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The Role of Sulfur in Biomineralization: Argopecten Irradians and the Impact of Ocean Acidification

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THE ROLE OF SULFUR IN BIOMINERALIZATION: *ARGOPECTEN IRRADIANS* AND THE IMPACT OF OCEAN ACIDIFICATION

A Thesis Presented

by

BRYANNA JOY BROADAWAY

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ABSTRACT

THE ROLE OF SULFUR IN BIOMINERALIZATION: *ARGOPECTEN IRRADIANS* AND THE IMPACT OF OCEAN ACIDIFICATION

December 2010

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The burning of fossil fuels, and other natural processes, has led to an increase of carbon dioxide (CO_2) in the atmosphere. The equilibration of atmospheric CO_2 with surface waters causes the ocean pH to decrease from the current value of 8.1. Evidence suggests that as the ocean becomes more alkaline, organisms such as the bay scallop, *Argopecten irradians*, will become more stressed due to increased energy demands to maintain shell deposition and growth. This stress, as hypothesized here, may cause the deposition of S-bearing organic macromolecules to aid in mineralization as well as the potential for sulfate substitution and the deposition of gypsum (calcium sulfate) as opposed to calcite. I hypothesize that scallops raised under pH conditions predicted for the end of the 21st century will have higher total S content which may be due to the

deposition of sulfate and/or deposition of high-S organic macromolecules. I will investigate this possibility as well as the possibility that the shift towards increased sulfur impacts the stable S isotopic composition of the inorganic and organic components of shells from organisms raised under high dissolved CO_2 conditions as compared to "normal" pH.

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INTRODUCTION

Several years of research performed at Mauna Loa, Hawaii by Keeling provided substantial information about carbon dioxide concentrations (CO_2) and isotopic abundances of atmospheric carbon 13 and carbon 12 (Keeling 1960). Keeling concluded that CO_2 was increasing in the atmosphere as a direct result of human activity. Recently, the relation between increased atmospheric CO_2 and ocean pH has been more clearly established as pH of the global ocean has declined by 0.1 pH units since preindustrial times (Orr 2005), such that current ocean water has an average pH of 8.08 (Potera 2010). This relation is based on the fact that as carbon dioxide increases in the atmosphere, it diffuses into the surface waters of the ocean due to partial pressure differences (Henry's Law). Through this air-sea gas exchange, atmospheric CO_2 enters the ocean where it either becomes dissolved or escapes, through evaporation, back into the atmosphere. Carbon dioxide, once dissolved in the surface waters, can form carbonic acid as a first step in the series of several reversible dissociation reactions that can release free hydrogen ions that form bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions (**Equation 1**).

The resultant increase in the dissolved free hydrogen ion (H^+) leads to a decline in pH (pH = -log [H^+]).

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow 2H^+ + CO_3^{2-} \quad \text{Equation 1}$$

The ability of the ocean to absorb atmospheric CO_2 emitted by both natural and anthropogenic sources depends largely on the extent of dissolution of calcium carbonate ($CaCO_3$) (Equation 2).

$$CaCO_3 \leftrightarrow CO_3^{2-} + Ca^{2+} \quad \text{Equation 2}$$

The formation and dissolution of $CaCO_3$ varies with the concentrations of both the calcium and carbonate ions through what is known as the saturation state (Ω) (Equation 3).

$$\Omega = \frac{[Ca^{2+}][CO_3^{2-}]}{K_{sp}} \quad \text{Equation 3}$$

The solubility product, K_{sp}, depends on pressure, temperature, salinity, and even the mineral phase of the calcium carbonate. Calcium carbonate occurs in a number of crystal polymorphs (same chemical composition, different crystal structure): calcite, aragonite, and vaterite; however, aragonite, under atmospheric pressure and temperature, is 50% more soluble than calcite (Mucci 1983).

The increase in dissolved [CO_2] and [H^+] coupled with the resultant decrease in pH and [CO_3^{2-}], causes a change in the saturation state of calcite and aragonite due to shifts in thermodynamic equilibria. For ocean surface waters, pH~ 8.1, the carbonate

reactions are reversible and near equilibrium (Millero 2002) such that approximately 90% of the inorganic carbon is bicarbonate, 9% is carbonate, and 1% is dissolved carbon dioxide. However, the increasing $[H^+]$ causes a decrease in carbonate ion concentrations, $[CO_3^{2-}]$. Thus the projected 0.3 to 0.4 pH drop projected by future models for the 21st century, due to continued anthropogenic CO_2 inputs to the atmosphere, will result from an increase of $[H^+]$ by 150% and a decrease in the $[CO_3^{2-}]$ by 50%. The shift in ocean pH to less alkaline conditions is called ocean acidification (Borges 2010).

Anthropogenically induced ocean acidification may be attributed to increasing atmospheric carbon dioxide from fossil fuel combustion, deforestation, and other anthropogenic activities (Dore 2009). However, it is also important to realize that shifts in carbonate chemistry, which affect pH, are also caused by natural processes, such as sea floor spreading, methane clathrate emissions (Doney 2009), and changes in weathering rates of carbonate and silicate rocks (Kump 2000). Ocean pH has changed throughout Earth's history as a result of these processes (Kump 2000). For example, in the Permian (250 mya) and Cretaceous (65 mya), atmospheric CO_2 exceed current atmospheric concentrations leading to increased deposition of shallow water carbonates (Doney 2009; Ries 2010). It is also interesting to note that these two periods of lower ocean pH and high atmospheric CO_2 concentrations are coincident with the two largest mass extinctions (Permo-Triassic and KT). Changes in atmospheric CO_2 are, therefore, the result of a combination of complex Earth system interactions and are now further complicated by human activity.

One of the concerns regarding ocean acidification is how the concentration of calcium is closely related to salinity, such that the variation in Ω are largely determined by changes in the carbonate ion, which can be indirectly calculated from total alkalinity and dissolved inorganic carbon (Doney 2009). Regarding shell-forming organisms unless a shell is protected from direct contact with seawater by either the organism through an organic coating or some other mechanism, dissolution of shells composed of calcium carbonate will occur with $\Omega < 1.0$ (Doney 2009). Therefore, ocean acidification may have contributed to the mass extinctions by causing dissolution of various organisms' shells.

Since carbon dioxide is more soluble in colder waters than warmer waters, higher latitude areas are expected to see lower pH values (Feely 2004; Ries 2010). Though predictions of future carbon emissions vary, it is clear that to remain on our current trajectory will lead to further acidification of the ocean (IPCC 2007). Although much of the acidification is expected to occur in surface waters, deep waters will experience changes as well by virtue of the ocean conveyor belt and the sinking of surface waters at the poles and cycling of this water, at depth, to the equator where it rises again. By the 21st century, ocean pH is expected to decrease in deeper waters due to ocean mixing and transport of anthropogenic carbon dioxide to deep waters (Caldeira 2005). Since chemical weathering of rocks on the continents also affects carbonate speciation and pH, coastal waters, which are in direct contact with river input, may experience acidification changes before the deeper waters. Changes in surface and deep water pH may have

negative impacts on many marine organisms and on the biogeochemical cycling of carbon and possibly other essential elements (Orr 2005). Of particular concern to the proposed work is the impact of ocean acidification on coastal calcifiers, such as bivalves.

This research specifically focuses on a coastal calcifier, the bay scallop, *Argopecten irradian*. Currently, the bay scallop is a commercially important species supporting much of the shellfishing industry in Nantucket Bay. Historically, the bay scallop was found from the north shore of Cape Cod down to the Laguna Madre, Texas (Waller 1969). The Mid-Atlantic distribution of the bay scallop (Figure 1) extends from Cape Cod to New Jersey. It is relevant to say, however, that the abundance of the bay scallop has diminished from historic values possibly due to environmental changes and overfishing.

