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Influence of Diet on Element Incorporation in the Shells of Two Bivalve Molluscs: Argopecten irradians concentricus and Mercenaria mercenaria

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Influence of Diet on Element Incorporation in the Shells of Two Bivalve Molluscs:

Argopecten irradians concentricus and *Mercenaria mercenaria*

by

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Abstract

Recently, biogenic carbonates have received much attention as potential proxies of environmental change; however, a major pathway of elemental incorporation is often overlooked when making interpretations or designing experiments. This research experimentally examines the influence of diet on elemental composition in juvenile shells of the bay scallop, *Argopecten irradians concentricus,* and the northern quahog, *Mercenaria mercenaria*.

Exploratory trials were conducted using *Argopecten irradians concentricus* juveniles fed different algal diets: *Isochrysis, Chaetoceros, Pavlova, Tetraselmis*, or a mix of all four in a 2:1:2:2 ratio. No differences between the left and right valves were revealed, thus, subsequent analysis of the dietary influence on shell chemistry utilized both valves. Only Mg/Ca and K/Ca were significantly different between the diet groups, though different influences were determined.

Experiments with juvenile *Mercenaria mercenaria* compared shell chemistries among clams fed unicellular diets of *Isochrysis* sp. (CCMP1324), *Pavlova pinguis* (CCMP609), *Chaetoceros mulleri* (CCMP1316), *Isochrysis sp.* (CCMP1611) culture, *Pavlova sp.* (CCMP1209), or *Chaetoceros galvestonensis* (CCMP186), a mixed diet of all species in equal ratios (Mixed), or no food (starvation control). The results indicate

that diet can influence shell chemistry either directly or indirectly, with degree of influence varying by diet and mollusc species.

Additional information concerning the use of alternative element ratios and changes in the shell chemistry due to starvation-induced stress are also presented. Altogether, the present research provides valuable information concerning shell dynamics and potential diet-associated fluxes, thus demonstrating the need to consider the composition of dietary inputs when assessing environmental associations with elemental shell chemistries.

1. Introduction

The chemistry of biogenic carbonate shells is complex and influenced by a wide variety of processes. Consequently, many scientific applications are utilizing shell chemistries to interpret evolutionary, paleoenvironmental, environmental and chemical processes. To interpret trends in shell chemistries in response to environmental change, it is essential to understand how basic biological processes and environmental changes influence the elemental composition of a shell (Strasser et al., 2008).

Physical processes, especially the influence of temperature, are among the parameters most commonly considered as influencing shell chemistries, in part because rates of ion substitution can be strongly temperature dependent (Kennedy, 1969; Klein et al., 1996; Weiner and Dove, 2003; Schone, 2008; Strasser et al., 2008). Likewise, the chemistry of the water in which an organism was living and growing has been shown to influence shell chemistry (e.g., Panella and McClintock, 1968; Bryan, 1973; Carriker et al., 1980; Lorens and Bender, 1980; Rhoads and Lutz, 1980; Rainbow, 1993; Puten et al., 1996; Stecher et al., 1996; Leng and Pierce, 1999; Surge et al., 2003; Gilliken et al., 2006). Post-mortem processes (taphonomy) are also essential to consider, as are a variety of other processes, depending upon the organism considered, the environment in which it lives, and how it feeds.

Yet dietary contribution to elemental shell composition and variation of elemental constituents related to dietary algal composition are rarely if ever considered in studies and interpretations of shell chemistries. Thus, one of the primary mechanisms influencing the ratios of ions in the fluids from which a shell is precipitated is almost unknown.

This dissertation presents an examination of dietary influence on elemental concentrations in the shells of two commercially important species of bivalve. In Chapter 2, I examine the effects of the diet of young bay scallops, *Argopecten irradians concentricus,* on their shell chemistries. In Chapters 3-5, I report more extensive experiments that examined dietary and other associated influences on shell dynamics of *Mercenaria mercenaria*. To provide the background to understand how diet might influence shell chemistries, the basics of bivalve molluscan shell morphologies and shell deposition are reviewed in this introductory chapter.

The Bivalve Mollusc Shell

The outward and distinguishing feature of the bivalve mollusk is a shell made up of a pair of valves that provide protection and support for internal organs and processes (Carefoot and Donovan, 1995). Figure 1.1 depicts the general features of a marine bivalve molluscan shell.

The shell itself is an accretionary exoskeleton composed primarily of organics and calcium carbonate that are organized into layers. The outer-most layer is the organic periostracum, under which lies the mineralized portion of the shell, which usually is composed of aragonite, calcite or a combination of these minerals. The

extrapallial space separates the shell from the epithelial cells of the mantle. Figure 1.2 illustrates a partial section through a bivalve shell, showing its structure and relationship to the mantle epithelium.

The following sections provide information on the internal tissues associated with shell formation, the proposed mechanisms of shell formation, an overview of bivalve molluscan shell mineralogy, control of element incorporation, and a summary of previous findings related to elemental composition with regard to environmental and biologic influences.

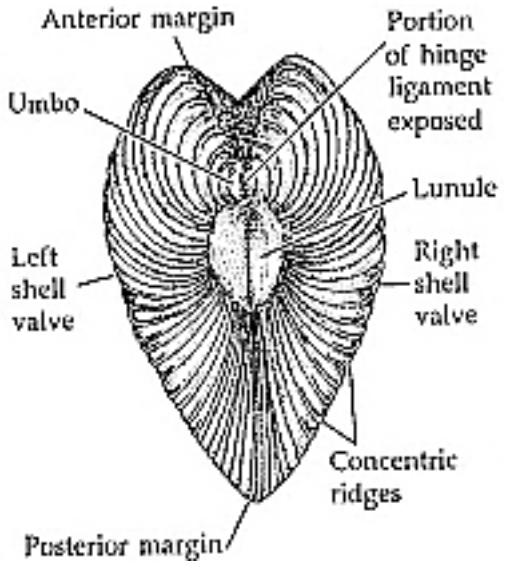
Bivalve shell deposition

The process of shell deposition is often discussed at two different levels: 1) the major players and process associated with the transfer of necessary materials into the staging area for shell deposition, and 2) the formation of the shell and nucleation of the composite crystals. Each level will be discussed separately.

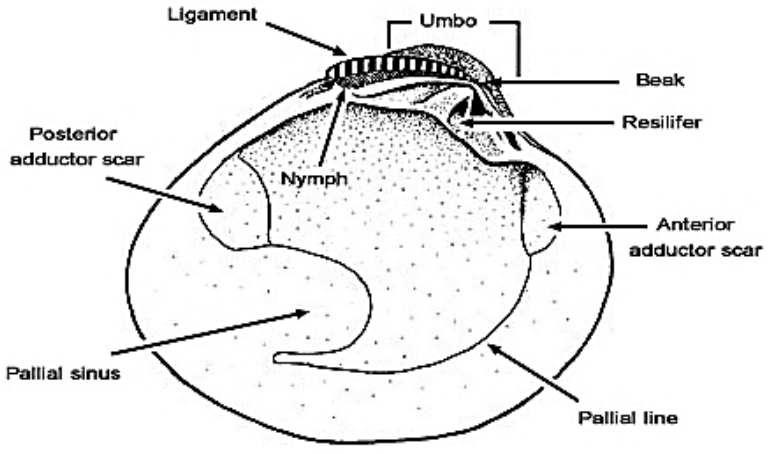
Tissues involved

Most sources refer to the tissues and spaces that are involved in shell deposition as “compartments”, with the mantle (outer mantle epithelium), extrapallial space, and the inner shell surface identified as the primary compartments (Crenshaw, 1980; Wheeler, 1992). The secondary compartment most often identified is the blood sinus of the mantle tissue; this is the major vessel for either food-derived or medium- (seawater) - derived materials to be transported to the mantle epithelium (Crenshaw, 1980).

The outer mantle epithelium is associated with the transfer and deposition of materials due to its position adjacent to both the extrapallial fluid and the blood sinus, which is positioned between the two epithelial layers. The role of the mantle epithelium in shell deposition is believed to be limited mostly to the marginal edge, based on the



A.



B.

Figure 1.1: General molluscan bivalve shell features. A. External shell features (Florida International University), B. Valve interior (Amqueddfa Cymru – National Museum Wales).

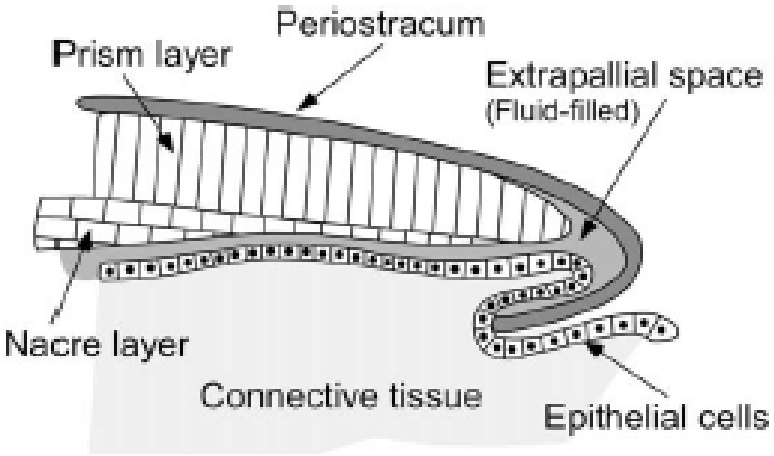


Figure 1.2: General molluscan bivalve shell structure relative to the extrapallial space and mantle epithelium (Jacob et al., 2008).

rate of shell deposition at the shell margin (Zischke et al., 1970; Wheeler and Wilbur, 1977) and upon high metabolic activity/respiratory rates described by studies such as Jodrey and Wilbur (1955). As such, the functional role of the outer mantle epithelium is further divided into two regions referred to as the central/proximal zone and the marginal zone; these zones are commonly illustrated in the histological classification of cell types and ultrastructure inherent to these cells (Crenshaw, 1980). The marginal zone, which is primarily associated with shell lengthening and thickening (Wheeler and Wilbur, 1977), is characterized by tall columnar cells with numerous mitochondria, well developed endoplasmic reticula and Golgi apparatus (Crenshaw, 1980). The central zone, which is more associated with acid-base metabolism and shell dissolution, is characterized by cuboidal epithelium with fewer mitochondria, less developed endoplasmic reticula, and minimal Golgi apparatus (Tsujii, 1968; Neff, 1972; Crenshaw, 1980). Transformation to columnar secretory cells can take place in the central zone to allow for shell repair (Tsujii, 1968).

The fluid-filled extrapallial space is the environment in which shell deposition takes place (Wilbur and Simkiss, 1972). The composition of the fluid is directly related to the metabolic activity of the outer mantle cavity, as hydrophobic barriers prevent direct association with the seawater (Crenshaw, 1980). As with the mantle, the pallial fluid can be categorized by the portion of the outer mantle with which it is associated. Though this is species specific, the outer mantle epithelium can attach at the pallial line to partition the pallial fluid into two unique components associated with the marginal and central zones (Crenshaw, 1980).

Shell formation

Shell formation is a complex process comprised of multiple stages and necessitating many biological products. Though the process has been studied for many years, there are still components that have not been accounted for and thus the exact mechanism is not fully understood.

The main components of the shell, and those consistently thought to be involved in the formation of the shell, are simply the organic matrix and calcium carbonate. Wilbur (1964) and Wilbur and Simkiss (1968) hypothesized that the organic matrix plays a significant role in shell formation as a depositional net that aids nucleation or that controls growth of the mineral. This idea led to different hypotheses related to shell formation. Bevelander and Nakahara (1969), for example, suggested that heterogeneous nucleation and growth occurs within established organic compartments (Figure 1.3). Studies such as Falini et al. (1996), however, suggest epitaxial growth of the mineral at active sites on the organic-matrix surface. Commonalities between the

hypotheses are specific to the molecules suggested to be actively involved in biomineralization and include chitin, acidic glycoproteins and silk-like proteins (Bevelander and Nakahara, 1980; Levi-Kalisman et al., 2001), with chitin determining crystal orientation of aragonite (Blank et al., 2003) and the acidic proteins controlling nucleation, polymorph, texture and morphology of the crystals (Mann et al., 1989; Weiner and Addadi, 1997; Levi-Kalisman, 2001). Figure 1.4 depicts the model of shell calcification from Levi-Kalisman et al. (2001). These descriptions, however, are based on development of primarily nacreous layers, as this has been the focus of shell mineralization studies. The following discussion will provide a general description of shell formation based on recent studies and accounting for differences between shell layers and in mineralogy.

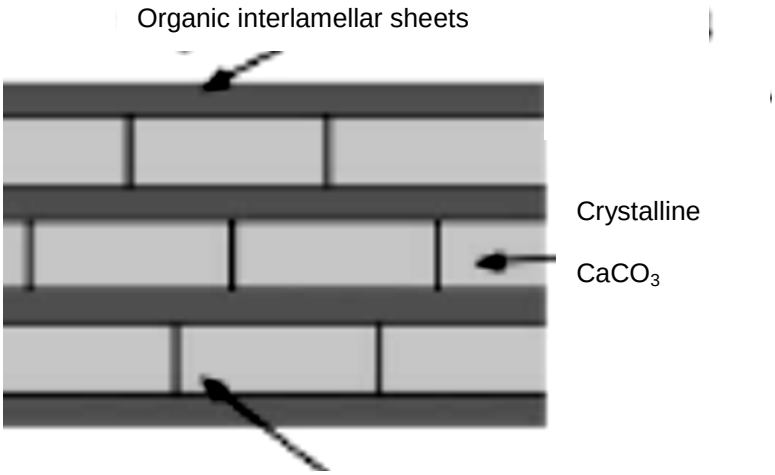


Figure 1.3: Nacre formation as described by Bevelander and Nakahara (1969), as interpreted in Jacob et al. (2008).

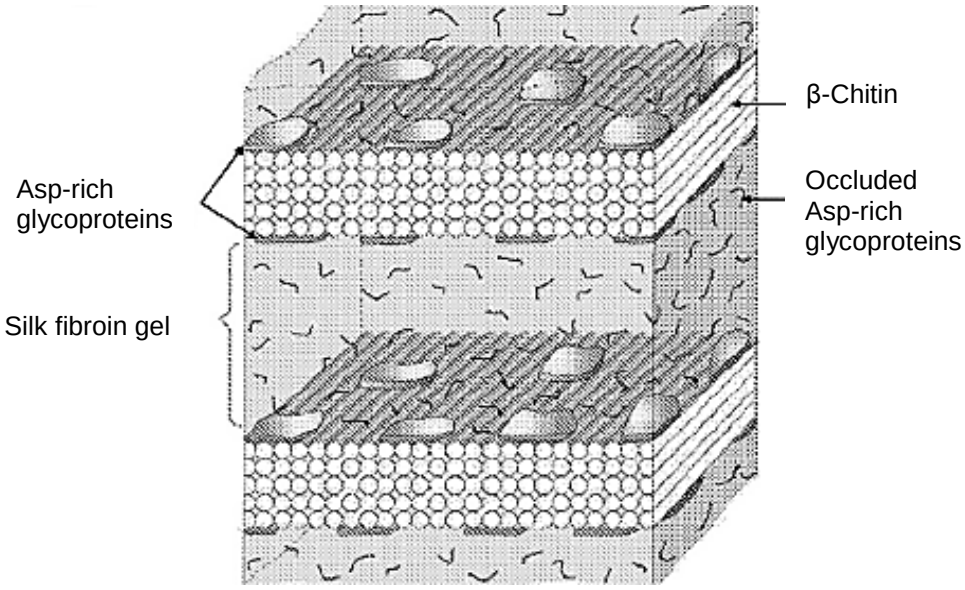


Figure 1.4: Model of shell calcification from Levi-Kalisman et al. (2001).

The depositional environment for mineral precipitation is isolated from the exterior environment (seawater) by the periostracum, which is the outermost layer of the bivalve shell comprised of highly cross-linked proteins, and the mantle epithelium (Addadi et al., 2006). This isolation allows for biological control of mineral formation (Simkiss and Wilbur, 1989; Weiner and Dove, 2003). This represents the first stage in shell formation.

The second stage is the secretion of necessary components into the extrapallial space and the construction of the organic matrix. The main components in the shell matrix are β-chitin, a hydrophobic silk protein, and other hydrophobic proteins, many of which are rich in aspartic acid (Addadi et al., 2006; Furuhashi et al., 2009). Cells of the outer mantle epithelium secrete a mixture of carbohydrates, proteins and lipids into the pallial space, all of which participate in the formation of the organic matrix (Lowenstam and Weiner, 1989; Gotliv et al., 2005). Addadi et al. (2006) suggest that the epithelial

cells secrete chitin first, which forms the general mold for the mineral, then assorted proteins, followed by silk protein in the form of a gel, which fills the chitin form and helps maintain spatial separation of lamellar sheets. In addition to the identified components, Meyer et al. (2007) identified specific phosphoproteins associated with secretory cells at the mantle edge, which they proposed to be significant in mineralization as phosphoprotein extracts have been shown to bind calcium-carbonate crystals while fixed in a hydrogel (Mount, 1999). The phosphoproteins, however, can also hinder crystal growth when in a solution (Wheeler et al., 1987).

Once the matrix (the form and foundation for mineralization) has been established, the mineral components are introduced. Vesicles containing amorphous calcium carbonate secreted from the mantle epithelial cells (Addadi et al., 2008), and/or hemocytes containing calcium carbonate crystals, deliver materials to the mineralization front (Mount et al., 2004). The presumed nacre-nucleation sites contain carboxylates and sulfates (Crenshaw and Ristedt, 1976; Nudelman et al., 2006), as seen in *Nautilus* shells. Similar sites have been seen in the shells of *Atrina rigida,* though less constricted to specific zones of the matrix (Nudelman et al., 2006). The acidic matrix components likely direct crystal formation through anionic domains (Sudo et al., 1997; Gotliv et al., 2005; Myers et al., 2007). Occluded material (acidic proteins) during mineralization is presumed to be responsible for different morphologies, as well as for alteration of solubility and mechanical characteristics (Addadi et al., 2006; Nudelman et al., 2007).

Prismatic layer formation is similar to that of nacre formation in origin and structure (Nakahara and Bevelander, 1970). The first stage is again the formation of an organic matrix juxtaposed to the periostracum and serving as a boundary for crystal

formation (Bevelander and Nakahara, 1969). These lamellae are then said to fragment and form envelopes within the compartments being formed. These envelopes contain dense extrapallial fluid (ground substance) enclosed during their formation. Crystal formation initiates within the envelopes at the inner margin with interprismatic material derived from the inner lamellae or ground substance (Nakahara and Bevelander, 1970). Nudelman et al. (2007) further describes the formation of prismatic layers atop an already formed mineral layer. In this process the first stage is suggested to be the deposition of a meshwork of chitin fibers. The next stage is the deposition of amorphous calcium carbonate onto the chitin fibers. The final stage is the crystallization of the calcium carbonate by epitaxial nucleation such that crystal orientation in each layer is maintained. Nudelman et al. (2007) further states that chitin fibers are occluded during mineralization. Figure 1.5 provides a schematic of the suggested mineralization mechanism for both prismatic layers and nacreous layers as described in Nudelman et al. (2007).

Biomineralization mechanisms remain speculative (Furuhashi et al., 2009). Lack of comparative research has prohibited a comprehensive view of molluscan shell formation (Furuhashi et al., 2009). While the structure of the bivalve shell has been generalized by many researchers, no attempt has been made to determine differences between even similarly constructed shells (Addadi et al., 2006). As illustrated in Kobayashi and Samata (2006), the components and molecular weight of the organic matrix are dependent upon the crystal structure and taxon of mollusc. Because the organic matrix has been shown to control mineralization, biological controls (genetic

controls) governing the organic matrix and thus crystal structure are an extremely important factor. It follows that not every molluscan shell would be the same.

Weiner and Traub (1980) suggested that chitin was not present in all molluscan shells, though since then, there has been a lack of research aimed at examining different taxa for chitin (Furuhashi et al., 2009). The presence of silk fibroin has not been verified in all molluscan shells (Ghiselin et al., 1967) and the protein structure lacks the helical and b-spirals characteristic of silk fibroin (Weiner and Traub, 1980). Absence of silk fibroin in prismatic layers was also suggested by Furuhashi et al. (2009).

The mechanisms for shell formation most likely differ in taxa adapted to different natural stresses (Crenshaw, 1980). Comprehensive research comparing a wide range of species and techniques will be necessary to determine all biological products associated with shell formation and the potentially numerous mechanisms of shell-layer mineralization based on evolution of the molluscan species (Crenshaw, 1980).

Bivalve shell mineralogy

The larval shell of a bivalve mollusc is constructed initially of amorphous calcium carbonate (Weiss et al., 2002), which is then transformed to aragonite. The larval shell of all bivalves is composed of aragonite with similar ultrastructure (Carriker and Palmer, 1979), implying that the larval shell is conserved through evolution (Taylor, 1973).

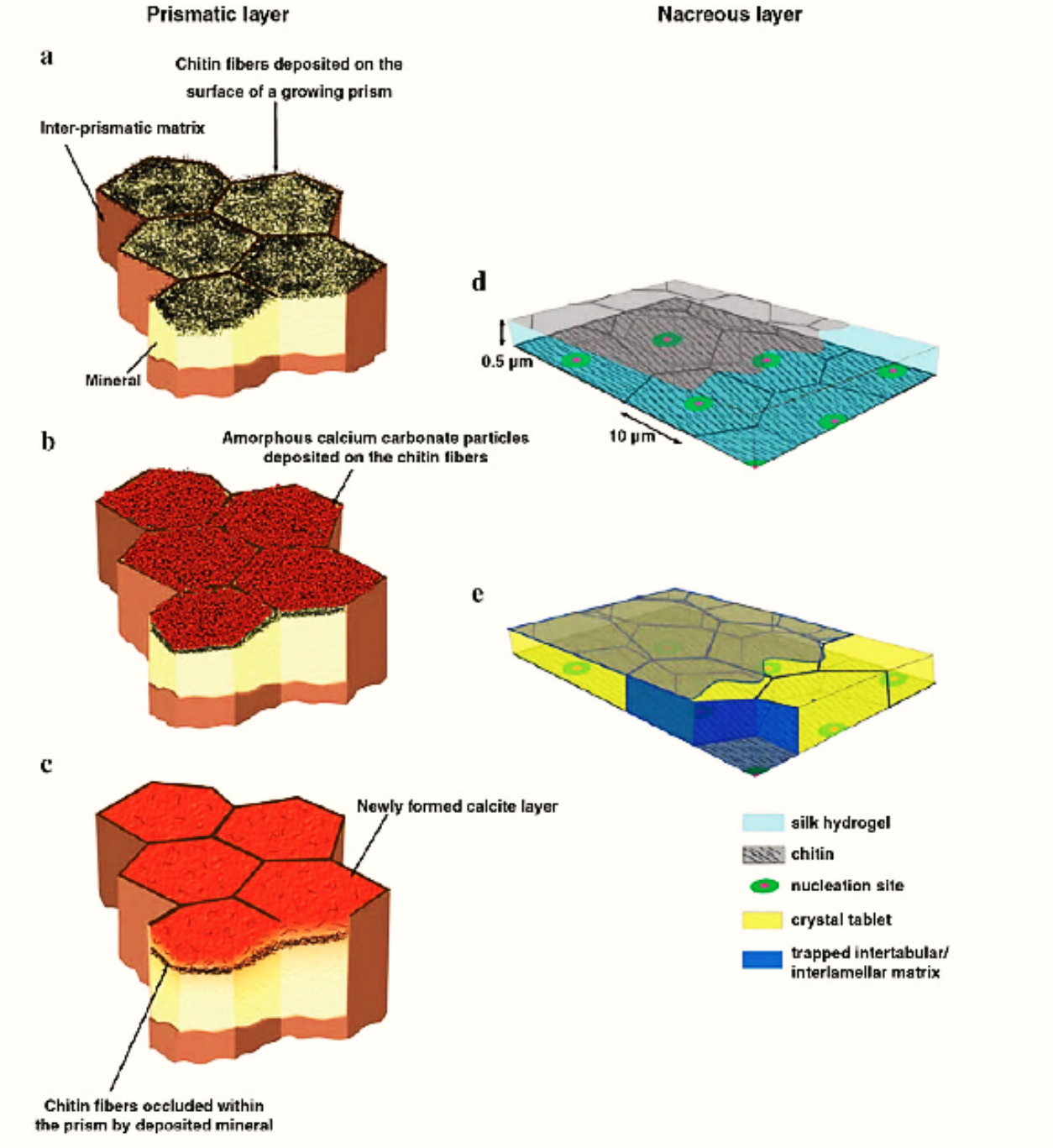


Figure 1.5: Model of shell calcification in both prismatic and nacreous layers (Nudelman et al., 2007).

The shells at later life stages are also under the influence of evolutionary adaptations, and as such, the mechanisms for deposition are genetically and biological controlled. For example, taxonomic differences can be found in the calcium carbonate mineral precipitated, the direction of allometric growth, shape of the skeleton, thickness, hardness, coloration, and other shell factors (Rhoades and Lutz, 1980). All bivalve shells, however, are composites of calcium carbonate polymorphs (calcite, aragonite, or possibly vaterite), and an organic matrix as previously identified, but the mineral structure of the calcium carbonate and combinations thereof are species specific (Kobayashi and Samata, 2006).

Carter (1980) identifies seven microstructure groups and five microstructure categories in the Bivalvia (Table 1.1). The primitive molluscan shell is assumed to have been composed of simple aragonitic prisms and nacre, as found in the presumed ancestral Monoplacophora (Taylor, 1973); variations have occurred through time to produce the listed groups and categories. Possible advantages to evolutionary changes in mineralogy, architecture and microstructure include breakage resistance, abrasion resistance, resistance to shell dissolution, fracture localization and deflection, flexibility, lower density, economy of secretion, and variable rates of vertical shell growth (Carter, 1980).

Minor and trace elements in bivalve shells

Many minor and trace elements can be incorporated into the calcium carbonate shell of bivalve mollusks (Brookes and Rumsby, 1965; Kobayashi, 1975). These elements can be associated with pigments (Foxx, 1966), part of the structural

components (Dodd, 1967), substituted for calcium or carbon, adsorbed, or associated with physiological and environmental factors (Rosenburg, 1980).

Table 1.1: Microstructure groups and categories with associated varieties and constituent microstructures (after Carter, 1980).

Microstructure Groups and principal varieties	**Microstructure categories and constituent microstructures**
Prismatic Simple prismatic Fibrous prismatic Spherulitic prismatic Composite prismatic	**Aragonitic Prismatic**
Spherulitic	**Calcitic prismatic**
Laminar Nacreous Regularly foliated	**Nacreous (aragonitic)**
Crossed Crossed lamellar Crossed acicular Complex crossed lamellar Crossed-matted/lineated	**Porcelaneous (aragonitic)** Aragonitic crossed lamellar Aragonitic crossed acicular Aragonitic complex crossed lamellar Aragonitic crossed-matted/lineated Aragonitic homogenous
Microstructure Groups and principal varieties	**Microstructure categories and constituent microstructures**
Homogenous Homogenous s.s. Granular	**Foliated (calcitic)** Regularly foliated Calcitic crossed lamellar = crossed foliated Calcitic complex crossed lamellar = complex crossed foliated
Isolated Spicules or spikes	
Isolated crystal morphotypes	

Incorporation of the elements that make up the shell can be achieved in two ways. The inner mantle tissue can uptake the elements from the surrounding environment or the elements can be derived from the ingestion of particles (feeding)

(Elinger, 1972). Both meet the same end; all elements are transferred to the blood sinus, are transported to the outer mantle epithelium and eventually into the pallial fluid for incorporation in the shell.

One major control of element incorporation is the chemical restriction associated with physical limitations of size for incorporation into the crystal lattice of the polymorphs of calcium carbonate. Aside from chemical restrictions, factors that determine the inclusion, distribution, or concentration of minor and trace elements range from ontogenetic regulation, physiological regulation, association with environmental dynamics, or can be seemingly random in nature (Rosenburg, 1980).

Magnesium can readily substitute for calcium in the crystal structure of calcite because of its relatively similar atomic radius, and has potential for environmental, ontogenetic, evolutionary and stoichiometric associations (Rosenberg, 1980). Strontium, in contrast, most readily substitutes for calcium in aragonite; Sr incorporation is widely studied as an indicator of temperature (Rosenburg 1980). The incorporation of both Mg and Sr can be influenced by many environmental factors. References to incorporation of other elements including manganese, boron, barium, lead, cadmium, iron, nickel, copper, zinc, as well as oxygen and carbon isotopes, are common in the scientific literature, with postulated influences including algal blooms altering media concentrations, temperature, salinity, ontogenetic variation, growth rate, tidal fluctuations, seasonal patterns, pollution, and sediment loading (e.g., Rucker and Valentine, 1961; Rosenburg, 1980; Carriker et al., 1996; Leng and Pearce, 1999; Putten et al., 2000; Lazareth et al., 2003). The studies, however, as noted by Rosenburg (1980), often assume cause and effect relationships without investigating all possible

factors in the elemental concentration and thus inconsistent results are common (Strasser et al., 2008).

Bivalve shells as environmental proxies

Ecological stresses can be recorded as changes in the chemistry of the molluscan bivalve shell. Their accretionary skeleton, sedentary nature, species-specific longevity, general hardiness, and preservation potential, make bivalves ideal candidates for studies of environmental influences on shell chemistry (Dodd, 1965; Phillips, 1977; Rhoads and Lutz, 1980). Presented here are some examples of experimental interpretations based on studies of shell-associated elements.

As noted earlier, Mg readily substitutes for Ca, especially in calcite, though studies have reported mixed results. Early researchers, including Chave (1954) and Wolfe et al. (1967), recognized the need for experiments to determine cause and effect relationships. Rosenburg (1980) demonstrated that Mg concentrations varied more in bivalves than other groups examined. Loren and Bender (1980) found possible confounding factors based on experimental conditions. Recent studies by Kleine et al. (1996) and Putten et al. (2000) have demonstrated that Mg concentrations covary with temperature, but Putten et al. (2000) also noted a sudden deviation indicating that the relationship was not constant. Other studies have correlated Mg with salinity (e.g., Milliman, 1974). Though observations relating Mg incorporation with environmental factors are inconclusive, evolutionary trends suggest biologically associated factors.

Strontium incorporation has been reported to be associated with multiple environmental factors though controversy surrounds these observations. Dodd (1965)

found that Sr increased with increasing temperature in the calcite layers in *Mytilus edulis*, and decreased with increasing temperature in the aragonitic shell layers. Thompson and Chow (1955), however, found no correlation with temperature in the multiple species they inspected. Hallam and Price (1968) surmised no correlation between growth rates and Sr, while Gilken et al. (2005) concluded that Sr was positively correlated with growth rate. Meanwhile, Hamer and Jenkins (2007) concluded that Sr was negatively correlated with growth rate. Stanton and Dodd (1970) concluded that Sr incorporation decreases with age, while Crisp (1975) found the opposite, and Strasser et al. (2008) reported mixed results.

Results with trace elements such as manganese have been equally problematic. Blanchard and Chasteen (1976) surmised the amount of substitution of Ca^{2+} by Mn^{2+} was correlated with tidal level, though the authors did not consider the oxygenation of the environment (Rosenburg, 1980). Crisp (1975) attempted to correlate the Mn concentration to salinity, while Strasser et al. (2008) correlated Mn/Ca levels with that of seawater concentration, potentially confounded by biological activity. Carre et al. (2006) suggested positive Mn/Ca association with growth rates while Strasser et al. (2006) showed negative correlation.

Multiple associations have been suggested for barium concentrations in shell, which is one of a very few elements potentially linked to algae dynamics thus far. Dorval (2007) suggested that Ba/Ca can be associated with salinity though more commonly, as in Stecher et al. (1996), Putten et al. (2000), Lazareth et al. (2007), and Thebault et al. (2009), is correlated with phytoplankton blooms (diatoms mostly). In the latter study, fresh water influences related to both phytoplankton blooms and increased Ba are

discussed as potential cofactors. Alternative environmental correlations were interpreted by Risk et al. (2010), who surmised a direct correlation of Ba in the shells of Chilean bivalves with local soil erosion. Coffey et al. (1997) also found possible correlation with fluvial inputs. Gillikin et al. (2006) linked dissolved concentrations of Ba with shell concentrations in *Mytilus edulis.*

Lead concentrations in shells are often correlated with the concentration in the natural environment, despite the complication of incorporation versus adsorption. Studies by both Ferrel et al. (1973) and Clarke et al. (1976) found a direct correlation between the lead concentration in the shell samples with that of the environment, though Clarke et al. examined the dead shells of *Corbicula manillensis,* while Ferrel et al. (1973) focused on the live shells of *Crassostrea virginica.* This presented the question of whether the measured lead was actually a component of the shell or simply adsorbed to the shell surface (Lutz and Rhoades, 1980). Other studies have discriminated between the dissolved and particulate phases of lead, including Pitts and Wallace (1994) who correlated lead in *Mya arenaria* shells with dissolved lead concentrations, while Borgoin (1990) associated the lead in *Mytilus edulis* shells with environmental concentrations of particulate Pb. More recently, Putten et al. (2000) found that their results were inconclusive and the lead concentrations in the shells of *Mytilus edulis* were not necessarily representative of the seasonal variability of environmental concentrations.

Objectives

The chemistry of the biogenic carbonate shell is clearly very complex (Wilbur, 1972). Carefully designed experimental studies are essential to understand the effects that environmental parameters and basic biological processes have on elemental components of the shell (Strasser et al., 2008). Moreover, all factors - such as diet – need to be included in the analyses to avoid misinterpretation and exclusion of primary contributors to shell chemistry.

This dissertation presents a first examination of the influence of algal diet on elemental concentrations in the shell of young bay scallops, *Argopecten irradians concentricus.* However, the major focus of my research is the detailed examination of dietary and other associated influences on shell chemistries of *Mercenaria mercenaria*. Similarly, few studies have determined if differences can occur between the two valves of a bivalve shell.

The elements analyzed in the shells of experimental *Argopecten* and *Mercenaria* include silver (Ag), aluminum (Al), arsenic (As), boron (B), beryllium (Be), calcium (Ca), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), potassium (K), lithium (Li), magnesium (Mg), manganese (Mn), molybdenum (Mo), sodium (Na), nickel (Ni), phosphorous (P), lead (Pb), scandium (Sc), selenium (Se), silica (Si), tin (Sn), strontium (Sr), titanium (Ti), thallium (Tl), vanadium (V) and zinc (Z). This suite of elements represents those available using the Perkin-Elmer 4300 DV ICP-OES in the USF Paleoclimatology, Paleoceanography Biogeochemistry Laboratory, but also includes the majority of elements frequently examined in calcium carbonate shells.

The following questions are addressed:

- Question 1. Do different algal diets contribute to unique elemental patterns in the bivalve shell?
- Question 2. Do diets based on different species of the same algal genus result in different elemental shell chemistries in bivalve molluscs? Furthermore, does origin of the algae affect associated elemental signatures?
- Question 3. Are elemental signatures distinct enough to differentiate the species of algae ingested? Is there a method which might increase the resolution between algal species ingested?
- Question 4. Do elemental signatures differ significantly between valves of a bivalve mollusc? If so, do differences suggest a genetic/evolutionary mechanism or possibly correlate with functionality?
- Question 5. Do elemental patterns contributed by diet to the bivalve molluscan shell depend on the mineralogy of the shell and species of bivalve?
- Question 6. Do the valves collected from dead bivalve molluscs (empty shell with no tissue) retain the elemental characteristics of the living population?

2. Elemental composition differences in the shell of juvenile *Argopecten irradians concentricus* fed different diets: a first look

Introduction

In general, the two pathways used for the incorporation of material into the pallial space [region of shell deposition (Wilbur and Simkiss, 1972)] are 1) direct internalization from the surrounding sea water or 2) feeding (Crenshaw, 1980). Though many studies concentrate on associations between the concentration of elements in seawater and the concentrations of those elements in the calcium carbonate shells of marine bivalves, little is known about the contribution of diet to elemental shell chemistry.

Ho et al. (2003) presented analysis of the cellular content of 15 phytoplankton species with regard to C, N, P, S, K, Mg, Ca, Sr, Fe, Mn, Zn, Cu, Co, Cd and Mo. In general, their study found that K concentrations are higher than seawater and Mg lower, except in the case of diatoms, where Mg and K are both relatively high. Ho et al. (2003) also repeated that the major nutrients C, N, P, S are variable with the average quotas of the organic biomass being similar to that of Redfield et al. (1963); and that the trace metals following the general pattern of Fe>Mn>Zn>Cu>Co=Cd>Mo. The coccolithophores examined by Ho et al. (2003) had higher Mn, Co, and Cd quotas compared to the diatoms; this was explained by the possible difference between metal requirements of oceanic (the coccolithophores) and neritic species (the diatoms). The

oceanic diatom examined, however, had low Fe, Mn, and Cu quotas and higher Co and Cd quotas compared to the coastal diatoms analyzed, thus lending support to the researcher's interpretation.

Besides the elemental content differences among algal taxonomic classes, researchers (e.g., Brown et al., 1997) have demonstrated varied nutritional value and biochemical composition of specific algae with regard to vitamin, amino acid, sugar, protein, and carbohydrate content, which may have implications for selective feeding upon certain algal classes. Bivalves such as the eastern oyster, *Crassostrea virginica*, select food particles based upon size, shape, concentration (Ward and Shumway, 2004) and biochemical composition, as demonstrated in the black lipped oyster (Brown et al., 1996).

Assimilation of material from ingested food particles is directly related to the food itself, i.e., size, biochemical composition, quantity and cell wall (Reinfelder and Fisher, 1991; Bayne, 1993). Furthermore, the partitioning of an element within an algal cell has been directly related to assimilation efficiencies of specific elements studied in *Mytilus edulis* shells and soft tissues (Wang and Fisher, 1996). Because some elements are more readily available for assimilation from certain algal cells than from others, selective feeding could potentially limit the elements internalized, providing for a mechanism of diet-associated elemental shell signatures related to the ecology of the organism and the specific cells being ingested.

The present research is a first look at the potential for variable elemental concentrations in the shell of *Argopecten irradians concentricus* due to differential diets provided in an aquacultural setting. The bay scallop, *Argopecten irradians concentricus*,

is a relatively fast growing hermaphroditic, filter-feeding bivalve with an average life span of 18 months. The shell consists of three layers: an inner and outer calcitic foliated layer, and a middle aragonitic crossed lamellar layer (Kennedy et al., 1969). Due to the short life span, the bay scallop is not considered an ideal experimental animal for long-term environmental association; however, the rapid growth rate and shell composed of both aragonite and calcite make the bay scallop a good specimen for exploratory research related to feeding dynamics.

Materials and Methods

Algal cultures

Six months prior to the start of the feeding trials, selected phytoplankton cultures were procured from Provasalli - Guillard Culture Center for Marine Phytoplankton [*Pavlova lutheri* (CCMP 1325)(Pl) and *Tetraselmis* sp. (CCMP 963)(Ttm)] or from Bay Shellfish Aquaculture [*Chaetoceros mulleri* (Cm) and *Isochrysis galbana* (TI)]. All cultures were acclimated to laboratory conditions for 48 hours prior to transfer to 150 ml sterile sea water for use as experimental stock cultures.

Stock and feeder cultures were maintained in a separate enclosure within the hatchery. Temperature was maintained at 22^0 C. Both standard and full spectrum fluorescent lights were used for illumination.

Specimen rearing and maintenance

Six adult bay scallops, *Argopecten irradians concentricus,* were spawned using thermal induction. Three million fertilized eggs were collected and placed in a 500L tank

with a heat source and filtered air supply. The resultant larvae were held in the same tank for 48 hours before the first water change occurred.

