for light absorption (Serodio et al. 2006, Cartaxana et al. 2011).

Similarly, *P. viridis* response times for irradiations of high intensity blue light resulted in significant differences in response times between leading and trailing irradiations, having response times that ranged from 12-14 s for leading end irradiations, and 171-225 s for trailing end irradiations (Figure 4). However, the leading and trailing end response times for exposure to red light were not significantly different. Specifically, red light stimulated response times for *P. viridis* that ranged from 142-182 s for leading end irradiations, and 97-153 s for trailing end irradiations (Figure 5). These results suggest that *P. viridis* has relatively little sensitivity to red light and therefore, does not likely use red wavelengths of light for positional cues, unlike *S. phoenicenteron* and *C. cuspidata*. Both *S. phoenicenteron* and *C. cuspidata* are sensitive to low red light, although *S. phoenicenteron* moves towards low red light and *C. cuspidata* moves away from it (Cohn & Disparti 1994, Cohn et al. 2004, Cohn et al. 2015). This difference in red light sensitivity might lead to spatial separation for these two species. The spectral ranges of *S. phoenicenteron* and *C. cuspidata*, that seem to stimulate motile responses, appear to be more similar than that for *P. viridis* and therefore, it is likely advantageous for these two species to exploit the differences in their light responses in order to partition resources and compete more effectively.

Previous research, along with these results, confirm that each of the diatom species used in this study has different sensitivities to light with particular wavelengths and intensities of light that attracts each species (Cohn & Disparti 1994, Cohn 2001). Specifically, *C. cuspidata* moves towards moderate blue light, *P. viridis* moves towards blue-green light, and *S. phoenicenteron* moves towards moderate blue light and low to moderate red light (Cohn & Disparti 1994, Cohn et al. 2004, Cohn et al. 2015). Such differences in responsiveness to various wavelengths, similar to the example for red wavelengths mentioned previously, may provide strong ecological stimuli that may drive these species to niche partition or stratify into different layers of their community.

In addition to species-specific responses to light wavelength and intensity, our data suggest that, at least with *S. phoenicenteron*, the presence or absence of other diatom species can also alter behavioral responses to light. In particular, *S. phoenicenteron* had response times to high intensity blue irradiations that were statistically longer in the presence of other species compared to single-species samples (Figure 8 and 9). The additional presence of *P. viridis* and *C. cuspidata* together or *C. cuspidata* alone significantly increased response times to blue light exposure for *S. phoenicenteron* compared to response times of *S. phoenicenteron* in single-species samples. The additional presence of *P. viridis* and *C. cuspidata* together resulted in a 3 fold increase and the additional presence of *C. cuspidata* alone resulted in a 2 fold increase (Figure 8 and 9).

The response times to blue light exposure at the leading end for *S. phoenicenteron* with the presence of *C. cuspidata* resulted in significant increases in response times compared to single-species samples of *S. phoenicenteron*. In contrast, the additional presence of *P. viridis* resulted in response times of *S. phoenicenteron* that were not significantly different from

response times of *S. phoenicenteron* single-species samples. These results suggest that *P. viridis* does not have an effect on the motility of *S. phoenicenteron* like *C. cuspidata* does.

Our data, analyzing the effect of irradiations as a function of the percentage of other species present, suggest that not only the species type but also the density of other species present in their immediate environment can alter diatom motility. In the experiments looking at the effect of relative abundance of *S. phoenicenteron* and *C. cuspidata* on their response times to high intensity blue light irradiation at the leading end, only *S. phoenicenteron* was significantly affected. The average response times of *S. phoenicenteron* increased as a function of increasing *C. cuspidata*, such that samples that contained 90% *C. cuspidata* cells showed a 4 fold increase in response times for *S. phoenicenteron* (Figure 10). These results suggest a dose-dependent effect of *C. cuspidata* on *S. phoenicenteron*, but not vice versa.

The altered direction change response times of *S. phoenicenteron* in the presence of *C. cuspidata*, suggest that the light sensitivity of some diatom species can change based on the composition of other species that are present in their community. *Craticula cuspidata* retained its same sensitivity to blue light, unlike *S. phoenicenteron*, suggesting this modified behavior is species-specific and not an altered response that all diatom species exhibit. The altered behavior of *S. phoenicenteron* suggests that there is only an interaction between these two species. Similar to the intrinsic species-specific light responses, the change in light responsiveness due to presence of other species is likely another way cells can partition their resources more effectively.