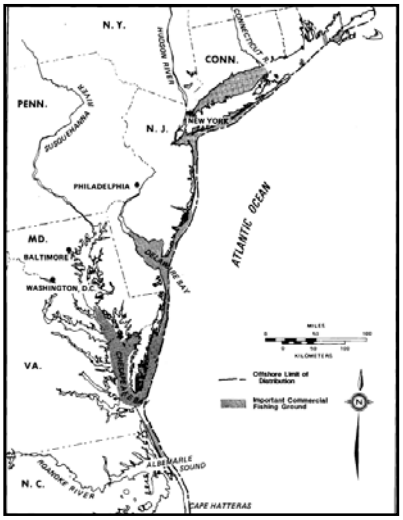


Figure 1: Mid-Atlantic distribution of the bay scallop (Fay 1983)

Bay scallops have been harvested since colonial times. In 1976, fisherman caught up to 946 metric tons of bay scallops along the Atlantic coast (Peters 1978). Bay scallops, compared to other sea scallops, are more economically beneficial to fishermen since they bring a higher price at market. This commercially important organism experiences changes in water quality found in estuaries (e.g., decline in pH) that are a consequence of direct anthropogenic forces that may affect the harvest of the bay scallop. The loss in harvest can be caused by overharvesting, but may be a result of environmental changes that stress different life stages of the bay scallop.

Bay scallops are hermaphrodites that release sperm in the water column to successfully fertilize an egg when water temperatures are between 15 and 20 degrees Celsius (Sastry 1963). Around 25 degrees Celsius, all the small larvae will be swimming within 48 hours and this stage is known as the trochophore larvae (Sastry 1965). Gradually, the scallop grows and secretes a shell to advance as a veliger larva, where it also begins feeding using ciliated velum (Sastry 1965). The energy obtained from feeding helps the organism to develop the straight-hinged veliger shell with curved umbones. After about 10 days, the organism develops a foot which will aid the scallop in movements, such as turning over. At this point, the scallop moves from the larval stage to the juvenile stage by replacing the velum with the development of gills to aid in respiration (Sastry 1965). Although water temperature is very critical at these stages, a lack of food supply will actually prevent the metamorphosis from larvae to juvenile (Belding 1910).

The juvenile stage is a very active stage for settling and shell growth. The young scallop attaches a fine thread called a byssus to a suitable substrate, such as ell grass, by sectretions made by a gland in the foot (Belding 1910). The substrate is very important at this stage of the scallop's life. For example, if a scallop finds itself in a silted substrate, it will attach to a epibenthic surface until it reaches approximately 25mm in length and then will drop to the bottom to improve survivability (Castagna 1975). Substrates can vary from stones, seaweeds, and oyster shells to filamentous algae, rope, and seagrasses in areas that are normally dominated by slow currents that enhance growth of the scallop. Once attached to a substrate, the juvenile will begin climbing and crawling using the foot and byssal threads until the muscle is strong enough to help aid in swimming. If for any reason the young scallop falls and is turned over on the left valve, the foot will be used to flip it over onto the right valve (Belding 1910). At this stage, there can be a lot of variation in shell color, which will disappear when the scallop enters the adult stage. Projected increases in atmospheric CO_2 may have detrimental effects on the bay scallop such that juveniles are unable to metamorphose into their adult years and may even cause stunted growth (Talmage 2009), which would have dramatic effects on the shellfishing industry.

The final stage of life for a scallop is the adult stage. At this time, the shell has formed radial ridges and furrows on the scallop shell, which will no longer increase in number with growth (Belding 1910). Another characteristic of the adult shell is the presence of the concentric ridge on the shell used by law enforcement to determine the

age of a scallop (Peters 1978). At this point in life, the scallop can still use byssal threads to attach to substrates, but it tends to settle into quiet waters protected from tides in under an average of 10 meters in depth. In the adult stage, the bay scallop effectively swims by pulsed expulsion of water from the cavity that alternates between the anterior and posterior gaps resulting in a zigzag pattern (Belding 1910). Swimming is used as a voluntary movement to escape unfavorable environmental conditions or predators (Belding 1910). However, it is important to realize that once the scallop is no longer resting on the bottom of the seafloor or attached to a substrate, it is possible for the organism to be moved by tides or currents. Just like in the juvenile stage, if the adult scallop gets flipped to its left valve, it will use the foot but at this stage also water expulsion to flip back over to the right valve (Belding 1910).

Although economic value of bay scallops with catches along the Atlantic coast has been worth millions of dollars annually is important, they also provide vital ecosystem services and are diminishing along the coast. Bay scallops funnel energy from planktonic species up the food chain to terrestrial predators and the dollar value of this service is estimated to far exceed their commercial value (Costanza 1997). The bay scallop was selected for the study of ocean acidification impacts in shells specifically because of their economic importance, the vulnerability of estuaries to human influences, and their unique role in coastal systems. It is important to realize that ocean acidification can affect the species at various life stages: larvae, juvenile, and adult.

Ries et al. (2010) investigated the impact of changes in pCO_2 on calcifiers, including the bay scallop. They found that bay scallops showed a net calcification decrease as the partial pressure of carbon dioxide was experimentally increased to 2856 ppm, leading to a decrease in the saturation state of aragonite to 0.7 units. Unlike other shellfish such as the blue crab, shrimp, lobster, and blue mussel whose shells are protected by an external organic layer, the bay scallop shell is in contact with ambient seawater soon after deposition of new shell (Ries 2010). Organisms that accreted shell that remained totally covered by an external layer exhibited greater resilience to elevated pCO_2 than organisms, such as the bay scallop (Ries 2010). Ries' study did not predict how the organism would be impacted with respect to survival or reproductive success, but the study does indicate that reduction in pH causes a negative response in net calcification. This research focusing on bay scallops will help management to anticipate the effects of anthropogenic ocean acidification in the decades and centuries ahead for the organism.

1.1. Marine Carbonate Cycle Through Time

Across geologically relevant time scales, calcium carbonate is cycled, primarily, through two modes: a transformation to a geological reservoir (burial) and a release from the reservoir (weathering). Currently, the surface of the ocean is over-saturated with carbonate causing precipitation and eventual exportation to the seafloor where it is buried in sediments. Organisms, such as coccolithophores, foraminifera, and corals, help change aqueous CO_2 to $CaCO_3$, primarily as the mineral aragonite, thereby exporting carbon

from the surface. Once in the sediments the material lithifies to marl, limestone, or other carbonate rock. This rock then can subduct into the mantle, where it melts and may, upon rising through the crust in the rift zones or orogenous zones, release CO_2 back into the atmosphere (volcanism) and retain the Ca in wollastonite ($CaSiO_3$) in the crystallized magma. The latter is common to the roots of mountain belts. If a carbonate rock or rock with wollastonite is obducted (uplifted onto the continent) and exposed to subaerial erosion and weather, the weathering of $CaCO_3$ and $CaSiO_3$ will draw down atmospheric CO_2. In $CaCO_3$ weathering, additional carbonate will be provided to the oceans via rivers (Figure 2). In both cases, the reactions produce additional acids (carbonic, silicic) which enhance weathering and continue to draw down CO_2. This process is only reversed when the dominant material being weathered on the continents is low in carbonate and/or wollastonite (e.g. high in organic matter). Indeed during times of low carbonate and high organic matter weathering atmospheric CO_2 increases (Vincent and Berger 1985). In sum, the cycling of CO_2 in the atmosphere, in geologic time, is defined by the complex interactions between orogenic activity, carbonate/wollastonite weathering, and carbonate burial rates.