Water was obtained from two 800 L settling tanks filled with water pumped from Tampa Bay and filtered using a sand filter, a diatomaceous earth filter, and two charcoal filters. The water pumped to the laboratory was further treated with an ultraviolet sterilizing unit and filtered a final time through a 1 µm sock filter. The day prior to a water change, an alternate 500L tank was filled and fitted with a heat source and filtered air supply. The larval tanks were maintained at 26^{O}C and salinity of 30±2.

The water was changed every other day. The larvae were retained on a 35 µm sieve for the first two water changes and a 75 µm sieve for the remainder of the larval stage. The larvae were transferred to the alternate rearing tank immediately at which time they received the first of four daily feedings (a mixture of algal species composed of *Isochrysis galbana, Pavlova lutheri, and Chaetoceros mulleri* in a 2:2:1 ratio). After the animals were transferred, the drained tanks were cleaned using a dilute glacial acetic acid solution, rinsed with tap water, cleaned again with a mild Alconox solution, and rinsed with Reverse Osmosis water. The tank was allowed to dry for 24 hours before being filled with bay water.

Once 1% of the larvae were at pediveliger stage, five clumps of faux grass made by bundling strips of black plastic were added for settling substrate. The water change methodology altered such that only 50% of the water was replaced every other day. This procedure was followed until the post-set scallops were 450 µm in size (Lu and Blake, 1999).

Feeding experiment

Scallops attaining 450 µm (shell height) were randomly collected, divided into five groups of 150, then further divided into groups of fifty to provide three replicate experimental groups for each of the five treatments. Each group was placed into a 22L tank with a heat source and filtered air supply. The same temperature and salinity parameters used during the rearing process were used during the experiment.

Each experimental group of scallops was treated identically except for diet received. Each of four groups received only one of the following algal species for nutritional supplement: *Isochrysis galbana, Pavlova lutheri, Chaetoceros mulleri,* or *Tetraselmis sp*. The fifth group was fed the same mixed diet received during the larval phase (*Isochrysis galbana, Pavlova lutheri, and Chaetoceros mulleri* in a 2:2:1 ratio).

All algal cultures used for feeding experiments were allowed to grow until a concentration of $4x10^6$ cells ml^{-1} was attained. Each treatment received a feeding of $1x10^5$ cells ml^{-1} per day for the first two days. Subsequently, the daily ration was increased by 10,000 cells per milliliter per day every two days. Using these guidelines, a volumetric measure of algae to be fed daily was determined and divided into three or four doses spread equally throughout the day.

The feeding experiment was terminated after thirty days. All living animals were collected, the valves separated and the tissue excised by use of a stainless steel scalpel. Each shell was then rinsed in RO water and lightly brushed to remove foreign organic matter. Up to thirty left valves per feeding group were then placed in sealed glass vials for elemental analysis by Inductively Coupled Plasma Optical Emission

Spectroscopy (ICP-OES) and for inspection by Energy Dispersive X-ray Spectroscopy (EDS x-ray analysis).

SEM microanalysis

A Hitachi 2000® scanning electron microscope with a Sun System electron dispersion system attachment was utilized for the x-ray analysis. Five valves from each of the five feeding groups were used for the EDS portion of the research. The clean and dried valves were adhered to carbon stubs such that the umbo of each valve faced in the same direction and the inner shell surface came into contact with the carbon tape. This orientation helped to minimize shadowing affects as well as time needed for the analysis.

The SEM was set for variable pressure imaging and the vacuum set for 25 Pa. A magnification of 60X was used with a 12.3 mm working distance. No aperture was utilized and both 5 KV and 15 KV beams were used on each valve with an initial x-ray time of 200 seconds.

Each valve was inspected point by point in five different regions along the leading marginal edge and at a sixth point at the umbo. All x-ray times were adjusted depending upon count rate acquired to make sure all data collected were comparable. The results of this procedure were utilized to calibrate the ICP unit based on the general detection limits of the EDS system and peaks present in the analysis.

ICP-OES

Centrifuge tubes, 15 ml, were cleaned by soaking in hot 2% trace pure nitric acid. After approximately 15 minutes, the tubes were removed from the cleaning solution and dried. Caps were placed on the tubes and the outside of the tube rinsed with MilliQ water to remove any acid residue from the handling surface.

The remaining valves from each feeding group were pooled into one sample per treatment per replicate. Each grouping was rinsed one more time in MilliQ water and then ground before being placed in the clean 15 ml centrifuge tubes and digested in 4 mls of 4% trace pure nitric acid after the methodology of He and Mai (2001). The inductively coupled plasma optical emission spectroscopy unit was calibrated for the inspection of a full sweep of trace elements, set for a linear fit, and run with a blank of 4% trace-pure nitric acid between every sample. Table 2.1 provides the detection and quantitation limits of the ICP-OES equipment used.

The resulting concentration measures from the inductively couple plasma optical emission spectroscope analyses were corrected using the determined dilution factors (mass of solute/ {mass of solute + mass of solvent}). The data were further transformed into element: calcium ratios.

Results

Survivorship

The highest survivorship was found in juvenile scallops fed the mixed diet and the *Pavlova pinguis* diet (96% and 98% respectively), as compared to the *Pavlova lutheri* and *Isochrysis galbana* with 84% and 88% respectively (Figure 2.1). The lowest

survivorships observed were the *Chaetoceros mulleri* (52%) and the *Tetraselmis sp.* (10%) feeding groups.

Table 2.1: Available Lines, Limits of Quantitation (LOQ), and Minimum Detection Limits (MDL) for the USF Paleoclimatology, Paleoceanography Biogeochemistry Laboratory - Perkin-Elmer 4300 DV ICP-OES

element	λ(nm)	LOQ* (ppb) 06/25/04 Axial View	MDL** (ppb) 06/25/04 Axial View	element	λ(nm)	LOQ* (ppb) 06/25/04 Axial View	MDL** (ppb) 06/25/04 Axial View
Ag	328.068	1.57	0.47	Mg	280.271	0.68	0.2
Al	396.153	5.35	1.6	Mg	285.213	1.21	0.36
As	188.979	95.76	28.73	Mn	257.61	0.16	0.05
B	249.677	16.6	4.98	Mo	202.031	8.81	2.64
Ba	233.527	0.63	0.19	Na	589.592	3.53	1.06
Be	313.107	0.25	0.08	Ni	231.604	2.79	0.84
Ca	315.887	19.56	5.87	P	213.617	56.21	16.86
Ca	317.933	4.44	1.33	Pb	220.353	13.08	3.92
Ca	422.673	2.21	0.66	Sb	206.836	46.45	13.93
Cd	228.802	1.94	0.58	Sc	361.383	0.16	0.05
Co	228.616	1.57	0.47	Se	196.026	150.12	45.04
Cr	267.716	0.54	0.16	Si	251.611	8.51	2.55
Cu	327.393	1.75	0.52	Sn	189.927	-	-
Fe	238.204	1.17	0.35	Sr	407.771	0.03	0.01
Hg	253.652	31.98	9.59	Sr	421.552	0.04	0.01
K	766.49	1.29	0.39	Ti	334.94	0.26	0.08
Li	670.784	0.07	0.02	Tl	190.801	54.62	16.39
Mg	279.077	4.2	1.26	V	290.88	0.81	0.24
Mg	279.553	0.38	0.11	Zn	206.2	6.59	1.98

* - Limit of Quantitation, 10s of reagent blank, n=20
** - Minimum Detection Limit, 3s of reagent blank, n=20

Growth

The highest growth rates were observed in scallops fed the mixed diet (control) and the *Pavlova pinguis* diet (Figure 2.2). On average, the specimens fed the mixed algal diet grew 110 µm per day while the scallops fed *Pavlova pinguis* grew on average 103 µm per day. The slowest growth rate observed was for scallops fed the *Tetraselmis* sp. at

52 µm per day. The growth rates on remaining diets were intermediate with the *Chaetoceros*-fed group growing on average 86 µm per day, *Pavlova lutheri*-fed scallops at 79 µm per day, and the *Isochrysis*-fed scallops 82 µm per day.

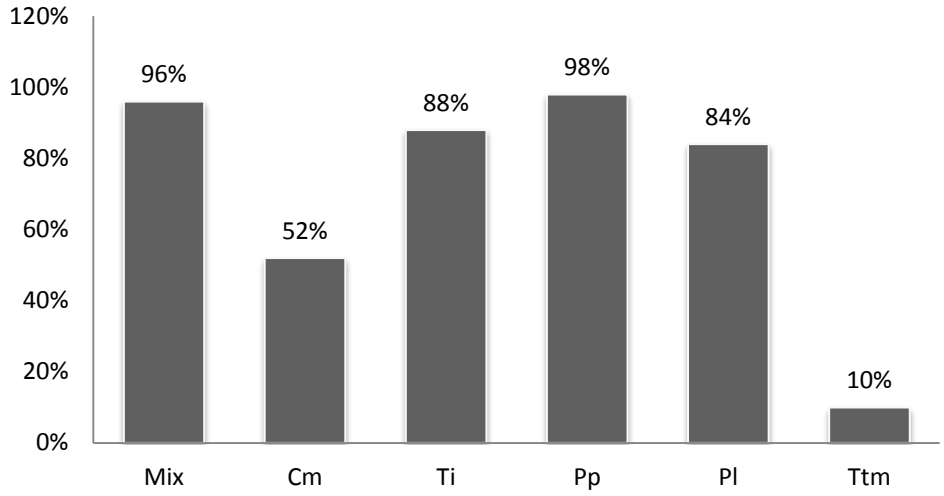


Figure 2.1: Percent survival of *Argopecten irradians concentricus* by diet received.

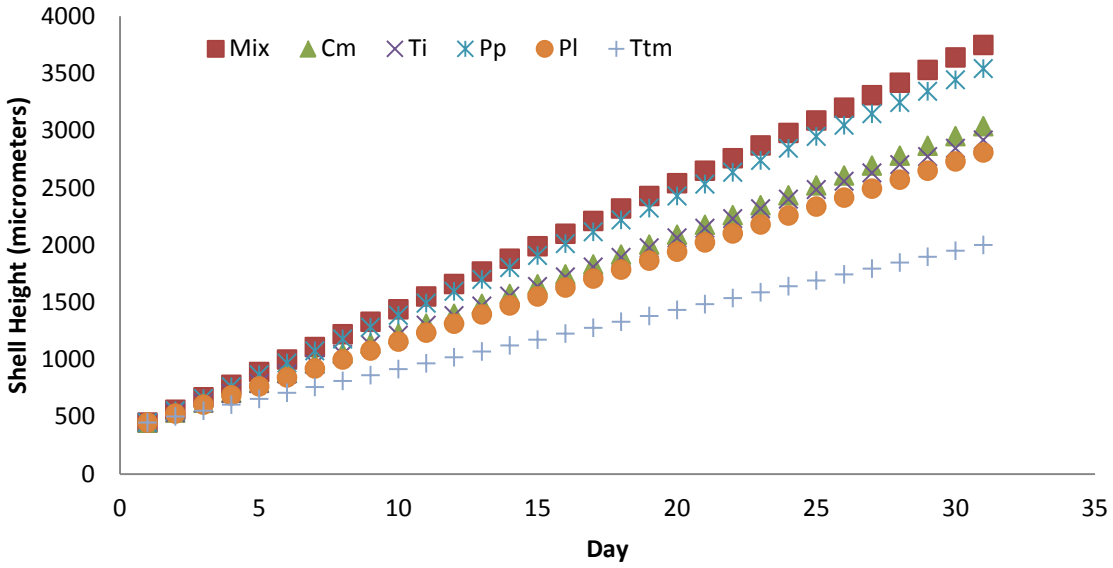


Figure 2.2: Growth rate (micrometers per day) of *Argopecten irradians concentricus* by diet received.

Elemental analysis (ICP-OES)

Complications during the analysis of the shell samples by Inductively Coupled Plasma Optical Emission Spectroscopy, greater than expected mortality in some of the feeding groups and variable valve mass restricted the available sample size to just two replicates. Figures 2.3 – 2.10 provide the element/calcium ratios for the scallop shell pools analyzed for the left and right valves, respectively, from feeding trials 1 and 3. Those elements originally included in the analysis but not reported were below the detection limits of the ICP-OES for all diets in both trials as were the values missing in the presented figures. The variability between the trials and valves is large for the majority of the element ratios examined. Differences in Fe/Ca (Figure 2.4), K/Ca (Figure 2.5), and Mg/Ca (Figure 2.6) are apparently influenced by diet. Due, impart, to the high variability, no trends are evident in the other elemental ratios amongst the diets.

Left versus right valve

The different valves were compared regardless of diet to provide a generalized comparison of the composition of the left and right valves of the bay scallop. All valve groups were analyzed by element/Ca ratio and the results are provided in Tables 2.2 and 2.3. The test used was based on the properties of the individual data sets. None of the elemental ratios were significantly different between the two valves.

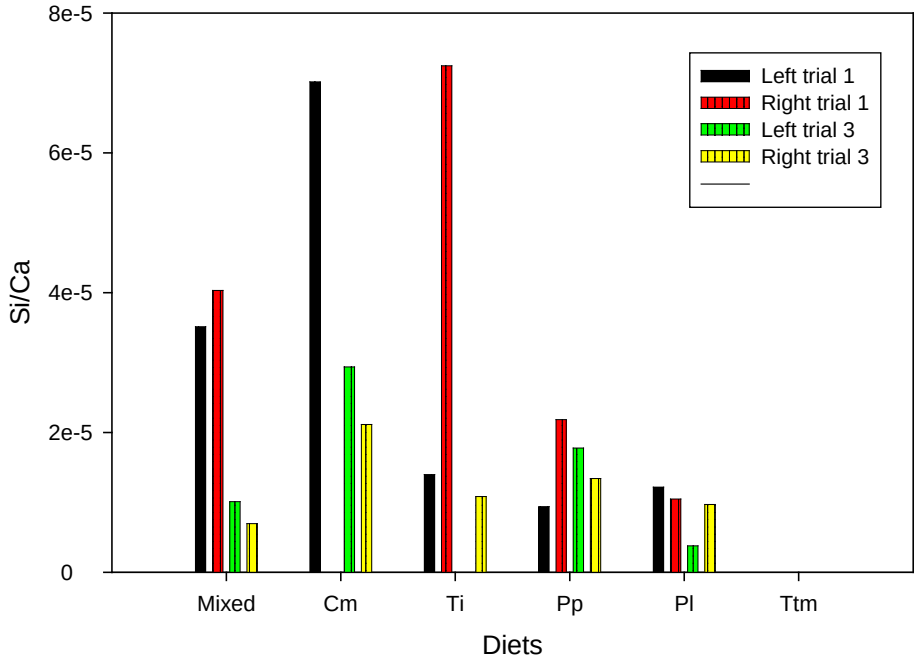


Figure 2.3: Si/Ca in pooled scallop shells by diet received. 1) Mixed algal diet, 2) *Chaetoceros mulleri* diet (Cm), 3) *Isochrysis galbana* diet (Ti), 4) *Pavlova pinguis* (Pp), 5) *Pavlova lutheri* diet (Pl), 6) *Tetraselmis* diet (Ttm).

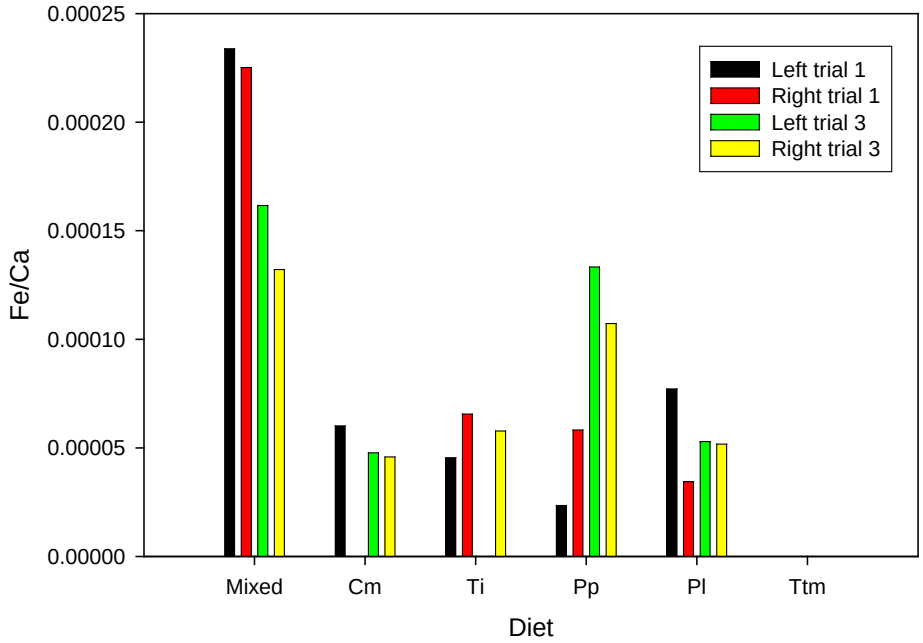


Figure 2.4: Fe/Ca in pooled scallop shells by diet received. 1) Mixed algal diet, 2) *Chaetoceros mulleri* diet (Cm), 3) *Isochrysis galbana* diet (Ti), 4) *Pavlova pinguis* (Pp), 5) *Pavlova lutheri* diet (Pl), 6) *Tetraselmis* diet (Ttm).

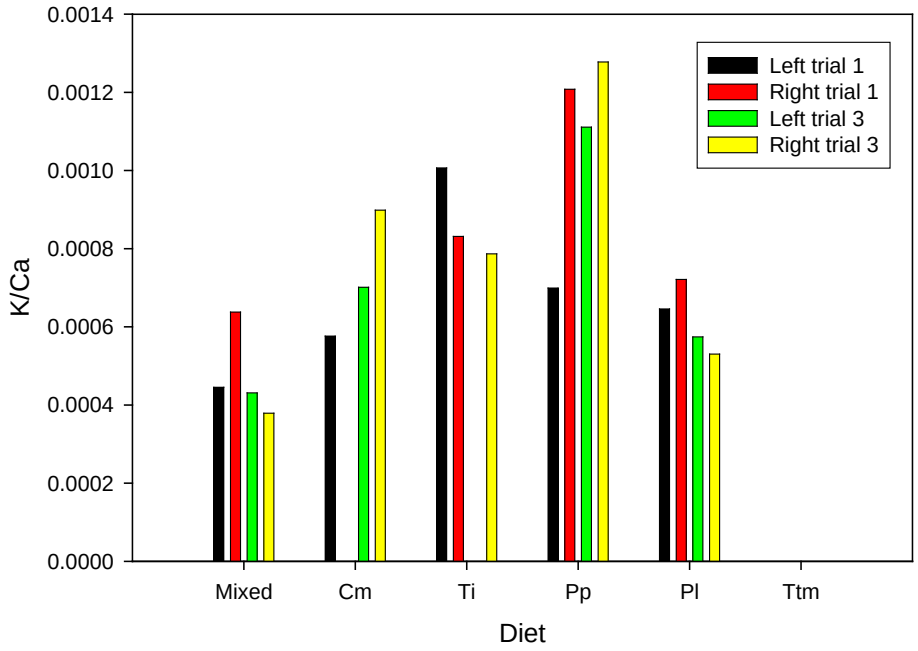


Figure 2.5: K/Ca in pooled scallop shells by diet received. 1) Mixed algal diet, 2) *Chaetoceros mulleri* diet (Cm), 3) *Isochrysis galbana* diet (Ti), 4) *Pavlova pinguis* (Pp), 5) *Pavlova lutheri* diet (Pl), 6) *Tetraselmis* diet (Ttm).

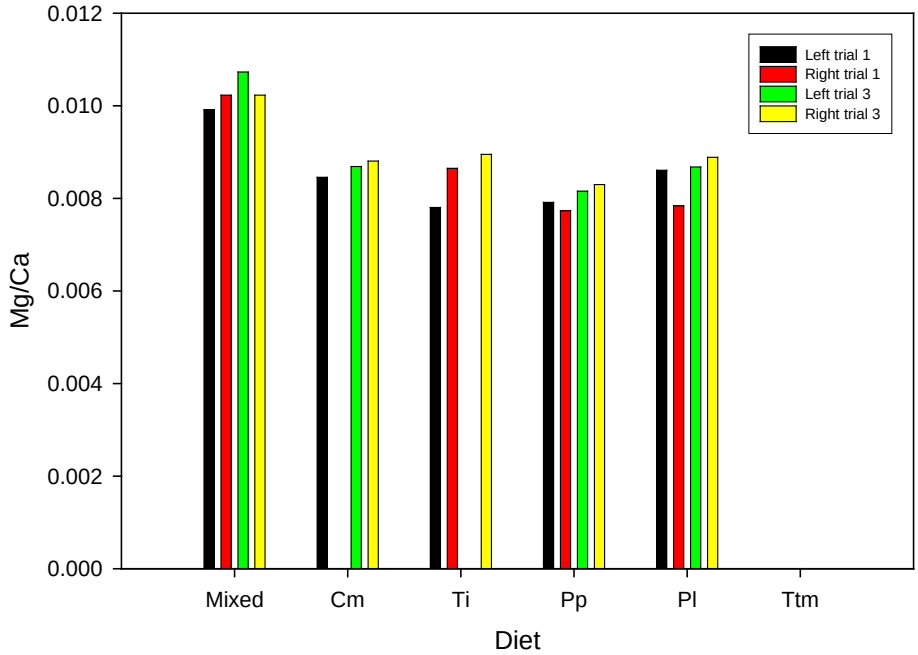


Figure 2.6: Mg/Ca in pooled scallop shells by diet received. 1) Mixed algal diet, 2) *Chaetoceros mulleri* diet (Cm), 3) *Isochrysis galbana* diet (Ti), 4) *Pavlova pinguis* (Pp), 5) *Pavlova lutheri* diet (Pl), 6) *Tetraselmis* diet (Ttm).

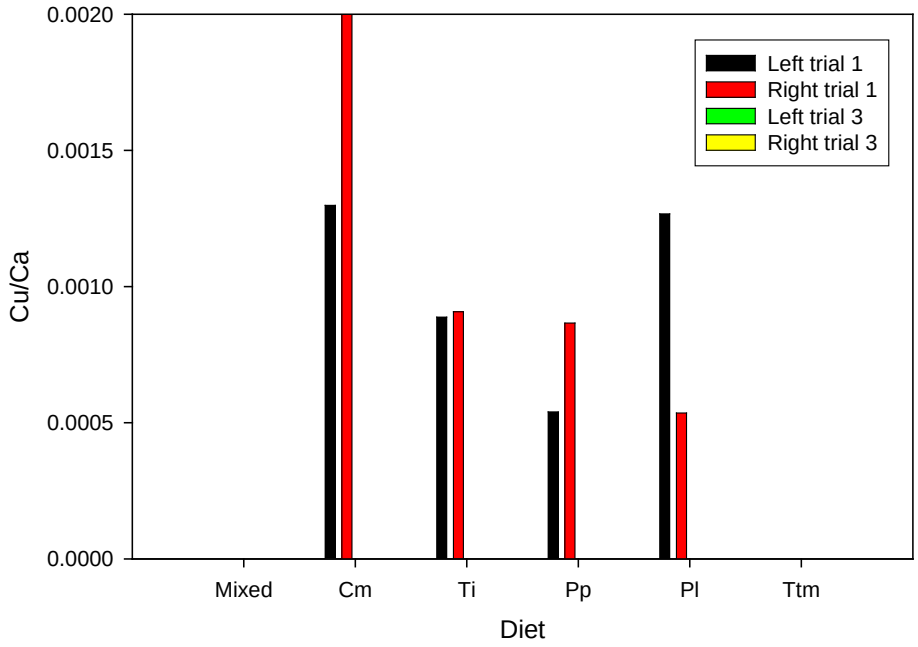


Figure 2.7: Cu/Ca in pooled scallop shells by diet received. 1) Mixed algal diet, 2) *Chaetoceros mulleri* diet (Cm), 3) *Isochrysis galbana* diet (Ti), 4) *Pavlova pinguis* (Pp), 5) *Pavlova lutheri* diet (Pl), 6) *Tetraselmis* diet (Ttm).

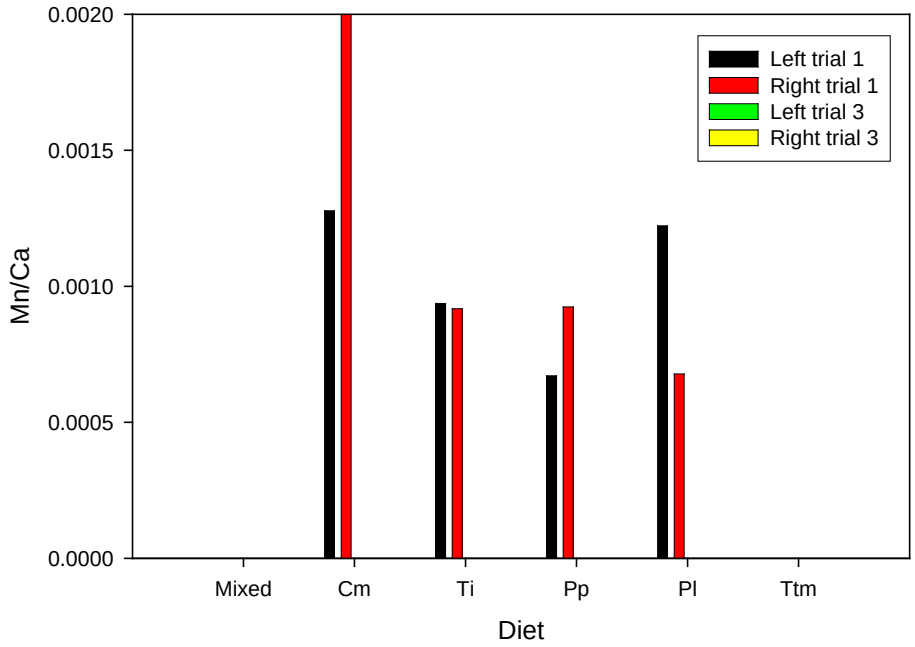


Figure 2.8: Mn/Ca in pooled scallop shells by diet received. 1) Mixed algal diet, 2) *Chaetoceros mulleri* diet (Cm), 3) *Isochrysis galbana* diet (Ti), 4) *Pavlova pinguis* (Pp), 5) *Pavlova lutheri* diet (Pl), 6) *Tetraselmis* diet (Ttm).

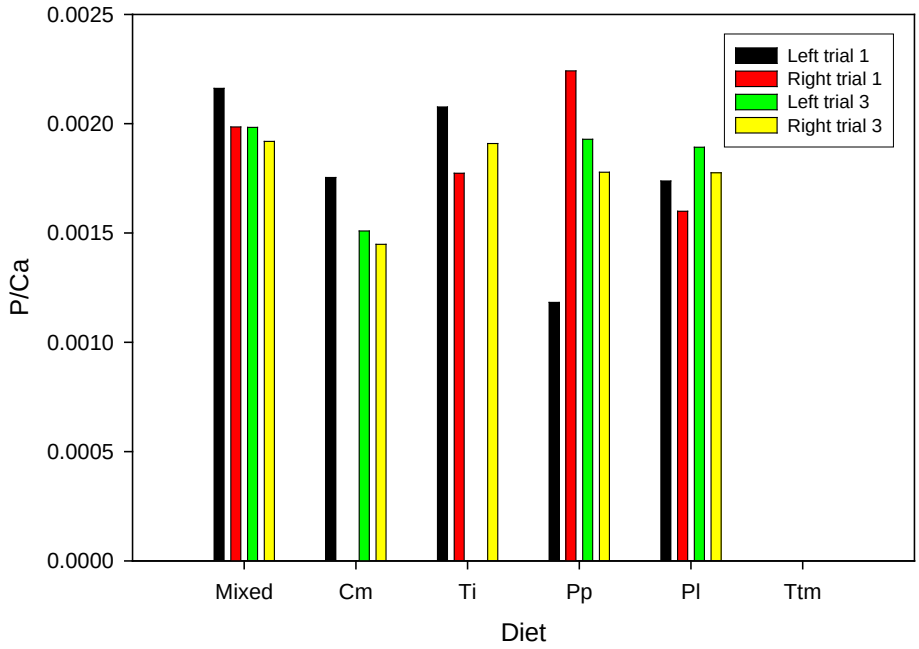


Figure 2.9: P/Ca in pooled scallop shells by diet received. 1) Mixed algal diet, 2) *Chaetoceros mulleri* diet (Cm), 3) *Isochrysis galbana* diet (Ti), 4) *Pavlova pinguis* (Pp), 5) *Pavlova lutheri* diet (Pl), 6) *Tetraselmis* diet (Ttm).

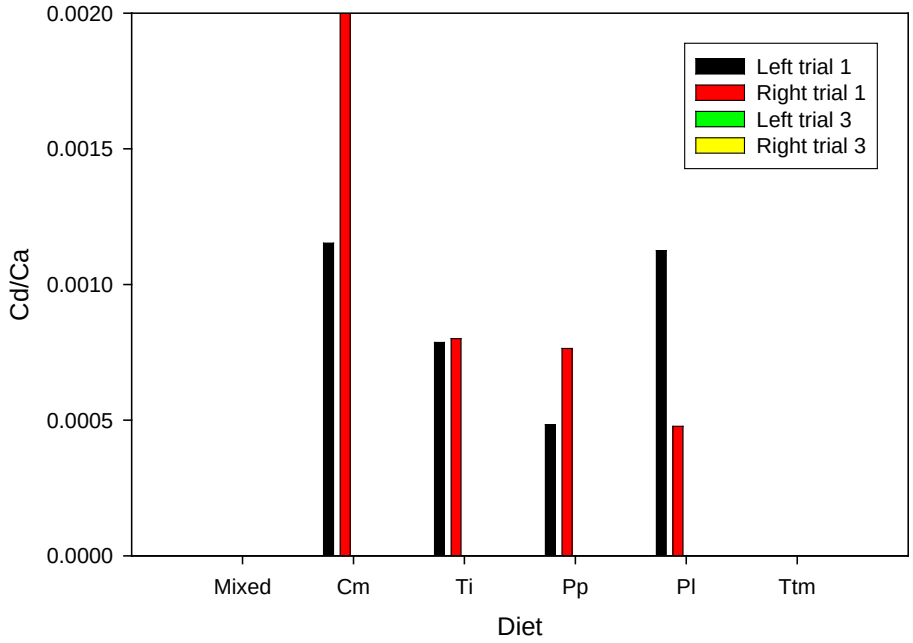


Figure 2.10: Cd/Ca in pooled scallop shells by diet received. 1) Mixed algal diet, 2) *Chaetoceros mulleri* diet (Cm), 3) *Isochrysis galbana* diet (Ti), 4) *Pavlova pinguis* (Pp), 5) *Pavlova lutheri* diet (Pl), 6) *Tetraselmis* diet (Ttm).

Differences in elemental composition by diet received

Because the left and right valves were determined not to be significantly different in regard to elemental composition, both valve pools per trial were analyzed together to increase the overall sample size sufficiently for statistical comparison. Two elemental ratios proved to be significantly different between various experimental diets, K/Ca and Mg/Ca. The results of the ANOVAs and post hoc pairwise analyses are provided in Tables 2.4 – 2.7, and shown in Figures 2.11 and 2.12.

Table 2.2: Mann-Whitney Rank Sum Test– scallop left versus right valve pools by element

Group	N	Missing	Median	25%	75%
Si/Ca-L	12	3	1.40E-05	9.92E-06	3.08E-05
Si/Ca-R	12	3	1.34E-05	1.03E-05	2.65E-05
Mann-Whitney U Statistic= 42.000					
T = 84.000 n(small)= 9 n(big)= 9 (P = 0.930)					
Fe/Ca-L	12	3	6.01E-05	4.72E-05	1.40E-04
Fe/Ca-R	12	3	5.82E-05	5.03E-05	1.13E-04
Mann-Whitney U Statistic= 39.000					
T = 87.000 n(small)= 9 n(big)= 9 (P = 0.930)					
Mg/Ca-L	12	3	8.61E-03	8.09E-03	8.99E-03
Mg/Ca-R	12	3	8.81E-03	8.18E-03	9.27E-03
Mann-Whitney U Statistic= 46.000					
T = 80.000 n(small)= 9 n(big)= 9 (P = 0.659)					
Cu/Ca-L	6	2	1.08E-03	7.14E-04	1.28E-03
Cu/Ca-R	6	2	8.87E-04	7.01E-04	1.00E-01
Mann-Whitney U Statistic= 7.000					
T = 19.000 n(small)= 4 n(big)= 4 P(est.)= 0.885 P(exact)= 0.886					
Mn/Ca-L	6	2	1.08E-03	8.04E-04	1.25E-03
Mn/Ca-R	6	2	9.21E-04	7.98E-04	9.04E-02
Mann-Whitney U Statistic= 7.000					
T = 19.000 n(small)= 4 n(big)= 4 P(est.)= 0.885 P(exact)= 0.886					
Cd/Ca-L	6	2	9.56E-04	6.35E-04	1.14E-03
Cd/Ca-R	6	2	7.82E-04	6.21E-04	8.84E-02
Mann-Whitney U Statistic= 7.000					
T = 19.000 n(small)= 4 n(big)= 4 P(est.)= 0.885 P(exact)= 0.886					

Table 2.3: t-test – scallop left versus right valve pools by element

Group Name	N	Missing	Mean	Std Dev	SEM
K/Ca-R	12	3	8.08E-04	2.93E-04	9.77E-05
K/Ca-L	12	3	6.88E-04	2.33E-04	7.75E-05
Difference	0.00012				
t = 0.962 with 16 degrees of freedom. (P = 0.350)					
95 percent confidence interval for difference of means: -0.000144 to 0.000384					
Group Name	N	Missing	Mean	Std Dev	SEM
P/Ca-L	12	3	1.80E-03	3.04E-04	1.01E-04
P/Ca-R	12	3	1.83E-03	2.28E-04	7.59E-05
Difference	-0.0000226				
t = -0.178 with 16 degrees of freedom. (P = 0.861)					
95 percent confidence interval for difference of means: -0.000291 to 0.000246					

The potassium ratio differed significantly between the *Pavlova pinguis* and Mixed diets, the *Pavlova pinguis* and *Pavlova lutheri* diets and the *Isochrysis galbana* and Mixed diets, establishing Pp > Mixed, Pp > Pl, and Ti > Mixed (Table 2.5). The magnesium ratio differed significantly between the Mixed diet and all the analyzed single algal species diets establishing Mixed > Cm, Ti, Pp, and Pl (Table 2.7).

Discussion

The Mixed algal diet produced the fastest growth rates and the second highest survivorship of juvenile bay scallops. The animals receiving the *Pavlova lutheri* and *Isochrysis galbana* both had similar survival rates as well as similar growth rates. The *Pavlova pinguis* and *Chaetoceros mulleri* diets did, however, deviate from the effect of the other single algal diets. *Pavlova pinguis* was associated with the highest survivorship and the second highest growth rate, thus was more similar to the mixed

diet. The *Chaetoceros mulleri* fed group had similar growth rates to *Pavlova lutheri* and *Isochrysis galbana*, but a low survivorship.

In general, diets composed of multiple algal species are superior to those composed of single algal species (Romberger and Epifanio, 1981; Albentosa et al., 1993; Brown et al., 1997) and the present study supports that trend. Diatoms, however, have been shown to be rich in fatty acids and support healthy growth rates and survival of cultured *Argopecten irradians* (Milke et al., 2006). In the present study, the initial size of the scallops used may have affected ingestion of the *C. mulleri* and thus their growth rate and survival.

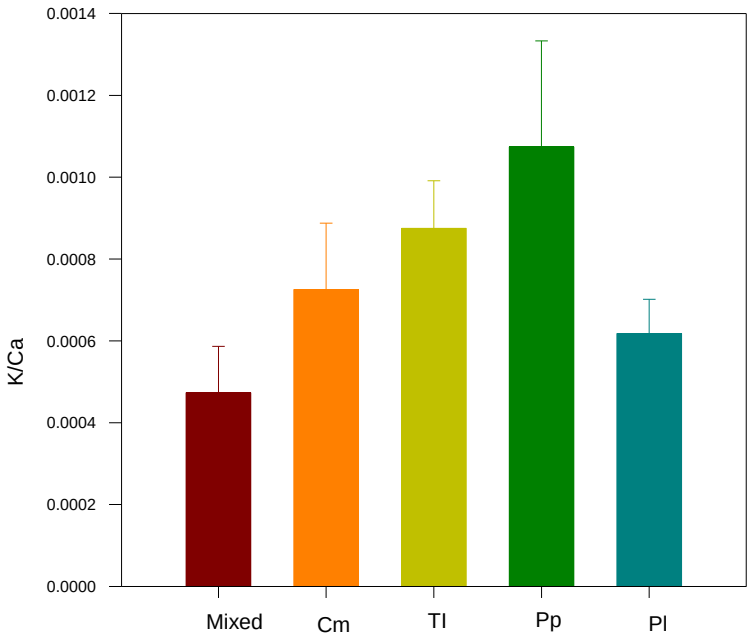


Figure 2.11: K/Ca versus diet received. The mean values for each of the diets are provided with the standard deviations.

Table 2.4: Results for analysis of variance of scallop valve pool K/Ca by diet.

Group Name	N	Missing	Mean	Std Dev	SEM
Mixed	5	1	4.73E-04	1.13E-04	5.67E-05
Cm	5	2	7.25E-04	1.62E-04	9.38E-05
Ti	5	2	8.75E-04	1.16E-04	6.71E-05
Pp	5	1	1.07E-03	2.59E-04	1.30E-04
Pl	5	1	6.18E-04	8.37E-05	4.19E-05
Source of Variation	DF	SS	MS	F	P
Between Groups	4	8.45E-07	0.000000211	8.059	0.002
Residual	13	3.41E-07	2.62E-08		
Total	17	1.19E-06			

Table 2.5: Results of pairwise comparison (Holm-Sidak method) of scallop valve pool K/Ca by diet.

Overall significance level = 0.05					
Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
Pp vs. Mixed	0.000601	5.248	0.000157	0.005	Yes
Pp vs. Pl	0.000456	3.985	0.00155	0.006	Yes
Ti vs. Mixed	0.000402	3.249	0.00634	0.006	Yes
Pp vs. Cm	0.000349	2.82	0.0145	0.007	No
Ti vs. Pl	0.000257	2.079	0.0579	0.009	No
Cm vs. Mixed	0.000252	2.039	0.0623	0.01	No
Pp vs. Ti	0.000199	1.61	0.131	0.013	No
Pl vs. Mixed	0.000145	1.263	0.229	0.017	No
Ti vs. Cm	0.00015	1.132	0.278	0.025	No
Cm vs. Pl	0.000108	0.87	0.4	0.05	No

The results of elemental analysis of the scallop valves were highly variable. Each trial and valve pool produced inconsistent trends for most all of the elements examined. Though the random design of the experiment potentially increased the differences reported between the left and right valves, the observed variability within each treatment was larger than anticipated. This, however, was similar to previous results with cultured bivalves (Carriker, 1996; Strasser et al., 2008). This consistent problem with cultured specimens complicates analysis and evaluation of elemental

trends in the shell, especially when attempting calibration-type experiments in the laboratory.