For example, both *C. cuspidata* and *S. phoenicenteron* are attracted to low blue light. However, *S. phoenicenteron* is also attracted to low red, unlike *C. cuspidata*. Therefore, the normal attraction of *S. phoenicenteron* to red light, along with the lowered sensitivity to blue

light in the presence of *C. cuspidata*, might drive *S. phoenicenteron* into a more red light favorable environment, allowing *C. cuspidata* to exploit blue light areas more effectively. This would allow each species to spatially partition themselves into different microhabitats where each species could use their resources most efficiently. However, there are still additional factors beyond their species-specific light sensitivities that should be examined to determine more conclusively what could potentially drive localization or stratification within their algal assemblages.

Unlike *S. phoenicenteron*, *C. cuspidata* and *P. viridis* had the same behavioral responses to light stimuli in the presence or absence of other species (Figures 4-7). These results indicate that there was no effect of multiple species being present on *C. cuspidata* and *P. viridis* motility. Our data suggest that alterations in their direction change response due to the presence of other species may not be a significant way by which these species resource partition. Localization or stratification of these two species within their algal assemblages might be due to different factors, although it is not clear if they stratify in nature.

Diatom species are known to stratify into different layers of their community, which is thought to be driven by a number of different environmental stimuli and physical characteristics (Litchman et al. 2007, Winder et al. 2009, Cibic et al. 2012). Therefore by looking at both physical and behavioral characteristics of these different diatom species one can make hypotheses as to how they might respond under different conditions. Our results do not show direct evidence of diatom stratification, however, the accumulation of information regarding the motile characteristics of these species allows us to begin to speculate on how these species might behave in their natural environment.

As previously described in examples above, behavioral responses that can lead to cell segregation are subject to the immediate conditions of the surrounding environment and can be modified. Behavioral aspects include responses to varying light and other environmental stimuli, motility differences on various substrata, and physical and chemical cues that can change due to the presence of other species. Our study focused on behavioral changes in response times to light irradiation for diatoms in the presence of other species, which helps us understand just one of the many ways that resource partitioning may occur.

Along with responsiveness to light, physical characteristics of the cell and their motion can also help species accomplish resource partitioning and stratification. Physical attributes of the cell are inherent to each species and often do not change. For example, cell size and shape, mucilage composition, and pattern of movement directed by the raphe (e.g. straight or curved), are all physical characteristics that do not rely on varying external conditions. These intrinsic characteristics can also affect the way species interact and compete with each other.

For instance, cell size and speed for these three diatom species are different and therefore, might play a role in how these species interact within their community. The small size and fast motile responsiveness to light stimuli of *C. cuspidata* suggest that these cells might be located near the surface of algal assemblage, where they can easily respond to unfavorable conditions and more easily move through the community. In contrast, both *P. viridis* and *S. phoenicenteron* have slightly larger cell sizes on average and respond slower to light stimuli compared to *C. cuspidata*, suggesting that these species might occupy lower layers of the community where fast responses to environmental conditions are not as crucial. Previous research has shown that cell size can play a role in stratification, where larger diatoms are found lower in the algal community compared to small ones (Baillie 1987, Litchman et al. 2007, Winder et al. 2009).

The inherent pattern of movement for diatoms can also influence the effectiveness of their motility responses within their environment. For instance, *P. viridis* moves in a curved pattern (Edgar & Pickett-Heaps 1983, Cohn & Weitzell 1996), unlike *C. cuspidata* and *S. phoenicenteron* that move in a straight path. This circular pattern of movement could potentially prevent *P. viridis* cells from responding to environmental cues, such as light, as effectively as *C. cuspidata* and *S. phoenicenteron*. Such data also suggest *P. viridis* does not exhibit the same motile behaviors as *C. cuspidata* and *S. phoenicenteron*, and would therefore likely localize different areas within their algal assemblage. Similar to these physical traits, behavioral characteristics that were not the focus in this study, might also affect how species within the same community differentiate their resource use.

Many organisms behave by using chemical cues when other species are present. This response can affect species around them, for example, some plants and certain types of algae, have chemoattractants that can change the responses of predators or prey in their environment by drawing them in or deterring them away (Bell & Mitchell 1972). Like other microorganisms, such as bacteria, some diatom species could potentially have the ability to send out chemical signals to inhibit the movement of other species. For instance, some bacteria use excitatory or inhibitory signals affecting the behavior of other species around them (Waters & Bassler 2005), a mechanism that is often cell density dependent. Such dependence is similar to the *C. cuspidata* and *S. phoenicenteron* relative abundance experiments in our research, where the increased proportion of *C. cuspidata* cells affected the motility responses of *S. phoenicenteron*. These cell density-dependent responses of *S. phoenicenteron* might be due to *C. cuspidata* secretion of either soluble factors or cell-specific mucilage.