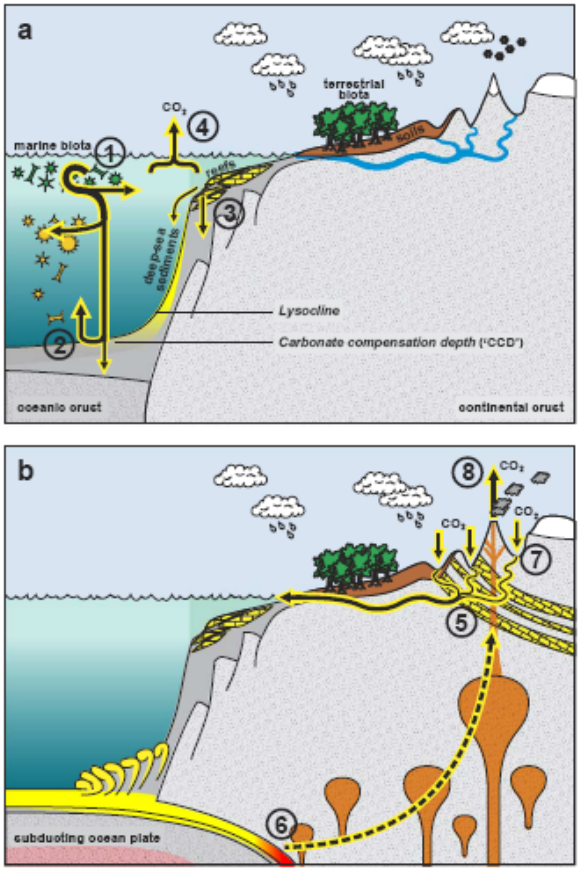


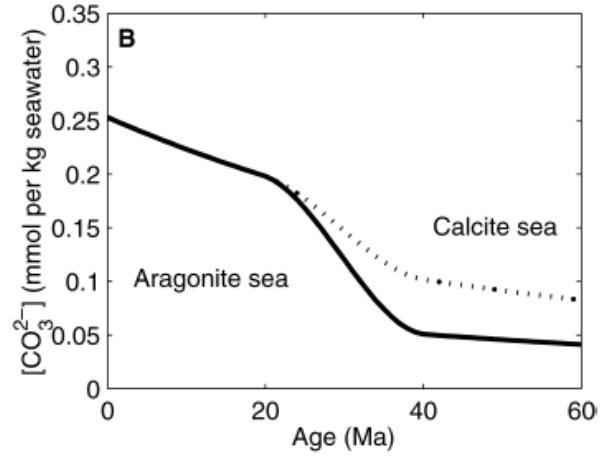
Figure 2: Global biogeochemical cycling of calcium carbonate. (a) $CaCO_3$ transformation and recycling to the geological reservoir. Step 1: Coccolithophores and foraminifera precipitate calcite in the open ocean. Step 2: Carbonate reaching the ocean bottom will dissolve in early diagenesis if the water is under-saturated and/or if organic matter flux is high. Step 3: $CaCO_3$ is precipitated by corals and shelly animals, primarily as the aragonite polymorph. Step 4: A net transfer of CO_2 from the ocean to the atmosphere occurs as $CaCO_3$ precipitation causes a higher pCO_2 at the surface. (b) $CaCO_3$ transformation, recycling, and return to surficial system. Step 5: Rifting and mountain-building episodes cause $CaCO_3$ laid down in shallow seas to be exposed to erosion. Step 6: Carbonates are subducted into the mantle and are thermally broke down, resulting in the creation of calcium silicates and a release of CO_2. Step 7: Silicate rocks are exposed to weathering. Step 8: CO_2 is emitted into the atmosphere through decarbonation closing the carbon cycle on very long time-scales. (Ridgwell 2005)

In addition to changes in carbonate burial rates and atmospheric pCO_2 concentrations across geologic time, the type of carbonate deposited in the oceans has

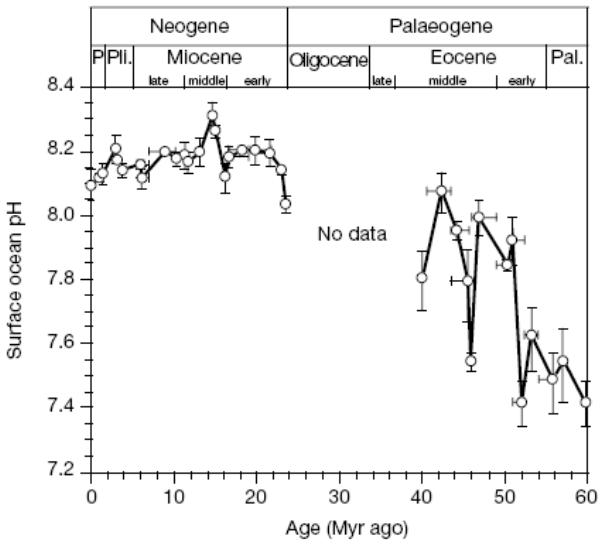
also changed through time. Atmospheric pCO_2 changes the primary mineralogy of oceanic carbonate sediments (Demicco 2003) precipitated to the ocean floor. Whether $CaCO_3$ precipitates as calcite or aragonite depends on the Mg/Ca ratio. Models predict whether a particular period in Earth's history can be considered an "aragonite sea" (Mg/Ca >2, 0-40 Ma) or a "calcite sea" (Mg/Ca <2, 40-60+ Ma) (Demicco 2003) (Figure 3). Demicco's results show that from 40 Ma to the present, aragonite had been the principle $CaCO_3$ precipitate with this conclusion based on the assumption that the ocean has been highly supersaturated with both aragonite and calcite. Supersaturation occurs because high Mg concentrations kinetically slow the nucleation and precipitation of low-magnesian calcite (<4 mol% $MgCO_3$). This kinetic barrier allows the $[Ca^{2+}][CO_3^{2-}]$ solubility product to rise, promoting the metastable nucleation of aragonite (Berner 1975).

The state of surface seawater saturation is unknown during times of calcite seas (40 – 60 Ma) (Demicco 2003). It has been postulated that seawater $[Mg^{2+}]$ was lower than current day values (Lowenstein 2001), such that the high $[Mg^{2+}]$ kinetic barrier to calcite precipitation was removed, and the solubility product of $[Ca^{2+}][CO_3^{2-}]$ was lower by four times, the current saturation state (Demicco 2003). However, it is accepted that reducing pH causes calcium carbonate saturation to lower in surface water (Caldeira 2005). The dramatic change in both surface ocean pH and carbonate concentration in the geological record is expected to be dramatic, because saturation states are also affected by temperature (Pearson 2000). The long term cooling at 50 Myr highlights the history

of Cenozoic cooling (Pearson 2000), and dramatic switch from aragonitic seas to calcitic seas. Therefore, if we remain on the current trend, there will be a shift from an "aragonite sea" to a "calcite sea."



(a)



(b)

Figure 3: (a) $[CO_3^{2-}]$ [(mol per kg seawater) vs. age (Ma). An aragonitic sea is represented from 0 – 40 Ma, such that it is supersaturated four times with respect to aragonite. From 40 – 60 Ma, the seawater is modeled as two times supersaturated with calcite (Demicco 2003). (b) Estimation of surface ocean pH from $\delta^{11}B_{cc}$ measurements with regards to age (Ma) (Pearson 2000).

Carbonate equilibria affects the pH of sea water, such that if the pH value is known, it is possible to calculate the aqueous CO_2 concentration (Pearson 2000). Concentration of aqueous CO_2, total dissolved inorganic carbon, total alkalinity, and pH are the four key variables that define carbonate chemistry. Therefore, pH may potentially be used as a proxy for carbonate chemistry of the ancient ocean (Figure 3) where higher pH values (>8.0) correspond to the aragonitic sea conditions found from 0 – 40 Ma. Lower pH values correspond to the time in which the ocean was assumed to be considered calcitic in nature. It is important to realize that CO_2, HCO_3^-, and CO_3^{2-} are a function of pH (Ridgwell 2005) (Figure 4, Equation 1, Equation 2, Equation 3). Thus, given the partial pressure of carbon dioxide, knowledge of the saturation state, the surface temperature of the water, and the major cation composition determines the entire aqueous carbonate system (Zeebe 2001). Therefore, understanding how the carbonate cycle is related to pH and its impact on bay scallops, we may be able to utilize fossil records to understand previous environmental conditions if bay scallops are available.

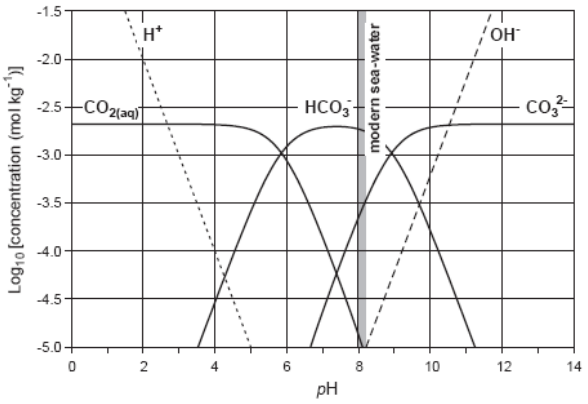


Figure 4: CO_2, HCO_3^-, CO_3^{2-}, OH^-, and H^+ as a function of pH (Ridgwell 2005)

1.2. Biomineralization and Ocean Acidification Impacts on Calcifiers

"Biomineralization" encompasses the study of biogenic minerals that contain various cations (Weiner 2003). The processes of biomineralization can be affected by the various ions controlling pH. Molluscan shells are elaborate structures comprised of an organic matrix and calcium carbonate crystals (Figure 6) with unique structural properties (Wilbur 1972), which are formed through the process of biomineralization. Deposition of calcium carbonate occurs throughout the life of the organism with rate, structure, and composition dependent on environmental and physiological factors (de Paula and Silveira 2009). Modes of calcium carbonate biomineralization across marine organisms are diverse (Aizenberg 2004), but can be broadly generalized.