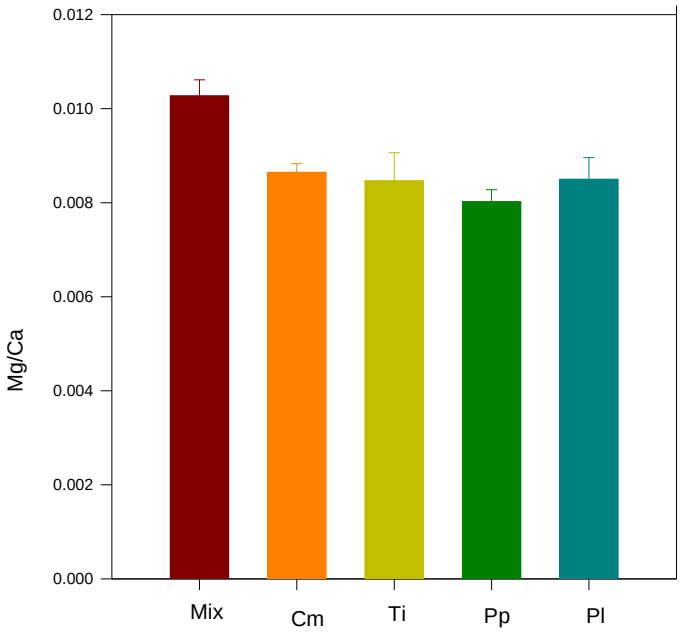


Figure 2.12: Mg/Ca versus diet received. The mean values for each of the diets are provided with the standard deviations.

Table 2.6: Results for analysis of variance of scallop valve pool Mg/Ca by diet.

Group Name	N	Missing	Mean	Std Dev	SEM
Mix	5	1	1.03E-02	3.37E-04	1.69E-04
Cm	5	2	8.65E-03	1.79E-04	1.03E-04
Ti	5	2	8.47E-03	5.95E-04	3.44E-04
Pp	5	1	8.02E-03	2.52E-04	1.26E-04
Pl	5	1	8.50E-03	4.59E-04	2.30E-04
Source of Variation	DF	SS	MS	F	P
Between Groups	4	0.0000119	0.00000297	19.892	<0.001
Residual	13	1.94E-06	0.000000149		
Total	17	0.0000138			

Table 2.7: Results of pairwise comparison (Holm-Sidak method) of scallop valve pool Mg/Ca by diet

Overall significance level = 0.05					
Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
Mix vs. Pp	0.00225	8.244	0.00000161	0.005	Yes
Mix vs. Pl	0.00177	6.493	0.0000203	0.006	Yes
Mix vs. Ti	0.00181	6.128	0.0000361	0.006	Yes
Mix vs. Cm	0.00163	5.511	0.0001	0.007	Yes
Cm vs. Pp	0.000626	2.122	0.0536	0.009	No
Pl vs. Pp	0.000478	1.751	0.103	0.01	No
Ti vs. Pp	0.000444	1.505	0.156	0.013	No
Cm vs. Ti	0.000182	0.577	0.574	0.017	No
Cm vs. Pl	0.000148	0.5	0.625	0.025	No
Pl vs. Ti	0.0000344	0.117	0.909	0.05	No

The initial hypothesis was that elemental incorporation should be significantly different based on diet, following the findings of Ho et al. (2003). The Mn/Ca, Cd/Ca, and Co/Ca ratios were expectd to differ between valves of scallops fed the the Coscinodiscophyceae (diatom) diet and the Prymnesiophycea (Ti, Pp, and Pl) diets, while Fe/Ca, Zn/Ca, and Cu/Ca were hypothesized to be most influenced by the *Tetraselmis* diet. The lack of data related to the *Tetraselmis* diet (high mortality restricting sample size and elemental concentrations below detection limits) prevented any examination of the elemental contribution to the shell. Evidence for influence of Mn/Ca, Cd/Ca/ and Co/Ca on the shells of the scallops fed *Chaetocerous*, *Pavlova* or *Isochrysis* was also inhibited by the inconsistency in the reults across trials and diets. The data sets related to the other elements were restricted in size by the lack of analytical results for trial 2.

To examine the overall differences in the elemental ratios between the left and right valves, the element/Ca ratio was examined regardless the diet received. Either a Mann-Whitney test or a standard T-Test was used to determine any difference between

the right and left valve relevant to the elemental ratios. All analyses indicated no significant differences in any of the elemental ratios compared to valve orientation. After probe analysis of the valves of the eastern oyster, Carriker et al. (1996) noted an increased concentration of most elements in the right valves examined and surmised the differences to be the result of structural differences, pigmentation, or mode of elemental incorporation (incorporation from bathing solution or transport to extrapallial fluid through tissues). The present research used element/Ca normalization, thus results are not fully comparable with the mentioned oyster study. Also, the left and right valves were selected randomly in the present study, meaning the left and right valves analyzed were not necessarily from the same scallops. Due to the combination of the different diet groups, the random selection of the valves, and pooled analysis groups; the examination of left and right valve differences is more a determinant of average experimental population dynamics related to elemental composition versus a direct comparison of left and right valves. As such, further experimentation is needed to formulate conclusive statements as to 1) right and left valve similarity, 2) whether differences noted in previous research compared to the present research are due to the comparison of an inequivalved bivalve and a more equivalved bivalve, or differences in analytical procedure, and 3) increased dissimilarity as a result of specific diets and metabolic effects.

Because the analyses of the left and right valves did not reveal any significant differences in the present study, both left and right valve pools were used in the analyses to determine differences among diets with respect to elemental shell composition. The increased sample size allowed for statistical analysis using a One

Way ANOVA and post hoc Holmes-Sidak method. Two elemental ratios were found to differ significantly between specific treatments: Mg/Ca and K/Ca.

Magnesium has been proposed to fluctuate with temperature in certain species of bivalve (Rucker and Valentine, 1961; Dodd, 1965; Rosenburg, 1980); though, more recent research suggests temperature and salinity have minor to no influence on Mg/Ca in other bivalve species (Carre et al., 2006; Strasser et al., 2008). In *Crassostrea virginica* (Carriker, 1996) and certain gastropods (Foster and Cravo, 2003), Mg has been shown to increase with size and through ontogeny. To eliminate two previously suggested influences on Mg/Ca, salinity and temperature were held constant during my experiments. The analyses revealed Mg/Ca for the Mixed diet was higher than all other diets tested. Ho et al. (2003) suggests that of the five taxonomic classes of algae examined in their study, diatoms had the largest Mg/P quota while the others were mostly similar. This trend was not evident in my study. The fact that the Mixed algal diet was the only diet to differ significantly from the other treatments further confounds the possibility that diet was directly responsible for the difference. As previously mentioned, Carriker (1996) suggested a relationship between size and Mg in the shell of *Crassostrea virginica*. Another study, Carre et al. (2006), has suggested a correlation between calcification rate and Mg/Ca. The present study does more closely support a correlation between Mg/Ca and growth rate or size. The Mg/Ca value for the Pp diet, however, is not consistent with this conclusion.

Carriker et al. (1996) concluded that differences in the potassium concentrations observed were possibly from ingestion of sedimentary particles containing potassium, though specific examination of the algal cells being ingested was not included. In the

present study, no sediments were added to any of the tanks as was done in Carriker et al. (1996) and water was treated in the same manner. The sole difference between experimental groups was the diet received, thus indicating an indirect biologic effect or a direct relationship with the algae ingested. Visually, it would appear that the Pp diet was correlated with the highest K/Ca; however, the only statistical differences were between the Pp and Mixed diets, the Pp and Pl diets, and the Ti and Mixed diets. The findings of Ho et al. (2003) suggested K is in higher concentration in algal cells than in seawater and that diatoms have a much higher K quota than the other algal classes examined. These finding are not fully translated to the shells of the bay scallops in the present study. There is no significant difference between any of the single algal taxonomic diets as related to K/Ca other than Pp vs. Pl. The finding that the mixed diet shells contain less K as compared to Ca than Pp or Ti diets cannot be explained by growth rates, but the lower K/Ca as compared to the other diets might be due to the decreased influence of any single algal species. The most notable difference in K/Ca between diets was the comparison of the Pp and Pl diets, as this suggests a difference between algal species and not just algal classes though growth conditions and where the culture isolate was derived could play a role in these differences as identified in Ho et al. (2003).

Conclusion

Much research has been aimed at elucidating trends that apply to paleothermometry, environmental conditions, or ecological conditions. Most methodologies that have compared a laboratory setting with natural conditions, however, have failed to directly address diet as a factor in elemental enrichment. The

results of the present research support the possibility that diet can contribute to the elemental composition of the shell of *Argopecten irradians concentricus*. Furthermore, algal species may contribute differently to shell composition. The actual mechanism, however, is uncertain and seemingly element specific. Clearly research is needed to distinguish biologic influences, including diet, to determine the dominant influences of elemental composition of shells and relationship with bivalve ecology and culture.

3. Elemental composition differences in the valves of juvenile *Mercenaria mercenaria* fed unique algal diets: implications for ecological patterns as well as for rearing bivalves for laboratory experiments and aquaculture

Introduction

The incorporation of elements into biominerals, including the shells of bivalves, is influenced by both geochemical and biological factors (Schone, 2008), but which elements and in what concentration are most often attributed to the physiochemistry of the surrounding water (Gillikin et al., 2006). Temperature or salinity are commonly assumed to influence shell chemistry, especially with respect to magnesium (Milliman, 1974; Putten et al., 2000), strontium (Dodd, 1965), manganese (Crisp, 1975), and barium (Dorval, 2007). Recently, however, researchers have begun to focus their attention towards mechanisms and pathways of incorporation of these elements. New research and experimental designs continue to elucidate those elements that are more likely influenced by environmental dynamics and those more biologically controlled, and thus, not dependable as environmental proxies (Stecher et al., 1996; Freitas et al., 2006).

In an assessment of element ratios between the digestive gland and gill tissues of *Laternula elliptica*, Poigner et al. (2012) suggested that assimilation of Al, Ca, Fe, K, Mn, and Mg is predominantly from the dissolved phase while elements such as Cd, Cu,

and Sr are assimilated through digestion of particulates. In another study, incorporation of Ba and Mo into the shells of *Pecten maximus* were examined with the conclusion that Ba enrichment was due to direct incorporation of dissolved Ba in proportion to the levels present in seawater while Mo enrichment was most likely associated with trophic uptake (Tabouret et al., 2012). Interestingly, the enrichment of Ba in scallop shells was delayed compared to maximum concentrations of the element in surrounding sea water which agreed with observations made by Stecher and Kogut (1999) and Ganeshram et al. (2003) that suggested dissolved Ba increased following specific algal blooms. Tabouret et al. (2012) postulated the increased concentration of dissolved Ba subsequent to algal blooms increased bioavailability which ultimately influenced incorporation into bivalve shells.

Based on the results of the previous chapter and conclusions of Carre et al. (2006), Strasser et al. (2006), Jenkins (2007), Strasser et al. (2008) and others, I postulated that the factors contributing to differences in elemental shell chemistry of *Argopecten irradians* fed unique diets was most likely associated with differences in growth rates caused by nutritional variation, time/ontogenetic factors, health/metabolic changes, or direct incorporation of elements associated with the diet received. I concluded that the difference in Mg/Ca was most evidently associated with growth rate, though confounded by other biological factors, while K/Ca was more directly influenced by the algal diet. The actual mechanisms, however, were uncertain and seemingly element specific.

The research presented in this chapter was designed to examine the influence of diet on the elemental shell chemistry of the hard shell clam, *Mercenaria mercenaria*.

This clam is a dioecious, filter-feeding, slower growing bivalve with an average life span of four to eight years (when considering fishing pressure/harvest), an estimated natural life span around 46 years (Peterson and Fegely, 1986), and suggested to have a potential maximum life span of at least 106 years (Ridgway et al., 2011). The shell of *M. mercenaria* is also composed of three distinct layers: an outer prismatic layer and a middle and inner homogenous layer (Panella and Mac Clintock, 1968). All three shell layers of the hard shell clam are aragonitic.

The results will be used to analyze differences in the elemental shell chemistry of clams fed diets of specific single algal species as well as clams fed a mixture of single algal species and starved individuals. The overall findings will be used to further determine the role diet plays in elemental shell chemistry of this marine bivalve. This work will aid in determining direct dietary influence on shell chemistry, species-specific effects and differences due to shell mineralogy, elemental composition change due to metabolic/health changes, growth-rate influence on shell chemistry, and the difference between left and right valves related to effects on the animal due to diet and associated biological factors.

Materials and Methods

Algae cultures

All species isolates were purchased from Provasalli - Guillard Culture Center for Marine Phytoplankton. *Isochrysis* sp. (CCMP1324), *Pavlova pinguis* (CCMP609), and *Chaetoceros mulleri* (CCMP1316), *Isochrysis sp.* (CCMP1611), *Pavlova sp.* (CCMP1209), and *Chaetoceros galvestonensis* (CCMP186) cultures were received

several months prior to the initiation of the feeding trials to allow for proper acclimation periods, ensure that adequate starter and stock cultures were available, as well as to allow sufficient time to determine the health of all cultures.

All cultures were maintained within enclosures in the laboratory under the same conditions. Temperature was maintained at approximately 22^0 C and both standard and full spectrum fluorescent lights were used for illumination. The stock cultures were maintained in three volumes including test tubes, 200 ml flasks (150 ml cultures), and 2 liter flasks (1500 ml culture) while feeding cultures were cultured in both 22 l K-Wall tubes and Carboys, with great care taken to ensure all feeding cultures were true monocultures.

Spawning and rearing

Thirty adult *Mercenaria mercenaria* were spawned using a flow through water table and thermal induction at the Bay Shellfish Aquaculture facilities in Palmetto, FL. The resultant fertilized eggs were transferred to a heated, aerated 800 l tank and left for 48 hours before the first water change and first feeding occurred. Similar to the scallop trials described in Chapter 2, an adjacent tank was prepared the day prior to ensure the temperature of the water and salinities were constant (28^0C and a salinity of 30+/- 2). After sieving the larvae, they were immediately placed into the other tank and received their first dose of food, which was a mix of *Chaetoceros* sp.*, Isochrysis* sp.*, and Pavlova sp.* The emptied tank was then cleaned using muriatic acid and Alconox, thoroughly rinsed, and filled with filtered, UV treated bay water. These changes occurred daily until metamorphosis of the clam larvae.

After metamorphosis, the settled clams were moved to a partial recirculating system (new water was always filtered and UV treated), divided equally between 12 downweller trays, and fed through continuous line systems with the same algal mixture received as larvae until they reached approximately 1-2 mm shell length. At that time, the clams were moved to a brown-water system supplied by natural water pulled from Tampa Bay until they reached an average of 4 mm shell length.

Feeding experiment

A subsample of the reared clams was sieved on a 4mm screen and all individuals retained were kept for the feeding trials and transferred to the Aquarium Laboratory at USF College of Marine Science. The clams were volumetrically counted and divided among 8 separate 500 L tanks and acclimated to laboratory conditions for 36 hrs. with no supplemental algae added.

Each of the tanks was fitted with a titanium heat source, two air lines, and a polyethylene mesh to suspend the clams from the bottom of the tank. Approximately 500 *M. mercenaria* were placed in each tank.

An approximate 25-50% water change occurred every other day and feedings (approximately 100,000-317,000 cells/ml per feeding) occurred three - four times daily with the overall volume fed dependent upon cell concentration of the individual cultures. If algae cultures were volume restricted for feeding on a specific day, the feeding volume was adjusted for all feeding groups such that equivalent cell concentrations were received. The feeding regimens were one of six single algal species diets - *Isochrysis* sp. (CCMP1324, TI), *Pavlova pinguis* (CCMP609, Pav609), *Chaetoceros*

mulleri (CCMP1316, Cm), *Isochrysis sp.* (CCMP1611, ISO) culture, *Pavlova sp.* (CCMP1209, Pav1209), or *Chaetoceros galvestonensis* (CCMP186, Cg) , as well as a mixed diet of all species in equal ratios (Mixed), or no food (starvation control, CTRL).

The water used for exchanges was pulled from Bayboro Harbor and filtered through a sand filter, two charcoal filters 10 micron sock filter and a five micron sock filter into two holding tanks fitted with a heat source and air supply (the DE filter used during the scallop experiments was eliminated to limit Si sources). On a maintenance day, the water in the two tanks was cycled through a UV sterilization unit and a one micron sock filter prior to use in the laboratory.

Salinity and temperature measurements were taken from both the holding tanks and the experimental tanks so that adjustments in the volume of water exchanged could be made to maintain temperature and salinity in the experimental units (28^0C+/- 1 and a salinity of 30+/-2). Each tank was siphoned to remove waste and the desired level of water. The tanks were then filled with the water passing again through the UV unit and another 1 micron sock filter. On non-water change days, small siphon tubes were used to eliminate as much accumulated waste as possible without significantly affecting water volume and introducing inter-tank differences beyond experimental variables. The first feeding occurred immediately following the water exchange or cleaning, with the remaining feedings spread as equally as possible through the day.

Once every two to three months, 25 individuals were randomly selected and removed from each of the tanks (January baseline collection, March, May, July, and October). All valves were immediately separated and the tissue excised. The valves

were then lightly brushed to remove debris, rinsed in distilled water, and dried before placing them individually in acid washed vials for storage until processed.

ICP analysis

The left and right valves were isolated from each of the individuals collected for processing during the first two collections. Each valve was individually measured and weighed on a micro scale, then quickly rinsed in a series of dishes – distilled water - 0.01% nitric acid – distilled water – milliQ water. After the shell was rinsed and dried, it was placed in an acid-cleaned vial and digested in approximately 4 ml of heated 5-6% trace-pure nitric acid. This procedure was repeated for all valves collected during the first two collections. The valves were then analyzed for silver (Ag), aluminum (Al), arsenic(As), barium (Ba), boron (B), beryllium (Be), calcium (Ca), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), potassium (K), lithium (Li), magnesium (Mg), manganese (Mn), molybdenum (Mo), sodium (Na), nickel (Ni), phosphorous (P), lead (Pb), scandium (Sc), selenium (Se), silica (Si), tin (Sn), strontium (Sr), titanium (Ti), thallium (Tl), vanadium (V) and zinc (Z) using the ICP-OES with blanks of 5% trace-pure nitric acid inserted between the valve samples. The detection limits of the ICP unit used are provided in Table 2.1.

The preparation procedure was slightly modified for the third and final collections. Rates of dissolution of the shells was noticeably influenced by size, as larger shells caused clogging of the ICP tubes, therefore, the left valves were placed in a muffle furnace at 400 degrees over night after they had been cleaned. The resultant ash was then reweighed and combined with the nitric acid in the vials for analysis. The right

valves were immediately placed in air-tight containers following cleaning and drying for later experimental use.

Statistical analysis

The results from the ICP-OES analysis were corrected using the established dilution factor per sample. The resultant concentrations were then sorted and evaluated based on the Relative Standard Deviation values (RSD), detection limitations of the ICP equipment, and returns from the associated blanks. All concentrations associated with RSD values greater than 10 were removed from the final data set. A statistical analysis was performed to compare the final data set with the data set containing the removed values to evaluate bias. The two data sets were not significantly different ($P = 0.87$) as a whole; as well, the concentrations per element were evaluated individually and found not to be significantly different except for zinc ($P > 0.05$).

The concentrations remaining in the final data set were transformed to element/calcium ratios for comparison. The element: calcium ratios were initially analyzed using one-way analysis of variance (ANOVA). However, due to the limitations of the data (normalization and variance); a Kruskal Wallis one-way ANOVA on ranks was employed. Dependent on the outcome of the ANOVA, further testing was performed using a post hoc multiple comparison procedure to assess the significant differences in shell chemistry between feeding groups. This procedure was also followed to examine the differences between left and right valve chemistry followed by a T-test for each diet group. Further analysis was then employed using a Pearson Correlation and Regression Analysis to determine the association of the elemental

differences observed with diet as compared to valve length, valve mass, time, and elemental interactions.

Results

Growth

To minimize alterations in growth patterns due to handling and associated stress, shell-length measurement were only taken during the random sampling of the individuals for shell chemistry analysis. As such, the length and mass measurements portray the size distribution of the clams examined subsequent to each collection and not the absolute growth rate of the different feeding groups. All figures and tables subsequent to this description use median values to illustrate the trends discussed to remain consistent with statistical analyses performed and presented throughout.

The number of samples (n) does not represent all individuals originally collected; instead, the sample number is based on all individuals that were successfully analyzed during elemental analysis (ICP-OES) and used in subsequent statistical analyses reported herein. For example, the minimum shell length observed in the baseline collection is 6 mm though individuals of 4-5mm were initially collected and assessed. Furthermore, the largest shells collected for the Mixed diet group are also not represented in the summaries or analyses.

As the baseline (January) collection occurred prior to the initiation of the feeding trials, each of the diet-specific statistics represent the March – October collections. In addition, inclusion of right valve summary statistics only occurs for the baseline, March

and May collections due to experimental design and planned later use of the right valves collected during July and October.

An initial random subsample of clams was used to provide a baseline measurement with which all measurements taken during the experiment have been compared to determine changes over time related to the experimental variables. Thus, the changes in shell length and valve mass for each feeding group are compared to the same base line values. Figure 3.1 depicts the median shell length of each feeding group by collection month. As apparent from the figure, shell length per diet for each of the collections was variable and do not depict a consistent increasing trend for any of the treatments.

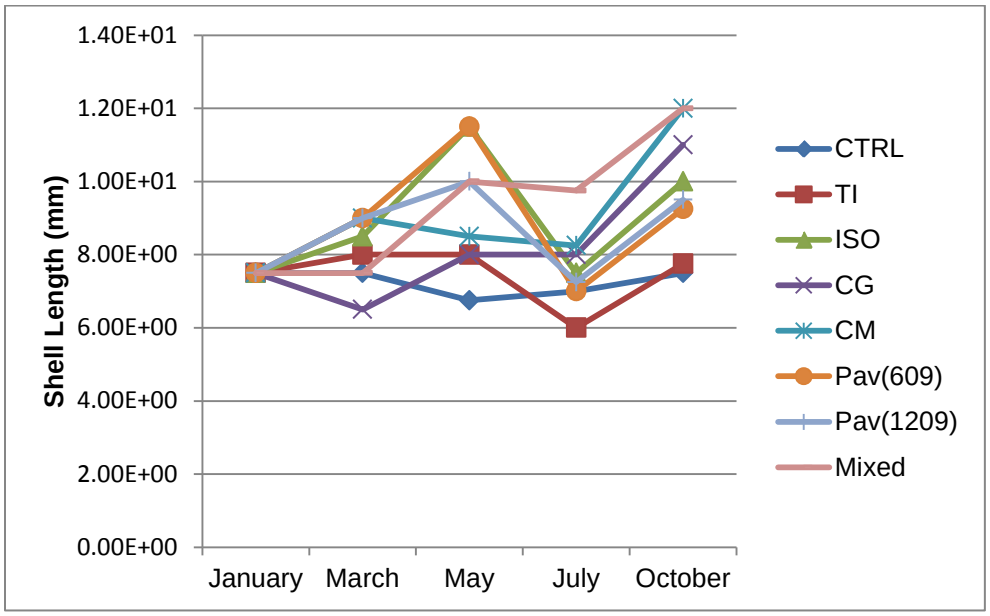


Figure 3.1: Shell length versus time by diet received.

Table 3.1A: Median left valve length (mm) by diet and month collected. The January collection period is equivalent to the baseline measurement with each subsequent month an experimental collection.

Length	January	March	May	July	October
CTRL	7.50	7.50	6.75	7.00	7.50
TI	7.50	8.00	8.00	6.00	7.75
ISO	7.50	8.50	11.50	7.50	10.00
CG	7.50	6.50	8.00	8.00	11.00
CM	7.50	9.00	8.50	8.25	12.00
Pav(609)	7.50	9.00	11.50	7.00	9.25
Pav(1209)	7.50	9.00	10.00	7.25	9.50
Mixed	7.50	7.50	10.00	9.75	12.00

Table 3.1B: Median right valve length (mm) by diet and month collected. The January collection period is equivalent to the baseline measurement with each subsequent month an experimental collection.

Length	January	March	May
CTRL	7.50	7.50	6.75
TI	7.50	8.00	8.00
ISO	7.50	8.50	11.50
CG	7.50	6.50	8.00
CM	7.50	9.00	8.50
Pav(609)	7.50	9.00	11.50
Pav(1209)	7.50	9.00	10.00
Mixed	7.50	7.50	10.00

The control group (CTRL) of starved clams (7.5 mm, 6.75 mm, 7.0 mm, and 7.5mm) did not exceed the baseline shell length (7.5mm) during the experiment. The TI fed clams exhibited a slight increase in shell length above the base line in the first collection (8.0 mm vs. 7.5 mm); however, none of the following collections exhibited a further increase – the specimens collected in May, in fact, had a median shell length below base line (6.0 mm vs. 7.5 mm). Only the Mixed diet (7.5 mm, 10 mm, 9.75 mm, and 12 mm) and CM diet (9.0 mm, 8.5 mm, 8.25 mm, and 12 mm) resulted in clams consistently above the base line shell length (7.5 mm). All remaining diet groups were variable in reference to baseline shell length, with at least one each whose median shell

length was equal to or below baseline. Table 3.1A provides the median shell length values/measurements by diet per collection month for comparison while Table 3.1B illustrates the similarity of the right valve lengths for the first three collections.

The median mass of the left valves by diet over time are illustrated in Figure 3.2 and presented in Table 3.2. All diets with the exception of CG (2.41E-02 g) show an increased median mass over baseline (3.72E-02 g) during the March collection. The valve median mass of the control group is below baseline during the May collection. In the July collection, the median valve mass for the control and TI fed groups were below that of the baseline group. In the October collection, the control group was again the only experimental group whose median valve mass was below that of the baseline while the other experimental groups valve mass followed the general pattern Mixed>CM>CG>ISO>Pav1209>Pav609>TI>Baseline.

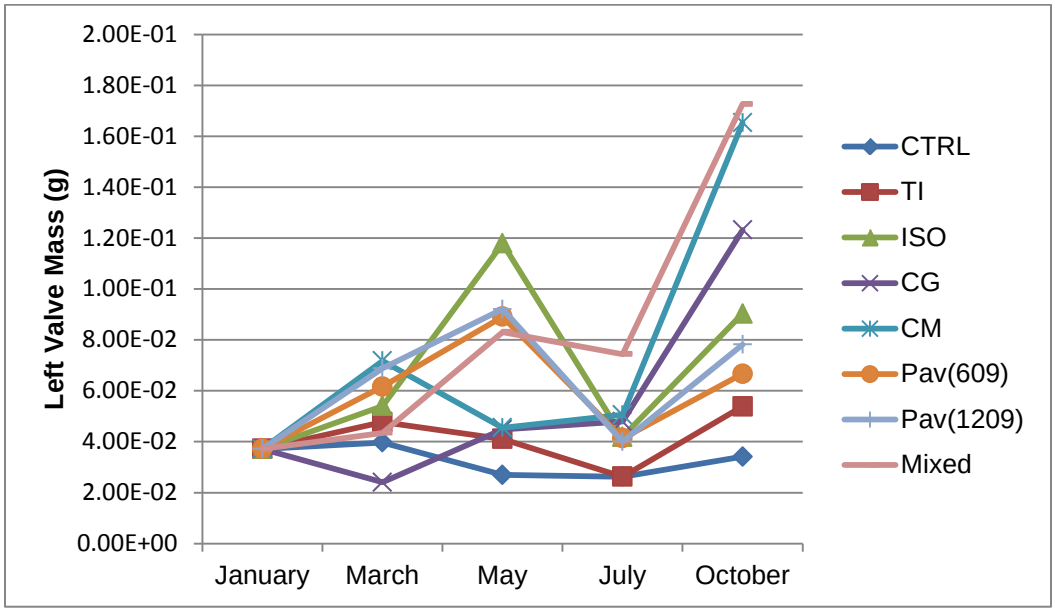


Figure 3.2: Median mass of the analyzed left valves by diet and month collected.

Table 3.2: Median mass (g) of the analyzed left valves by diet and month collected.

Mass	January	March	May	July	October
Ctrl	0.0372	0.0397	0.0270	0.0263	0.0341
TI	0.0372	0.0477	0.0411	0.0263	0.0539
ISO	0.0372	0.0540	0.1180	0.0419	0.0903
CG	0.0372	0.0241	0.0448	0.0479	0.1230
CM	0.0372	0.0717	0.0455	0.0506	0.1650
Pav(609)	0.0372	0.0617	0.0891	0.0414	0.0666
Pav(1209)	0.0372	0.0688	0.0921	0.0400	0.0782
Mixed	0.0372	0.0435	0.0831	0.0744	0.1730

The mass of the right valves collected and analyzed during the first two collections post- baseline measurements follow similar trends to that of the left valves collected during the same months. The median mass of the right valves per diet and collection are depicted in Figure 3.3 and presented in Table 3.3.

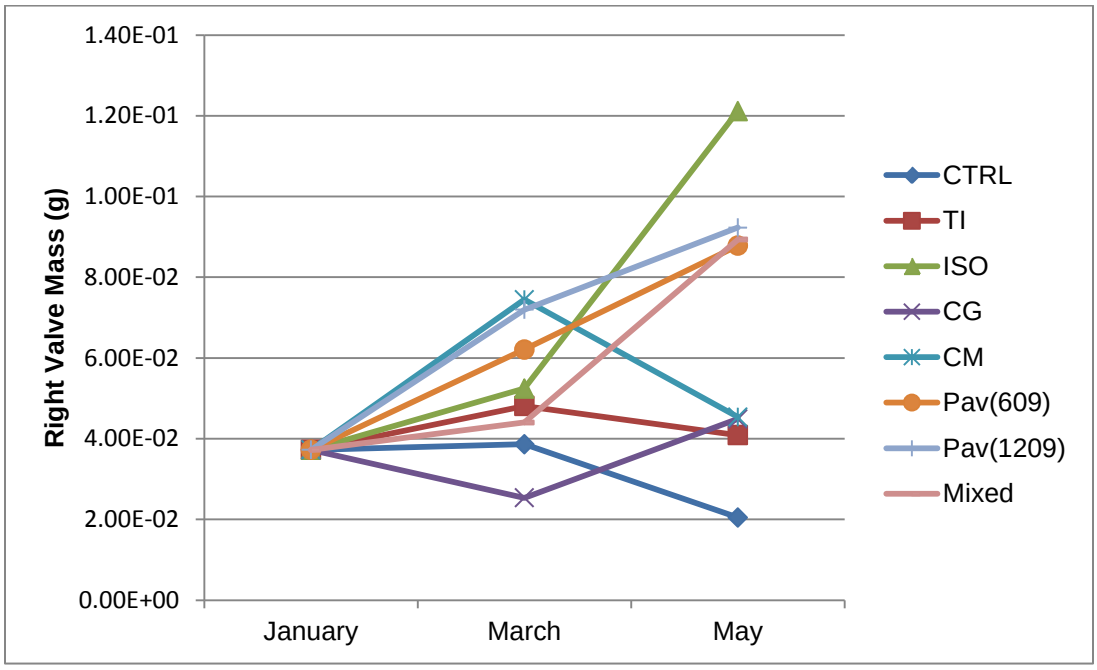


Figure 3.3: Median mass of the right valves by diet and month collected.

Again, the median mass for each diet is above baseline except CG in the first collection. The only median mass below baseline during the second collection was the control group with the remaining groups showing the general ranking of ISO>Pav1209>Mixed>Pav609>CM>CG>TI>Baseline.

Table 3.3: Median mass (g) of the right valves by diet and month collected.

Mass	January	March	May
Ctrl	0.0372	0.0386	0.0205
TI	0.0372	0.0481	0.0409
ISO	0.0372	0.0524	0.1210
CG	0.0372	0.0254	0.0449
CM	0.0372	0.0745	0.0454
Pav(609)	0.0372	0.0621	0.0878
Pav(1209)	0.0372	0.0720	0.0923
Mixed	0.0372	0.0440	0.0893

Shell length and valve mass by diet

The statistical analysis of the shell length measurements established that the collected valve composites for six of the experimental diets (Cm, Cg, Pav609, Pav1209, ISO, and Mixed) were significantly larger than the control group. The total collections for both the Baseline valves and those for the TI fed group were not significantly different from the control group's shell length. The shell length was not significantly different between any of the other diet comparisons, and importantly, no feeding group was significantly different from the baseline. There were, however, three comparisons which resulted in significant differences when all collected valves were included: Mixed vs. TI, Mixed vs. Baseline, and Cm vs. Baseline. Figures 3.4A and 3.4B illustrate the median shell length of the collected left and right valves, respectively, by treatment.

Tables 3.4A and 3.4B provide the summary of results for the statistical analyses performed.

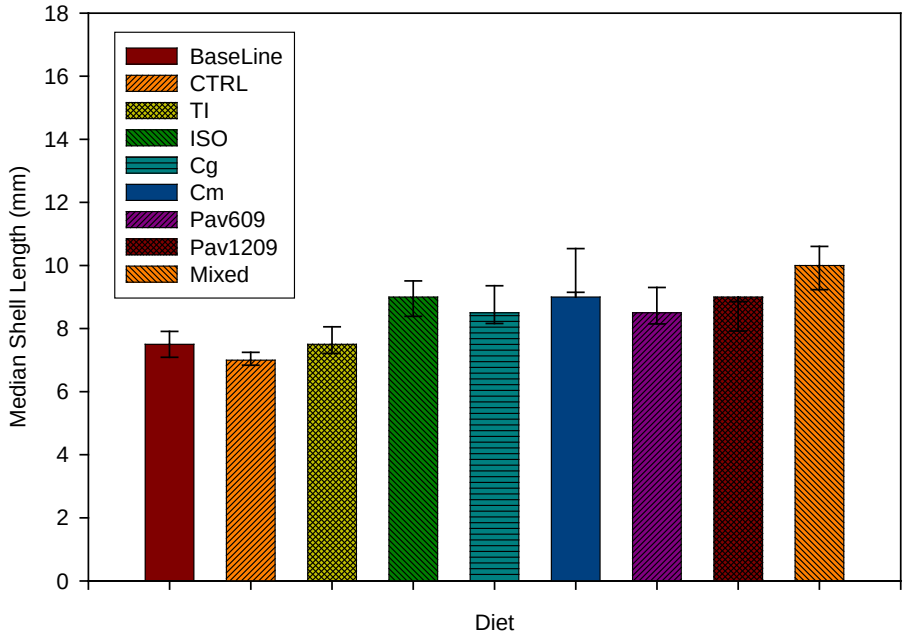


Figure 3.4A: Median left valve length by treatment.

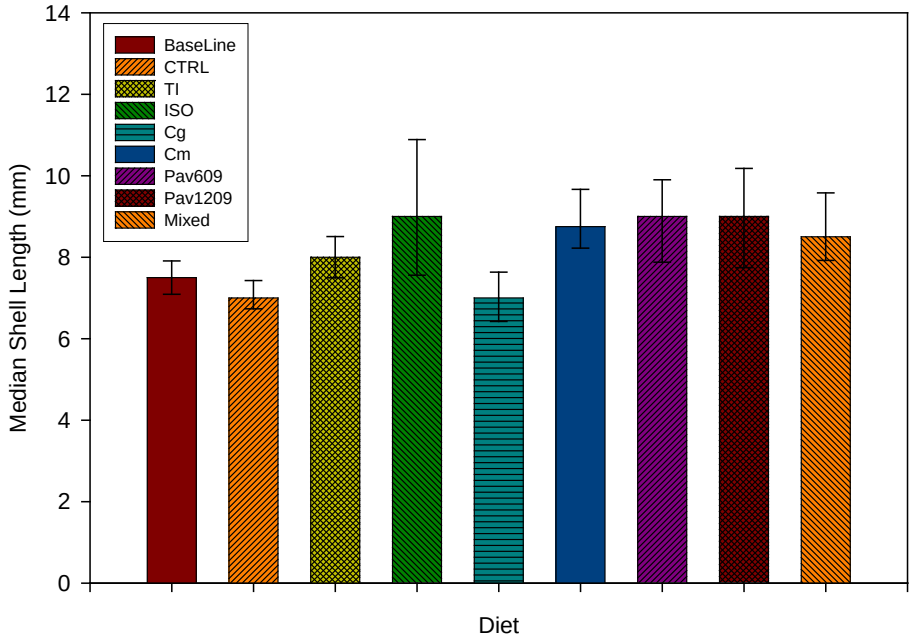


Figure 3.4B: Median right valve length by treatment.

Table 3.4A: Results for the Kruskal-Wallis analysis of shell length among diets.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
Baseline	14	1	7.50	7.00	8.00
CTRL	61	1	7.00	6.50	7.50
TI	57	1	7.50	6.25	8.50
ISO	50	1	9.00	7.38	10.63
Cg	59	1	8.50	7.00	10.00
Cm	64	1	9.00	7.63	11.38
Pav(609)	59	1	8.50	7.00	10.00
Pav(1209)	54	1	9.00	7.00	9.50
Mixed	68	1	10.00	7.00	12.00
H = 75.9 with 8 degrees of freedom (P = <0.001)					

Table 3.4B: Results for post hoc pair wise comparisons of shell length between diets.

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Mixed vs. CTRL	167.61	6.84	Yes
Cm vs. CTRL	164.79	6.63	Yes
ISO vs. CTRL	132.33	4.99	Yes
Cg vs. CTRL	112.51	4.43	Yes
Pav(609) vs. CTRL	111.67	4.40	Yes
Pav(1209) vs. CTRL	104.07	4.01	Yes
Mixed vs. Baseline	127.48	3.05	No
Mixed vs. TI	120.38	4.82	No
Mixed vs. Pav(1209)	63.54	2.51	No
Mixed vs. Pav(609)	55.94	2.26	No
Mixed vs. Cg	55.10	2.23	No
Mixed vs. ISO	35.28	1.36	No
Mixed vs. Cm	2.82	0.12	No
Cm vs. Baseline	124.66	2.97	No
Cm vs. TI	117.56	4.64	No
Cm vs. Pav(1209)	60.72	2.36	No
Cm vs. Pav(609)	53.12	2.12	No
Cm vs. Cg	52.28	2.08	No
Cm vs. ISO	32.46	1.24	No
ISO vs. Baseline	92.20	2.14	No
ISO vs. TI	85.10	3.16	No

Table 3.4B (Continued)

Comparison	Diff of Ranks	Q	P<0.05
ISO vs. Pav(1209)	28.26	1.04	No
ISO vs. Pav(609)	20.66	0.77	No
ISO vs. Cg	19.82	0.74	No
Cg vs. Baseline	72.38	1.71	No
Cg vs. TI	65.28	2.53	No
Cg vs. Pav(1209)	8.44	0.32	No
Cg vs. Pav(609)	0.84	0.03	No
Pav(609) vs. Baseline	71.54	1.69	No
Pav(609) vs. TI	64.44	2.50	No
Pav(609) vs. Pav(1209)	7.60	0.29	No
Pav(1209) vs. Baseline	63.94	1.50	No
Pav(1209) vs. TI	56.84	2.15	No
TI vs. CTRL	47.22	1.84	No
TI vs. Baseline	7.10	0.17	No
Baseline vs. CTRL	40.13	0.95	No

Analyses of the left valve shell mass by experimental diet (Table 3.5) established significant differences among ten diets with subsequent ranking as follows: Mixed > CTRL, Mixed > Baseline, Mixed > TI, Cm > CTRL, Cm > Baseline, Cm > TI, ISO > CTRL, Pav(1209) > CTRL, Pav(609) > CTRL, Cg > Ctrl. No other comparisons were significant. These results are similar to those for analysis of shell length in that the Mixed, Cm, Cg, ISO, Pav609 and Pav1209 diets show significantly increased median values above the control. In addition, however, both the mixed diet and Cm diet proved to have larger median masses compared to the baseline and TI diet. Figure 3.6 depicts the median masses of each diet per collection and the summary of the statistical analyses are presented in Tables 3.5A and 3.5B.