Mucilage composition from one species may also change the motile behavior of other species within the environment by physically changing the substratum. Mucilage secretion is central to diatom motility, providing a way for diatoms to adhere to surfaces as well as using those adhered connections to produce movement. Diatom mucilage is composed of different polysaccharides and proteins that are unique to each species (Hoagland et al. 1993, Myklestad 1999, Chiovitti et al. 2006, Poulsen et al. 2014). The exact chemical nature of mucilage for many diatom species is unknown due to the difficulty in proper isolation techniques that retain the molecular composition of mucilage. However, more recent studies have developed new methods for characterizing the molecular components (Poulsen et al. 2014), which may allow for the mucilage composition of more species to be determined. Mucilage from different species present in the environment could potentially affect motile characteristics, such as cell speed. For instance, the mucilage from one species might physically slow the movement of other species around them by affecting the surface they move on. This could be what is happening with *C. cuspidata*, where the mucilage secreted by *C. cuspidata* hinders *S. phoenicenteron* ability to respond quickly. Therefore, the dose-dependent effect might be due to the increase of mucilage from *C. cuspidata* as the relative concentration of these cells increases.

Secreted mucilage from different diatom species, as well as other environmental conditions, might also have an effect on adhesion properties, ultimately making adhesion to substrata weaker or stronger. Previous research, using the same three species of diatoms used in this study, has determined that temperature, as well as the presence of multiple species, can affect adhesion strength and motility of diatoms (Cohn et al. 2003). Specifically, Cohn et al. (2003) determined that presence of other species reduced some species speed and attachment to the substrata. Our research only focused on responses of diatoms to light exposure and therefore,

further experiments should be performed in order to determine if the presence of other species specifically affects adhesion.

Research that allows us to study inter-species reactions to a variety of different conditions are crucial to understanding how diatoms behave in their complex environment. However, due to the nature of the sample collection in our study, there was no way of determining the degree to which the three species were stratified within the sediment of their natural environment. All three of these species were collected together in a large-scale accumulation of sediment and specific core samples were not taken. Therefore, what wavelengths of light each species was exposed to or where each species was specifically located within their community could not be determined. Future lines of research could focus on ways to accurately measure stratification of diatoms in their natural environment and how such stratification correlates with other diatom characteristics, both physical and behavioral. A better understanding of how closely related species behave in confined areas gives one more insight to the ways in which species partition their resources. Below are suggestions for future experiments that could help answer some of the questions that arose from the results of this study.

For instance, it was hypothesized that cell size and speed might influence where different diatom species are located within the community. To determine if these factors effect diatom stratification, core samples (i.e. tube that can be sealed off) could be taken of natural algal assemblages in pond sediment, and analyzed (Battarbee et al. 2001). Then separate experiments could be performed from the core samples. One method for determining how cell size affects stratification is by fixing core samples, for example in alcohol, which keeps the cells from migrating within the sample. After fixing, different layers could be removed and analyzed to determine diatom abundance for each species. Then cell size could be correlated with the area in

the core sample that they were found. To determine if cell speed effects stratification, diatom species could be isolated from different layers of unfixed core samples and put on glass slide chambers and observed under a microscope where cell speed could be recorded. Previous experiments have determined that diatoms migrate faster in large grain size sediments compared to small grain size sediments (Du et al. 2010). To ensure sediment size is not a determining factor for cell speed, core samples would have the same sediment type. Previous experiments that have determined average cell speed for some diatom species could be compared to new motility experiments to confirm each species average speed. Then one could examine the relationship between the average cell speed of each diatom species with their location within the core sample. If our speculation is correct, the results would show accumulation of slow, large diatoms near the bottom of algal assemblages and accumulation of fast, small diatoms near the surface.

Field studies could also focus on diatom stratification over time to determine if distribution and abundance of diatoms in algal assemblages changes during the day or over the year (Coelho et al. 2011, Cibic et al. 2012). One possible experiment could involve placing clean rock samples in the water where algae could accumulate. Then the rock samples could be collected at various times (e.g. day, night, summer, winter) or under different conditions (e.g. rain, sunshine) to determine what species settles on them and if distribution changes over time. The rock samples could also be fixed (i.e. embedded in plastic) (Johnson et al. 1996) and layers could be shaved off to identify where different species are located within the assemblages. This method could be used to determine the first colonizers of an algal community by correlating species abundance with the area they were found in the sample.