Primarily, the shell formation of a bivalve, such as *Argopecten irradians*, is controlled by the mantle (Lowenstam 1989). The groove at the mantle edge is the site of periostracum formation in molluscs. The periostracum is thought to provide protection

from the environment, isolate tissues from the surrounding environment, and act as the substrate upon which mineralization is initiated (Clark 1974). This component of a shell contains fibrous proteins, carbohydrates, and lipids that help in the formation of the prismatic layer (Checa 2000). A developed bivalve mollusc shell is composed of multiple layers: the periostracum, prismatic layer, nacre, and myostracum (Figure 5). In a typical shell, the spatial distribution of these individual layers is complex. The myostracum are always aragonitic, and as a shell grows, the muscle attachment areas leave behind the myostracum layer that is covered by inner shell layers (Lowenstam 1989).

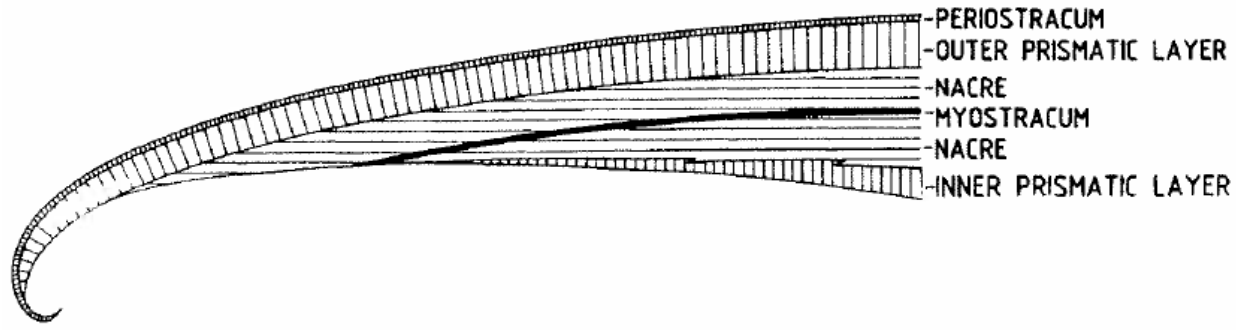


Figure 5: Transverse section of a bivalve shell (Lowenstam 1989)

In molluscs, two major polymorphs of calcium carbonate, aragonite and calcite (Figure 6), dominate shell composition (Lutts 1960) although there are eight known polymorphs of calcium, seven which are crystalline and one that is amorphous. There has been some debate as to why a less stable aragonite crystal is formed over calcite. Aragonite typically requires high pressures, whereas calcite is considered the low pressure polymorph (Boettcher and Wyllie 1968). The chosen polymorph may be a result

of competition with Mg, thermodynamics, the organic matrix structure, or the influence of carbonic anhydrase (Sarashina 1998). The lack of carbonic anhydrase in calcitic shells seems to support the hypothesis that the enzyme is used in aragonite formation by sequestering the carbonate ions to the site of crystal growth (Sarashina 1998). The innermost layer, nacre (Figure 6), is usually composed of aragonite that has a higher tendency to accommodate large cations, such as Sr^{2+} (1.18 Å), Pb^{2+} (1.19 Å), and Ba^{2+}(1.35 Å) (Berkovitch-Yellin 1985). The outer layer, commonly referred to as the prismatic layer, is composed of either calcite or aragonite depending on the animal species (de Paula and Silveira 2009), but is typically calcitic in *Argopecten irradians* (Ries 2010). Due to the crystal structure of calcite, only smaller cations like Mg^{2+} (0.72 Å) and Fe^{2+} (0.61 Å or 0.78 Å) (Berkovitch-Yellin 1985) can fit within the interstitial spaces of the crystal lattice.

In addition to inorganic crystals, the shell contains many organic components. A crossed-lamellar layer was shown to lie between the nacre and the oblique crystals of the prismatic layer in fossil scallops (Runnegar 1984; de Paula and Silveira 2009). The interlamellar matrix is primarily composed of β-chitin, with little evidence of silk-like proteins (Addadi 2006). The types of proteins present play an important role on how the crystal lattice is formed with regards to carbonate crystal structure and what metals are present. Amino acids, peptides, and proteins accelerate shell growth, and control the elemental composition of calcite through hydrophilicity of carboxyl-rich biomolecules (Fu 2005). In order for calcification to occur, the organism must sequester and stabilize

amorphous calcium carbonate until one or more triggers promote the transformation to the final crystalline phase (Dove 2010).

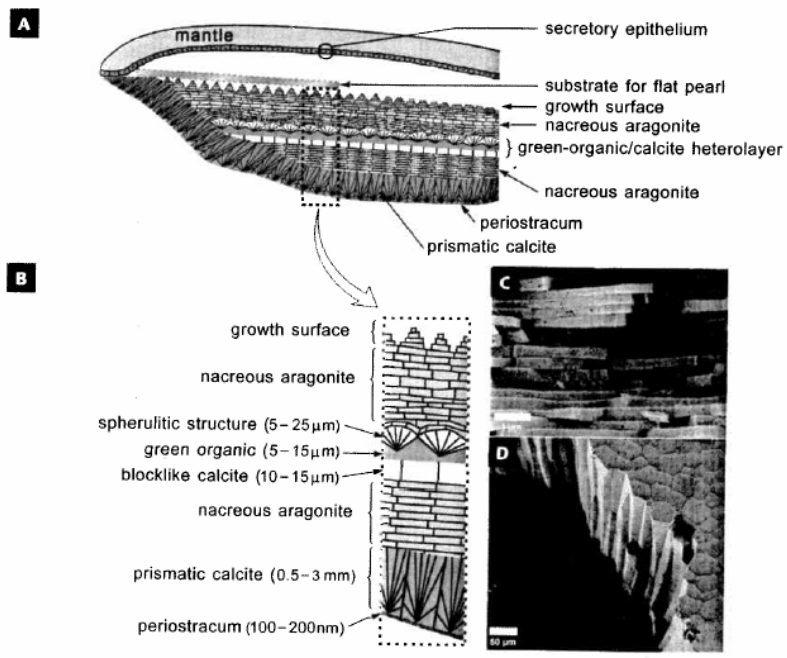


Figure 6: (a) Shell and mantle of the bivalve, *Atrina rigida* (red abalone). (b) A closer look at the $CaCO_3$ that is interlayered with organic matrix, which provides a substrate for new growth. (c) SEM image showing aragonite tables in the nacreous layer. (d) The outer layer of the shell composed as a prismatic calcite (Dove 2010)

The basic process of biomineralization has been generalized across species to either be "biologically induced" (Lowenstam 1981) or "biologically controlled" (Mann 1983). The "biologically induced" portion of biomineralization accounts for the part of nucleation and mineral growth that the organism has little to no control over as it is commonly dictated by the particular surrounding environment (Frankel 2003). However,

it is the "biologically controlled" process that organisms use to control the mineralization process. The "biologically controlled" processes can be divided into three distinct areas based on the location where the cells responsible are located: extracellularly, intercellularly, or intracellularly (Weiner 2003). Intracellular mineralization occurs within a specialized vesicle and directs the nucleation within the cell where the compartment is capable of controlling the biomineral morphology and composition (Weiner 2003). Intercellular mineralization is the least common, occurring primarily in single-celled organisms, where mineralization occurs between cells forming, for example, an exoskeleton (Weiner 2003). The final distinct area where mineralization can occur is extracellular: a cell produces a macromolecular matrix, commonly composed of proteins, polysaccharides, or glycoproteins, outside of the cell that will become the site of mineralization (Weiner 2003). Extracellular biomineralization will be the focus of this proposal.