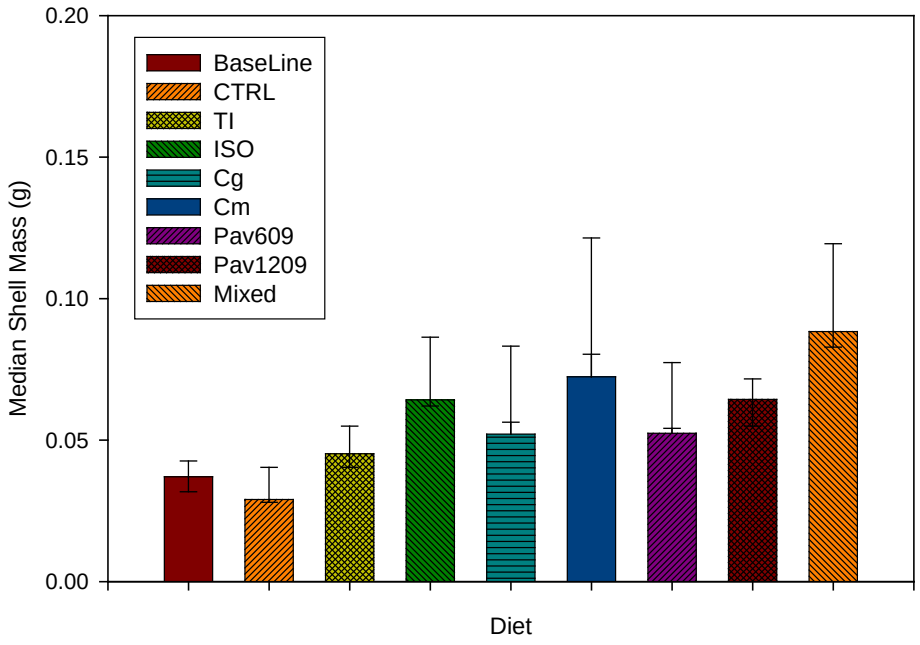


Figure 3.5: Median left valve masses by treatment

Table 3.5A: Results of analyses of left valve mass by treatment.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
Baseline	14	1	0.037	0.0336	0.0419
CTRL	61	1	0.029	0.023	0.0405
TI	57	1	0.0452	0.0263	0.0572
ISO	50	1	0.0642	0.0414	0.102
Cg	59	1	0.0521	0.0311	0.102
Cm	64	1	0.0724	0.0421	0.13
Pav(609)	59	1	0.0524	0.0336	0.0814
Pav(1209)	54	1	0.0644	0.0367	0.0823
Mixed	68	1	0.0884	0.0361	0.139
H = 81.0 with 8 degrees of freedom (P = <0.001)					

Figure 3.5B: Results of post hoc pairwise analyses of left valve mass and treatment.

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Mixed vs. CTRL	175.408	7.159	Yes
Mixed vs. Baseline	143.769	3.441	Yes
Mixed vs. TI	105.857	4.241	Yes
Cm vs. CTRL	174.527	7.019	Yes
Cm vs. Baseline	142.888	3.403	Yes
Cm vs. TI	104.976	4.147	Yes
ISO vs. CTRL	149.806	5.644	Yes
Pav(1209) vs. CTRL	129.437	4.981	Yes
Pav(609) vs. CTRL	119.158	4.695	Yes
Cg vs. CTRL	118.693	4.676	Yes
Mixed vs. Cg	56.716	2.294	No
Mixed vs. Pav(609)	56.25	2.275	No
Mixed vs. Pav(1209)	45.972	1.814	No
Mixed vs. ISO	25.602	0.988	No
Mixed vs. Cm	0.881	0.0364	No
Cm vs. Cg	55.835	2.226	No
Cm vs. Pav(609)	55.369	2.207	No
Cm vs. Pav(1209)	45.091	1.755	No
Cm vs. ISO	24.721	0.942	No
ISO vs. Baseline	118.167	2.748	No
ISO vs. TI	80.255	2.976	No
ISO vs. Cg	31.113	1.163	No
ISO vs. Pav(609)	30.648	1.146	No
ISO vs. Pav(1209)	20.37	0.746	No
Pav(1209) vs. Baseline	97.798	2.292	No
Pav(1209) vs. TI	59.885	2.267	No
Pav(1209) vs. Cg	10.744	0.41	No
Pav(1209) vs. Pav(609)	10.278	0.392	No
Pav(609) vs. Baseline	87.519	2.069	No
Pav(609) vs. TI	49.607	1.921	No
Pav(609) vs. Cg	0.466	0.0182	No
Cg vs. Baseline	87.054	2.058	No
Cg vs. TI	49.142	1.903	No
TI vs. Ctrl	69.551	2.716	No
TI vs. Baseline	37.912	0.893	No
Baseline vs. CTRL	31.639	0.75	No

Figure 3.6 depicts the median mass of the collected right valves per diet in each of the collections. During statistical analyses, it was determined that the valve mass of clams in each the Cm, ISO, Mixed, Pav(1209), and Pav(609) treatments was significantly different from the CTRL group (Tables 3.6A and 3.6B). Additionally, Pav(1209) vs. Cg was determined to be significant. No other comparisons were significant in terms of valve mass and diet received. The general trend that valve mass of clams fed unique diets increased compared to the control, as seen in both the valve length comparisons and left valve mass, is again supported by data for the right valve mass. The main difference from previously presented comparisons is that of Pav1209 versus Cg.

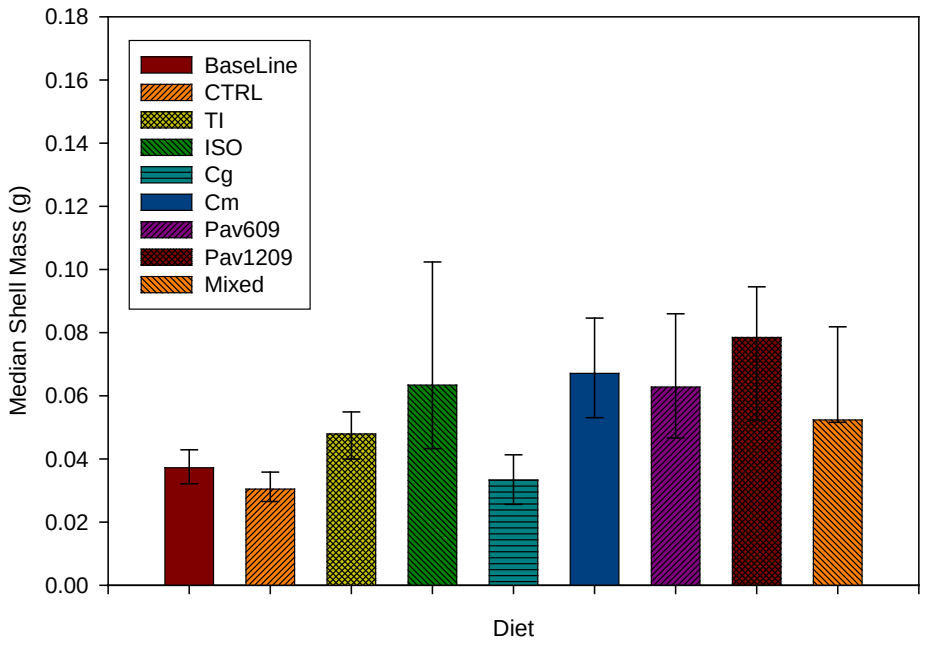


Figure 3.6: Median mass of the collected right valves by treatment.

Table 3.6A: Results of analysis of right valve mass comparison by treatment.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
Baseline	14	1	0.0372	0.0336	0.043
CTRL	26	1	0.0305	0.0219	0.0417
TI	17	1	0.0479	0.0402	0.0538
ISO	10	1	0.0634	0.0405	0.11
Cg	19	1	0.0333	0.0203	0.0448
Cm	27	1	0.0671	0.0375	0.0836
Pav(609)	19	1	0.0628	0.0345	0.0809
Pav(1209)	14	1	0.0785	0.0549	0.0932
Mixed	31	1	0.0523	0.0337	0.0918
H = 38.3 with 8 degrees of freedom (P = <0.001)					

Table 3.6B: Results of post hoc pairwise comparisons of right valve shell mass by diet.

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Cm vs. CTRL	54.746	4.018	Yes
ISO vs. CTRL	61.122	3.233	Yes
Mixed vs. CTRL	49.217	3.736	Yes
Pav(1209) vs. Cg	59.543	3.363	Yes
Pav(1209) vs. CTRL	64.054	3.851	Yes
Pav(609) vs. CTRL	51.456	3.422	Yes
Baseline vs. Cg	10.697	0.604	No
Baseline vs. CTRL	15.208	0.914	No
Cg vs. CTRL	4.511	0.3	No
Cm vs. Baseline	39.538	2.393	No
Cm vs. Cg	50.235	3.368	No
Cm vs. Mixed	5.529	0.424	No
Cm vs. Pav(609)	3.291	0.221	No
Cm vs. TI	15.534	1.005	No
ISO vs. Baseline	45.915	2.177	No
ISO vs. Cg	56.611	2.851	No
ISO vs. Cm	6.376	0.339	No
ISO vs. Mixed	11.906	0.644	No
ISO vs. Pav(609)	9.667	0.487	No
ISO vs. TI	21.91	1.081	No
Mixed vs. Baseline	34.009	2.106	No
Mixed vs. Cg	44.706	3.083	No

Table 3.6B (Continued)

Comparison	Diff of Ranks	Q	P<0.05
Mixed vs. TI	10.004	0.664	No
Pav(1209) vs. Baseline	48.846	2.56	No
Pav(1209) vs. Cm	9.308	0.563	No
Pav(1209) vs. ISO	2.932	0.139	No
Pav(1209) vs. Mixed	14.837	0.919	No
Pav(1209) vs. Pav(609)	12.598	0.712	No
Pav(1209) vs. TI	24.841	1.368	No
Pav(609) vs. Baseline	36.248	2.047	No
Pav(609) vs. Cg	46.944	2.895	No
Pav(609) vs. Mixed	2.239	0.154	No
Pav(609) vs. TI	12.243	0.733	No
TI vs. Baseline	24.005	1.322	No
TI vs. Cg	34.701	2.076	No
TI vs. CTRL	39.212	2.518	No

Differences between the left and right valves: valve length and mass

Further analyses were performed to determine the difference between the valves within each of the feeding groups during the baseline, March and May collections in reference to length and mass. Three separate comparative analyses were performed: 1) an overall comparison of left and right valve independent of time and diet, 2) a comparison of the baseline valves and all experimental valves, and 3) comparison within each individual group.

The left and right valves were similar in length when considering all valves measured with no significant difference found (P=0.233) between neither left and right valve composites nor between the left and right valve lengths of the baseline and feeding group composite (P=0.334). The valve lengths within each feeding group were found to be virtually identical (P=1.00).

Figure 3.7 depicts the masses of both the left and right valves collected for each diet by month. The statistical comparisons revealed no significant differences between the composited right and left valves (P=0.877), the left and right valves for the experimental and base line groups (P=0.118), or the left and right valves within the specific feeding groups. Tables 3.7-3.8 provide the summary of the statistical tests associated with the comparisons of valve mass.

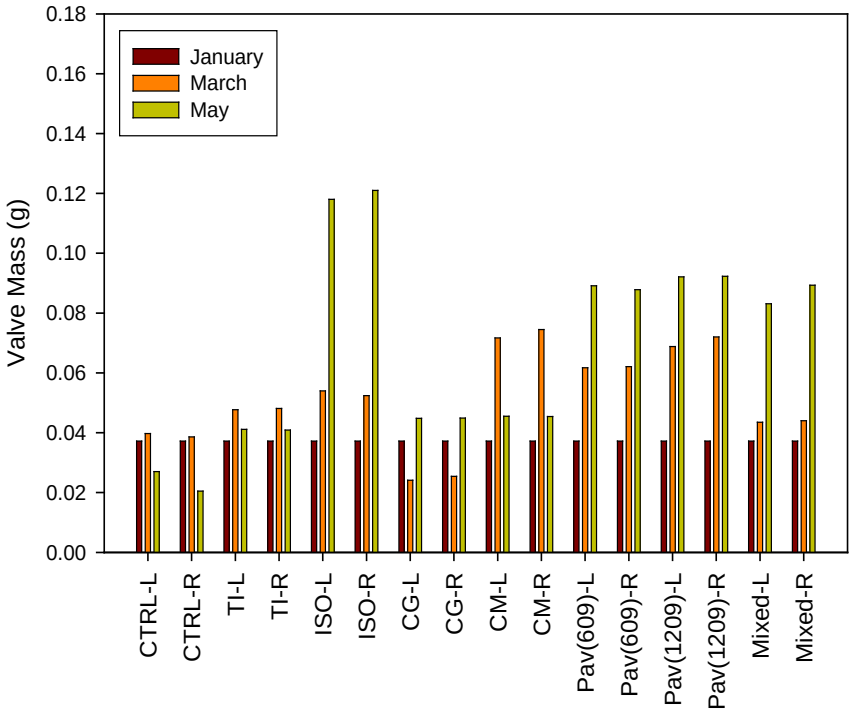


Figure 3.7.Median left (L) and right (R) valve mass by diet received**.**

Table 3.7A: Results of analyses of all valves collected, left vs. right valve mass.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
Left - all	169	1	0.0445	0.0314	0.0721
Right All	169	1	0.0445	0.03	0.0741
H = 0.024 with 1 degrees of freedom (P = 0.877)					

Table 3.7B: Summary results of analyses of baseline data versus experimental composite.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
Baseline-Left	14	1	0.037	0.0336	0.0419
Baseline-Right	14	1	0.0372	0.0336	0.043
Experimental-Left	156	1	0.045	0.0303	0.0748
Experimental-Right	156	1	0.0454	0.0294	0.0752
H = 5.87 with 3 degrees of freedom (P = 0.118)					

Table 3.8A: Summary of results for diet specific left and right valve comparisons.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
Baseline-Left	14	1	0.037	0.0336	0.0419
Baseline-Right	14	1	0.0372	0.0336	0.043
CTRL-Left	26	1	0.0321	0.0229	0.0429
CTRL-Right	26	1	0.0305	0.0219	0.0417
TI-Left	17	1	0.0467	0.04	0.0557
TI-Right	17	1	0.0479	0.0402	0.0538
ISO-Left	10	1	0.0642	0.0422	0.107
ISO-Right	10	1	0.0634	0.0405	0.11
Cg-Left	19	1	0.0372	0.0192	0.0445
Cg-Right	19	1	0.0333	0.0203	0.0448
Cm-Left	27	1	0.0642	0.0395	0.0833
Cm-Right	27	1	0.0671	0.0375	0.0836
Pav(609)-Left	19	1	0.0626	0.0336	0.0814
Pav(609)-Right	19	1	0.0628	0.0345	0.0809
Pav(1209)-Left	14	1	0.0777	0.0542	0.0934
Pav(1209)-Right	14	1	0.0785	0.0549	0.0932
Mixed-Left	31	1	0.0516	0.0339	0.0908
Mixed-Right	31	1	0.0523	0.0337	0.0918
H = 74.7 with 17 degrees of freedom (P = <0.001)					

Table 3.8B: Results of post hoc pairwise analyses of diet specific left and right valve comparisons.

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Baseline-Right vs. Baseline-Left	1.192	0.0313	No
Cg-Left vs. Cg-Right	0.278	0.00858	No
Cm-Left vs. Cm-Right	5.577	0.207	No
CTRL-Left vs. CTRL-Right	12.1	0.44	No
ISO-Right vs. ISO-Left	0.167	0.00364	No
Mixed-Right vs. Mixed-Left	2.583	0.103	No
Pav(1209)-Right vs. Pav(1209)-Left	4.231	0.111	No
Pav(609)-Right vs. Pav(609)-Left	0.444	0.0137	No
TI-Right vs. TI-Left	1.25	0.0364	No

Elemental shell chemistry

Due to the results from the ICP-OES and the post- processing procedures, described previously, the total number of shells (n) is specific to each experimental diet and element ratio analyzed. Only the concentrations of boron, barium, cadmium, calcium, cobalt, copper, iron, potassium, lithium, magnesium, manganese, nickel, potassium, lead, silica, strontium, and zinc were detected from each single valve sample overall, so, only these elements are reported herein. Subsequent analyses were further limited by the observed variability and the different number of quantifiable observations for each element by diet which restricted reporting of differences in cadmium, cobalt, copper, nickel and lead.

The associated chemistries are provided for all collections for the left valves while the right valve chemistries are only available for the Baseline, March and May collections. Figures 3.8 – 3.20 depict the median element/Ca for each diet by collection month. The baseline measures for P/Ca and Sr/Ca, all Cd/Ca, all Ni/Ca, and Co/Ca for the right valves are not depicted due to measurement complications or elimination during post-processing as previously described.

The provided graphics (Figures 3.8-3.20) illustrate the variability in element/calcium ratios among the different diets and collections. Visually, it is evident that many of the reported ratios decreased from baseline during the course of the experiment. It is also obvious that there is not a clear trend over time with multiple diets showing random spikes in specific ratios.

Some of the observed elemental ratios do appear to remain relatively stable (i.e., B/Ca) throughout the experimental time frame regardless of diet received, while others are extremely variable. In many cases, the left and right valves do not mirror one another, though usually representative of the same clams.

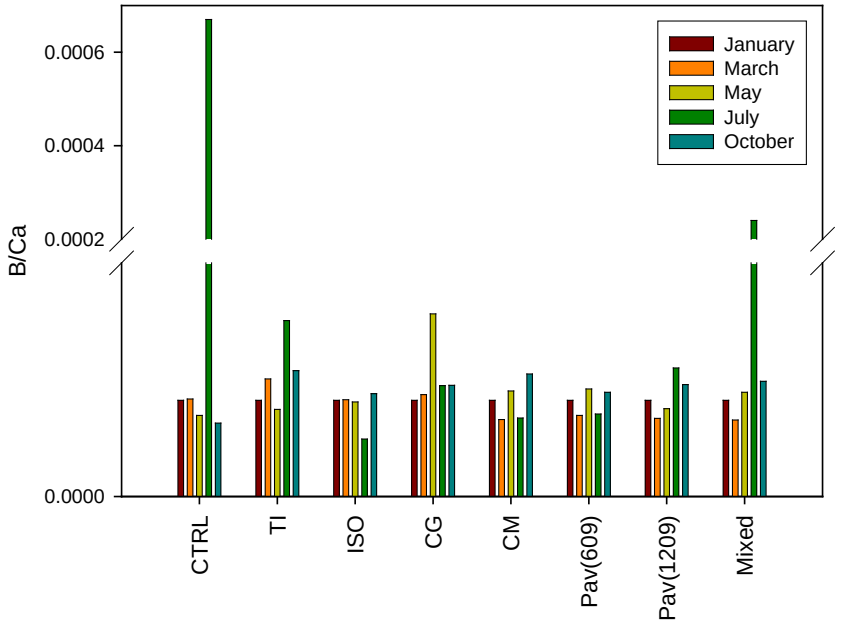


Figure 3.8A: Median B/Ca versus diet received for the collected left valves by collection month. The feeding groups are labeled as follows: starvation control = CTRL, TI = *Isochrysis* sp. (CCMP1324), ISO = *Isochrysis sp.* (CCMP1611), Pav609 = *Pavlova pinguis* (CCMP609), Pav1209 = *Pavlova sp.* (CCMP1209), *CG* = *Chaetoceros galvestonensis* (CCMP186), CM = *Chaetoceros mulleri* (CCMP609), and Mixed = the mixed diet of all species.

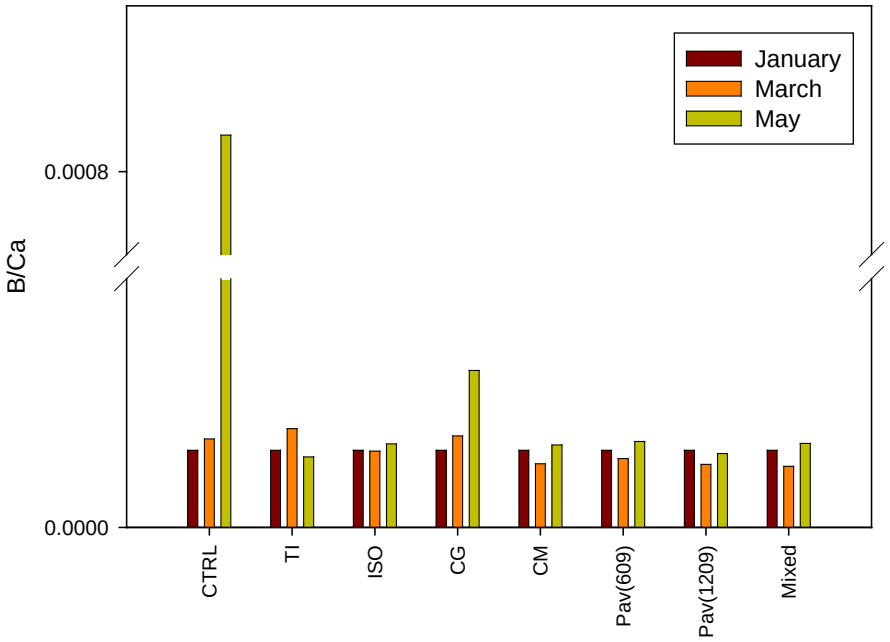


Figure 3.8B: Median B/Ca versus diet received for the collected right valves by collection month. The diet labels are the same as those in Fig. 3.8A.

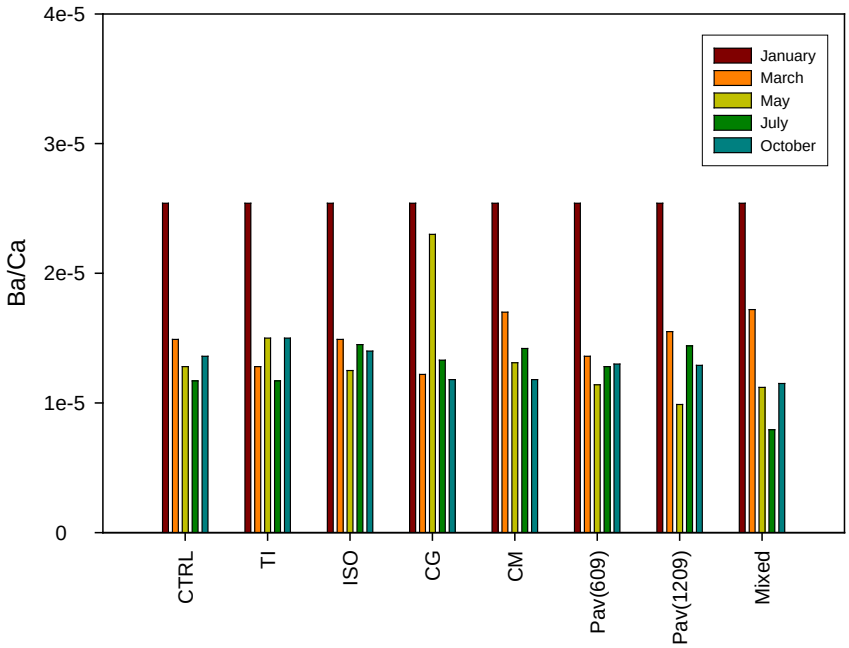


Figure 3.9A: Median Ba/Ca versus diet received for the collected left valves by collection month. The diet labels are the same as those in Fig. 3.8A.

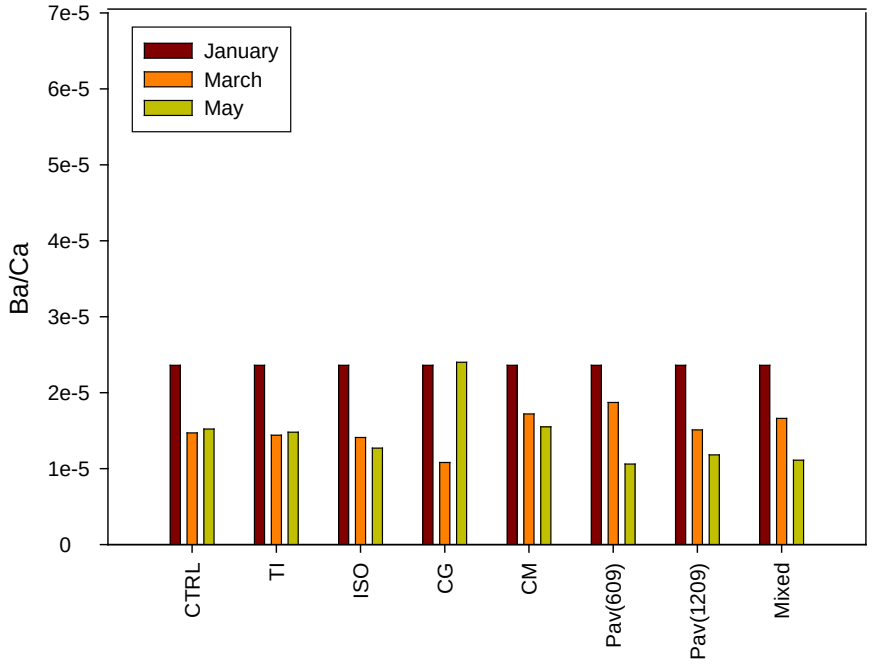


Figure 3.9B: Median Ba/Ca versus diet received for the collected right valves by collection month. The diet labels are the same as those in Fig. 3.8A.

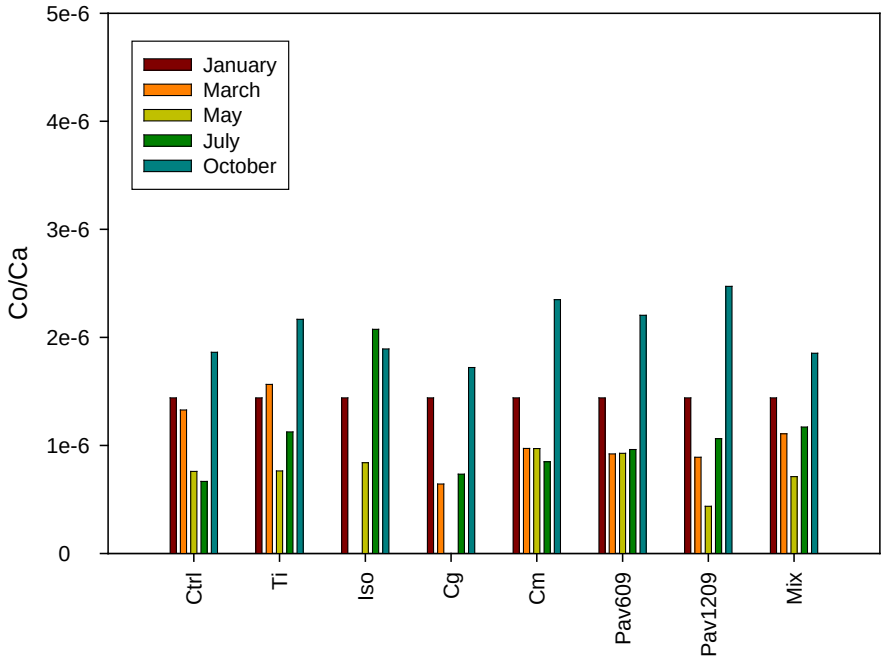


Figure 3.10: Median Co/Ca versus diet received for the collected left valves by collection month. The diet labels are the same as those in Fig. 3.8A.

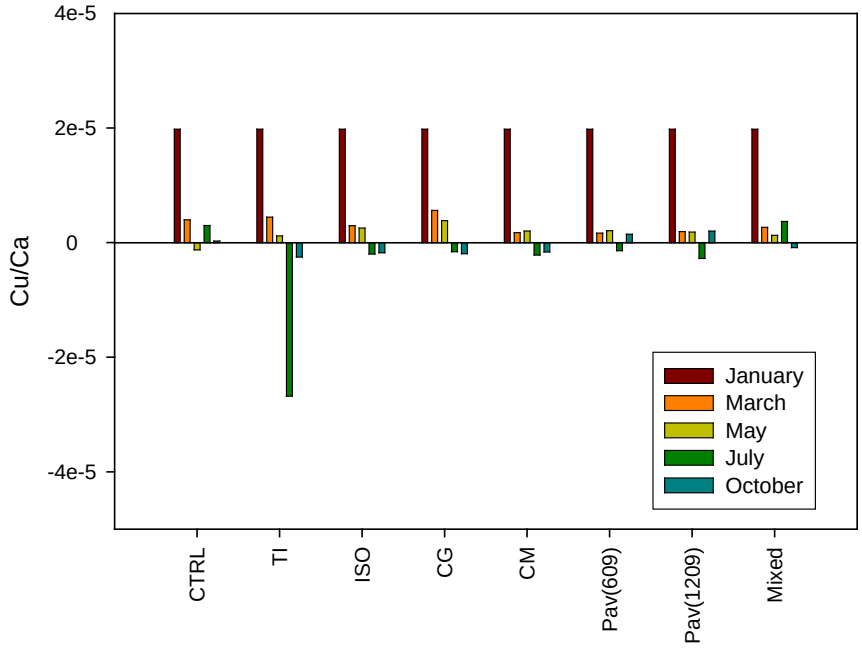


Figure 3.11A: Median Cu/Ca versus diet received for the collected left valves by collection month. The diet labels are the same as those in Fig. 3.8A.

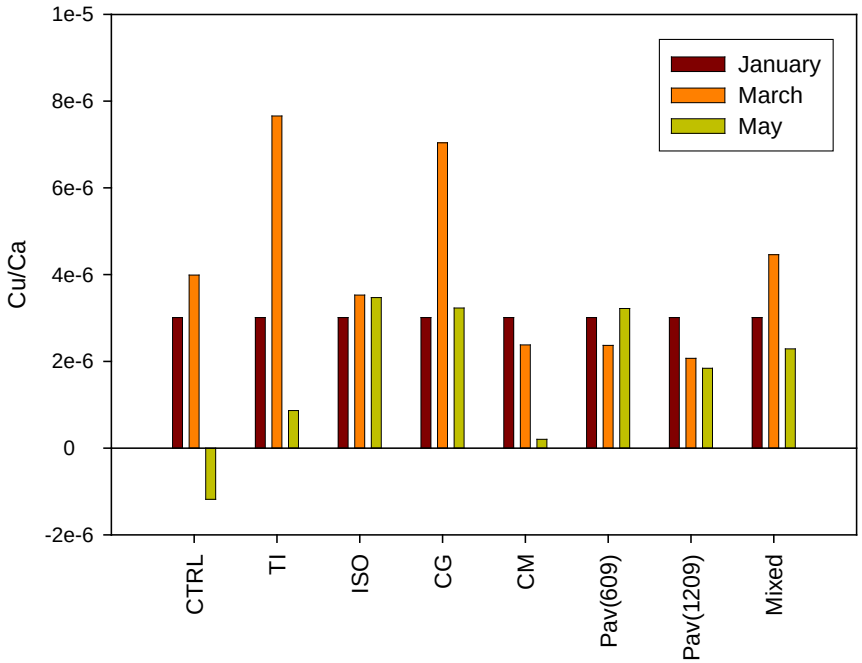
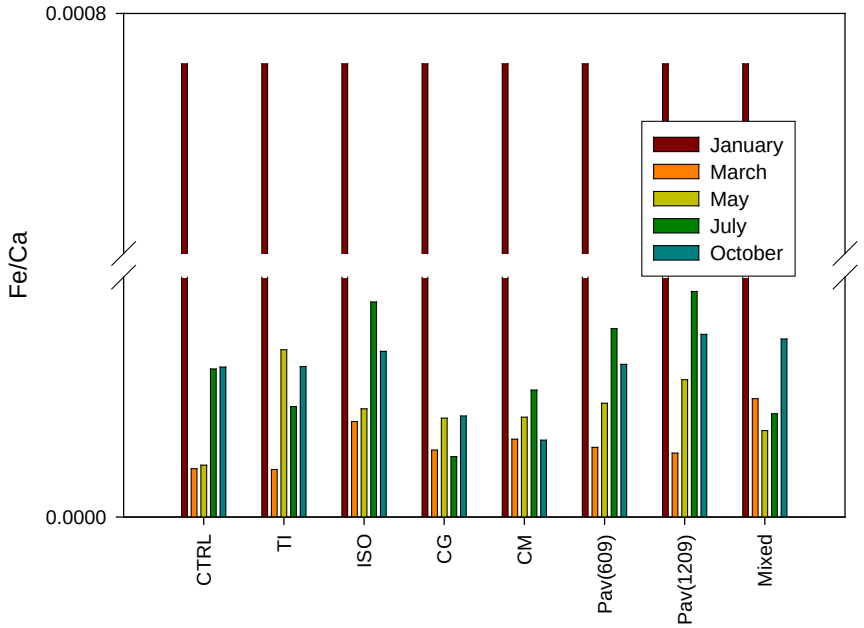
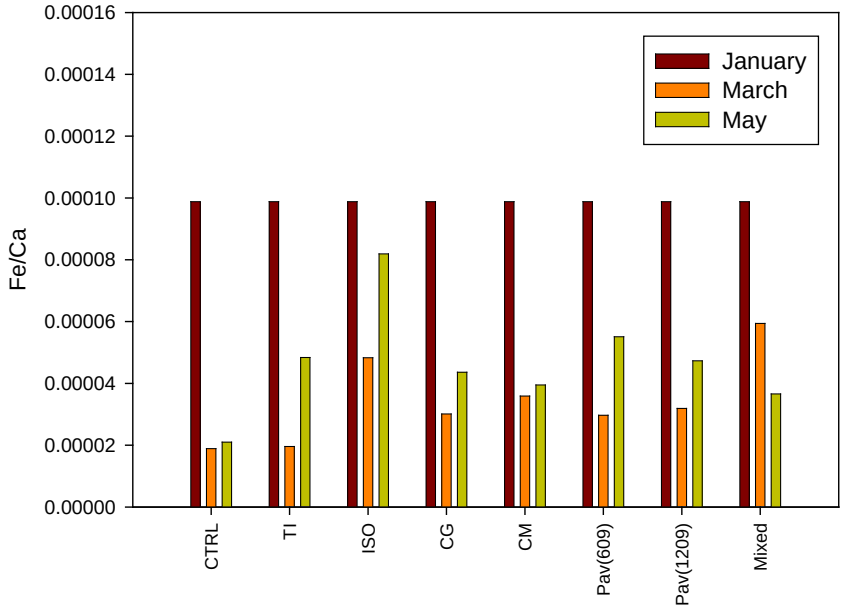


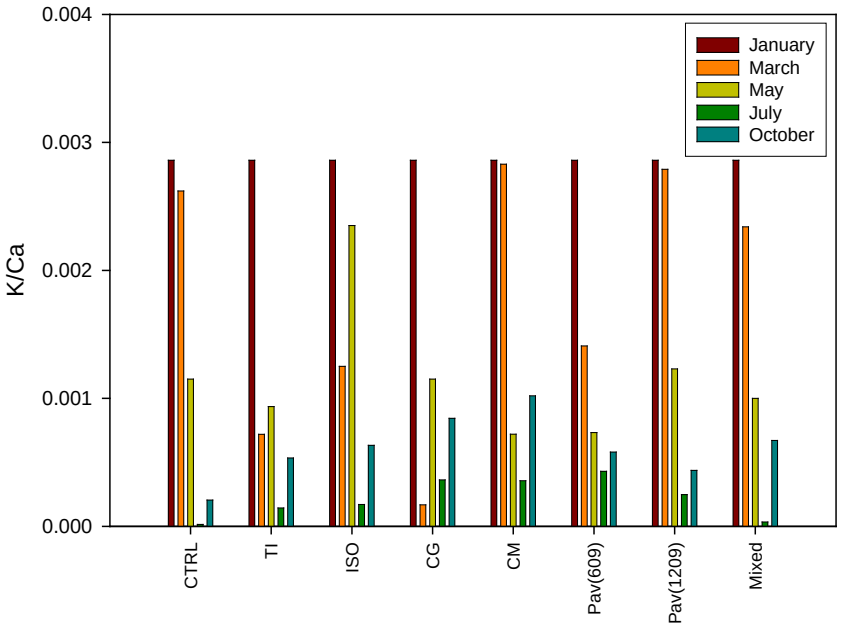
Figure 3.11B: Median Cu/Ca versus diet received for the collected right valves by collection month. The diet labels are the same as those in Fig. 3.8A.



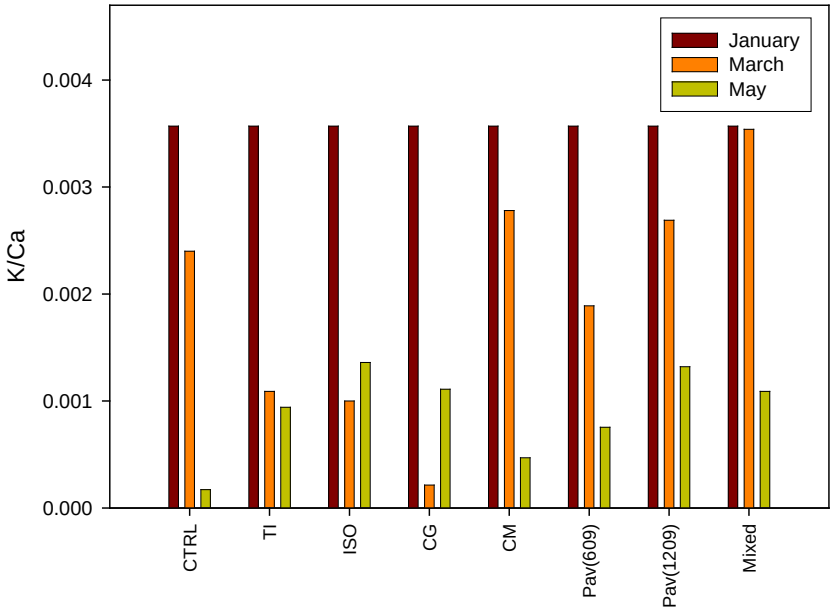
3.12A: Median Fe/Ca versus diet received for the collected left valves by collection month. The diet labels are the same as those in Fig. 3.8A.



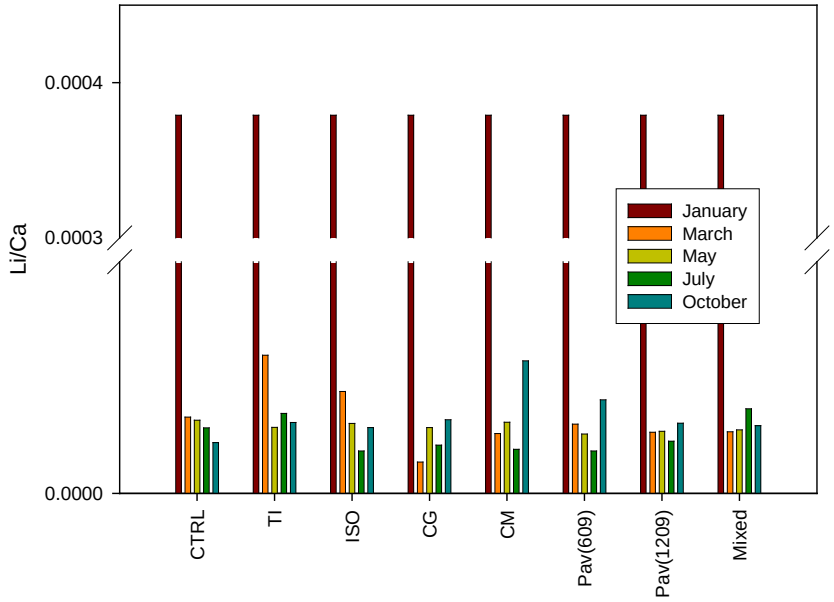
3.12B: Median Fe/Ca versus diet received for the collected right valves by collection month. The diet labels are the same as those in Fig. 3.8A.