Motility experiments could also be performed to determine if pattern of movement affects where different diatom species are located within their assemblage (i.e. accumulation into specific light areas). For example, *P. viridis* is known to have circular path orientation and is somewhat less responsive to light conditions, unlike *S. phoenicenteron* and *C. cuspidata* that move in a straight path (Cohn 2001). The data from this study suggest that *P. viridis* might be less able to move into otherwise generally favorable light areas. Light spot experiments could be performed to determine if each diatom species accumulates into light areas of different wavelengths and intensities. One method could involve placing potentially favorable light spots (i.e. low to moderate intensity of different wavelengths of light) on a microscope and testing rates of cell accumulation into these spots in both single-species and multi-species samples. Pattern of movement for each species could then be correlated with light spot accumulation of cells. If our hypothesis is correct, species that move in a curved fashion would respond more slowly and have little accumulation into light spots compared to species that have a straight path orientation.

Analytical chemical tests, as well as motility experiments, could be performed to determine if soluble factors affect cell movement. Since *S. phoenicenteron* had slower response times in the presence of *C. cuspidata* these two species soluble fractions could be compared. For example, single-species of *S. phoenicenteron* and *C. cuspidata*, and mixed-species (e.g. *S. phoenicenteron* and *C. cuspidata* together) slides could be prepared (i.e. sealed chamber with diatom medium). Then the fluid within the slide chambers could be removed and chemical tests (e.g. Mass spectrometry, IR, HPLC) could be performed to determine if there are particular chemicals within the solution that differ between single-species and multi-species samples. This test could then be followed by motility experiments, where motile *C. cuspidata* cells could be

placed in a slide chamber for a period of time and then the solution from that chamber could be isolated. That solution could be transferred to a single-species sample of motile *S. phoenicenteron* cells and irradiation experiments could be performed to determine if response times have changed compared to single-species solution samples of *S. phoenicenteron*. These fluid experiments could be performed multiple times with samples that contain different amounts of *C. cuspidata* cells to see if there is a dose-dependent effect. The soluble fraction could then be correlated with the reduction of movement seen in *S. phoenicenteron*.

Motility experiments could also be performed in order to determine how one species' mucilage reacts with the presence of another species mucilage. For instance, to see if *C. cuspidata* mucilage affects *S. phoenicenteron* movement, motility experiments could be performed on *S. phoenicenteron* with the mucilage of *C. cuspidata* present. *Craticula cuspidata* cells could be placed on a cover slip for a period of time (i.e. to deposit mucilage) and then every cell along with the fluid solution (to rule out soluble factors) could be removed. *Stauroneis phoenicenteron* cells could then be placed on the same cover slip and irradiation experiments could be performed to determine if motility responses are different from samples with only mucilage from *S. phoenicenteron*. This experiment could also be performed multiple times with different amounts of *C. cuspidata* cells to determine if there is a dose-dependent effect. Then one could correlate the average cell speed with the mucilage present in the sample. This would help us better understand if the presence of other species or the mucilage of other species itself is the main determinant for the altered motility of *S. phoenicenteron*.

Adhesion experiments could be performed in order to determine if adhesion strength is affected by the presence of other species. Previous research has already shown that multiple species can reduce adhesion (Cohn et al. 2003), however, samples were not prepared with

different ratios of diatom species like this study has. Therefore, one could repeat the earlier study using different ratios of diatom species. For example, single-species and mixed-species samples could be prepared on glass slides and then the slides could be inverted and the length of time each species stays attached to the surface could be recorded. Adhesion strength for each species could then be correlated with the presence of other species in the sample. A significant difference in attachment time between single and mixed-species samples would show evidence that adhesion strength is affected by the presence of other species.

By doing these kinds of studies we can begin to determine the differences between species interactions, physical characteristics, and environmental stimulus that can drive and affect resource partitioning. While many past studies have focused on diatom motility by observing individual species, a better understanding of multispecies interactions should be explored. By studying diatom motility in the presence of other species, a better understanding of their true behavior in a more natural environment can begin to be developed. The ecological success of diatoms relies on environmental conditions they find themselves in, including the nature and abundance of other species present in the community. Thus, the relative distribution other types of algae like Cyanobacteria, Rhodophyta, Chlorophyta, as well as diatoms themselves can affect the overall success and distribution of diatoms within a particular ecosystem. Therefore, studies should also consider how non-diatom species might affect diatom motility.

It is well known that the ability to maintain diversity and withstand a great variety of disturbances in any ecosystem is a sign of a healthy, stable community (McCann 2000, Cleland 2011). Disturbances that lead to an overabundance of competition can effect growth of populations, limit diversity within populations and communities, and can lead to extinction of

species (weaker species gets excluded from resources). By segregating resource use, similar species can exploits slightly different resources, where each species spends less time competing and therefore can expend this energy more efficiently on growth, cell division, accumulation of nutrients, and reproduction. Through studies such as this one, we can better understand the ways that diatoms interact with each other and their environmental conditions, and what different microenvironments each species occupies. Such research will allow us to determine the conditions for healthy and stable aquatic communities.

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