In extracellular biomineralization, the macromolecular matrix typically contains proteins that have high proportions of acidic amino acids (such as aspartate) and phosphorylated group members (Weiner 1979). A specific protein, MSP-1, has been identified as a major component of the soluble matrix responsible for shell building in a scallop (*Patinopecten yessoensis*). This protein contains high proportions of serine, glycine, and aspartate residues (Sarashina 1998). In order to achieve mineralization extracellularly, cells actively pump cations into a surrounding region where supersaturation is maintained by ion diffusion (Simkiss 1986) or ions passively diffuse in

response to electroneutrality driven by pH gradients to achieve "on-site" nucleation (Simkiss 1976). The components needed to begin extracellular biomineralization are procured from ingested particles, or possibly from contact with the ambient water through adsorption on the gills (Klunder 2008).

It is assumed that the raw materials are transported to the site of deposition and stored temporarily in membrane-bound vesicles and then re-dissolved with this process – maintain the equilibrium required for shell deposition (Weiner 2003). In terms of stages, mineralization of a shell is assumed to occur through (1) the assembly of the matrix, (2) the first-formed mineral phase, (3) the nucleation of individual aragonite tables in nacre, and (4) finally the growth to form mature shells (Addadi 2006).

The mantle of a shell is the organ responsible for shell formation (Lowenstam 1989). In phase 1 of shell formation, the matrix is assembled through the secretion of chitin, by mantle cells, into the extracellular space. Void spaces are then filled by gel-like "silk" protein phase (Addadi 2006). The chitin then becomes ordered through self-assembly and its final orientation dictates how mature nacre cells will be oriented (Weiner 2003). In order for phase 2 (first-formed mineral) to occur, vesicles holding amorphous calcium carbonate are delivered (Loste 2004) to the area of matrix formation. Lipid membranes isolate the amorphous calcium carbonate from the aqueous stage where the silk gel inhibits spontaneous mineralization (Loste 2004). At this point, magnesium, phosphate, and certain proteins can also inhibit crystallization (Addadi 2006). A special

carbonic anhydrase enzyme has been found in mollusc shells (Miyamoto 1996) in the matrix that inhibits crystallization by reducing the rate of mineralization.

For nucleation of aragonite to occur (phase 3), carboxylates surrounded by a ring rich in sulfates probably from aspartate-rich proteins orientate the amorphous calcium carbonate (Crenshaw 1976); it is here where aspartate-rich proteins are assumed to attract the calcium ions to the site (Addadi 2006). Finally, in the fourth phase, the crystals grow vertically until it reaches the chitin, and then grows laterally (Addadi 2006). In order for shell growth to continue, it is presumed that the silk proteins are only present in areas of new growth (Addadi 2006).

As mentioned previously magnesium, phosphate, and certain proteins may prevent uncontrolled crystallization. Inhibition of calcite nucleation caused by Mg may be overcome by secretion of S-bearing organics (e.g. cystein, methionine, or chonroitin) which aid mineralization (Freitas 2009). Sulfur is also found in the inorganic material, as sulfate, which can act as a substitute for carbonate groups (Kontrec 2004). The latter suggests that sulfate will substitute for the carbonate in shells where the carbonate species is limited for biomineralization. The fact that sulfur concentrations, both in the organic and inorganic components, may increase in shells deposited during stress suggests that sulfur concentrations may be a useful biomarker for shell deposition under conditions of stress, such as a decrease in pH. It is this supposition that forms the basis of the proposed research.

Sulfur found in the organic components of shells is primarily concentrated in chondroitin sulfate (polysaccharide), and either cysteine or methionine amino acids, whereas in the inorganic mineral fraction it would be expected to reside as calcium sulfate (Rosenberg 1991). Enhanced production of S-rich organic material, as well as crystallization of calcium sulfate, is energetically costly and should only occur when the organism is under stress conditions such as would occur should the pH drop below that favorable for calcium carbonate crystallization (Freitas 2009). One thing is clear; stress can disrupt "normal" shell deposition causing a change in the elemental composition of shells, including sulfur (Freitas 2009).

With regards to *Pecten diegensis* (San Diego scallop), shell deposition for calcite occurs in the extra-pallial space that is exposed to ambient seawater periodically at the shell margin, and is initiated beneath the periostracum rather than against existing shell material (Clark 1974). Since mineralization is controlled by nucleation, crystal shape and size, crystallization microenvironment, and polymorphism (Aizenberg 2004; Berman 2008), the process can be affected by stresses that impact the physiology of the organism. The adjustments made by organisms are referred to as vital effects. Vital effects are commonly classified as either kinetic (uptake, transport, or even deposition of the mineral formation) or taxonomic (species dependent) (Weiner 2003). In *M. edulis* (mussel species), shell material deposited under stress conditions had high S:Ca and Mg:Ca ratios (Lorens 1980) suggesting an increase in Mg levels in the extra-pallial fluid that inhibited

calcite nucleation and crystal growth (Berner 1975) and further supporting the impact of stress on shell biomineralization.

As an extension of understanding the overall effects of pH-induced stress on biomineralization, trace metal chemistry may also change based on the sulfur composition, and the organic matrix, of a shell along with a changing environment. Takesue (2008) showed that the trace element chemistry of *C. amurensis* (Asian clam) shells is temporally variable. Between the right and left valve trace element chemistry was well correlated in newer growth regions but not in older regions of the shell. This suggests changes in mineral and/or organic composition across the life of the organism. These changes could be related to physiology (including stress) changes in environment, and/or changes in shell composition due to aging. Here, I hypothesize that sulfur may be the key to unlocking the impact of environmentally-induced stress on the biomineralization process and understanding how changes, such as those observed by Takesue, might occur.

If polymorphism and crystal morphology are controlled by a protein-rich organic phase (Pokroy 2006), it is possible that sulfur concentrations in shells could be in proportion to the matrix content assuming sulfur contribution is coming from proteins, acidic macromolecules, etc. (Rosenberg 1991). However, though the expectation is that sulfur concentrations should track the organic content of the shell, there is potential for the inorganic material to also contain sulfur in the form of calcium sulfate (gypsum). Gypsum is stable under aqueous conditions and occurs, almost exclusively, in the shells

of marine species (Bosselmann 2008), but, to my knowledge, is not known to occur in bivalve shells. Despite no published evidence of occurrence in bivalve shells, there is no reason to presume that it does not occur, even in low abundance, in shells deposited under stress given that sulfur concentrations in the organic-phase do increase when the organism is subjected to environmental stress. I propose to investigate the potential of sulfur to reveal information about biomineralization under conditions of low pH, such as predicted under the future scenarios for ocean acidification. In so doing a new biomarker of environmental stress may be developed that may be applied to relic and/or fossil shells for environmental reconstruction.

In addition to using sulfur concentrations as biomarkers for stress it is possible to use other environmentally-induced biomarkers. For example, let us assume that, under ocean acidification conditions, sulfur concentrations will increase in the organic portion due to increased deposition of sulfur-bearing polysaccharides or amino acids (Rosenberg 1991). In the estuarine bivalve, *Macoma blathica,* we know that other environmental changes such as increased copper concentrations can be detected based on the ratio of the amino acids taurine to glycine; the ratio of taurine:glycine was capable of measuring the stress the animal had undergone (Hummel 1996). This taurine:glycine ratio has also been used in *Mytilus edulis* exposed to copper (Hummel 1994). Taurine contains sulfur and so, in the proposed research, I will use the taurine:glycine ratio to monitor ocean acidification/pH induced stress. Therefore, the proposed research should indicate

whether sulfur increases in the shell can be caused by ocean acidification, when metal concentrations are held constant.

CHAPTER 2

PROPOSED RESEARCH

Null Hypothesis: Ocean acidification does not impact the sulfur chemistry (concentration and isotopic composition) in the growing edge of bivalve shells.

Evidence suggests that as oceans become less alkaline, organisms such as the bay scallop, *Argopecten irradians,* will become more stressed due to increased energy demands to maintain shell deposition and growth. As in *M. edulis* (mussels) this stress may, as hypothesized here, cause the deposition of S-bearing organic macromolecules to aid in mineralization as well as the potential for sulfate substitution and the deposition of gypsum (calcium sulfate) as opposed to calcite. I hypothesize that total sulfur content measured in the growing edge of shells from current populations can serve as a baseline to which I will reference laboratory studies of scallops raised under high dissolved CO_2 conditions. It is further hypothesized that scallops raised under pH conditions predicted for the end of the 21st century will have higher total S content which may be due to the deposition of sulfate and/or deposition of high-S organic macromolecules. I will investigate this possibility as well as the possibility that the shift towards increased sulfur impacts the stable S isotopic composition of the inorganic and organic components of

shells from organisms raised under high dissolved CO_2 conditions as compared to "normal" pH.