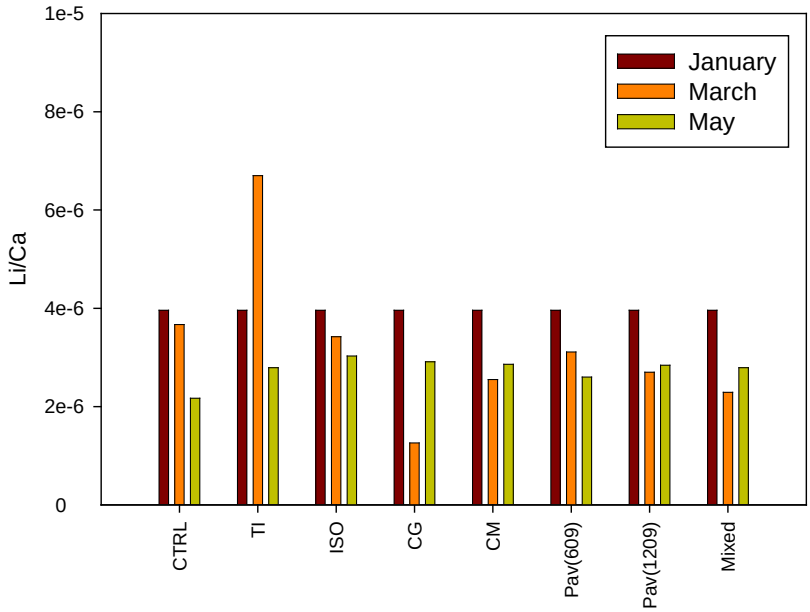
3.13A: Median K/Ca versus diet received for the collected left valves by collection month. The diet labels are the same as those in Fig. 3.8A.



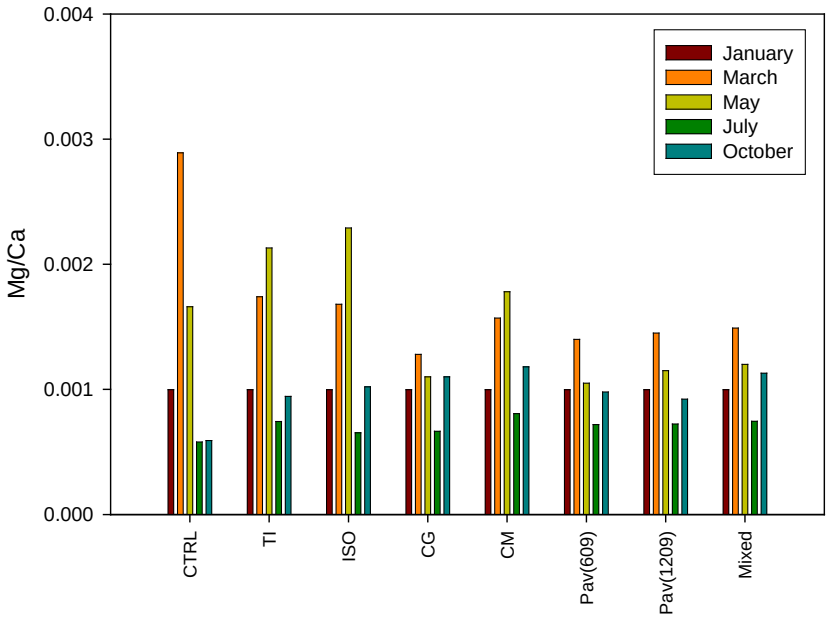
3.13B: Median K/Ca versus diet received for the collected right valves by collection month. The diet labels are the same as those in Fig. 3.8A.



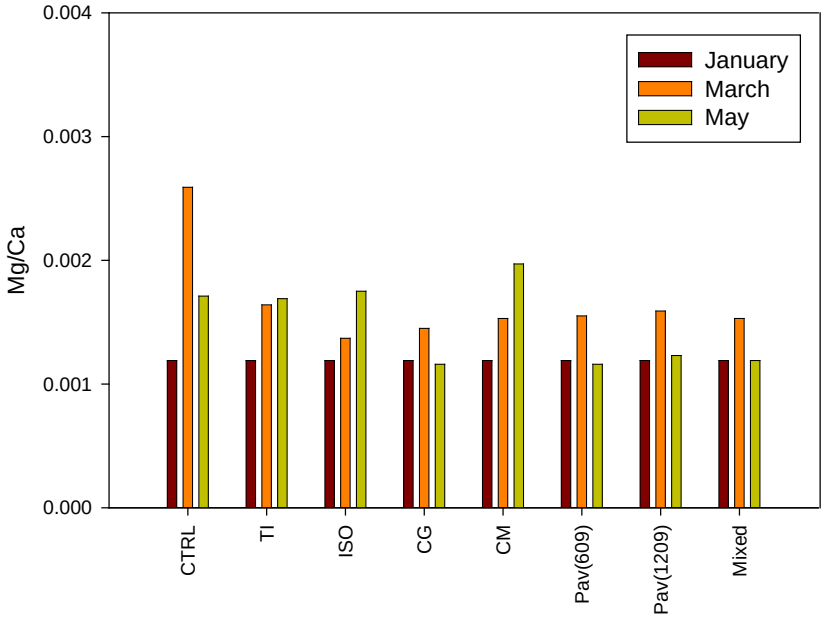
3.14A: Median Li/Ca versus diet received for the collected left valves by collection month. The diet labels are the same as those in Fig. 3.8A.



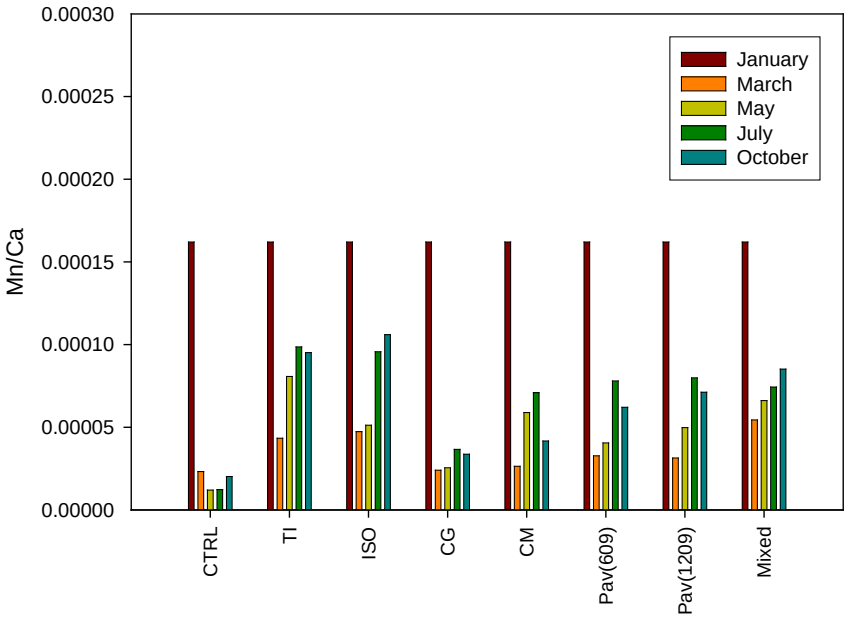
3.14B: Median Li/Ca versus diet received for the collected right valves by collection month. The diet labels are the same as those in Fig. 3.8A.



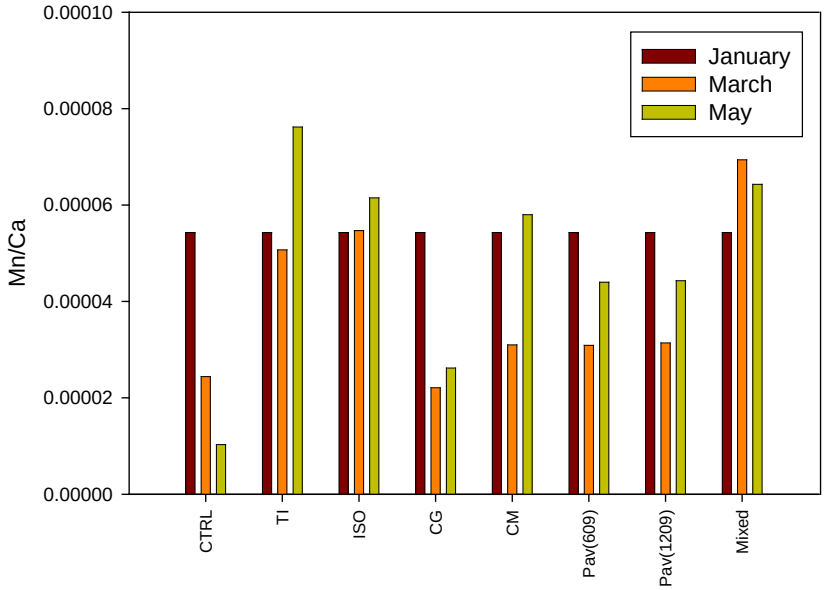
3.15A: Median Mg/Ca versus diet received for the collected left valves by collection month. The diet labels are the same as those in Fig. 3.8A.



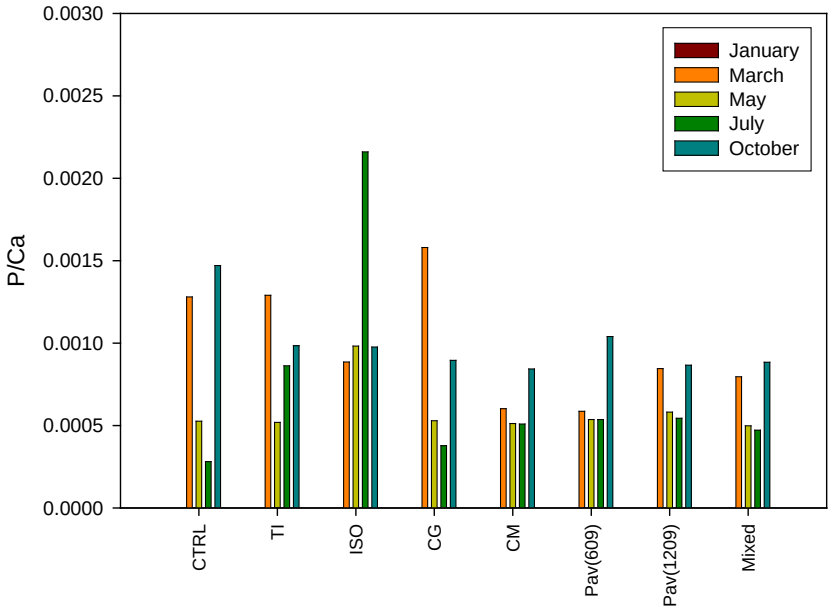
3.15B: Median Mg/Ca versus diet received for the collected right valves by collection month. The diet labels are the same as those in Fig. 3.8A.



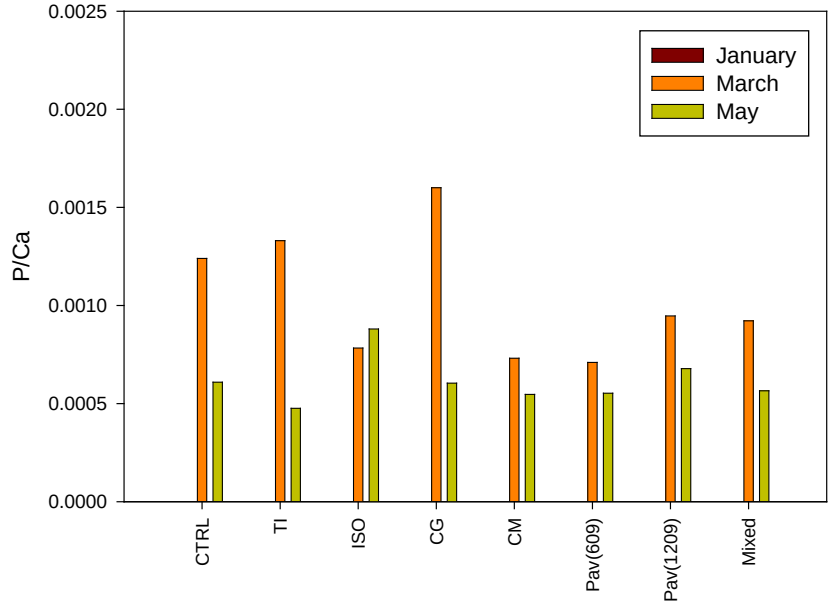
3.16A: Median Mn/Ca versus diet received for the collected left valves by collection month. The diet labels are the same as those in Fig. 3.8A.



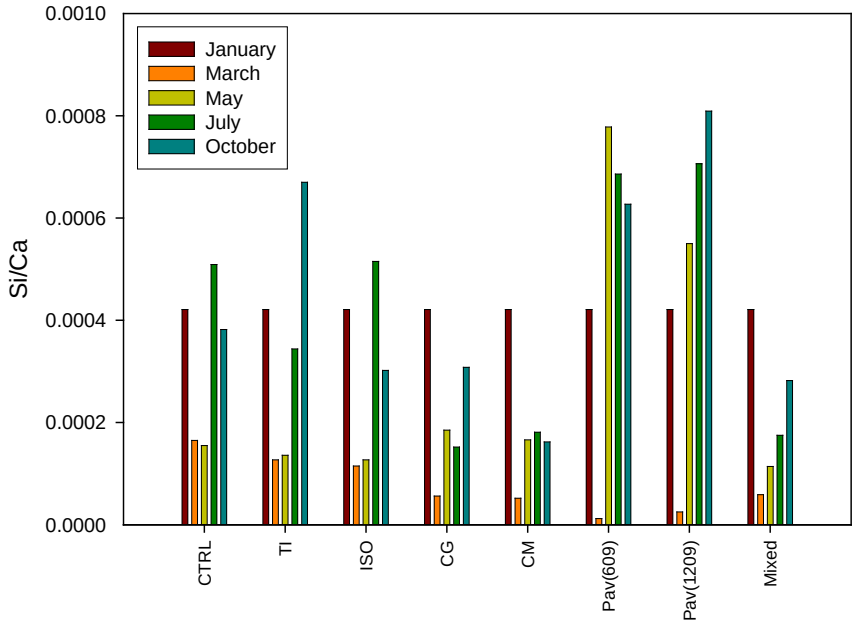
3.16B: Median Mn/Ca versus diet received for the collected right valves by collection month. The diet labels are the same as those in Fig. 3.8A.



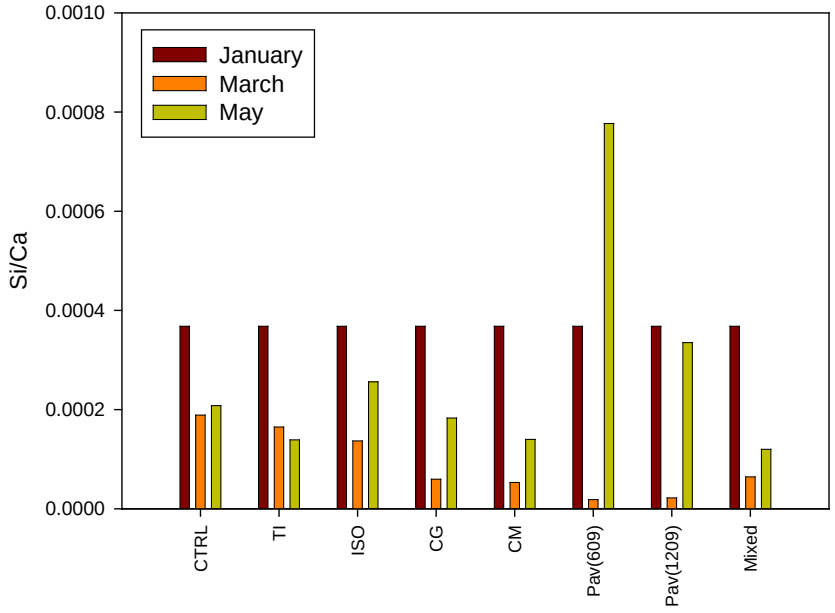
3.17A: Median P/Ca versus diet received for the collected left valves by collection month. The diet labels are the same as those in Fig. 3.8A.



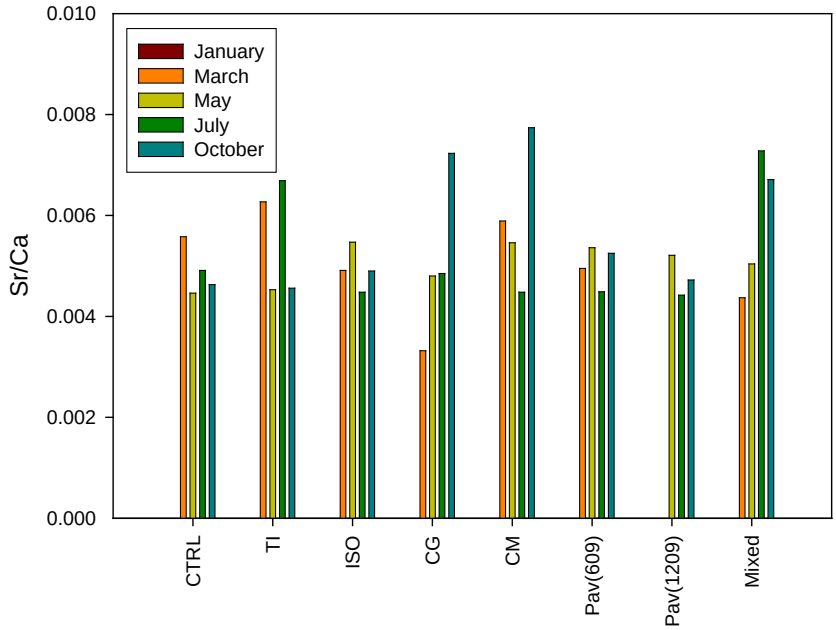
3.17B: Median P/Ca versus diet received for the collected right valves by collection month. The diet labels are the same as those in Fig. 3.8A.



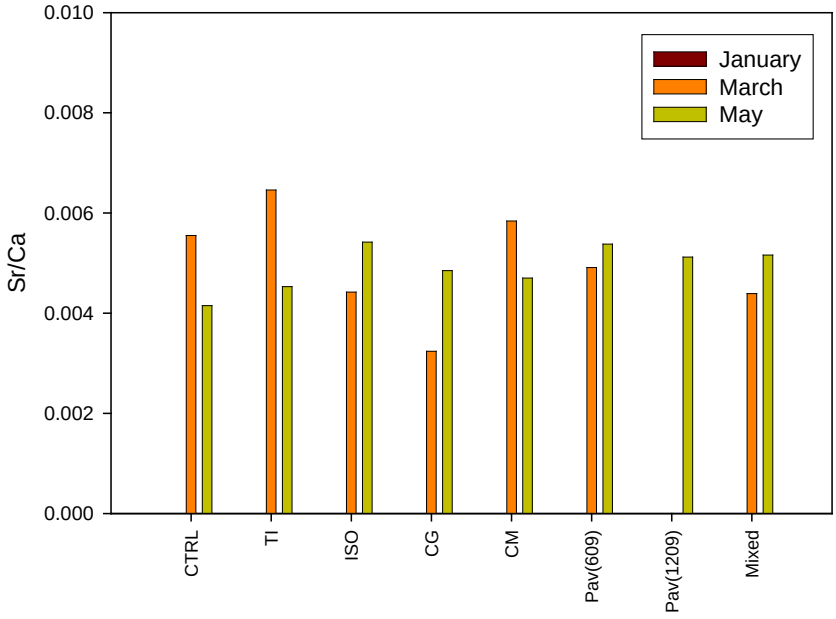
3.18A: Median Si/Ca versus diet received for the collected left valves by collection month. The diet labels are the same as those in Fig. 3.8A.



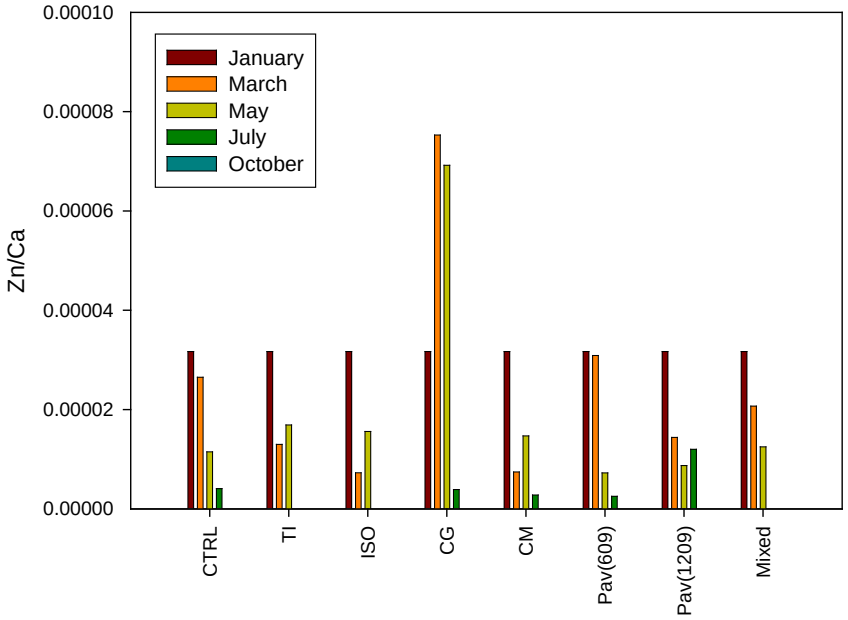
3.18B: Median Si/Ca versus diet received for the collected right valves by collection month. The diet labels are the same as those in Fig. 3.8A.



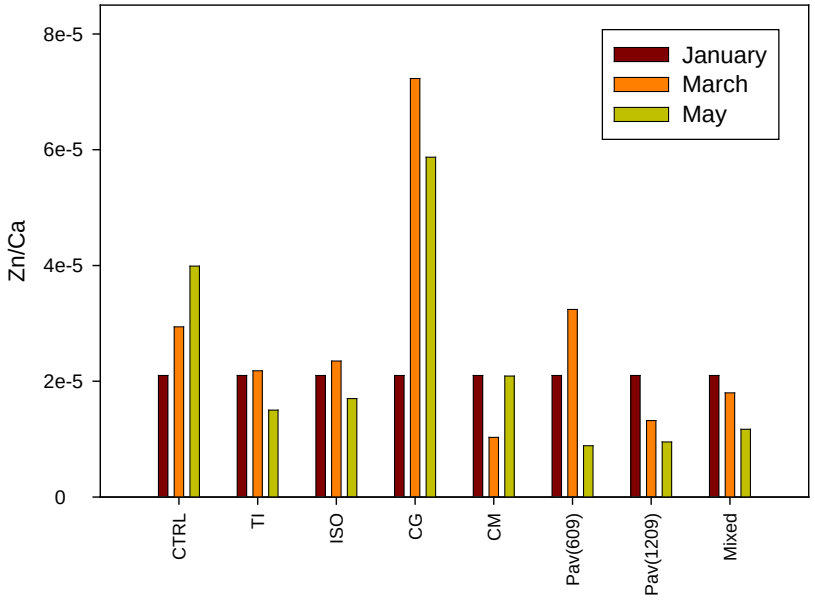
3.19A: Median Sr/Ca versus diet received for the collected left valves by collection month. The diet labels are the same as those in Fig. 3.8A.



3.19B: Median Sr/Ca versus diet received for the collected right valves by collection month. The diet labels are the same as those in Fig. 3.8A.



3.20A: Median Zn/Ca versus diet received for the collected left valves by collection month. The diet labels are the same as those in Fig. 3.8A.



3.20B: Median Zn/Ca versus diet received for the collected right valves by collection month. The diet labels are the same as those in Fig. 3.8A.

Statistical comparison of elemental shell chemistry between left and right valves

Two levels of analysis for examination of possible differences between left and right valve shell chemistry were conducted:1) all left and right valves were compared regardless of diet received to determine any chemical differences that might be expected at a population or species level, and 2) the right and left valves specific to the diet received to determine any differences in shell chemistry resultant of experimental variables and procedures as well as determine dominant influences observed during the first left versus right valve analysis.

Analysis of all collected left and right valves resulted in only two ratios being determined significantly different in regard to valve orientation – Ni/Ca and Zn/Ca. The summary of statistical tests is presented in Tables 3.9 and 3.10. The difference in Ni/Ca between valves was based on fewer than 20 left and right valves, which were representative of only a few diets. Though a significant difference was determined ($P<0.05$), these results were primarily attributable to differences between the Control and Baseline with the other diets unevenly represented with regard to valve orientation. Zinc measurements were, on the other hand, represented for all diets and in each collection. A P value of <0.05 was determined from the Dunn's method pairwise analysis and established right valve Zn/Ca > left valve Zn/Ca.

When each individual diet was examined with regard to the differences between left and right valve elemental chemistry, significant differences were observed in both the Control (CTRL) and *Chaetoceros mulleri* (CM) diet groups (Table 3.11). Three ratios, B/Ca, Si/Ca and Zn/Ca, were identified as being significantly higher in the right valve compared to the left valve in the Control ($P=0.001$, 0.046, and 0.002 respectively).

Only the Ba/Ca ratio was identified as significantly different between valves (P=0.030) in the CM group, though Li/Ca (P=0.053) was borderline. The right valve was again identified as having a higher ratio.

Table 3.9: Results for left versus right valve comparisons with respect to Ni/Ca.

Ni					
One Way Analysis of Variance					
Normality Test:	Passed	(P = 0.831)			
Equal Variance Test:	Passed	(P = 0.256)			
Group Name	N	Missing	Mean	Std Dev	SEM
Left	143	124	9.04E-07	4.58E-07	1.05E-07
Right	152	137	4.74E-07	3.45E-07	8.91E-08
Source of Variation	DF	SS	MS	F	P
Between Groups	1	1.55E-12	1.55E-12	9.138	0.005
Residual	32	5.44E-12	1.70E-13		
Total	33	7.00E-12			
Power of performed test with alpha = 0.050: 0.803					
All Pairwise Multiple Comparison Procedures (Holm-Sidak method):					
Overall significance level = 0.05					
Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
Left vs. Right	4.31E-07	3.023	0.0049	0.05	Yes

Table 3.10: Results for left versus right valve comparisons with respect to Zn/Ca.

Zn					
One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
Left	167	35	1.65E-05	1.08E-05	3.37E-05
Right	167	46	2.30E-05	1.35E-05	3.79E-05
H = 3.88 with 1 degrees of freedom. (P = 0.049)					
All Pairwise Multiple Comparison Procedures (Dunn's Method) :					
Comparison	Diff of Ranks	Q	P<0.05		
Left vs. Right	18.137	1.969	Yes		

All associated P-values for the statistical tests used for each diet and elemental ratio are provided in Table 3.11. The median ratio values with 95% confidence intervals are depicted in Figures 3.21 – 3.24 for the CTRL and CM examinations. As well, Tables 3.12 – 3.15 provide the statistical test summaries for the CTRL and CM left and right valve group comparisons.

Table 3.11: Significance of diet-specific left and right valve comparisons.

Bl	**Left vs. Right P value**	**Tl**	**Left vs. Right P value**	**ISO**	**Left vs. Right P value**
B	0.391	***B***	0.609	***B***	0.821
Ba	0.892	***Ba***	0.102	***Ba***	0.735
Cd	Na	***Cd***	Na	***Cd***	Na
Co	0.747	***Co***	1.000	***Co***	Na
Cu	1.000	***Cu***	0.640	***Cu***	0.314
Fe	0.765	***Fe***	0.877	***Fe***	0.158
K	0.807	***K***	0.954	***K***	0.245
Li	0.765	***Li***	0.765	***Li***	0.885
Mg	0.977	***Mg***	0.721	***Mg***	0.229
Mn	0.840	***Mn***	0.369	***Mn***	0.209
Ni	Na	***Ni***	Na	***Ni***	Na
P	Na	***P***	0.598	***P***	0.994
Pb	Na	***Pb***	Na	***Pb***	Na
Si	0.887	***Si***	0.841	***Si***	0.102
Sr	Na	***Sr***	0.554	***Sr***	0.996
Zn	0.840	***Zn***	0.910	***Zn***	0.284
CG	**Left vs. Right P value**	**CM**	**Left vs. Right P value**	**Pav609**	**Left vs. Right P value**
B	0.580	***B***	0.309	***B***	0.728
Ba	0.937	***Ba***	**0.030**	***Ba***	0.817
Cd	Na	***Cd***	Na	***Cd***	Na
Co	Na	***Co***	0.410	***Co***	0.451
Cu	0.397	***Cu***	0.297	***Cu***	0.381
Fe	0.669	***Fe***	0.750	***Fe***	0.531
K	0.856	***K***	0.489	***K***	0.385
Li	0.978	***Li***	0.053	***Li***	0.753
Mg	0.319	***Mg***	0.705	***Mg***	0.197
Mn	0.812	***Mn***	0.922	***Mn***	0.978
Ni	Na	***Ni***	Na	***Ni***	Na

Table 3.11 (Continued)

CG	**Left vs. Right P value**	**CM**	**Left vs. Right P value**	**Pav609**	**Left vs. Right P value**
P	0.517	***P***	0.962	***P***	0.394
Pb	Na	***Pb***	Na	***Pb***	Na
Si	0.740	***Si***	0.421	***Si***	0.717
Sr	0.809	***Sr***	0.091	***Sr***	0.994
Zn	0.812	***Zn***	0.125	***Zn***	0.869
Pav1209	Left vs. Right P value	**CTRL**	Left vs. Right P value	**Mixed**	Left vs. Right P value
B	0.716	***B***	**0.001**	***B***	0.418
Ba	0.505	***Ba***	0.222	***Ba***	0.554
Cd	Na	***Cd***	Na	***Cd***	Na
Co	0.391	***Co***	0.418	***Co***	0.189
Cu	0.878	***Cu***	0.231	***Cu***	0.323
Fe	0.915	***Fe***	0.831	***Fe***	0.845
K	0.837	***K***	0.421	***K***	0.796
Li	0.864	***Li***	0.171	***Li***	0.687
Mg	0.779	***Mg***	0.614	***Mg***	0.829
Mn	0.887	***Mn***	0.771	***Mn***	0.580
Ni	Na	***Ni***	0.123	***Ni***	Na
P	0.608	***P***	0.561	***P***	0.473
Pb	Na	***Pb***	Na	***Pb***	Na
Si	0.878	***Si***	**0.046**	***Si***	0.845
Sr	0.561	***Sr***	0.985	***Sr***	0.798
Zn	0.918	***Zn***	**0.002**	***Zn***	0.820

Comparison of elemental shell chemistry by to diet

Statistical comparisons of differences in shell chemistry between experimental diets were performed using the composite of all month collections by diet on both the left and right valves. Kruskal-Wallis One way Analysis of Variance on Ranks was used, followed by appropriate post-hoc pairwise examinations to determine if there were significant differences among diets with regard to elemental shell chemistry and which elements, if any, were influenced. It is important to recognize that the comparison of right valves only included individuals from the Baseline, March and May collections, whereas, the left valve comparisons include individuals from all experimental collections

January – October as time and growth influences must be closely examined during interpretation.

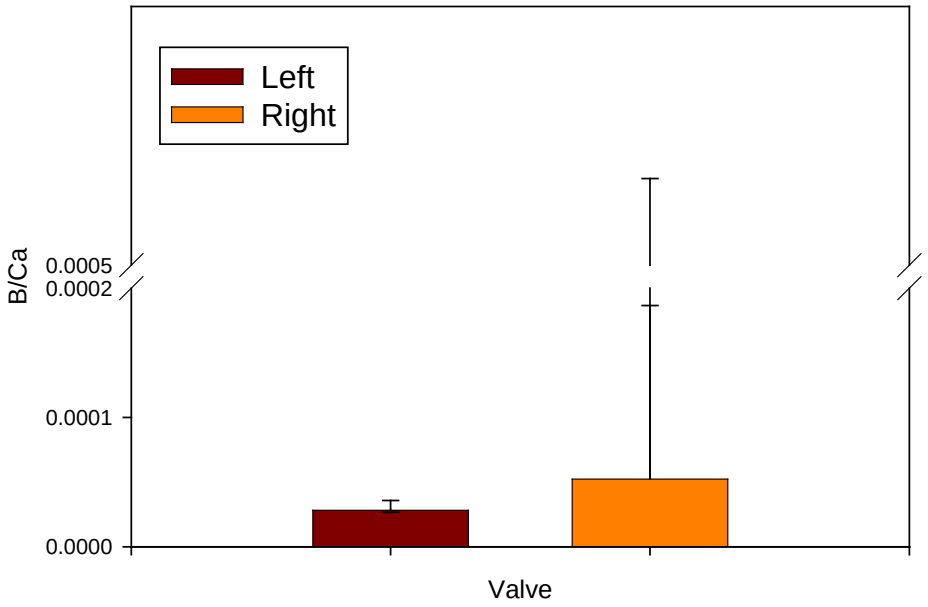


Figure 3.21: Left versus right valve for the Control feeding group with regard to B/Ca.

Table 3.12: Results of the comparison of the left and right valves from the Control group with regard to B/Ca.

CTRL-B					
t-test					
Normality Test:	Failed	(P < 0.050)			
Mann-Whitney Rank Sum Test					
Group	N	Missing	Median	25%	75%
Left	26	1	2.83E-05	2.40E-05	3.22E-05
Right	26	1	5.23E-05	3.09E-05	7.90E-04
Mann-Whitney U Statistic= 510.000					
T = 440 n(small)= 25 n(big)= 25 (P = <0.001)					

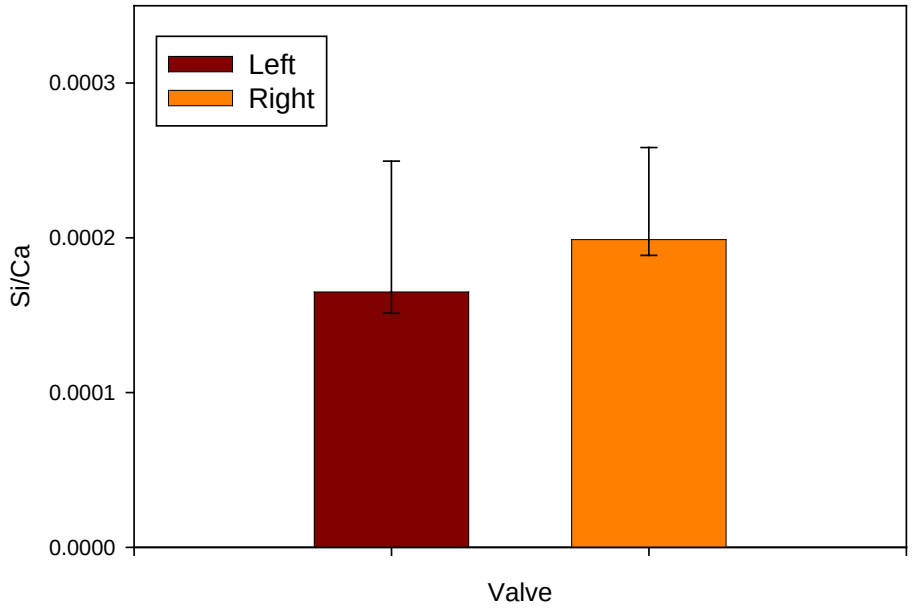


Figure 3.22: Left versus right valve for the Control feeding group with regard to Si/Ca.

Table 3.13: Results of the comparison of the left and right valves from the Control group with regard to Si/Ca.

CTRL-Si					
t-test					
Normality Test:	Failed	(P < 0.050)			
Mann-Whitney Rank Sum Test					
Group	N	Missing	Median	25%	75%
Left	26	1	1.65E-04	1.42E-04	2.30E-04
Right	26	1	1.99E-04	1.73E-04	2.47E-04
Mann-Whitney U Statistic= 416.000					
T = 534 n(small)= 25 n(big)= 25 (P = 0.046)					

Inadequate sample sizes preclude analyses for Cd/Ca, Cu/Ca, Ni/Ca, and Pb/Ca. Comparisons P/Ca and Sr/Ca are included despite baseline comparisons not being available.

Ten differences based on diet were identified for B/Ca of the left valves: Cg vs. ISO, Cg vs. Pav609, Mixed vs. ISO, Mixed vs. Pav609, TI vs. Baseline, TI vs. Cm, TI vs.

CTRL, TI vs. ISO, TI vs. Pav1209, and TI vs. Pav609 (Table 3.16B). The median values and associated 95% confidence intervals are provided in Figure 3.25, and the analyses summaries are provided in Tables 3.16 and 3.17. The analyses support the

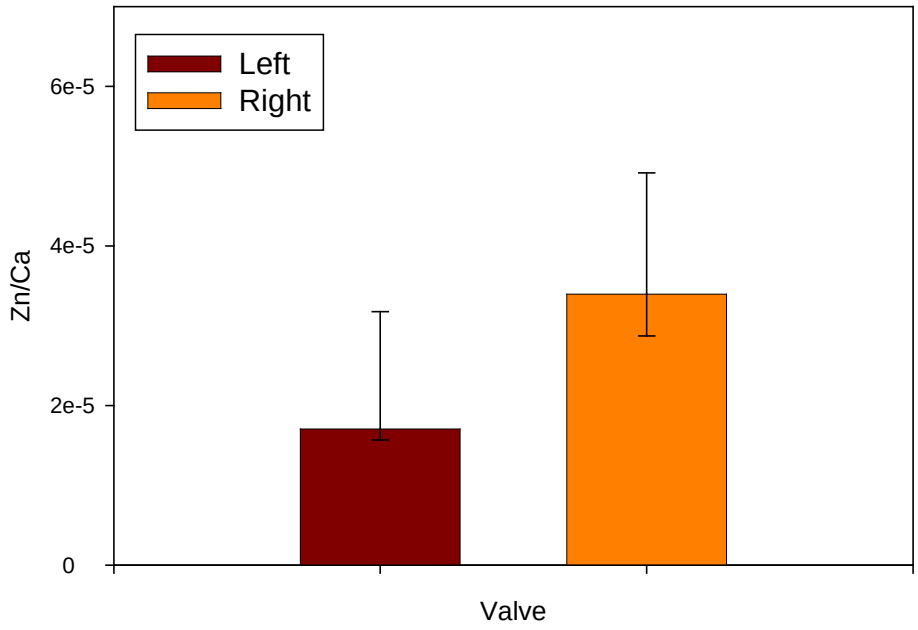


Figure 3.23: Left versus right valve for the Control feeding group with regard to Zn/Ca.

Table 3.14: Results of the comparison of the left and right valves from the Control group with regard to Zn/Ca.

CTRL-Zn					
t-test					
Normality Test:	Failed	(P < 0.050)			
Mann-Whitney Rank Sum Test					
Group	N	Missing	Median	25%	75%
Left	26	2	1.71E-05	1.15E-05	3.16E-05
Right	26	1	3.40E-05	2.44E-05	4.68E-05
Mann-Whitney U Statistic= 454.000					
T = 446 n(small)= 24 n(big)= 25 (P = 0.002)					

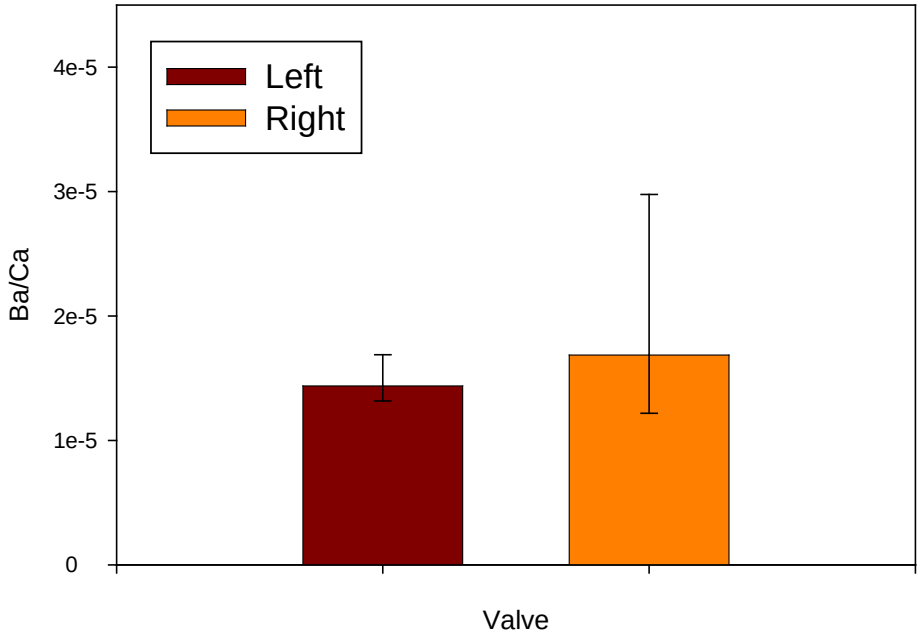


Figure 3.24: Left versus right valve for the CM feeding group with regard to Ba/Ca.

Table 3.15: Results of the comparison of the left and right valves from the CM group with regard to Ba/Ca.

CM-Ba					
t-test					
Normality Test:	Failed	(P < 0.050)			
Mann-Whitney Rank Sum Test					
Group	N	Missing	Median	25%	75%
Left	27	9	1.44E-05	1.23E-05	1.70E-05
Right	27	9	1.69E-05	1.53E-05	1.81E-05
Mann-Whitney U Statistic= 231.000					
T = 264 n(small)= 18 n(big)= 18 (P = 0.030)					

following ranking: Cg> ISO and Pav609, Mixed> ISO and Pav609, and finally, TI>Baseline, Cm, CTRL, ISO, Pav609, and Pav1209.

The comparison of right valve B/Ca between experimental diet groups determined that only the CTRL diet was significantly different from the Baseline, Cm,

Mixed, Pav1209, and Pav609 diets (Table 17B). The associated ranking is as follows: CTRL > Baseline, Cm, Mixed, Pav1209, and Pav609.

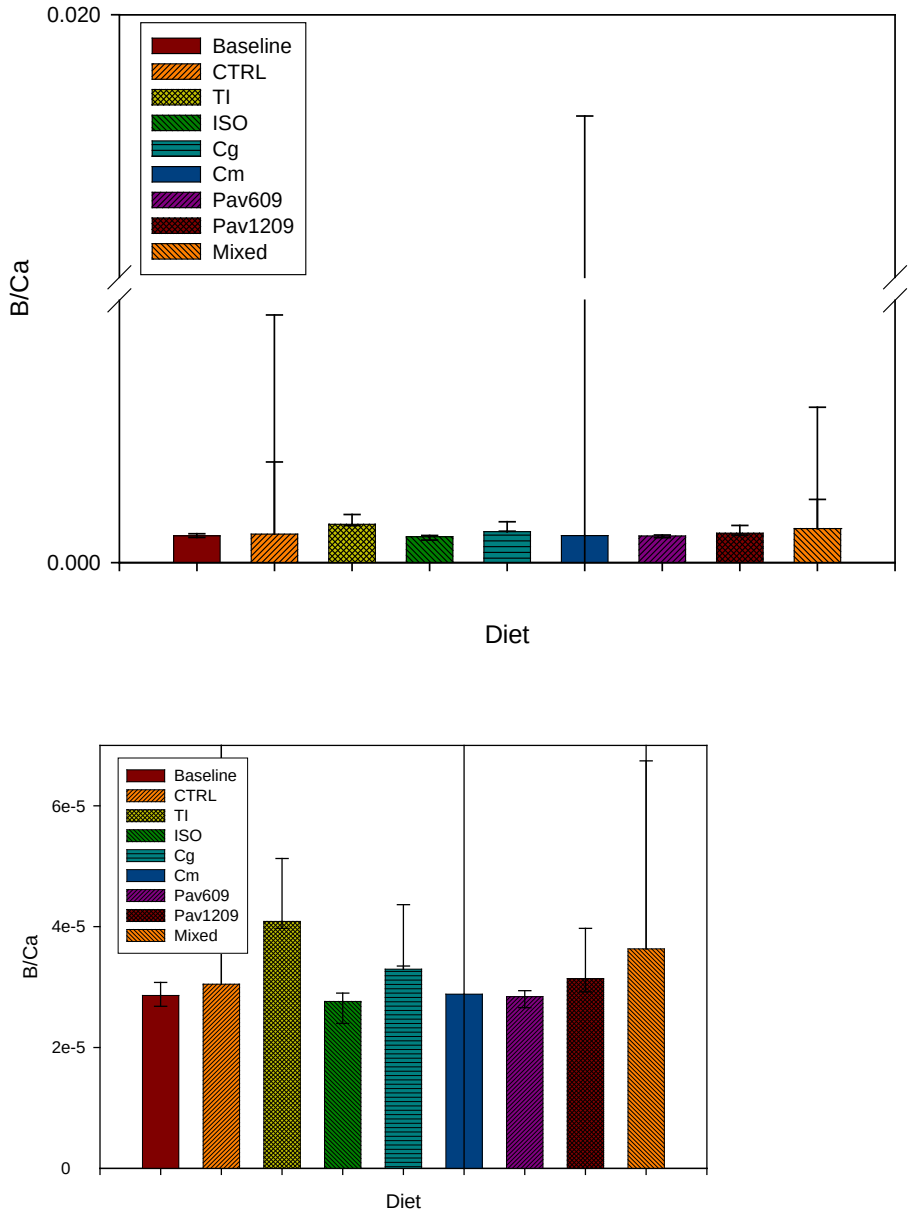


Figure 3.25: Left valve median B/Ca values compared to diet. Error bars depict the 95% confidence intervals and diets are represented as follows: Baseline = baseline/January collection, starvation control = CTRL, TI = *Isochrysis* sp. (CCMP1324), ISO = *Isochrysis sp.* (CCMP1611), Pav609 = *Pavlova pinguis* (CCMP609), Pav1209 = *Pavlova sp.* (CCMP1209), *Cg* = *Chaetoceros galvestonensis* (CCMP186), Cm = *Chaetoceros mulleri* (CCMP609), and Mixed = the mixed diet of all species. The lower graph is a close up of the first to allow a closer examination not limited by the error bar range.

Table 3.16A: Results of analysis of variance of left valve B/Ca among experimental diets. Diets are represented as previously identified in Figure 3.25.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
Baseline	14	1	2.86E-05	2.71E-05	3.17E-05
CTRL	61	1	3.05E-05	2.39E-05	5.23E-05
TI	54	4	4.09E-05	3.13E-05	5.39E-05
ISO	50	1	2.76E-05	1.92E-05	3.20E-05
Cg	58	3	3.30E-05	2.64E-05	3.92E-05
Cm	64	8	2.88E-05	2.44E-05	3.70E-05
Pav609	59	7	2.84E-05	2.39E-05	3.18E-05
Pav1209	52	1	3.14E-05	2.59E-05	3.96E-05
Mixed	53	13	3.63E-05	2.91E-05	1.35E-04
H = 61.1 with 8 degrees of freedom (P = <0.001)					

Table 3.16B: Results of pairwise comparison of left valve B/Ca between experimental diets. Diets are represented as previously identified in Figure 3.25.

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Cg vs. ISO	87.071	3.6	Yes
Cg vs. Pav609	76.485	3.212	Yes
Mixed vs. ISO	123.566	4.71	Yes
Mixed vs. Pav609	112.981	4.363	Yes
TI vs. Baseline	124.238	3.241	Yes
TI vs. Cm	103.325	4.313	Yes
TI vs. CTRL	75.717	3.212	Yes
TI vs. ISO	149.516	6.041	Yes
TI vs. Pav1209	87.759	3.582	Yes
TI vs. Pav609	138.931	5.697	Yes
Baseline vs. ISO	25.278	0.658	No
Baseline vs. Pav609	14.692	0.385	No
Cg vs. Baseline	61.793	1.627	No
Cg vs. Cm	40.88	1.749	No
Cg vs. Ctrl	13.271	0.577	No
Cg vs. Pav1209	25.313	1.058	No
Cm vs. Baseline	20.913	0.552	No
Cm vs. ISO	46.191	1.918	No
Cm vs. Pav609	35.606	1.502	No
CTRL vs. Baseline	48.522	1.288	No

Table 3.16B (Continued)

Comparison	Diff of Ranks	Q	P<0.05
CTRL vs. Cm	27.608	1.207	No
CTRL vs. ISO	73.8	3.113	No
CTRL vs. Pav1209	12.042	0.514	No
CTRL vs. Pav609	63.214	2.71	No
Mixed vs. Baseline	98.288	2.501	No
Mixed vs. Cg	36.495	1.426	No
Mixed vs. Cm	77.375	3.036	No
Mixed vs. Ctrl	49.767	1.98	No
Mixed vs. Pav1209	61.809	2.377	No
Pav1209 vs. Baseline	36.48	0.954	No
Pav1209 vs. Cm	15.566	0.653	No
Pav1209 vs. ISO	61.758	2.508	No
Pav1209 vs. Pav609	51.172	2.109	No
Pav609 vs. ISO	10.586	0.432	No
TI vs. Cg	62.445	2.596	No
TI vs. Mixed	25.95	0.994	No

Table 3.17A: Results of analysis of variance of right valve B/Ca among experimental diets. Diets are represented as previously identified in Figure 3.25.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
Baseline	14	2	2.79E-05	2.68E-05	2.99E-05
CTRL	29	1	4.61E-05	3.08E-05	7.77E-04
TI	17	7	3.42E-05	2.73E-05	3.81E-05
ISO	10	1	3.02E-05	2.40E-05	3.25E-05
Cg	19	1	3.31E-05	2.67E-05	3.87E-05
Cm	27	9	2.87E-05	2.41E-05	3.10E-05
Pav609	19	9	2.62E-05	2.22E-05	3.11E-05
Pav1209	14	1	2.35E-05	2.11E-05	2.67E-05
Mixed	31	13	2.98E-05	2.57E-05	3.18E-05
H = 38.9 with 8 degrees of freedom (P = <0.001)					

Table 3.17B: Results of pairwise comparison of right valve B/Ca between experimental diets. Diets are represented as previously identified in Figure 3.25.

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
CTRL vs. Baseline	44.833	3.298	Yes
CTRL vs. Cm	41.583	3.493	Yes
CTRL vs. Mixed	38.194	3.208	Yes
CTRL vs. Pav1209	71.865	5.434	Yes
CTRL vs. Pav609	50.35	3.469	Yes
Baseline vs. Pav1209	27.032	1.714	No
Baseline vs. Pav609	5.517	0.327	No
Cg vs. Baseline	23.139	1.576	No
Cg vs. Cm	19.889	1.514	No
Cg vs. ISO	13.111	0.815	No
Cg vs. Mixed	16.5	1.256	No
Cg vs. Pav1209	50.171	3.498	No
Cg vs. Pav609	28.656	1.844	No
Cm vs. Baseline	3.25	0.221	No
Cm vs. Pav1209	30.282	2.111	No
Cm vs. Pav609	8.767	0.564	No
CTRL vs. Cg	21.694	1.822	No
CTRL vs. ISO	34.806	2.305	No
CTRL vs. TI	20.25	1.395	No
ISO vs. Baseline	10.028	0.577	No
ISO vs. Cm	6.778	0.421	No
ISO vs. Mixed	3.389	0.211	No
ISO vs. Pav1209	37.06	2.169	No
ISO vs. Pav609	15.544	0.859	No
Mixed vs. Baseline	6.639	0.452	No
Mixed vs. Cm	3.389	0.258	No
Mixed vs. Pav1209	33.671	2.348	No
Mixed vs. Pav609	12.156	0.782	No
Pav609 vs. Pav1209	21.515	1.298	No
TI vs. Baseline	24.583	1.457	No
TI vs. Cg	1.444	0.0929	No
TI vs. Cm	21.333	1.373	No
TI vs. ISO	14.556	0.804	No
TI vs. Mixed	17.944	1.155	No
TI vs. Pav1209	51.615	3.114	No
TI vs. Pav609	30.1	1.708	No

The median Ba/Ca value for left valves by diet is depicted in Figure 3.26. Analysis of variance and post hoc pairwise analysis revealed 10 significant differences based on diet: Baseline vs. Cg, Baseline vs. Cm, Baseline vs. CTRL, Baseline vs. ISO, Baseline vs. Mixed, Baseline vs. Pav1209, Baseline vs. Pav609, Baseline vs. TI, ISO vs. Mixed, and TI vs. Mixed (Tables 3.18A and 3.18B). The ranking of these diet groups is as follows: Baseline > Cg, Cm, CTRL, ISO, Mixed, Pav1209, Pav609, and TI, as well as, TI and ISO > Mixed.

The comparison of Ba/Ca values between diets using the right valves resulted in four significant differencest: Baseline vs. CTRL, Baseline vs. ISO, Baseline vs. Mixed, and Baseline vs. TI. These differences support Baseline Ba/Ca > CTRL, ISO, Mixed, and TI. Summaries of the statistical results have been provided in Tables 3.19A and 3.19B.

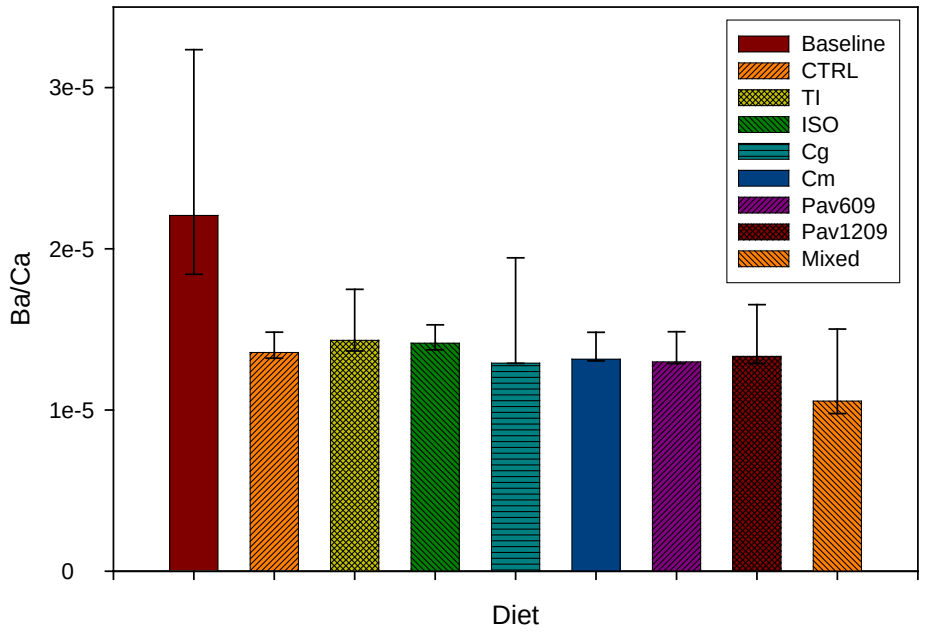


Figure 3.26: Left valve median Ba/Ca values compared to diet. Error bars depict the 95% confidence intervals and diets are represented as previously identified in Figure 3.25.

Table 3.18A: Results of analysis of variance of left valve Ba/Ca among diets. Diets are represented as previously identified in Figure 3.25.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
Baseline	14	1	2.21E-05	1.83E-05	2.65E-05
CTRL	61	1	1.36E-05	1.17E-05	1.51E-05
TI	54	8	1.43E-05	1.18E-05	1.69E-05
ISO	50	1	1.41E-05	1.31E-05	1.58E-05
Cg	58	3	1.29E-05	1.15E-05	1.56E-05
Cm	64	9	1.32E-05	1.18E-05	0.000015
Pav609	59	7	0.000013	1.18E-05	1.46E-05
Pav1209	52	1	1.33E-05	1.15E-05	1.55E-05
Mixed	53	9	1.06E-05	7.99E-06	1.53E-05
H = 50.7 with 8 degrees of freedom (P = <0.001)					

Table 3.18B: Results of pairwise comparison of left valve Ba/Ca between diets. Diets are represented as previously identified in Figure 3.25.

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Baseline vs. Cg	189.568	5.004	Yes
Baseline vs. Cm	183.077	4.833	Yes
Baseline vs. Ctrl	177.76	4.731	Yes
Baseline vs. ISO	145.832	3.806	Yes
Baseline vs. Mixed	253.509	6.538	Yes
Baseline vs. Pav1209	177.587	4.653	Yes
Baseline vs. Pav609	189.269	4.969	Yes
Baseline vs. TI	149.946	3.886	Yes
ISO vs. Mixed	107.677	4.221	Yes
TI vs. Mixed	103.562	3.998	Yes
Cg vs. Mixed	63.941	2.574	No
Cm vs. Cg	6.491	0.277	No
Cm vs. Mixed	70.432	2.835	No
Cm vs. Pav609	6.192	0.261	No
CTRL vs. Cg	11.808	0.515	No
CTRL vs. Cm	5.317	0.232	No
CTRL vs. Mixed	75.748	3.107	No
CTRL vs. Pav609	11.509	0.495	No
ISO vs. Cg	43.736	1.813	No
ISO vs. Cm	37.245	1.544	No

Table 3.18B (Continued)

Comparison	Diff of Ranks	Q	P<0.05
ISO vs. Ctrl	31.928	1.35	No
ISO vs. Pav1209	31.755	1.292	No
ISO vs. Pav609	43.437	1.776	No
ISO vs. TI	4.114	0.163	No
Pav1209 vs. Cg	11.981	0.502	No
Pav1209 vs. Cm	5.49	0.23	No
Pav1209 vs. Ctrl	0.174	0.00742	No
Pav1209 vs. Mixed	75.922	3.004	No
Pav1209 vs. Pav609	11.683	0.483	No
Pav609 vs. Cg	0.299	0.0126	No
Pav609 vs. Mixed	64.24	2.553	No
TI vs. Cg	39.621	1.614	No
TI vs. Cm	33.13	1.35	No
TI vs. Ctrl	27.814	1.155	No
TI vs. Pav1209	27.64	1.107	No
TI vs. Pav609	39.323	1.582	No

Table 3.19A: Results of analysis of variance of right valve Ba/Ca among diets. Diets are represented as previously identified in Figure 3.25.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
Baseline	14	2	2.36E-05	1.87E-05	2.70E-05
Ctrl	29	1	1.49E-05	1.33E-05	1.77E-05
TI	17	7	1.46E-05	1.01E-05	1.49E-05
ISO	10	1	1.28E-05	1.24E-05	1.71E-05
Cg	19	1	1.75E-05	1.08E-05	2.27E-05
Cm	27	9	1.69E-05	1.53E-05	1.81E-05
Pav(609)	19	9	1.44E-05	1.23E-05	1.96E-05
Pav(1209)	14	1	1.41E-05	1.25E-05	2.22E-05
Mixed	31	11	1.20E-05	1.04E-05	1.65E-05
H = 30.4 with 8 degrees of freedom (P = <0.001)					

Table 3.19B: Results of pairwise comparison of right valve Ba/Ca between diets. Diets are represented as previously identified in Figure 3.25.

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Baseline vs. CTRL	46.869	3.398	Yes
Baseline vs. ISO	57.306	3.25	Yes
Baseline vs. Mixed	71.383	4.89	Yes
Baseline vs. TI	66.833	3.904	Yes
Baseline vs. Cg	39.417	2.645	No
Baseline vs. Cm	31.139	2.09	No
Baseline vs. Pav1209	43.353	2.709	No
Baseline vs. Pav609	46.383	2.709	No
Cg vs. Ctrl	7.452	0.617	No
Cg vs. ISO	17.889	1.096	No
Cg vs. Mixed	31.967	2.461	No
Cg vs. Pav1209	3.936	0.27	No
Cg vs. Pav609	6.967	0.442	No
Cg vs. TI	27.417	1.739	No
Cm vs. Cg	8.278	0.621	No
Cm vs. CTRL	15.73	1.302	No
Cm vs. ISO	26.167	1.603	No
Cm vs. Mixed	40.244	3.098	No
Cm vs. Pav1209	12.214	0.839	No
Cm vs. Pav609	15.244	0.967	No
Cm vs. TI	35.694	2.264	No
CTRL vs. ISO	10.437	0.681	No
CTRL vs. Mixed	24.514	2.094	No
CTRL vs. TI	19.964	1.355	No
ISO vs. Mixed	14.078	0.877	No
ISO vs. TI	9.528	0.519	No
Pav1209 vs. CTRL	3.516	0.262	No
Pav1209 vs. ISO	13.953	0.805	No
Pav1209 vs. Mixed	28.031	1.968	No
Pav1209 vs. Pav609	3.031	0.18	No
Pav1209 vs. TI	23.481	1.396	No
Pav609 vs. CTRL	0.486	0.033	No
Pav609 vs. ISO	10.922	0.595	No
Pav609 vs. Mixed	25	1.615	No
Pav609 vs. TI	20.45	1.144	No
TI vs. Mixed	4.55	0.294	No

Statistical comparison of diet groups as relates to Co/Ca could only be performed on the left valves (Figure 3.27). Two significant differences were found: ISO > Mixed and TI > Mixed (Tables 3.20A and 3.20B).

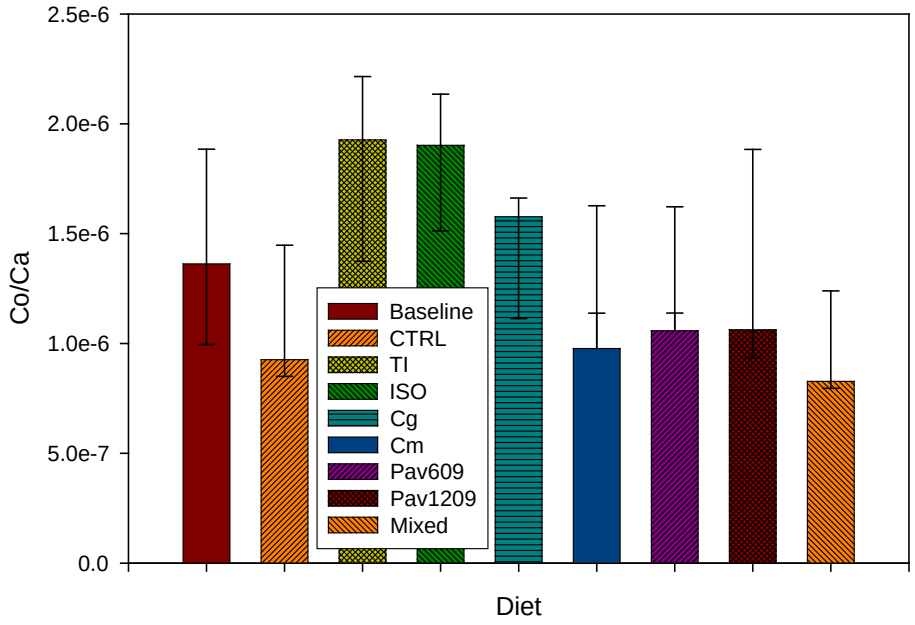


Figure 3.27: Left valve median Co/Ca values compared to diet. Error bars depict the 95% confidence intervals and diets are represented as previously identified in Figure 3.25.

Table 3.20A: Results of analysis of variance of left valve Co/Ca among diets. Diets are represented as previously identified in Figure 3.25.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
Baseline	12	7	1.36E-06	1.17E-06	1.71E-06
CTRL	59	44	9.26E-07	7.46E-07	1.39E-06
TI	53	44	1.93E-06	1.45E-06	2.21E-06
ISO	48	35	1.90E-06	1.70E-06	2.12E-06
Cg	57	40	1.58E-06	7.36E-07	1.79E-06
Cm	63	31	9.77E-07	8.69E-07	1.91E-06
Pav609	58	33	1.06E-06	9.26E-07	1.94E-06
Pav1209	52	38	1.06E-06	8.91E-07	2.38E-06
Mixed	53	35	8.27E-07	7.05E-07	1.17E-06
H = 22.6 with 8 degrees of freedom (P = 0.004)					

Table 3.20B: Results of pairwise comparison of left valve Co/Ca between diets. Diets are represented as previously identified in Figure 3.25.

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
ISO vs. Mixed	59.701	3.826	Yes
TI vs. Mixed	57.778	3.301	Yes
Baseline vs. Cg	16.8	0.77	No
Baseline vs. Cm	12.144	0.589	No
Baseline vs. CTRL	32.133	1.452	No
Baseline vs. Mixed	43.578	2.011	No
Baseline vs. Pav1209	9.586	0.429	No
Baseline vs. Pav609	9.24	0.44	No
Cg vs. CTRL	15.333	1.01	No
Cg vs. Mixed	26.778	1.847	No
Cm vs. Cg	4.656	0.362	No
Cm vs. CTRL	19.99	1.49	No
Cm vs. Mixed	31.434	2.489	No
CTRL vs. Mixed	11.444	0.764	No
ISO vs. Baseline	16.123	0.715	No
ISO vs. Cg	32.923	2.085	No
ISO vs. Cm	28.267	2.005	No
ISO vs. CTRL	48.256	2.971	No
ISO vs. Pav1209	25.709	1.557	No
ISO vs. Pav609	25.363	1.73	No
ISO vs. TI	1.923	0.103	No
Pav1209 vs. Cg	7.214	0.466	No
Pav1209 vs. Cm	2.558	0.186	No
Pav1209 vs. CTRL	22.548	1.415	No
Pav1209 vs. Mixed	33.992	2.225	No
Pav609 vs. Cg	7.56	0.561	No
Pav609 vs. Cm	2.904	0.254	No
Pav609 vs. CTRL	22.893	1.635	No
Pav609 vs. Mixed	34.338	2.591	No
Pav609 vs. Pav1209	0.346	0.0242	No
TI vs. Baseline	14.2	0.594	No
TI vs. Cg	31	1.754	No
TI vs. Cm	26.344	1.629	No
TI vs. CTRL	46.333	2.563	No
TI vs. Pav1209	23.786	1.299	No
TI vs. Pav609	23.44	1.407	No

Figure 3.28 depicts the left valve Fe/Ca median values by diet received. In all, 15 comparisons were determined to be significant as relates to diet-specific Fe/Ca: Baseline vs. Cg, Baseline vs. Cm, Baseline vs. CTRL, Baseline vs. Mixed, ISO vs. Cg, ISO vs. Cm, ISO vs. CTRL, ISO vs. Mixed, Pav1209 vs. Cg, Pav1209 vs. Cm, Pav1209 vs. CTRL, Pav1209 vs. Mixed, Pav609 vs. Cg, Pav609 vs. CTRL, and TI vs. Cg. Based on the analyses, the following rankings are supported (Table 3.21A, B): Baseline > Cg, Cm, CTRL, and Mixed; ISO > Cg, Cm, CTRL, and Mixed; Pav1209 > Cg, Cm, CTRL, and Mixed; Pav609 > Cg and CTRL; and finally, TI > Cg.

The assessment of Fe/Ca in the right valves (Table 3.22A, B) revealed significant differences between four diet pairs: Baseline vs. CTRL, Baseline vs. TI, ISO vs. CTRL, and Mixed vs. CTRL. The ratios of the shells from the Baseline, ISO, and Mixed treatments were higher than those from CTRL group, and the ratios from the Baseline higher than those from the TI treatment.

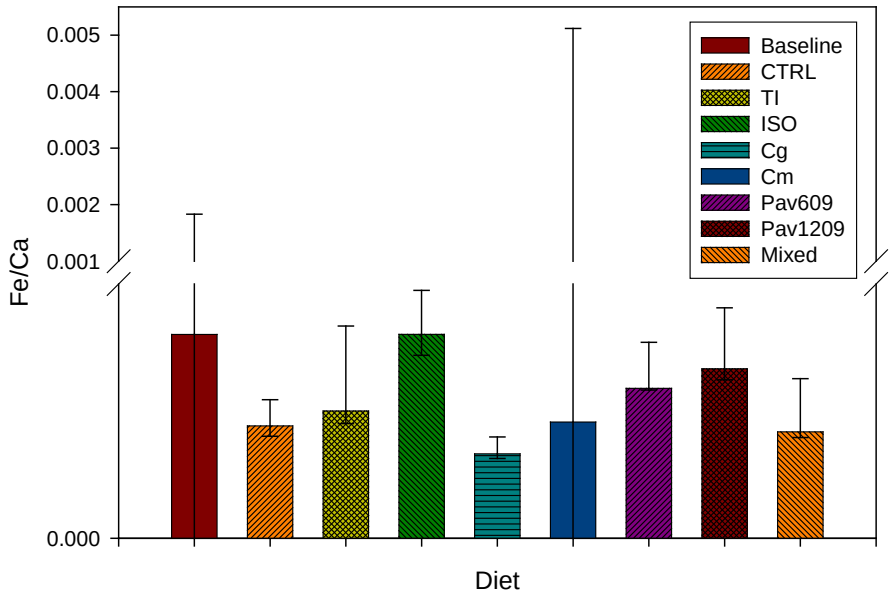


Figure 3.28: Left valve median Fe/Ca values compared to diet. Error bars depict the 95% confidence intervals and diets are represented as previously identified in Figure 3.25.

Table 3.21A: Results of analysis of variance of left valve Fe/Ca among diets. Diets are represented as previously identified in Figure 3.25.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
Baseline	14	1	8.01E-05	5.74E-05	7.70E-04
CTRL	61	1	4.42E-05	2.22E-05	6.41E-05
TI	54	7	5.00E-05	3.85E-05	7.05E-05
ISO	50	2	8.02E-05	5.55E-05	9.67E-05
Cg	58	3	3.32E-05	2.50E-05	4.29E-05
Cm	64	14	4.57E-05	2.84E-05	6.40E-05
Pav609	59	7	5.89E-05	4.75E-05	8.22E-05
Pav1209	52	5	6.66E-05	4.57E-05	9.26E-05
Mixed	53	12	4.18E-05	3.29E-05	5.67E-05
H = 93.0 with 8 degrees of freedom (P = <0.001)					

Table 3.21B: Results of pairwise comparisons of left valve Fe/Ca between diets. Diets are represented as previously identified in Figure 3.25.

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Baseline vs. Cg	189.99	5.161	Yes
Baseline vs. Cm	127.154	3.422	Yes
Baseline vs. CTRL	136.821	3.747	Yes
Baseline vs. Mixed	129.398	3.406	Yes
ISO vs. Cg	177.836	7.543	Yes
ISO vs. Cm	115	4.768	Yes
ISO vs. Ctrl	124.667	5.393	Yes
ISO vs. Mixed	117.244	4.619	Yes
Pav1209 vs. Cg	144.858	6.109	Yes
Pav1209 vs. Cm	82.021	3.382	Yes
Pav1209 vs. CTRL	91.688	3.943	Yes
Pav1209 vs. Mixed	84.265	3.303	Yes
Pav609 vs. Cg	130.375	5.647	Yes
Pav609 vs. CTRL	77.205	3.414	Yes
TI vs. Cg	87.347	3.684	Yes
Baseline vs. ISO	12.154	0.326	No
Baseline vs. Pav1209	45.133	1.207	No
Baseline vs. Pav609	59.615	1.611	No
Baseline vs. TI	102.643	2.744	No

Table 3.21B (Continued)

Comparison	Diff of Ranks	Q	P<0.05
Cm vs. Cg	62.836	2.694	No
Cm vs. CTRL	9.667	0.423	No
Cm vs. Mixed	2.244	0.0892	No
Ctrl vs. Cg	53.17	2.386	No
ISO vs. Pav1209	32.979	1.346	No
ISO vs. Pav609	47.462	1.986	No
ISO vs. TI	90.489	3.694	No
Mixed vs. Cg	60.592	2.46	No
Mixed vs. CTRL	7.423	0.307	No
Pav1209 vs. Pav609	14.483	0.603	No
Pav1209 vs. TI	57.511	2.336	No
Pav609 vs. Cm	67.538	2.857	No
Pav609 vs. Mixed	69.782	2.799	No
Pav609 vs. TI	43.028	1.791	No
TI vs. Cm	24.511	1.011	No
TI vs. Ctrl	34.177	1.47	No
TI vs. Mixed	26.755	1.049	No

Table 3.22A: Results of analysis of variance of right valve Fe/Ca among diets. Diets are represented as previously identified in Figure 3.25.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
Baseline	14	2	9.88E-05	5.51E-05	5.04E-04
CTRL	29	1	2.10E-05	1.67E-05	3.46E-05
TI	17	7	2.49E-05	1.86E-05	4.84E-05
ISO	10	1	7.83E-05	3.86E-05	1.01E-04
Cg	19	1	3.75E-05	2.89E-05	5.45E-05
Cm	27	9	3.73E-05	2.54E-05	6.75E-05
Pav609	19	9	3.18E-05	2.47E-05	5.65E-05
Pav1209	14	1	3.19E-05	2.34E-05	5.03E-05
Mixed	31	11	4.16E-05	2.97E-05	6.51E-05
H = 39.1 with 8 degrees of freedom (P = <0.001)					

Table 3.22B: Results of pairwise comparisons of right valve Fe/Ca between diets. Diets are represented as previously identified in Figure 3.25.

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Baseline vs. CTRL	74.488	5.4	Yes
Baseline vs. TI	65.117	3.804	Yes
ISO vs. CTRL	57.683	3.765	Yes
Mixed vs. CTRL	39.221	3.351	Yes
Baseline vs. Cg	45.75	3.07	No
Baseline vs. Cm	44.194	2.966	No
Baseline vs. ISO	16.806	0.953	No
Baseline vs. Mixed	35.267	2.416	No
Baseline vs. Pav1209	51.532	3.22	No
Baseline vs. Pav609	52.317	3.056	No
Cg vs. CTRL	28.738	2.379	No
Cg vs. Pav1209	5.782	0.397	No
Cg vs. Pav609	6.567	0.416	No
Cg vs. TI	19.367	1.228	No
Cm vs. Cg	1.556	0.117	No
Cm vs. CTRL	30.294	2.508	No
Cm vs. Pav1209	7.338	0.504	No
Cm vs. Pav609	8.122	0.515	No
Cm vs. TI	20.922	1.327	No
ISO vs. Cg	28.944	1.773	No
ISO vs. Cm	27.389	1.678	No
ISO vs. Mixed	18.461	1.15	No
ISO vs. Pav1209	34.726	2.003	No
ISO vs. Pav609	35.511	1.933	No
ISO vs. TI	48.311	2.63	No
Mixed vs. Cg	10.483	0.807	No
Mixed vs. Cm	8.928	0.687	No
Mixed vs. Pav1209	16.265	1.142	No
Mixed vs. Pav609	17.05	1.101	No
Mixed vs. TI	29.85	1.928	No
Pav1209 vs. CTRL	22.956	1.711	No
Pav1209 vs. Pav609	0.785	0.0467	No
Pav1209 vs. TI	13.585	0.808	No
Pav609 vs. CTRL	22.171	1.505	No
Pav609 vs. TI	12.8	0.716	No
TI vs. CTRL	9.371	0.636	No

Comparison of the diet groups with regard to K/Ca in the left valves (Figure 3.29) initially revealed significant differences (P=0.011) within the dataset (Table 3.23A); however, no pairwise comparisons revealed significant differences between the individual diets (Table 3.23B). One comparison, CTRL vs. Baseline, did result in a Difference of Ranks of 111 and associated Q value of 3.04, which was assessed further and the labeled insignificance regarded as questionable. Comparison of diets with regard to K/Ca in the collected right valves did result in two significant pairwise comparisons (Tables 3.24A,B): Baseline > Cg and Pav1209 > Cg.

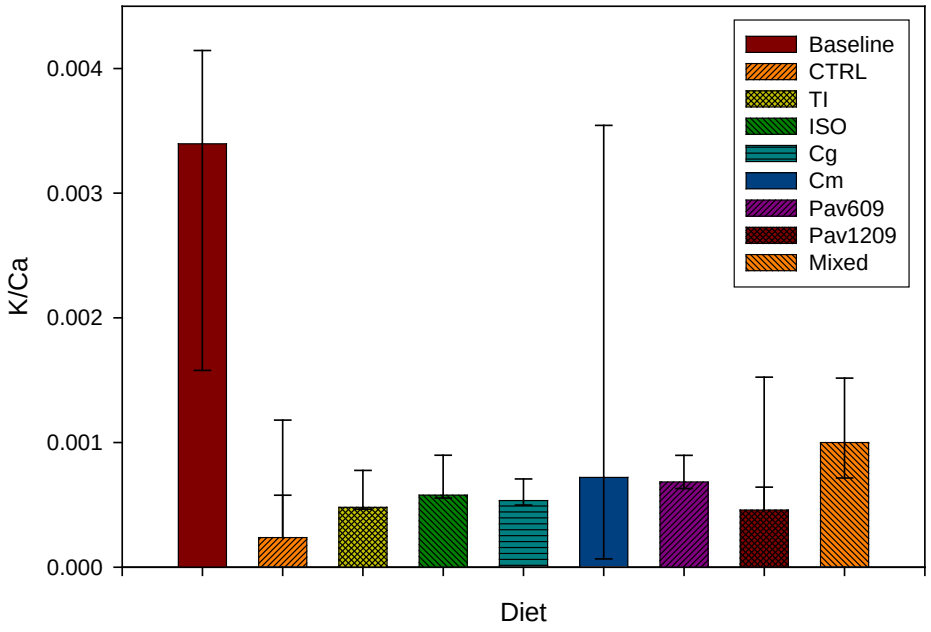


Figure 3.29: Left valve median K/Ca values compared to diet. Error bars depict the 95% confidence intervals and diets are represented as previously identified in Figure 3.25.

Table 3.23A: Results of analysis of variance of left valve K/Ca among diets. Diets are represented as previously identified in Figure 3.25.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
Baseline	14	1	3.40E-03	3.72E-06	4.72E-03
CTRL	61	1	2.38E-04	2.25E-05	1.49E-03
TI	54	13	4.80E-04	3.18E-04	8.12E-04
ISO	50	1	5.78E-04	2.83E-04	8.34E-04
Cg	58	4	5.34E-04	2.85E-04	8.46E-04
Cm	64	7	7.20E-04	3.61E-04	1.27E-03
Pav609	59	5	6.84E-04	4.40E-04	9.13E-04
Pav1209	52	5	4.58E-04	3.33E-04	1.21E-03
Mixed	53	15	1.00E-03	4.29E-04	1.26E-03
H = 19.9 with 8 degrees of freedom (P = 0.011)					

Table 3.23B: Results of pairwise comparisons of left valve K/Ca between diets. Diets are represented as previously identified in Figure 3.25.