The consequences of shifting from calcite to sulfate mineralization would also be that, due to differences in crystal shape, the trace element composition would also be distinct since the interstitial spaces will change. It may then be possible to use the crystal chemistry of sulfur-enriched shells to reveal stress conditions provided so we can resolve the trace element chemistry differences between sulfate and carbonate matrices.

Sulfur isotopic composition should help elucidate whether the sulfur that is present in the shell is impacted by vital effects in much the same way carbon isotopes are (Schidlowski 1988). Few studies exist regarding the sulfur isotope composition of shells and none, to my knowledge, are available for the organic matrix. In sediments we know that, under reducing conduits, the stable isotopes ^{32}S and ^{34}S are discriminated such that the daughter sulfides are isotopically fractionated with respect to the parent sulfate, with sulfides being depleted in ^{34}S (Thode 1951). This process, in sediments, is biologically mediated by sulfur-reducing bacteria such that depletion of ^{34}S is biological in origin. We know that sulfur isotopic fractionation depends on temperature, bacteria, supply of electron donors, and availability of sulfate. We do not know if, during shell formation, when sulfur-containing amino acids, etc. are deposited if this process fractionates sulfur isotopes. It is expected that the local environment of deposition would be maintained under slightly reducing conditions to enable deposition of acid macromolecules. If this

does occur, we may see slight fractionation of sulfur isotope composition in the organic matrix of the shells to an increase in ^{32}S.

CHAPTER 3

MATERIALS AND METHODS

3.1. Control

In the summer of 2009, thirty-two sites in the Nantucket Harbor were sampled over the course of 4 weeks. At sixteen of the sites, thirty scallops were collected for analysis. All sites had surface and bottom waters collected using trace metal clean techniques, separate water for nutrient data from the surface, and a sediment grab. In the last week of the sampling, juvenile scallops, *Argopecten irradians*, were large enough to be visible and be collected (total of 31 spat). The site locations and amount of scallops collected were determined by the town of Nantucket (Figure 6). Water quality measurements for each site were measured: pH, salinity, temperature, conductivity, total dissolved solids, and dissolved oxygen. These samples will serve as a baseline for the bay scallops current conditions.

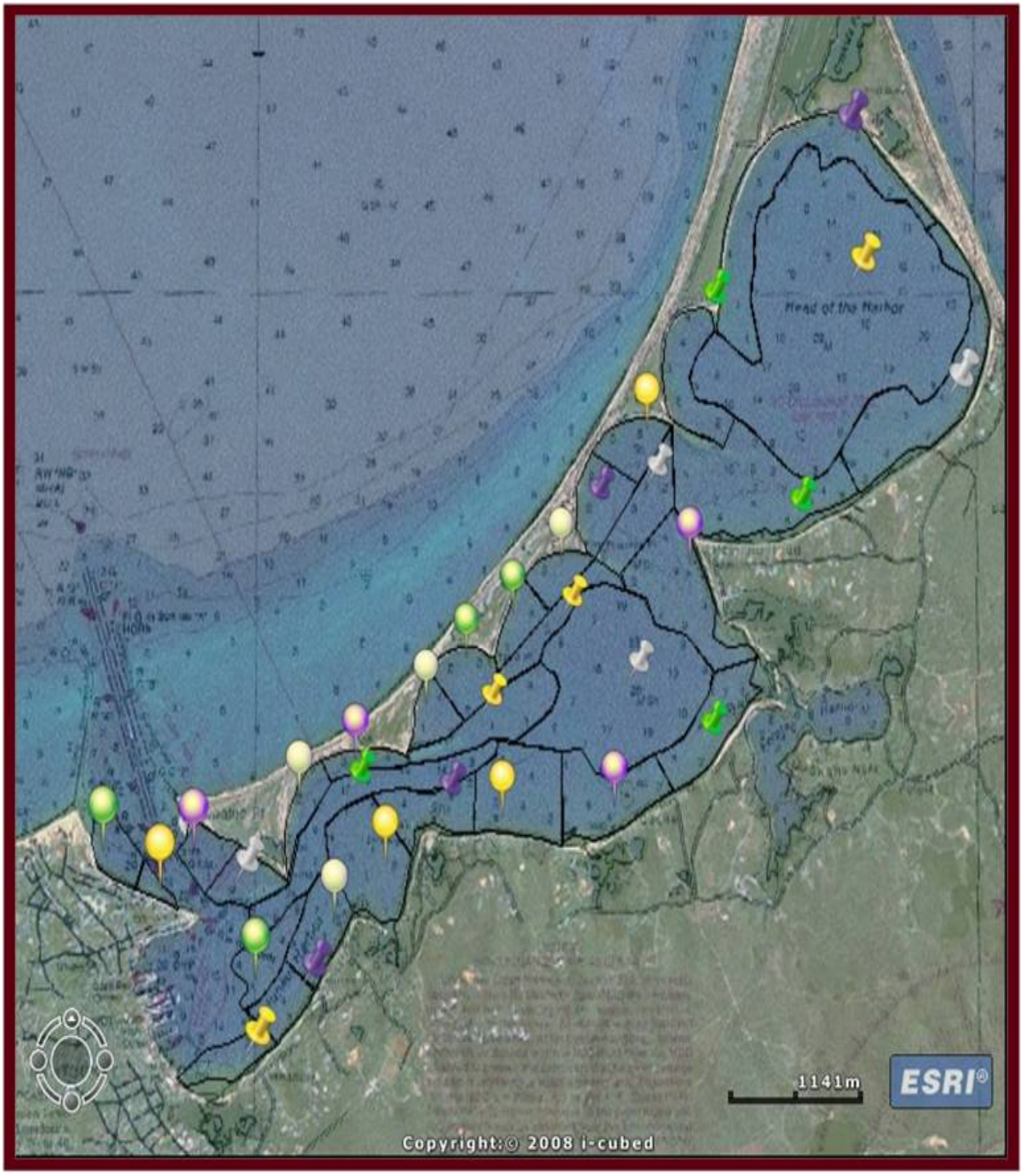


Figure 7: Nantucket Harbor site locations visited during the summer of 2009 (July 21st – Aug 12th). Round push pins represent locations where scallops were counted and

collected, 30 were taken for analysis. Push pins were monitored for scallop populations, but no samples were taken.

A full factorial design will be implemented to be able to address the effects of individual factors alongside interrelationship between the factors. Sample analysis will be done in random order and in replicate. From this collection, the following data can be measured: trace and major metals in water, nutrients, total suspended solids, non-volatile suspended solids, ash free dry mass, shells (for both metals and sulfur concentrations), and sediments (composition and trace metals).

3.2. Experimental Growth Conditions

Argopecten irradians will be collected from the Nantucket Harbor that are about 5 mm in shell height from the umbo to the leading edge, and then grown in four 38-Liter glass aquaria filled with 0.2 μm filtered seawater obtained from Boston Harbor. In order to ensure water with appropriate salinity concentrations, it may be necessary to take a boat to gather water solutions (salinity should be between 25% and 28%) Four air-CO_2 gas mixtures will be equilibrated (400, 600, 900, 3000 ppm pCO_2) continuously. The aquaria seawater temperatures will be maintained at 24°C (typical summer month temperature for wild bay scallop environments, Summer 2009) using electric heaters. Fixing the temperature of the water also maintains aragonite saturation between tanks. Based on previously published experiments, tanks will be illuminated for 10 hours/day at 213 W/m^2 (T8, 8000K aquarium spectrum lamps) (Ries 2010). The aquarium and filtration systems will be covered with plastic wrap to facilitate gas equilibration and

minimization of evaporative water-loss. Each tank will be given 290 mg wet weight puréed frozen brine shrimp and 10 mg dry weight puréed green algae for every 5 bay scallops present.