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Baseline vs. Cm	45.128	1.23	No
Baseline vs. Pav609	55.499	1.505	No
Baseline vs. Pav1209	69.951	1.87	No
Baseline vs. Mixed	42.462	1.107	No
Baseline vs. CTRL	111.162	3.044	No
Baseline vs. TI	92.535	2.435	No
Baseline vs. ISO	76.686	2.059	No
Baseline vs. Cg	89.295	2.421	No
Cm vs. Pav609	10.37	0.457	No
Cm vs. Pav1209	24.823	1.055	No
Cm vs. CTRL	66.033	2.991	No
Cm vs. TI	47.407	1.939	No
Cm vs. ISO	31.558	1.357	No
Cm vs. Cg	44.167	1.948	No
Pav609 vs. Pav1209	14.452	0.607	No
Pav609 vs. CTRL	55.663	2.486	No
Pav609 vs. TI	37.036	1.498	No
Pav609 vs. ISO	21.187	0.9	No
Pav609 vs. Cg	33.796	1.471	No
Pav1209 vs. CTRL	41.211	1.772	No

Table 3. 23B (Continued)

Comparison	Diff of Ranks	Q	P<0.05
Pav1209 vs. TI	22.584	0.885	No
Pav1209 vs. ISO	6.735	0.276	No
Pav1209 vs. Cg	19.344	0.812	No
Mixed vs. Cm	2.667	0.107	No
Mixed vs. Pav609	13.037	0.516	No
Mixed vs. Pav1209	27.489	1.056	No
Mixed vs. CTRL	68.7	2.776	No
Mixed vs. TI	50.073	1.863	No
Mixed vs. ISO	34.224	1.326	No
Mixed vs. Cg	46.833	1.853	No
TI vs. CTRL	18.627	0.77	No
ISO vs. CTRL	34.476	1.5	No
ISO vs. TI	15.849	0.627	No
ISO vs. Cg	12.609	0.535	No
Cg vs. CTRL	21.867	0.977	No
Cg vs. TI	3.24	0.131	No

Table 3.24A: Results of analysis of variance of right valve K/Ca among diets. Diets are represented as previously identified in Figure 3.25.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
Baseline	14	2	3.57E-03	1.32E-03	4.85E-03
Ctrl	29	2	1.42E-03	1.87E-04	2.41E-03
TI	17	7	1.02E-03	4.79E-04	1.44E-03
ISO	10	2	1.35E-03	9.65E-04	1.54E-03
Cg	19	1	3.35E-04	1.88E-04	1.31E-03
Cm	27	6	7.16E-04	4.16E-04	2.04E-03
Pav(609)	19	6	1.59E-03	8.92E-04	2.03E-03
Pav(1209)	14	1	2.17E-03	1.36E-03	4.01E-03
Mixed	31	10	1.26E-03	8.67E-04	1.52E-03
H = 23.3 with 8 degrees of freedom (P = 0.003)					

Table 3.24B: Results of pairwise comparisons of right valve K/Ca between diets. Diets are represented as previously identified in Figure 3.25.

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Baseline vs. Cg	51.278	3.322	Yes
Pav(1209) vs. Cg	59.406	3.94	Yes
Baseline vs. Cm	33.143	2.211	No
Baseline vs. CTRL	31.148	2.167	No
Baseline vs. ISO	26.333	1.393	No
Baseline vs. Mixed	22.429	1.496	No
Baseline vs. Pav(609)	15.026	0.906	No
Baseline vs. TI	39.133	2.206	No
Cm vs. Cg	18.135	1.363	No
Cm vs. TI	5.99	0.376	No
Ctrl vs. Cg	20.13	1.597	No
Ctrl vs. Cm	1.995	0.165	No
Ctrl vs. TI	7.985	0.521	No
ISO vs. Cg	24.944	1.417	No
ISO vs. Cm	6.81	0.396	No
ISO vs. CTRL	4.815	0.289	No
ISO vs. TI	12.8	0.651	No
Mixed vs. Cg	28.849	2.168	No
Mixed vs. Cm	10.714	0.838	No
Mixed vs. CTRL	8.72	0.723	No
Mixed vs. ISO	3.905	0.227	No
Mixed vs. TI	16.705	1.05	No
Pav1209 vs. Baseline	8.128	0.49	No
Pav1209 vs. Cm	41.271	2.823	No
Pav1209 vs. Ctrl	39.276	2.809	No
Pav1209 vs. ISO	34.462	1.851	No
Pav1209 vs. Mixed	30.557	2.09	No
Pav1209 vs. Pav(609)	23.154	1.425	No
Pav1209 vs. TI	47.262	2.712	No
Pav609) vs. Cg	36.252	2.404	No
Pav609 vs. Cm	18.117	1.239	No
Pav609 vs. Ctrl	16.123	1.153	No
Pav609 vs. ISO	11.308	0.607	No
Pav609 vs. Mixed	7.403	0.506	No
Pav609 vs. TI	24.108	1.384	No
TI vs. Cg	12.144	0.743	No

Figure 3.30 illustrates the left valve median Li/Ca values by diet. Statistical comparison of the diet groups suggests significant differences between 11 of the groups (Tables 3.25A, B): Baseline vs. Cg, Baseline vs. Cm, Baseline vs. CTRL, Baseline vs. ISO, Baseline vs. Mixed, Baseline vs. Pav1209, Baseline vs. Pav609, TI vs. Cg, TI vs. Cm, TI vs. ISO, and TI vs. Pav1209. The determined relationships are as follows: Baseline> Cg, Cm, CTRL, ISO, Mixed, Pav1209, Pav609; TI > Cg, Cm, ISO, and Pav1209.

The statistical comparison of the diet groups with regard to right valve Li/Ca (Tables 3.26A, B) identified six significant differences: Baseline vs. Cg, Baseline vs. Cm, Baseline vs. CTRL, Baseline vs. Mixed, Baseline vs. Pav1209, TI vs. Cg. The relationships established are as follows: Baseline > Cg, Cm, CTRL, Mixed, Pav1209 and TI> Cg.

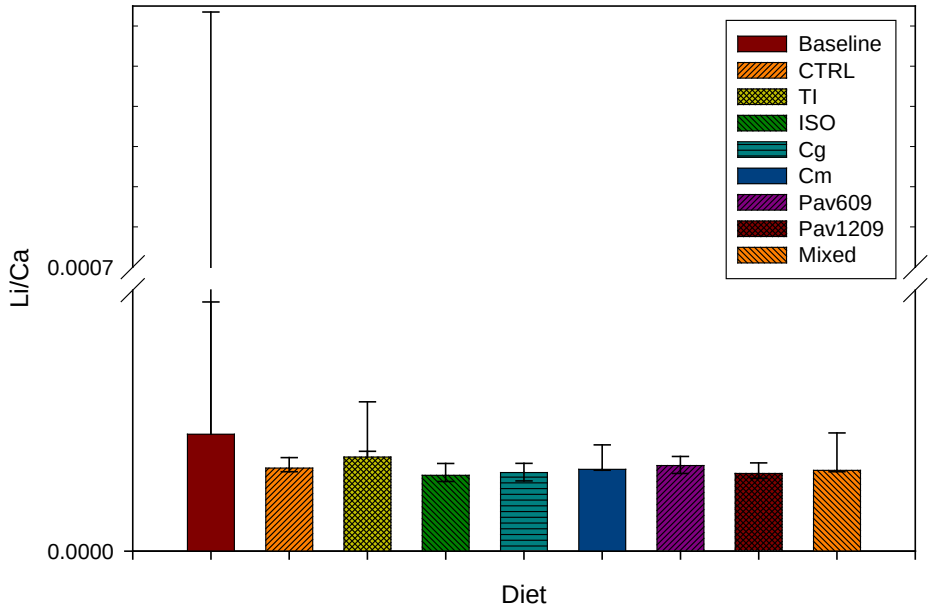


Figure 3.30: Left valve median Li/Ca values compared to diet. Error bars depict the 95% confidence intervals and diets are represented as previously identified in Figure 3.24.

Table 3.25A: Results of analysis of variance of left valve Li/Ca among diets. Diets are represented as previously identified in Figure 3.25.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
Baseline	14	1	4.03E-06	3.86E-06	9.15E-04
Ctrl	61	1	2.86E-06	2.20E-06	3.30E-06
TI	54	12	3.24E-06	2.84E-06	4.19E-06
ISO	50	1	2.61E-06	1.94E-06	3.06E-06
Cg	58	5	2.71E-06	1.95E-06	3.25E-06
Cm	64	8	2.82E-06	2.08E-06	3.32E-06
Pav(609)	59	6	2.95E-06	1.87E-06	3.89E-06
Pav(1209)	52	5	2.68E-06	2.34E-06	3.27E-06
Mixed	53	14	2.78E-06	2.45E-06	3.13E-06
H = 48.1 with 8 degrees of freedom (P = <0.001)					

Table 3.25B: Results of pairwise comparisons of left valve Li/Ca between diets. Diets are represented as previously identified in Figure 3.25.

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Baseline vs. Cg	183.868	4.989	Yes
Baseline vs. Cm	160.536	4.379	Yes
Baseline vs. CTRL	154.383	4.238	Yes
Baseline vs. ISO	196.204	5.281	Yes
Baseline vs. Mixed	157.692	4.135	Yes
Baseline vs. Pav1209	171.617	4.599	Yes
Baseline vs. Pav609	158.566	4.302	Yes
TI vs. Cg	102.392	4.162	Yes
TI vs. Cm	79.06	3.253	Yes
TI vs. ISO	114.728	4.582	Yes
TI vs. Pav1209	90.141	3.565	Yes
Baseline vs. TI	81.476	2.156	No
Cg vs. ISO	12.336	0.523	No
Cm vs. Cg	23.332	1.022	No
Cm vs. ISO	35.668	1.531	No
Cm vs. Pav1209	11.081	0.47	No
CTRL vs. Cg	29.485	1.314	No
CTRL vs. Cm	6.152	0.278	No
CTRL vs. ISO	41.821	1.824	No
CTRL vs. Mixed	3.309	0.135	No

Table 3.25B (Continued)

Comparison	Diff of Ranks	Q	P<0.05
CTRL vs. Pav1209	17.234	0.743	No
CTRL vs. Pav609	4.183	0.186	No
Mixed vs. Cg	26.176	1.042	No
Mixed vs. Cm	2.843	0.114	No
Mixed vs. ISO	38.512	1.507	No
Mixed vs. Pav1209	13.925	0.54	No
Mixed vs. Pav609	0.874	0.0348	No
Pav1209 vs. Cg	12.251	0.513	No
Pav1209 vs. ISO	24.587	1.011	No
Pav609 vs. Cg	25.302	1.094	No
Pav609 vs. Cm	1.97	0.0863	No
Pav609 vs. ISO	37.638	1.595	No
Pav609 vs. Pav1209	13.051	0.547	No
TI vs. CTRL	72.907	3.043	No
TI vs. Mixed	76.216	2.878	No
TI vs. Pav609	77.09	3.134	No

Table 3.26A: Results of analysis of variance of right valve Li/Ca among diets. Diets are represented as previously identified in Figure 3.25.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
Baseline	14	2	3.96E-06	3.80E-06	4.71E-04
CTRL	29	2	2.88E-06	2.33E-06	3.68E-06
TI	17	7	5.72E-06	2.79E-06	7.15E-06
ISO	10	2	3.09E-06	3.02E-06	3.65E-06
Cg	19	1	2.40E-06	1.16E-06	3.11E-06
Cm	27	9	2.62E-06	2.55E-06	3.03E-06
Pav609	19	7	2.97E-06	2.56E-06	3.21E-06
Pav1209	14	1	2.79E-06	2.47E-06	3.26E-06
Mixed	31	13	2.76E-06	2.50E-06	2.83E-06
H = 41.0 with 8 degrees of freedom (P = <0.001)					

Table 3.26B: Results of pairwise comparisons of right valve Li/Ca between diets. Diets are represented as previously identified in Figure 3.25.

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Baseline vs. Cg	74.306	5.06	Yes
Baseline vs. Cm	64.25	4.375	Yes
Baseline vs. CTRL	50.398	3.687	Yes
Baseline vs. Mixed	67.528	4.598	Yes
Baseline vs. Pav1209	57.122	3.621	Yes
TI vs. Cg	54.622	3.515	Yes
Baseline vs. ISO	27.708	1.541	No
Baseline vs. Pav609	48.333	3.005	No
Baseline vs. TI	19.683	1.167	No
Cm vs. Cg	10.056	0.766	No
Cm vs. Mixed	3.278	0.25	No
CTRL vs. Cg	23.907	1.994	No
CTRL vs. Cm	13.852	1.155	No
CTRL vs. Mixed	17.13	1.429	No
CTRL vs. Pav1209	6.724	0.505	No
ISO vs. Cg	46.597	2.783	No
ISO vs. Cm	36.542	2.182	No
ISO vs. Ctrl	22.69	1.43	No
ISO vs. Mixed	39.819	2.378	No
ISO vs. Pav1209	29.413	1.661	No
ISO vs. Pav609	20.625	1.147	No
Mixed vs. Cg	6.778	0.516	No
Pav1209 vs. Cg	17.184	1.198	No
Pav1209 vs. Cm	7.128	0.497	No
Pav1209 vs. Mixed	10.406	0.726	No
Pav609 vs. Cg	25.972	1.769	No
Pav609 vs. Cm	15.917	1.084	No
Pav609 vs. CTRL	2.065	0.151	No
Pav609 vs. Mixed	19.194	1.307	No
Pav609 vs. Pav1209	8.788	0.557	No
TI vs. Cm	44.567	2.868	No
TI vs. CTRL	30.715	2.106	No
TI vs. ISO	8.025	0.429	No
TI vs. Mixed	47.844	3.079	No
TI vs. Pav1209	37.438	2.259	No
TI vs. Pav609	28.65	1.698	No

Comparison of left valve Mg/Ca between experimental diets (Tables 3.27A, B) revealed no significant differences, probably as a consequence of high variability (Figure 3.31). Comparison of right valve Mg/Ca with regard to diet (Tables 3.28A, B) revealed significant differences between three of the diets: CTRL < Baseline, CTRL < Cg, and CTRL < Mixed.

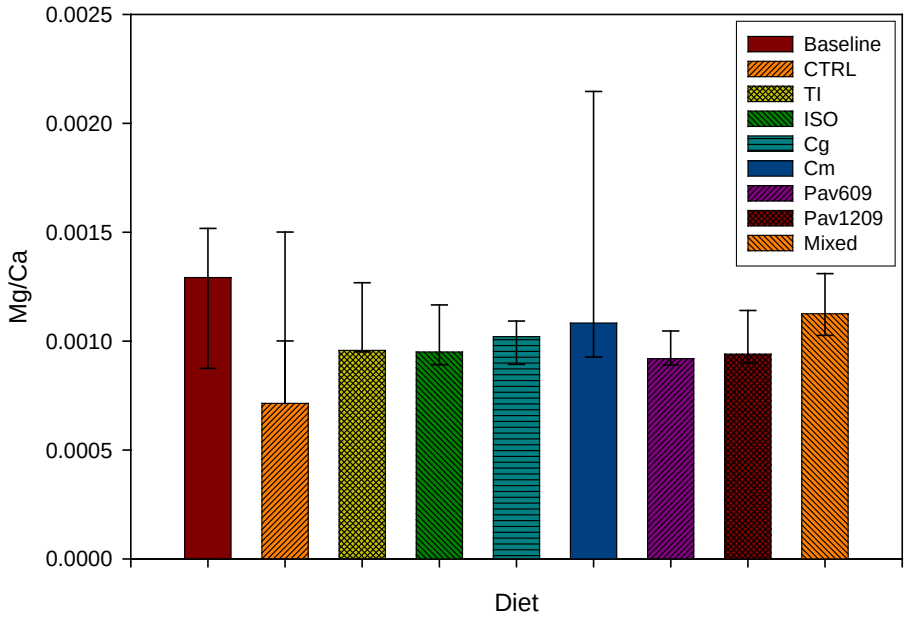


Figure 3.31: Left valve median Mg/Ca values compared to diet. Error bars depict the 95% confidence intervals and diets are represented as previously identified in Figure 3.25.

Table 3.27A: Results of analysis of variance of left valve Mg/Ca among diets. Diets are represented as previously identified in Figure 3.25.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
Baseline	12	2	1.29E-03	1.13E-03	1.43E-03
CTRL	61	1	7.14E-04	5.33E-04	1.80E-03
TI	54	6	9.57E-04	8.38E-04	1.16E-03
ISO	50	1	9.50E-04	7.58E-04	1.17E-03

Table 3.27A (Continued)

Group	N	Missing	Median	25%	75%
Cg	58	3	1.02E-03	6.92E-04	1.20E-03
Cm	64	7	1.08E-03	8.40E-04	1.57E-03
Pav609	59	6	9.20E-04	7.60E-04	1.07E-03
Pav1209	52	3	9.41E-04	7.54E-04	1.15E-03
Mixed	53	12	1.13E-03	8.10E-04	1.40E-03
H = 20.0 with 8 degrees of freedom (P = 0.010)					

Table 3.27B: Results of pairwise comparisons of left valve Mg/Ca between diets. Diets are represented as previously identified in Figure 3.25.

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Baseline vs. Cg	89.582	2.137	No
Baseline vs. Cm	34.109	0.816	No
Baseline vs. CTRL	105.983	2.544	No
Baseline vs. ISO	90.294	2.134	No
Baseline vs. Mixed	44.21	1.028	No
Baseline vs. Pav1209	86.784	2.051	No
Baseline vs. Pav609	94.826	2.255	No
Baseline vs. TI	78.975	1.863	No
Cg vs. CTRL	16.402	0.72	No
Cg vs. ISO	0.712	0.0297	No
Cg vs. Pav609	5.245	0.223	No
Cm vs. Cg	55.473	2.406	No
Cm vs. CTRL	71.875	3.186	No
Cm vs. ISO	56.185	2.365	No
Cm vs. Mixed	10.101	0.404	No
Cm vs. Pav1209	52.675	2.217	No
Cm vs. Pav609	60.718	2.609	No
Cm vs. TI	44.866	1.878	No
ISO vs. CTRL	15.689	0.668	No
ISO vs. Pav609	4.533	0.188	No
Mixed vs. Cg	45.372	1.803	No
Mixed vs. CTRL	61.774	2.5	No
Mixed vs. ISO	46.084	1.785	No
Mixed vs. Pav1209	42.574	1.649	No
Mixed vs. Pav609	50.617	1.995	No
Mixed vs. TI	34.765	1.34	No
Pav1209 vs. Cg	2.798	0.117	No
Pav1209 vs. CTRL	19.2	0.818	No

Table 3.27B (Continued)

Comparison	Diff of Ranks	Q	P<0.05
Pav1209 vs. ISO	3.51	0.142	No
Pav1209 vs. Pav609	8.043	0.333	No
Pav609 vs. CTRL	11.157	0.485	No
TI vs. Cg	10.607	0.44	No
TI vs. CTRL	27.008	1.144	No
TI vs. ISO	11.319	0.457	No
TI vs. Pav1209	7.809	0.315	No
TI vs. Pav609	15.851	0.652	No

Table 3.28A: Results of analysis of variance of right valve Mg/Ca among diets. Diets are represented as previously identified in Figure 3.25.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
Baseline	14	4	1.30E-03	1.14E-03	1.47E-03
CTRL	29	1	1.90E-03	1.69E-03	2.71E-03
TI	17	4	1.69E-03	1.30E-03	2.26E-03
ISO	10	2	1.54E-03	1.30E-03	1.78E-03
Cg	19	1	1.30E-03	1.07E-03	1.58E-03
Cm	27	6	1.72E-03	1.41E-03	2.13E-03
Pav609	19	6	1.50E-03	1.24E-03	1.75E-03
Pav1209	14	1	1.58E-03	1.23E-03	1.68E-03
Mixed	31	10	1.26E-03	1.15E-03	1.52E-03
H = 27.5 with 8 degrees of freedom (P = <0.001)					

Table 3.28B: Results of pairwise comparisons of right valve Mg/Ca between diets. Diets are represented as previously identified in Figure 3.25.

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
CTRL vs. Baseline	51.864	3.352	Yes
CTRL vs. Cg	46.575	3.67	Yes
CTRL vs. Mixed	48.036	3.962	Yes
Cg vs. Baseline	5.289	0.319	No
Cg vs. Mixed	1.46	0.108	No
Cm vs. Baseline	37.543	2.326	No
Cm vs. Cg	32.254	2.391	No
Cm vs. ISO	10.893	0.624	No
Cm vs. Mixed	33.714	2.601	No

Table 3.28B (Continued)

Comparison	Diff of Ranks	Q	P<0.05
Cm vs. Pav1209	15.297	1.032	No
Cm vs. Pav609	16.374	1.105	No
CTRL vs. Cm	14.321	1.181	No
CTRL vs. ISO	25.214	1.497	No
CTRL vs. Pav1209	29.618	2.101	No
CTRL vs. Pav609	30.695	2.177	No
CTRL vs. TI	14.234	1.01	No
ISO vs. Baseline	26.65	1.338	No
ISO vs. Cg	21.361	1.197	No
ISO vs. Mixed	22.821	1.308	No
ISO vs. Pav1209	4.404	0.233	No
ISO vs. Pav609	5.481	0.29	No
Mixed vs. Baseline	3.829	0.237	No
Pav1209 vs. Baseline	22.246	1.259	No
Pav1209 vs. Cg	16.957	1.109	No
Pav1209 vs. Mixed	18.418	1.243	No
Pav1209 vs. Pav(609)	1.077	0.0654	No
Pav609 vs. Baseline	21.169	1.198	No
Pav609 vs. Cg	15.88	1.039	No
Pav609 vs. Mixed	17.341	1.17	No
TI vs. Baseline	37.631	2.13	No
TI vs. Cg	32.342	2.116	No
TI vs. Cm	0.0879	0.00593	No
TI vs. ISO	10.981	0.582	No
TI vs. Mixed	33.802	2.28	No
TI vs. Pav1209	15.385	0.934	No
TI vs. Pav609	16.462	0.999	No

The Mn/Ca median values of the collected left valves for each diet group are depicted in Figure 3.32. Fifteen of the diet group comparisons were significant (Tables 3.29A, B) with regard to Mn/Ca associated with the collected left valves - Baseline vs. CTRL, Cg vs. CTRL, Cm vs. CTRL, ISO vs. Cg, ISO vs. Cm, ISO vs. CTRL, Mixed vs. Cg, Mixed vs. Ctrl, Pav1209 vs. Cg, Pav1209 vs. Ctrl, Pav609 vs. Cg, Pav609 vs. CTRL, TI vs. Cg, TI vs. Cm, TI vs. CTRL. The relationships determined are as follows:

CTRL< Baseline, Cg, Cm, ISO, Mixed, Pav1209, Pav609, TI; Cg< ISO, Mixed, Pav1209, Pav609, TI; Cm<ISO, TI.

Comparison of the right valve Mn/Ca between diet groups revealed 12 significant differences (Tables 3.30A, B): Baseline vs. Cg, Baseline vs. CTRL, Cm vs. Cg, Cm vs. CTRL, ISO vs. Cg, ISO vs. CTRL, Mixed vs. Cg, Mixed vs. CTRL, Mixed vs. Pav 609, Mixed vs. Pav1209, TI vs. Cg, TI vs. CTRL. The subsequent ranking of these diet comparisons is as follows: Cg < Baseline, Cm, ISO, Mixed, TI; CTRL < Baseline, Cm, ISO, Mixed, TI; Mixed> Pav609, Pav1209.

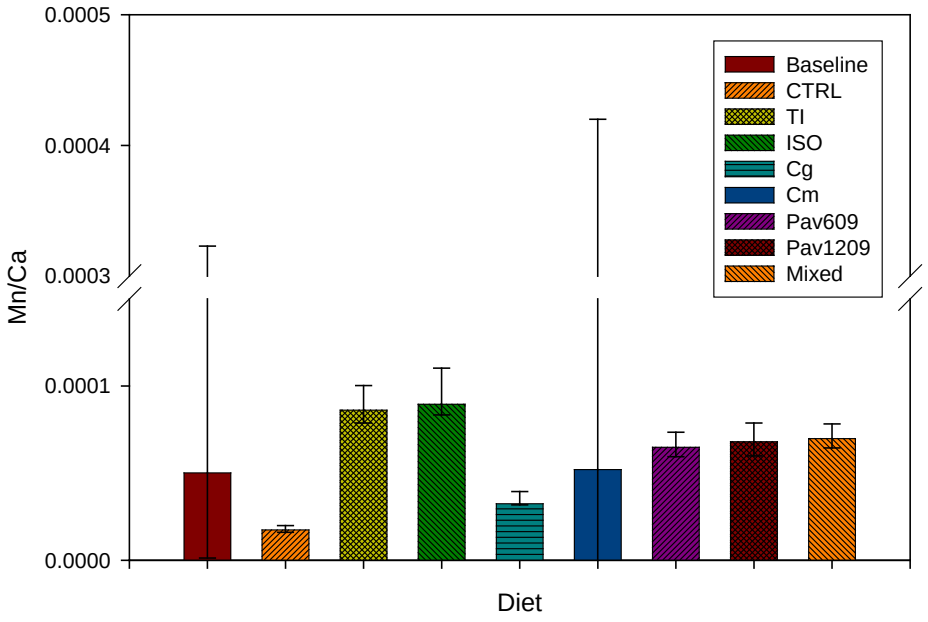


Figure 3.32: Left valve median Mn/Ca values compared to diet. Error bars depict the 95% confidence intervals and diets are represented as previously identified in Figure 3.25.

Table 3.29A: Results of analysis of variance of left valve Mn/Ca among diets. Diets are represented as previously identified in Figure 3.25.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
Baseline	14	2	5.01E-05	3.18E-05	2.00E-04
CTRL	61	1	1.74E-05	1.20E-05	2.21E-05
TI	54	5	8.61E-05	6.26E-05	1.13E-04
ISO	50	1	8.96E-05	6.89E-05	1.11E-04
Cg	58	3	3.25E-05	2.71E-05	4.02E-05
Cm	64	7	5.20E-05	4.13E-05	7.28E-05
Pav609	59	6	6.48E-05	5.15E-05	7.64E-05
Pav1209	52	4	6.80E-05	5.04E-05	8.40E-05
Mixed	53	11	6.98E-05	5.68E-05	8.27E-05
H = 231 with 8 degrees of freedom (P = <0.001)					

Table 3.29B: Results of pairwise comparisons of left valve Mn/Ca between diets. Diets are represented as previously identified in Figure 3.25.

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Baseline vs. CTRL	180.983	4.659	Yes
Cg vs. CTRL	79.458	3.465	Yes
Cm vs. CTRL	176.584	7.773	Yes
ISO vs. Cg	199.64	8.274	Yes
ISO vs. Cm	102.513	4.284	Yes
ISO vs. CTRL	279.097	11.801	Yes
Mixed vs. Cg	147.371	5.855	Yes
Mixed vs. CTRL	226.829	9.179	Yes
Pav1209 vs. Cg	130.213	5.367	Yes
Pav1209 vs. CTRL	209.671	8.815	Yes
Pav609 vs. Cg	125.581	5.312	Yes
Pav609 vs. CTRL	205.038	8.855	Yes
TI vs. Cg	186.354	7.723	Yes
TI vs. Cm	89.227	3.729	Yes
TI vs. Ctrl	265.812	11.239	Yes
Baseline vs. Cg	101.526	2.594	No
Baseline vs. Cm	4.399	0.113	No
Cm vs. Cg	97.127	4.183	No
ISO vs. Baseline	98.114	2.48	No
ISO vs. Mixed	52.269	2.024	No

Table 3.29B (Continued)

Comparison	Diff of Ranks	Q	P<0.05
ISO vs. Pav1209	69.426	2.783	No
ISO vs. Pav609	74.059	3.042	No
ISO vs. TI	13.286	0.535	No
Mixed vs. Baseline	45.845	1.14	No
Mixed vs. Cm	50.244	2.012	No
Mixed vs. Pav1209	17.158	0.661	No
Mixed vs. Pav609	21.79	0.859	No
Pav1209 vs. Baseline	28.688	0.724	No
Pav1209 vs. Cm	33.087	1.375	No
Pav1209 vs. Pav609	4.632	0.189	No
Pav609 vs. Baseline	24.055	0.613	No
Pav609 vs. Cm	28.454	1.214	No
TI vs. Baseline	84.828	2.144	No
TI vs. Mixed	38.983	1.509	No
TI vs. Pav1209	56.141	2.251	No
TI vs. Pav609	60.773	2.497	No

Table 3.30A: Results of analysis of variance of right valve Mn/Ca among diets. Diets are represented as previously identified in Figure 3.25.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
Baseline	14	2	5.43E-05	3.56E-05	2.32E-04
Ctrl	29	1	1.83E-05	1.23E-05	2.49E-05
TI	17	4	5.60E-05	4.94E-05	7.79E-05
ISO	10	1	6.15E-05	4.54E-05	6.95E-05
Cg	19	1	2.46E-05	1.97E-05	3.16E-05
Cm	27	8	5.04E-05	3.63E-05	6.00E-05
Pav (609)	19	7	3.80E-05	2.89E-05	4.73E-05
Pav(1209)	14	1	3.51E-05	2.76E-05	4.47E-05
Mixed	31	11	6.67E-05	6.22E-05	6.99E-05
H = 91.5 with 8 degrees of freedom (P = <0.001)					

Table 3.30B: Results of pairwise comparisons of right valve Mn/Ca between diets. Diets are represented as previously identified in Figure 3.25.

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Baseline vs. Cg	54.694	3.518	Yes
Baseline vs. CTRL	71.738	4.984	Yes
Cm vs. Cg	48.83	3.559	Yes
Cm vs. CTRL	65.874	5.313	Yes
ISO vs. Cg	60.333	3.543	Yes
ISO vs. Ctrl	77.377	4.841	Yes
Mixed vs. Cg	74.878	5.525	Yes
Mixed vs. CTRL	91.921	7.527	Yes
Mixed vs. Pav609	49.433	3.245	Yes
Mixed vs. Pav1209	50.638	3.407	Yes
TI vs. Cg	67.701	4.459	Yes
TI vs. Ctrl	84.745	6.053	Yes
Baseline vs. Cm	5.864	0.381	No
Baseline vs. Pav609	29.25	1.718	No
Baseline vs. Pav1209	30.455	1.824	No
Cg vs. CTRL	17.044	1.352	No
Cm vs. Pav 609	23.386	1.52	No
Cm vs. Pav1209	24.591	1.638	No
ISO vs. Baseline	5.639	0.307	No
ISO vs. Cm	11.503	0.681	No
ISO vs. Pav609	34.889	1.897	No
ISO vs. Pav1209	36.094	1.995	No
Mixed vs. Baseline	20.183	1.325	No
Mixed vs. Cm	26.047	1.949	No
Mixed vs. ISO	14.544	0.869	No
Mixed vs. TI	7.177	0.483	No
Pav609 vs. Cg	25.444	1.637	No
Pav609 vs. CTRL	42.488	2.952	No
Pav609 vs. Pav1209	1.205	0.0722	No
Pav1209 vs. Cg	24.239	1.597	No
Pav1209 vs. CTRL	41.283	2.949	No
TI vs. Baseline	13.006	0.779	No
TI vs. Cm	18.87	1.257	No
TI vs. ISO	7.368	0.407	No
TI vs. Pav609	42.256	2.531	No
TI vs. Pav1209	43.462	2.656	No

The median P/Ca values for the collected left valves and associated diet are depicted in Figure 3.33. No baseline data are available for this elemental ratio. Ten diet comparisons with regard to P/Ca were determined significant (Tables 3.31A, B): ISO vs. Cg, ISO vs. Cm, ISO vs. CTRL, ISO vs. Mixed, ISO vs. Pav1209, ISO vs. Pav609, ISO vs. TI, TI vs. Cg, TI vs. Cm, and TI vs. Mixed. The subsequent ranking of these diet groups is as follows: ISO> Cg, Cm, CTRL, Mixed, Pav1209, Pav609, TI and TI> Cg, Cm and Mixed.

Analysis of variance related to the right valve P/Ca per diet also identified significant differences within the dataset (P=0.006, Table 3.32A). The post hoc pairwise analysis, however, revealed no significant difference between any of the individual diet groups (Table 3.32B).

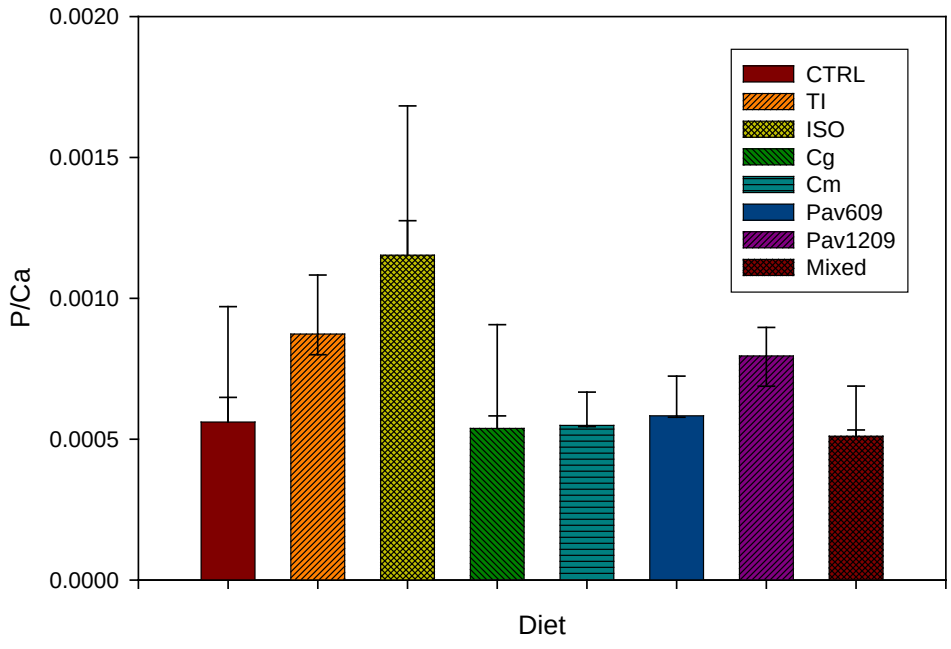


Figure 3.33: Left valve median P/Ca values compared to diet. Error bars depict the 95% confidence intervals and diets are represented as previously identified in Figure 3.25.

Table 3.31A: Results of analysis of variance of left valve P/Ca among diets. Diets are represented as previously identified in Figure 3.25.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
CTRL	61	6	5.61E-04	3.02E-04	1.37E-03
TI	53	13	8.73E-04	6.10E-04	1.15E-03
ISO	50	7	1.15E-03	9.35E-04	2.09E-03
Cg	57	10	5.38E-04	3.62E-04	8.93E-04
Cm	62	18	5.49E-04	4.80E-04	6.66E-04
Pav609	58	18	5.83E-04	4.97E-04	7.82E-04
Pav1209	52	12	7.95E-04	5.63E-04	9.05E-04
Mixed	53	12	5.11E-04	4.41E-04	7.38E-04
H = 81.7 with 7 degrees of freedom (P = <0.001)					

Table 3.31B: Results of pairwise comparisons of left valve P/Ca between diets. Diets are represented as previously identified in Figure 3.25.

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
ISO vs. Cg	142.666	6.682	Yes
ISO vs. Cm	150.024	6.915	Yes
ISO vs. Ctrl	133.924	6.502	Yes
ISO vs. Mixed	153.753	6.962	Yes
ISO vs. Pav1209	96.195	4.328	Yes
ISO vs. Pav609	133.42	6.003	Yes
ISO vs. TI	72.22	3.249	Yes
TI vs. Cg	70.446	3.237	Yes
TI vs. Cm	77.805	3.52	Yes
TI vs. Mixed	81.533	3.626	Yes
Cg vs. Cm	7.359	0.347	No
Cg vs. Mixed	11.087	0.513	No
Cm vs. Mixed	3.728	0.17	No
CTRL vs. Cg	8.741	0.435	No
CTRL vs. Cm	16.1	0.787	No
CTRL vs. Mixed	19.828	0.95	No
Pav1209 vs. Cg	46.471	2.135	No
Pav1209 vs. Cm	53.83	2.435	No
Pav1209 vs. Ctrl	37.73	1.794	No
Pav1209 vs. Mixed	57.558	2.56	No
Pav1209 vs. Pav609	37.225	1.645	No

Table 3.31B (Continued)

Comparison	Diff of Ranks	Q	P<0.05
Pav609 vs. Cg	9.246	0.425	No
Pav609 vs. Cm	16.605	0.751	No
Pav609 vs. Ctrl	0.505	0.024	No
Pav609 vs. Mixed	20.333	0.904	No
TI vs. Ctrl	61.705	2.935	No
TI vs. Pav1209	23.975	1.06	No
TI vs. Pav609	61.2	2.705	No

Table 3.32A: Results of analysis of variance of right valve P/Ca among diets. Diets are represented as previously identified in Figure 3.25.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
CTRL	26	1	8.23E-04	6.38E-04	1.39E-03
TI	17	7	8.75E-04	4.76E-04	1.56E-03
ISO	10	1	7.96E-04	6.94E-04	1.33E-03
Cg	19	1	9.91E-04	6.06E-04	1.88E-03
Cm	27	9	5.60E-04	4.65E-04	6.12E-04
Pav609	19	9	6.51E-04	4.49E-04	8.70E-04
Pav1209	14	1	9.23E-04	6.63E-04	1.10E-03
Mixed	31	11	6.03E-04	4.66E-04	9.19E-04
H = 20.0 with 7 degrees of freedom (P = 0.006)					

Table 3.32B: Results of pairwise comparisons of right valve P/Ca between diets. Diets are represented as previously identified in Figure 3.25.

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
CTRL vs. Cm	33.791	3.066	No
CTRL vs. Mixed	24.73	2.312	No
CTRL vs. Pav609	24.98	1.873	No
CTRL vs. TI	4.48	0.336	No
Cg vs. CTRL	3.042	0.276	No
Cg vs. Cm	36.833	3.099	No
Cg vs. ISO	0.278	0.0191	No
Cg vs. Mixed	27.772	2.398	No
Cg vs. Pav609	28.022	1.993	No
Cg vs. Pav1209	2.722	0.21	No

Table 3.32B (Continued)

Comparison	Diff of Ranks	Q	P<0.05
Cg vs. TI	7.522	0.535	No
ISO vs. CTRL	2.764	0.199	No
ISO vs. Cm	36.556	2.512	No
ISO vs. Mixed	27.494	1.921	No
ISO vs. Pav609	27.744	1.694	No
ISO vs. Pav1209	2.444	0.158	No
ISO vs. TI	7.244	0.442	No
Mixed vs. Cm	9.061	0.782	No
Mixed vs. Pav609	0.25	0.0181	No
Pav609 vs. Cm	8.811	0.627	No
Pav1209 vs. CTRL	0.32	0.0262	No
Pav1209 vs. Cm	34.111	2.629	No
Pav1209 vs. Mixed	25.05	1.972	No
Pav1209 vs. Pav609	25.3	1.687	No
Pav1209 vs. TI	4.8	0.32	No
TI vs. Cm	29.311	2.085	No
TI vs. Mixed	20.25	1.467	No
TI vs. Pav609	20.5	1.286	No

The median Si/Ca values by diet for the left valves collected are depicted in Figure 3.34. Significant differences were revealed in ten diet group comparisons with regard to left valve Si/Ca (Tables 3.33A, B), and the following ranks established: Mixed < Baseline, CTRL, ISO, Pav1209, Pav609, TI; Pav1209 > Cg, Cm; Pav609 > Cg, Cm.

The comparisons of right valves collected with regard to Si/Ca revealed significant differences in nine comparisons (Tables 3.34A, B): Baseline vs. Cg, Baseline vs. Cm, Baseline vs. Mixed, Baseline vs. Pav609, Baseline vs. Pav1209, Ctrl vs. Cg, CTRL vs. Mixed, CTRL vs. Pav609, and CTRL vs. Pav1209. Based on these assessments, the subsequent rankings of the diet groups are as follows: Baseline > Cg, Cm, Mixed, Pav609, Pav1209 and CTRL > Cg, Mixed, Pav609, and Pav1209.