The experimental gas mixture will be introduced to each tank with mass flow controllers that enter a 6-inch micro-porous air-stone secured to the base of the aquaria at approximately 30 cm depth (Ries 2010). Measurements of aquaria water quality (dissolved pCO_2, alkalinity, dissolved organic carbon, salinity, temperature, and pH) will be performed weekly. Specimens will be collected from Nantucket Sound (approximately 5 mm), and transported back to the University of Massachusetts Boston, where they will be placed in holding tanks equilibrated with ambient pCO_2 (~409ppm). After 2 weeks, surviving specimens will be transferred to the experimental tanks (initially at ambient pCO_2) for another 2 weeks before experiments are begun. The day the experiments begin a time zero sample of scallops will be collected as the control for the tank experiment.

3.3. Measurement of Calcification

Null hypothesis: The buoyant weight method and dry shell measurements for carbonate quantification loss yield statistically similar results.

In order to observe the impact on growth under different dissolved pCO_2 conditions, calcification rates will be determined by buoyant weight (Ries 2010). Specimens will be suspended at 15 cm depth within the tank by a hook attached to a

bottom-loading scale and weights determined at the beginning and end of the experiment. The change in buoyant weight is meant to reflect total calcification, without including calcium carbonate loss through dissolution, molting, or abrasion and will be referred to as "net calcification/dissolution rate" (Ries 2010). Dry $CaCO_3$ weight (shell weight) will be taken at the end of the experiment and will be compared to final buoyant weight to determine the relation between these two measurements. The coefficient of determination (R^2) and the p-value will be used to determine if there is a statistically significant relationship between calcification weight during exposure and total calcification. The average %-error in the regressions quantifies the potential errors from tissue densities that may result from this experimental design. As long as the linear regression values show excellent correlation defined as an $R^2 > 0.90$ and a p-value < 0.01, the buoyant weight method will be accepted as a measurement of growth based on calcification. Shell length (dorsal to ventral), width (left to right), and height (anterior to posterior) will be measured at the beginning and the end of the experiment as a measurement of growth and used as an indicator of growth if buoyant weight is not found to be correlated to the dry $CaCO_3$ weight (shell weight). Using the regression estimates the variability in the buoyant method using the amount of $CaCO_3$ in the shell as a predictor and therefore, an estimator for growth.

3.4. Calcium Carbonate Polymorphism Identification

Null hypothesis: There are no peaks at $713cm^{-1}$ in the organic fraction.

Many different instrumental methods can be used to determine the mineral composition of a shell. For example, FTIR-ATR (Fourier Transform Infrared – Attenuated Total Reflectance) microscopy can identify the presence of aragonite by doublet peaks that appear at 713 and 700 cm^{-1} and calcite by a single peak at 713 cm^{-1} (Figure 7) (Vagenas 2003). FTIR analysis on *P. cubensis* shells demonstrated the insoluble organic matrix by indicating bands representing three different amide bands, sulfate peak, polysaccharide bands, and a phosphate band (de Paula and Silveira 2009). The sulfate identification probably correlated to the 1240 cm^{-1}. The polymorph mineralogy can also be confirmed using x-ray diffraction and scanning electron microscopy. It is important to assess polymorphism to identify changes attributable to lower pH. If polymorphism occurs and there are changes in mineral composition occurring during growth, we will have additional insight into the impact of ocean acidification on bivalves.

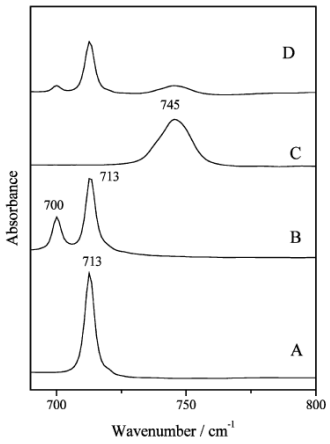


Figure 8: Absorption FT-IR spectra of (a) calcite with peaks at 713 wavenumbers, (b) aragonite with peaks at 700 and 713 wavenumbers (c) vaterite with a peak at 745, and (d) a mixture of 0.2 mg calcite, 0.1 mg aragonite, and 0.15 mg vaterite (Vagenas 2003)

3.5. Metal associated with Organic Fraction

Null hypothesis: The sum of large cations (Sr, Pb, and Ba) concentrations are the same as the sum of small cations (Mg and Fe).

As mentioned previously, the structure, specifically the lattice spacing, of different polymorphs will also have an effect on the trace metals present in the shell. Portions of the shell dominated by aragonite should have large cations, such as Sr^{2+} (1.18 Å), Pb^{2+} (1.19 Å), and Ba^{2+}(1.35 Å) present. However, portions of the shell containing primarily calcite should contain relatively lower concentrations of large cations and high concentrations of smaller ions present, such as Mg^{2+} and Fe^{2+}. I will test for a relation between polymorph/mineral composition and trace element size composition using Pearson's correlation test assuming normal distribution of data. Data may require normalization to perform this test. If the data cannot be normalized, I will test this

hypothesis using Spearman's Rank correction. Mineral composition, and to some extent organic naceral composition, will be determined by the FTIR-ATR microscopy.

In order to prepare samples for FTIR-ATR microscopy analysis a shell will have all the flesh removed and placed in pure glacial acetic acid to remove any tissue that may remain for 1 minute followed by a rinse in demineralized water (18 mΩ). The shell will be placed in a polyester resin, crystal bond, and thin sectioned from the umbo to the leading ventral edge (Figure 9) using an IsoMist saw with diamond tipped blades for juveniles. Once the thin section is removed from the rest of the shell, it will be polished, rinsed with dimineralized water (18 mΩ) then ethanol, air-dried, and lastly photographed. The same sample preparation can be used for Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS). Primary mineral composition (e.g. aragonite, calcite, aragonite + sulfate, and calcite + sulfate) will be recorded and samples categorized by mineral type.

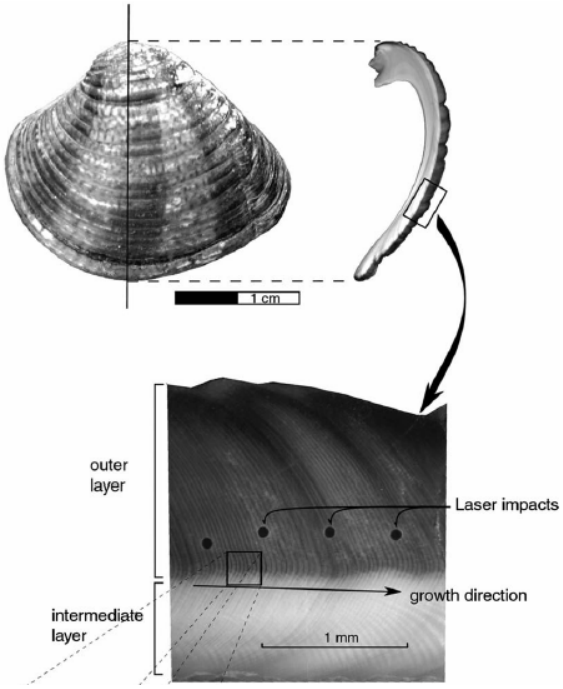


Figure 9: Thin section cut from the umbo to the leading edge and example of where laser analysis will occur used to analyze juveniles (Carré, Bentaleb et al. 2006)

The region of shell analyzed for mineral composition will be analyzed for trace element composition using LA-ICP-MS. Elements to be quantified and isotopes to be monitored include: Ca (43, 46), Sr(86, 87, 88), Pb(206, 207, 208), Ba(135, 137, 138), Mg(24, 25), S(32, 33, 34, 35) and Fe(54, 56) based on the expected incorporation into the shell matrix. In order to quantify, matrix matched standards are essential. A series of 7 calcium carbonate standards containing concentrations between 0.1 and 500 μg/g of the elements of interest (Ca, Sr, Pb, Ba, Mg, S, and Fe) will be prepared. Pure calcite will be dissolved in 18 MΩ water and acidified with ultra pure nitric acid. This aqueous solution will then be spiked with the appropriate metals to achieve the desired concentrations.

Subsequent calcium carbonate re-preicipitation is performed by raising the pH above 7.0 by adding aqueous ammonia. Ammonia bicarbonate can be added to compensate for carbonate loss (Barats 2007). The precipitate is then filtered at 0.45 μm and dried at ambient temperature for one week under a laminar bench hood. The precipitates will be homogenized and pressed at 7 MPa for 2 minutes and stored in a dessicator. A blank sample is made to address contamination in this procedure.

The metal concentrations obtained from the LA-ICP-MS will be normalized to calcium to account for instrumental drift during the analysis, and then ranked from most abundant (Rank 1) to least abundant (Rank 5). Using the net calcification rate, mineral classification and Ca-normalized trace element data, I will test the hypothesis that calcification rate (response variable) is effected by mineralogy and trace element composition. A linear regression will be used to determine the correlation between the matrix composition and associated metals in an effort to determine how much variation in shell composition (y-axis) can be explained by the various trace metals.

Adult shells collected from Nantucket in the summer of 2009 will only be tested for chemistry on the edge. The shells will be cleaned as mentioned previously. However, the edge of each shell will be removed with a Dremel tool and crushed to a mesh size of 60 or less. This powder will be pooled with all of the shells collected from the same site prior to analysis. After FTIR-ATR analysis, the powder will be digested with nitric acid and analyzed as a solution for total metal in the aliquot.

3.6. Measurement of Total Sulfur in Shell

Null Hypothesis: The shells taken from less alkaline tanks and more alkaline tanks have the same total sulfur concentration present in the shell matrix.

I hypothesize that decreased pH values, due to increased dissolved pCO_2, will cause stress on *Argopecten irradian,* such that in order to maintain shell integrity, the organism will utilize sulfur containing proteins as a biological mediator. The total sulfur concentration will be higher in growing edge of the shell. Total sulfur composition will be monitored by LA-ICP-MS, however, the ability of this technique to accurately quantify sulfur is limited. Therefore, total sulfur composition will be more accurately measured using an elemental analyzer (ECS 4010 by Costech). Shell from the leading edge will be sampled and from earlier stages in growth and ground using a mortar and pestle. The samples will be transferred to the elemental analyzer sampling containers where they will be weighed on a microbalance. Sulfanilamide and 2,5-Bis-(5-tert-butyl-benzoaxzol-2yl) thiophene will be used as standards to quantify sulfur in the shells. S data will be tested for normality using the Kolmogrov-Smirnov normality test in order to implement the statistical tests presented. If the data set is shown to be normal, a t-test will indicate rather the differences between the control and experimental tank averages are significantly above random variations. The null hypothesis will be accepted at a p-value < 0.05. Alternatively, if the null is rejected, the organisms will be statistically shown to have different concentrations of total sulfur.

3.7. Measurement of Sulfur Isotopes (32 and 34)

Null hypothesis: The shell from experimentally less alkaline tanks is no different than the shell from other tanks with higher alkalinity.

Sulfur (^{34}S) has shown little to no enrichment in animals with respect to diet in tissues (Lajtha 1994). It may be that S isotopic composition of the shell and individual inorganic and organic fractions may record episodes of stress which would not be complicated, as is the case with some trace elements, by diet. Therefore, the organic fractions of the shell should have enriched values of ^{32}S than the corresponding inorganic fractions, since ^{32}S is preferentially used in metabolic processes. I hypothesize that, under stress, an organism begins to increase the production of S-enriched proteins to support shell deposition, and therefore it may produce discernible fractionation of S isotopes. S isotopic composition of the growing edge of shells will be analyzed using an isotope ratio mass spectrometer. Shells will be prepared as they were for the elemental analysis where they are ground for homogenization. The elemental analyzer will be interfaced with an isotope ratio mass spectrometer to determine relative abundances of ^{32}S and ^{34}S. A Canyon Diablo Triolite (CDT) standard will be used for sulfur, and values will be corrected for the influence of oxygen isotopes. $\delta^{34}S$ of control and experimental organisms will be compared using two-sample t-test to test for differences in isotopic composition. If differences in $\delta^{34}S$ are found to be significant between control and experimental samples and between experimental conditions, I will test for a relation

between $\delta^{34}S$ and pH using a two-way ANOVA. If appropriate, a multivariate model that combines $\delta^{34}S$, pH, and pCO_2 data will be developed to identify whether there are predictive relations between pH and $\delta^{34}S$.

To ensure that the organic fraction is being analyzed it is possible to use a leaching technique to separate the components. The shell being analyzed will be ground into a fine powder (<60 mesh). Adapting a leaching technique designed to isolate mineral fractions from sedimentary rocks ((Abanda 2006), Figure 9), I will isolate the carbonate, sulfate, and organic fractions of the shells. This leaching technique will be applied for all hypotheses presented.

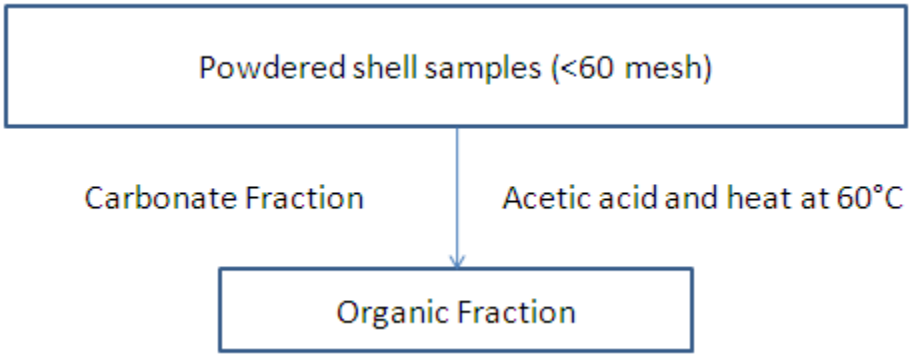


Figure 10: Flow chart of sequential leaching techniques used to isolate the organic fraction in shale that will be applied to the shells (Abanda 2006)

With respect to this experimental design, the sulfide fraction and organic fraction are of greatest interest. The residue obtained after the silicate fraction will be analyzed before and after treatment with aqua regia to see how much oxidation is occurring. This

can lead to an enrichment of organic matter in the sulfide fraction. Each of the fractions will be tested for sulfur content to see at what stage the sulfur is leaching. A comparative standard that is either a composed of shale or limestone will be used for trace metal concentrations and total sulfur concentrations. Each fraction will be tested for isotopic ^{32}S and ^{34}S, total sulfur, and trace metal if enough mass is available. Total sulfur will be determined by a CHNS analyzer.

CHAPTER 4

RESULTS

I hypothesize that ocean acidification will lead to increased sulfur utilization during shell deposition in bivalves, and that this increased S utilization will be recorded in the organic shell components as distinguished by the leaching techniques Figure 10. I am using *Argopecten irradians* (bay scallop) as my model organism to test ocean acidification effects on elemental composition, including sulfur. If the results from total sulfur analysis indicate an increase in sulfur concentration in exposed organisms it may be that total shell elemental sulfur concentrations can be used as a proxy for high dissolved pCO_2 induced stress. By exploring not only total S composition, but also assessing the S composition of the organic and inorganic shell components, the changes in mineralogy, calcification rate, trace element chemistry, and $\delta^{34}S$ of the growing edge of shells the results of this data are expected to fill vital gaps in our understanding of biomineralization in bivalves, specifically how biomineralization is mediated by an organism and how sulfur is used to maintain shell growth. If changes in shell composition are directly relatable to dissolved pCO_2 condition during growth, the data resulting from this work will also provide a unique tool for retrospective assessment of

dissolved pCO_2 changes in relic and fossil shells. If S is utilized in a different way during shell growth under conditions of high dissolved pCO_2, these data will also enable a more comprehensive assessment of water chemistry to include information about changes in bioavailable sulfur and sulfate in the ocean. Specific to the model organism, the bay scallop is exposed to ambient seawater during the process of shell growth that may lack protective organic layers, such that the bay scallop is likely to be more sensitive to changes in pH caused by increased dissolved pCO_2.

Given that ocean acidification is happening and is expected to worsen over coming years, predicting the impact of this process on high risk species such as the bay scallop and, and more broadly, on coastal bivalves is crucial to our ability to identify at-risk organisms and to predict the impact of these changes not only on the organisms themselves, but on the economic systems which depend on them (e.g. shell fish industries, food industries). Moreover, through understanding the role of sulfur in biomineralization, the results of this research will lend significant insight into the potential impacts of ocean acidification on an often overlooked component of biogeochemical cycles, sulfur.

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