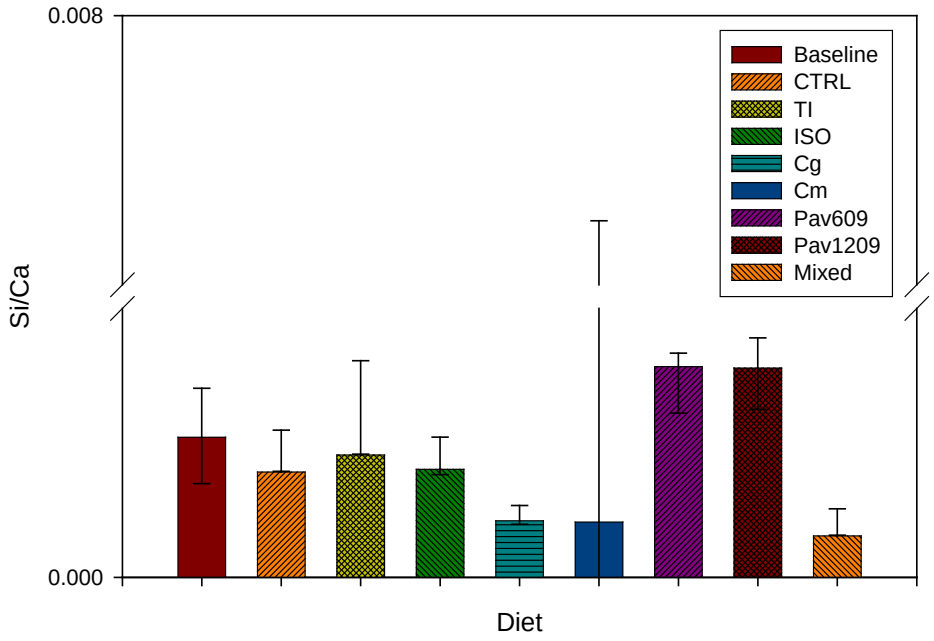


Figure 3.34: Left valve median Si/Ca values compared to diet. Error bars depict the 95% confidence intervals and diets are represented as previously identified in Figure 3.25.

Table 3.33A: Results of analysis of variance of left valve Si/Ca among diets. Diets are represented as previously identified in Figure 3.25.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
Baseline	10	1	4.17E-04	2.93E-04	5.26E-04
CTRL	61	1	3.13E-04	1.66E-04	5.09E-04
TI	54	7	3.64E-04	2.28E-04	7.26E-04
ISO	50	1	3.21E-04	2.13E-04	4.98E-04
Cg	58	3	1.68E-04	1.24E-04	2.36E-04
Cm	64	8	1.64E-04	9.96E-05	2.69E-04
Pav609	59	7	6.27E-04	4.74E-04	7.29E-04
Pav1209	52	4	6.22E-04	4.17E-04	8.29E-04
Mixed	53	12	1.23E-04	9.46E-05	1.95E-04
H = 109 with 8 degrees of freedom (P = <0.001)					

Table 3.33B: Results of pairwise comparisons of left valve Si/Ca between diets. Diets are represented as previously identified in Figure 3.25.

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Baseline vs. Mixed	146.965	3.313	Yes
CTRL vs. Mixed	113.37	4.642	Yes
ISO vs. Mixed	111.609	4.375	Yes
Pav1209 vs. Cg	149.294	6.271	Yes
Pav1209 vs. Cm	137.324	5.793	Yes
Pav1209 vs. Mixed	171.874	6.706	Yes
Pav609 vs. Cg	148.677	6.378	Yes
Pav609 vs. Cm	136.707	5.89	Yes
Pav609 vs. Mixed	171.258	6.804	Yes
TI vs. Mixed	137.492	5.338	Yes
Baseline vs. Cg	124.384	2.87	No
Baseline vs. Cm	112.415	2.597	No
Baseline vs. CTRL	33.594	0.78	No
Baseline vs. ISO	35.356	0.809	No
Baseline vs. TI	9.473	0.216	No
Cg vs. Mixed	22.581	0.908	No
Cm vs. Cg	11.969	0.523	No
Cm vs. Mixed	34.55	1.395	No
CTRL vs. Cg	90.789	4.035	No
CTRL vs. Cm	78.82	3.52	No
CTRL vs. ISO	1.762	0.0759	No
ISO vs. Cg	89.028	3.76	No
ISO vs. Cm	77.059	3.269	No
Pav1209 vs. Baseline	24.91	0.569	No
Pav1209 vs. CTRL	58.504	2.507	No
Pav1209 vs. ISO	60.266	2.462	No
Pav1209 vs. Pav609	0.617	0.0256	No
Pav1209 vs. TI	34.383	1.39	No
Pav609 vs. Baseline	24.293	0.558	No
Pav609 vs. CTRL	57.887	2.535	No
Pav609 vs. ISO	59.649	2.486	No
Pav609 vs. TI	33.766	1.392	No
TI vs. Cg	114.911	4.8	No
TI vs. Cm	102.942	4.318	No
TI vs. CTRL	24.122	1.027	No
TI vs. ISO	25.883	1.052	No

Table 3.34A: Results of analysis of variance of right valve Si/Ca among diets. Diets are represented as previously identified in Figure 3.25.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
Baseline	10	1	3.68E-04	2.94E-04	5.58E-04
CTRL	29	1	2.03E-04	1.76E-04	3.10E-04
TI	17	8	1.50E-04	1.11E-04	1.90E-04
ISO	10	1	1.40E-04	1.26E-04	2.56E-04
Cg	19	1	7.78E-05	5.92E-05	1.72E-04
Cm	27	9	8.49E-05	5.32E-05	2.90E-04
Pav 609	19	9	3.34E-05	9.35E-06	1.18E-04
Pav1209	14	1	3.39E-05	2.02E-05	1.05E-04
Mixed	31	11	1.06E-04	7.17E-05	1.32E-04
H = 46.9 with 8 degrees of freedom (P = <0.001)					

Table 3.34B: Results of pairwise comparisons of right valve Si/Ca between diets. Diets are represented as previously identified in Figure 3.25.

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Baseline vs. Cg	59.833	3.775	Yes
Baseline vs. Cm	54.778	3.456	Yes
Baseline vs. Mixed	58.456	3.751	Yes
Baseline vs. Pav609	77.056	4.319	Yes
Baseline vs. Pav1209	79.786	4.739	Yes
CTRL vs. Cg	39.385	3.358	Yes
CTRL vs. Mixed	38.007	3.344	Yes
CTRL vs. Pav609	56.607	3.958	Yes
CTRL vs. Pav1209	59.338	4.554	Yes
Baseline vs. CTRL	20.448	1.374	No
Baseline vs. ISO	36.778	2.009	No
Baseline vs. TI	40.222	2.198	No
Cg vs. Pav609	17.222	1.125	No
Cg vs. Pav1209	19.953	1.412	No
Cm vs. Cg	5.056	0.391	No
Cm vs. Mixed	3.678	0.292	No
Cm vs. Pav609	22.278	1.455	No
Cm vs. Pav1209	25.009	1.77	No
CTRL vs. Cm	34.329	2.927	No
CTRL vs. ISO	16.329	1.098	No

Table 3.34B (Continued)

Comparison	Diff of Ranks	Q	P<0.05
CTRL vs. TI	19.774	1.329	No
ISO vs. Cg	23.056	1.455	No
ISO vs. Cm	18	1.136	No
ISO vs. Mixed	21.678	1.391	No
ISO vs. Pav609	40.278	2.258	No
ISO vs. Pav1209	43.009	2.555	No
ISO vs. TI	3.444	0.188	No
Mixed vs. Cg	1.378	0.109	No
Mixed vs. Pav609	18.6	1.237	No
Mixed vs. Pav1209	21.331	1.542	No
Pav 609 vs. Pav1209	2.731	0.167	No
TI vs. Cg	19.611	1.237	No
TI vs. Cm	14.556	0.918	No
TI vs. Mixed	18.233	1.17	No
TI vs. Pav609	36.833	2.065	No
TI vs. Pav1209	39.564	2.35	No

Again there was no baseline data for the examination of Sr enrichment. With regard to comparison of Sr/Ca in the left valves (Figure 3.35), significant differences were revealed to exist between four diet groupings: Cm vs. ISO, Cm vs. Pav1209, Cm vs. Pav609, and Mixed vs. Pav1209. The subsequent ranking of these comparisons (Tables 35A, B) is as follows: Cm > ISO, Pav609, Pav1209 and Mixed > Pav1209. In terms of right valve comparisons of Sr/Ca by diet (Tables 36A, B), only the TI vs. CG comparison proved significant, and the TI group revealed to have a higher Sr/Ca than that from the Cg group.

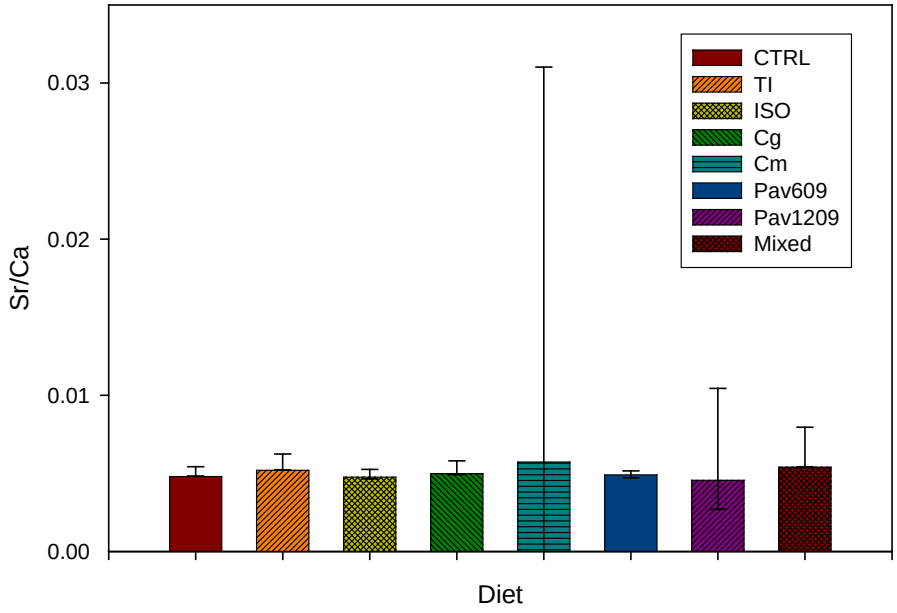


Figure 3.35: Left valve median Sr/Ca values compared to diet. Error bars depict the 95% confidence intervals and diets are represented as previously identified in Figure 3.25.

Table 3.35A: Results of analysis of variance of left valve Sr/Ca among diets. Diets are represented as previously identified in Figure 3.25.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
CTRL	61	1	4.80E-03	4.48E-03	5.46E-03
TI	54	5	5.20E-03	4.54E-03	6.30E-03
ISO	50	1	4.77E-03	4.19E-03	5.84E-03
Cg	58	3	4.99E-03	4.11E-03	6.50E-03
Cm	64	7	5.73E-03	4.51E-03	7.48E-03
Pav609	59	1	4.91E-03	4.34E-03	5.36E-03
Pav1209	52	10	4.56E-03	4.06E-03	5.21E-03
Mixed	53	6	5.41E-03	4.59E-03	6.68E-03
H = 28.9 with 7 degrees of freedom (P = <0.001)					

Table 3.35B: Results of pairwise comparisons of left valve Sr/Ca between diets. Diets are represented as previously identified in Figure 3.25.

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Cm vs. ISO	73.511	3.131	Yes
Cm vs. Pav1209	102.218	4.171	Yes
Cm vs. Pav609	71.485	3.18	Yes
Mixed vs. Pav1209	90.414	3.533	Yes
Cg vs. CTRL	12.385	0.55	No
Cg vs. ISO	25.006	1.056	No
Cg vs. Pav1209	53.713	2.175	No
Cg vs. Pav609	22.98	1.013	No
Cm vs. Cg	48.505	2.129	No
Cm vs. CTRL	60.889	2.731	No
Cm vs. Mixed	11.804	0.497	No
Cm vs. TI	23.47	1	No
CTRL vs. ISO	12.621	0.544	No
CTRL vs. Pav1209	41.329	1.704	No
CTRL vs. Pav609	10.595	0.477	No
ISO vs. Pav1209	28.707	1.133	No
Mixed vs. Cg	36.701	1.533	No
Mixed vs. CTRL	49.086	2.091	No
Mixed vs. ISO	61.707	2.508	No
Mixed vs. Pav609	59.681	2.523	No
Mixed vs. TI	11.666	0.474	No
Pav609 vs. ISO	2.026	0.0866	No
Pav609 vs. Pav1209	30.733	1.259	No
TI vs. Cg	25.035	1.057	No
TI vs. CTRL	37.42	1.612	No
TI vs. ISO	50.041	2.055	No
TI vs. Pav1209	78.748	3.107	No
TI vs. Pav609	48.015	2.053	No

The final elemental ratio analyzed was Zn/Ca. In the comparisons of the left valves (Figure 3.36), only two significant differences between diets were revealed – ISO vs. Baseline and ISO vs. Cg. In both cases, the left valve Zn/Ca was lower in the ISO diet group (Tables 3.37A, B). In the comparisons of the right valves by diet with regard

to Zn/Ca (Tables 3.38A, B), four significant differences between diets were revealed and the following ranking established: Cg and CTRL > Mixed and Pav1209.

Table 3.36A: Results of analysis of variance of right valve Sr/Ca among diets. Diets are represented as previously identified in Figure 3.25.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
CTRL	29	1	4.89E-03	4.33E-03	5.86E-03
TI	17	3	6.40E-03	5.05E-03	7.42E-03
ISO	10	1	4.79E-03	4.25E-03	5.88E-03
Cg	19	1	3.70E-03	3.24E-03	5.04E-03
Cm	27	7	4.99E-03	4.57E-03	5.51E-03
Pav (609)	19	2	4.95E-03	4.19E-03	5.28E-03
Pav(1209)	14	11	5.12E-03	4.90E-03	5.69E-03
Mixed	31	6	4.81E-03	4.38E-03	5.82E-03
H = 21.0 with 7 degrees of freedom (P = 0.004)					

Table 3.36B: Results of pairwise comparisons of right valve Sr/Ca between diets. Diets are represented as previously identified in Figure 3.25.

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
TI vs. Cg	61.722	4.461	Yes
CTRL vs. Cm	1.507	0.133	No
CTRL vs. Cg	33.329	2.841	No
CTRL vs. Mixed	6.167	0.577	No
CTRL vs. Pav609	8.137	0.682	No
CTRL vs. ISO	5.94	0.399	No
Cm vs. Cg	31.822	2.523	No
Cm vs. Mixed	4.66	0.4	No
Cm vs. Pav609	6.629	0.518	No
Cm vs. ISO	4.433	0.284	No
TI vs. CTRL	28.393	2.234	No
TI vs. Cm	29.9	2.21	No
TI vs. Mixed	34.56	2.667	No
TI vs. Pav609	36.529	2.607	No
TI vs. Pav1209	19	0.769	No
TI vs. ISO	34.333	2.07	No

Table 3.36B (Continued)

Comparison	Diff of Ranks	Q	P<0.05
Mixed vs. Cg	27.162	2.263	No
Mixed vs. Pav609	1.969	0.161	No
Pav609 vs. Cg	25.193	1.919	No
Pav1209 vs. CTRL	9.393	0.398	No
Pav1209 vs. Cm	10.9	0.453	No
Pav1209 vs. Cg	42.722	1.764	No
Pav1209 vs. Mixed	15.56	0.656	No
Pav1209 vs. Pav609	17.529	0.721	No
Pav1209 vs. ISO	15.333	0.592	No
ISO vs. Cg	27.389	1.728	No
ISO vs. Mixed	0.227	0.015	No
ISO vs. Pav609	2.196	0.137	No

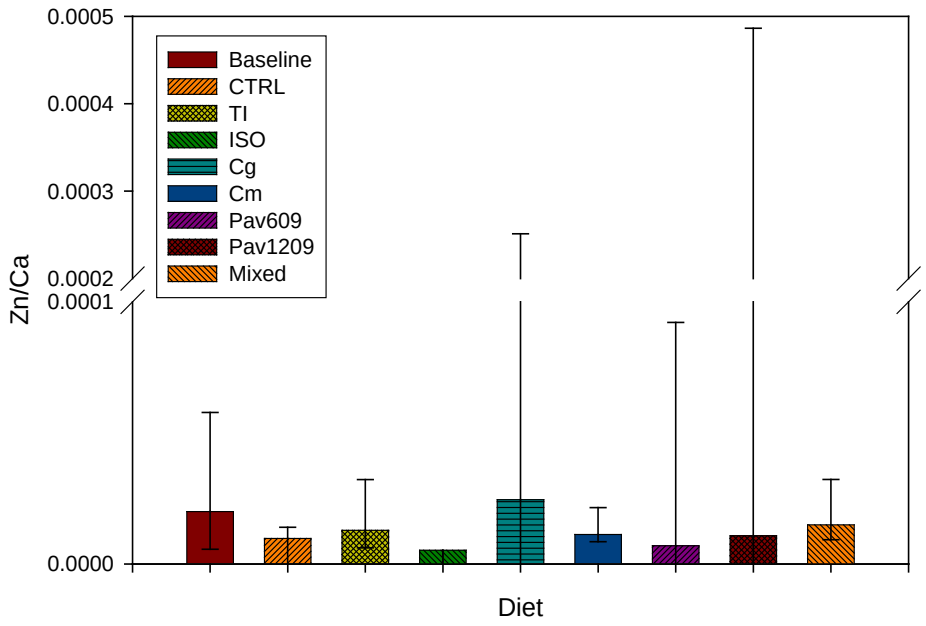


Figure 3.36: Left valve median Zn/Ca values compared to diet. Error bars depict the 95% confidence intervals and diets are represented as previously identified in Figure 3.25.

Table 3.37A: Results of analysis of variance of left valve Zn/Ca among diets. Diets are represented as previously identified in Figure 3.25.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
Baseline	14	2	2.00E-05	1.61E-05	2.80E-05
CTRL	58	17	9.74E-06	3.23E-06	1.96E-05
TI	35	21	1.28E-05	9.36E-06	2.58E-05
ISO	25	11	5.27E-06	-2.54E-04	1.18E-05
Cg	39	7	2.45E-05	4.28E-06	8.13E-05
Cm	45	27	1.12E-05	7.03E-06	1.59E-05
Pav609	39	17	6.96E-06	2.59E-06	1.98E-05
Pav1209	20	5	1.07E-05	9.01E-06	2.02E-05
Mixed	29	10	1.49E-05	9.21E-06	2.37E-05
H = 20.2 with 8 degrees of freedom (P = 0.010)					

Table 3.37B: Results of pairwise comparisons of left valve Zn/Ca between diets. Diets are represented as previously identified in Figure 3.25.

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Baseline vs. ISO	72.357	3.398	Yes
Cg vs. ISO	57.42	3.311	Yes
Baseline vs. Cg	14.938	0.815	No
Baseline vs. Cm	37.056	1.837	No
Baseline vs. CTRL	46.378	2.611	No
Baseline vs. Mixed	20.816	1.043	No
Baseline vs. Pav1209	25.367	1.21	No
Baseline vs. Pav609	44.136	2.272	No
Baseline vs. TI	32.714	1.536	No
Cg vs. Cm	22.118	1.387	No
Cg vs. CTRL	31.441	2.463	No
Cg vs. Mixed	5.878	0.375	No
Cg vs. Pav1209	10.429	0.616	No
Cg vs. Pav609	29.199	1.948	No
Cg vs. TI	17.777	1.025	No
Cm vs. CTRL	9.322	0.609	No
Cm vs. ISO	35.302	1.83	No
Cm vs. Pav609	7.081	0.412	No
CTRL vs. ISO	25.979	1.551	No
Mixed vs. Cm	16.24	0.912	No

Table 3.37B (Continued)

Comparison	Diff of Ranks	Q	P<0.05
Mixed vs. CTRL	25.562	1.702	No
Mixed vs. ISO	51.541	2.704	No
Mixed vs. Pav1209	4.551	0.243	No
Mixed vs. Pav609	23.321	1.376	No
Mixed vs. TI	11.898	0.624	No
Pav1209 vs. Cm	11.689	0.618	No
Pav1209 vs. Ctrl	21.011	1.286	No
Pav1209 vs. ISO	46.99	2.336	No
Pav1209 vs. Pav609	18.77	1.036	No
Pav1209 vs. TI	7.348	0.365	No
Pav609 vs. CTRL	2.242	0.157	No
Pav609 vs. ISO	28.221	1.525	No
TI vs. Cm	4.341	0.225	No
TI vs. CTRL	13.664	0.816	No
TI vs. ISO	39.643	1.938	No
TI vs. Pav609	11.422	0.617	No

Table 3.38A: Results of analysis of variance of right valve Zn/Ca among diets. Diets are represented as previously identified in Figure 3.25.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
Baseline	14	2	2.10E-05	1.41E-05	2.62E-05
CTRL	27	1	3.35E-05	2.30E-05	4.63E-05
TI	17	12	1.50E-05	1.25E-05	2.11E-05
ISO	10	5	1.70E-05	1.30E-05	2.60E-05
Cg	19	1	7.23E-05	3.03E-05	8.46E-05
Cm	24	9	2.03E-05	1.79E-05	2.67E-05
Pav609	19	9	2.43E-05	1.51E-05	6.42E-05
Pav1209	14	1	1.32E-05	9.40E-06	2.02E-05
Mixed	29	11	1.25E-05	9.75E-06	2.17E-05
H = 43.9 with 8 degrees of freedom (P = <0.001)					

Table 3.38B: Results of pairwise comparisons of right valve Zn/Ca between diets. Diets are represented as previously identified in Figure 3.25.

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Cg vs. Mixed	63.667	5.401	Yes
Cg vs. Pav1209	60.158	4.674	Yes
CTRL vs. Mixed	42.47	3.917	Yes
CTRL vs. Pav1209	38.962	3.244	Yes
Baseline vs. Cm	1.117	0.0815	No
Baseline vs. ISO	8.85	0.47	No
Baseline vs. Mixed	21.528	1.634	No
Baseline vs. Pav1209	18.019	1.273	No
Baseline vs. TI	14.45	0.768	No
Cg vs. Baseline	42.139	3.197	No
Cg vs. Cm	43.256	3.499	No
Cg vs. CTRL	21.197	1.955	No
Cg vs. ISO	50.989	2.852	No
Cg vs. Pav609	32.889	2.358	No
Cg vs. TI	56.589	3.166	No
Cm vs. ISO	7.733	0.423	No
Cm vs. Mixed	20.411	1.651	No
Cm vs. Pav1209	16.903	1.261	No
Cm vs. TI	13.333	0.73	No
CTRL vs. Baseline	20.942	1.697	No
CTRL vs. Cm	22.059	1.924	No
CTRL vs. ISO	29.792	1.725	No
CTRL vs. Pav609	11.692	0.889	No
CTRL vs. TI	35.392	2.05	No
ISO vs. Mixed	12.678	0.709	No
ISO vs. Pav1209	9.169	0.493	No
ISO vs. TI	5.6	0.25	No
Pav609 vs. Baseline	9.25	0.611	No
Pav609 vs. Cm	10.367	0.718	No
Pav609 vs. ISO	18.1	0.934	No
Pav609 vs. Mixed	30.778	2.207	No
Pav609 vs. Pav1209	27.269	1.833	No
Pav609 vs. TI	23.7	1.224	No
Pav1209 vs. Mixed	3.509	0.273	No
TI vs. Mixed	7.078	0.396	No
TI vs. Pav1209	3.569	0.192	No

Correlation analysis: time, growth, and element association

Because the dataset being analyzed was shown to have different degrees of normality dependent upon the variables being examined, two separate correlation analyses were conducted: 1) Pearson Product Moment Correlation and 2) Spearman' Correlation. These tests were used to further examine the association of elemental composition (element/Ca ratios) with 1) time (month of collection), 2) growth (shell length and mass), and 3) other elements present.

Table 3.39A provides the Correlation Coefficients, associated P-value, and Sample size (n) for each correlation determined significant with regard to element ratio association with time, shell length and valve mass using the Pearson's correlation procedure. Ranking of the strength of association was based on weak <0.3, moderate 0.3-0.7, and strong >0.7. The Ba/Ca, Co/Ca, K/Ca, Li/Ca, Mg/Ca, and Zn/Ca ratios all showed a weak to moderate association with time with the only positive correlation revealed for Co/Ca (Table 3.39A, B). Barium/calcium, Co/Ca, Mg/Ca, and Ni/Ca were associated with length and mass with directionality element specific (Table 3.39B). The Ni/Ca associations, as with the prior analyses, however, were based on a small sample size and considered indeterminate.

The Spearman's correlation procedure was performed after the Pearson correlation analysis on the same dataset (Table 3.40). A different strategy for classifying level of association was used: moderate association was assumed 0.4-0.6 and weak and strong either side of the moderate. Those comparisons showing significant association with time were length, mass, B/Ca, Ba/Ca, Co/Ca, Cu/Ca, Fe/Ca, K/Ca, Mg/Ca, Mn/Ca, P/Ca, Si/Ca, and Zn/Ca. Associations with length were shown to

exist in variable degrees with mass, Ba/Ca, Co/Ca, Fe/Ca, K/Ca, Li/Ca, Mg/Ca, Ni/Ca, P/Ca, Sr/Ca, and Zn/Ca. Lastly, associations with mass were revealed for Ba/Ca, Co/Ca, Fe/Ca, K/Ca, Li/Ca, Mg/Ca, Ni/Ca, P/Ca, Sr/Ca, and Zn/Ca.

After consideration of the descriptive statistics, data plots, and results of the correlation analyses, the most appropriate and best fit function was determined. Table 3.41 identifies the best fit identified between monotonic and linear relationships by variable. It is important to understand that these associations and descriptions are specific to the dataset being used and overall results of the present study.

Further analyses were performed to determine if any associations were evident between the different elemental ratios examined during the course of this study. Again, a Pearson correlation and Spearman correlation were performed due to the mixed nature of the data set. Many of the comparisons show a moderate to strong association within the context of the Pearson correlation procedure with Fe, Mg, Mn, Si, and Sr being most often seemingly associated with the other elemental ratios. The majority of the associations identified was positive and summarized in Table 3.42. For comparison, the results of the Spearman correlations are provided in Table 3.43. It is evident that there is more often an association between the ratios than not. This interpretation, however, is specific to the findings of the present study.

Discussion

The dominant focus of past research has been aimed at determining chemical signatures in bivalve shells that are capable of being used as environmental proxies. Researchers, however, have frequently noted apparent confounded trends in these

Table 3.39A: Pearson correlation analysis results of significance for element/calcium ratio associations with time, shell length, and valve mass. The correlation coefficient (Corr Coef), the P value (P), and the number of samples examined (n) are provided. The shading variations indicate level of association based on the correlation coefficient (light shade = weak, medium shade = moderate, dark shade = strong).

		Length	Mass	Ba	Co	K	Li	Mg	Ni	Zn
	Corr Coef	0.253	0.304	-0.301	0.666	-0.277	-0.216	-0.319		-0.285
	P	1.30E-07	1.79E-10	2.90E-09	2.96E-18	8.68E-08	3.35E-05	3.23E-10		3.70E-04
Time	n	422	422	373	132	362	362	370		152
	Corr Coef		0.906	-0.261	0.295			0.112	-0.564	
	P		6.03E-181	4.07E-08	2.56E-04			2.12E-02	2.19E-03	
Length	n		480	428	149			425	27	
	Corr Coef			-0.227	0.345			0.0961	-0.511	
	P			2.00E-06	1.69E-05			4.77E-02	6.49E-03	
Mass	n			428	149			425	27	

Table 3.39B: Direction of association for initial Pearson correlations

	time	length	mass	Ba	Co	K	Li	Mg	Ni	Zn
time		+	+	-	+	-	-	-	-	-
length			+	-	+			+	-	
mass				-	+			+	-	

Table 3.40 Spearman's correlation analysis results of significance for element/calcium ratio associations with time, shell length, and valve mass.

		Len	mass	B	Ba	Co	Cu	Fe	K	Li	Mg	Mn	Ni	P	Si	Sr	Zn
	CC	0.216	0.252	0.174	-0.186	0.63	-0.439	0.356	-0.415		-0.435	0.269		0.17	0.502		-0.687
	P Value	0	0	0	0	0	0	0	0		0	0		0.003	0		0
time	n	422	422	374	373	132	237	361	362		370	373		303	365		152
	CC		0.957		-0.305	0.235		-0.153	0.44	0.311	0.381		-0.626	0.154		0.645	-0.174
	P Value		0		0	0.004		0.0017	0	0	0		0	0.004		0	0.0182
length	n		480		428	149		416	416	415	425		27	350		420	184
	CC				-0.306	0.258		-0.14	0.447	0.296	0.371		-0.611	0.185		0.641	-0.18
	P Value				0	0.002		0.0042	0	0	0		0	0		0	0.0144
mass	n				428	149		416	416	415	425		27	350		420	184

Table 3.41 Best fit correlation function for data description.

Linear	Ba/Ca	Co/Ca	Li/Ca	Mg/Ca	Ni/Ca						
Monotonic	length	mass	B/Ca	Cu/Ca	Fe/Ca	K/Ca	P/Ca	Si/Ca	Sr/Ca	Zn/Ca	

Table 3.42: Significant element/calcium ratio correlation results – Pearson correlation. Weak associations have been highlighted in light grey; moderate in a shade darker and the strong associations the darkest shade. The correlation coefficient (Corr Coef), the P value (P), and the number of samples examined (n) are provided in order.

	Co/Ca	Fe/Ca	K/Ca	Li/Ca	Mg/Ca	Mn/Ca	P/Ca	Si/Ca	Sr/Ca	Zn/Ca
B/Ca	-0.175	0.997	0.925		0.83	0.985	-0.231	0.999	0.983	
	0.0326	0	2.92E-173		1.54E-107	6.818E-322	0.0000135	0	1.35E-291	
	149	413	408		418	420	348	417	395	
Ba/Ca			0.217							0.314
			9.91E-06							0.0000141
			409							184
Co/Ca							0.516	0.268	0.251	-0.499
							2.71E-09	0.00102	0.00322	0.0000146
							117	147	136	68
Fe/Ca			0.915	0.84	0.829	0.989	0.118	0.999	0.997	
			5.15E-159	5.90E-108	3.99E-105	0	0.0289	0	0	
			401	400	410	413	344	411	383	
K/Ca					0.905	0.894	0.227	0.914	0.959	
					1.30E-154	4.97E-145	0.0000299	7.16E-160	4.19E-212	
					412	413	333	405	386	
Li/Ca						0.718			0.554	
						1.24E-66			2.36E-32	
						413			385	
Mg/Ca						0.809	0.233	0.823	0.842	
						2.79E-99	0.0000115	5.67E-104	8.58E-108	
						423	346	417	396	
Mn/Ca							0.116	0.995	0.99	
							0.0302	0	0	
							347	418	397	
P/Ca										
Si/Ca									0.995	-0.16
									0	0.0312
									391	181

Table 3.43 Significant element/calcium ratio correlation results – Spearman correlation. Weak associations have been highlighted in light grey; moderate in a shade darker and the strong associations the darkest shade. The correlation coefficient, The P value, and the number of samples examined provided in order (Top to bottom) for each comparison.

	Ba/Ca	Co/Ca	Cu/Ca	Fe/Ca	K/Ca	Li/Ca	Mg/Ca	Mn/Ca	Ni/Ca	P/Ca	Si/Ca	Sr/Ca	Zn/Ca
	-0.186	0.167			-0.102	0.425			0.4	-0.166	0.186	0.412	
	0	0.042			0.0393	0			0.0384	0.0019	0	0	
B/Ca	424	149			408	409			27	348	417	395	
			0.162	0.294				0.15				-0.31	0.353
			0.0081	0				0.0021				0	0
Ba/Ca			267	412				419				394	184
				0.291		0.449		0.185		0.59	0.371	0.267	
				0		0		0.0244		0	0	0.0017	
Co/Ca				143		149		148		117	147	136	
					0.192	0.25	0.417	-0.24			-0.194		0.563
					0.0019	0	0	0			0.0016		0
Cu/Ca					259	260	268	272			264		132
					-0.356	-0.152	-0.276	0.617	0.465	0.115	0.723	-0.288	-0.17
					0	0.0023	0	0	0.0168	0.0329	0	0	0.0209
Fe/Ca					401	400	410	413	26	344	411	383	184
						0.386	0.818	-0.194		0.317	-0.331	0.382	0.434
						0	0	0		0	0	0	0
K/Ca						408	412	413		333	405	386	182
							0.523			0.108		0.589	0.363
							0			0.0496		0	0
Li/Ca							410			334		385	179
								-0.143		0.313	-0.306	0.388	0.573
								0.0033		0	0	0	0
Mg/Ca								423		346	417	396	181
										0.143	0.372	-0.14	
										0.0078	0	0.0052	
Mn/Ca										347	418	397	
													0.224
													0.00355
P/Ca													169
													-0.43
													0
Si/Ca													181

Table 3.43 (Continued)

	Ba/Ca	Co/Ca	Cu/Ca	Fe/Ca	K/Ca	Li/Ca	Mg/Ca	Mn/Ca	Ni/Ca	P/Ca	Si/Ca	Sr/Ca	Zn/Ca
													-0.159
													0.0473
Sr/Ca													156

signatures due to biologic factors (Rosenberg, 1980; Wheeler, 1992; Carre et al., 2006; Strasser et al., 2008). Research specific to biologic controls and influences on shell chemistry is limited despite the assertion that elemental composition is highly dependent on these factors (Wheeler, 1992).

The results presented in this chapter elaborate on the potential influences of specific algal diets on the elemental shell chemistry of *Mercenaria mercenaria* under controlled conditions. Overall, the differences observed in this study can be categorized as influenced by pre experimental factors, seemingly biologic/metabolic factors not assessed, assumed metabolic factors due to stress or nutritional variance, or directly associated with diet received. All factors aside from pre experimental influences can be related to the study design and project variables which ultimately link the observations to diet; however, direct influences and indirect influences have been regarded as separate due to project scope and reliance on previous findings to explain observations outside the analytical design.

Shell length and mass

In general diets composed of multiple algal species have been shown to be superior to those composed of single algal species (Romberger and Epifanio, 1981; Albentosa et al., 1993; Brown et al., 1997). Diatoms, however, have been shown to be

rich in fatty acids (Brown et al., 1997) and support healthy growth rates and survival of cultured *Argopecten irradians* (Milke et al., 2006), though Prymnesiophytes such as *Pavlova* and *Isochrysis* species have also been shown to be relatively rich in fatty acids (Brown et al., 1997). In fact, Prymnesiophytes were shown to have the highest percentage of saturated fatty acids which were suggested to be more beneficial to *C. gigas* (the pacific oyster) larval growth compared to polyunsaturated fatty acids (Thompson et al., 1993). The results of the present study seemingly supported the noted benefit of mixed algal diets and the correlation with growth rates and diatom inclusion; however, previous observations associated with Prymnesiophyte diets were not duplicated. Replication of results determined for different species of bivalve is not necessarily expected due to different requirements of the animal being examined (Brown, 2002) as well nutritional value of the microalgae fed can change due to differences in culture conditions (Enright et al., 1986).

In my experiment, clams fed the Mixed diet or *Chaetoceros mulleri* (CCMP1316)[Cm] diet exhibited the most consistent increase in shell length. The *Chaetoceros galvestonensis* (CCMP186)[Cg] fed group exhibited the next most consistent shell length increase following the second collection which was suspected to be a consequence of algal strain culture problems prior to the March collection. The *Isochrysis specie*s (CCMP1611) [ISO], *Pavlova specie*s (CCMP1209)[Pav1209], and *Pavlova pinguis* (CCMP609)[Pav609] fed clams exhibited moderate to poor shell length increase with *Isochrysis* sp. (CCMP1324)[TI] fed clams and the control group [CTRL] exhibiting virtually no growth during the experiment. All feeding groups except for the *Isochrysis* sp. (CCMP1324)[TI] fed group differed significantly from the control group in

terms of overall shell length. Regarding the valves used in the elemental chemistry analyses, median collected shell length never significantly exceeded the baseline measurements. When the same comparisons were made using all valves collected throughout the experiment, however, the Mixed and *Chaetoceros mulleri* diets were found to promote significant growth over baseline.

The valve mass followed the same trends as the shell length in regard to the overall mass increase of the left valves collected. The right valves collected and subsequently analyzed for the purposes of this experiment exhibited a different trend where ISO>Pav1209>Mixed>Pav609>Cm>Cg>TI; however, the right valves did not differ significantly from the left and were only specifically measured during the first two trials, thus did not reflect changes occurring through the entire experiment. Statistically, the left valve mass for both the mixed and Cm diet groups exceeded those of the control and baseline valves. The remaining groups, excluding TI, significantly differed only from the control group.

Overall, only the Cm and Mixed diet groups exhibited significant growth throughout the experiment. This is an important consideration. It has been suggested variable growth or shifts in shell morphology could possibly affect shell deposition and elemental shell chemistry of the valves of bivalve molluscs (Rhoades and Lutz, 1980) with one of the associated factors of shell form being food supply (Stanley, 1970). Additionally, past research has identified potential associations between growth and element incorporation, i.e. Strontium (Gilken et al., 2005), Manganese (Carre et al.; 2006; Strasser et al., 2006), and Magnesium (Carriker, 1996).

Left versus right valve elemental chemistry

Due to the observed variation between left and right shell mass, as well as previous observations in the eastern oyster having increased elemental concentration in the right valve (Carriker et al., 1996), element/Ca ratios were compared between the left and right valves of the experimental clams. A step-wise approach was taken during analysis to develop a more comprehensive picture of the elemental dynamics between the shell valves.

In the previous chapter, no significant differences were found between the right and left valve groups of *Argopecten irradians.* This result did not support the original hypothesis, but was suggested as a function of the more equivalved nature of the scallops as compared to observations made by Carriker et al. (1996) in reference to the eastern oyster, *Crassostrea virginica*. The other possibilities explaining the results being different than expected were, 1. the use of element/Ca ratios to normalize concentration for the examination as compared to the previously identified oyster study which examined straight elemental concentrations or 2. the analytical methodology was insufficient to identify any differences between valves if present. In the current study, *Mercenaria mercenaria*, an equivalved bivalve, was examined using similar analytical procedures which provided for somewhat different results from that of the scallop study. Both Ni/Ca and Zn/Ca differed significantly between the left and right valves; however, the nickel ratio comparison was based, overall, on only a few diets and not representative of all clams analyzed. A notable difference in the experimental design was the addition of a starvation control in the present clam study which contributed an additional influence for consideration – starvation and associated stress.

When the left and right valves were examined specific to diet received, two diet groups exhibited significant differences with respect to four different element/Ca ratios. The right valves from the Control were determined to have a higher ratio value with respect to B/Ca, Si/Ca and Zn/Ca as compared to the left valve. The right valves of the Cm group had a higher ratio with respect to Ba/Ca as compared to the left. This similar to the observations of Carriker et al. (1996), enrichment was specific to the right valve in each case.

The enrichment of elements or of specific elements in the right valve could be due to a genetic disposition such as remnant biologic controls for torsion (personal communication with Sandra E. Shumway) or potentially, metabolic differences between the mantle tissues associated with each valve. The difference between the valves being a function of metabolic change is partially supported by the results of the control group. Three separate elemental ratios differed between the left and right valves compared to only one in the Cm group and zero in the other groups. Additionally, the difference determined in the composite comparison was mirrored by one of the differences in the diet specific comparison - Zn/Ca. The control group valves were most probably the dominant influence of the Zn/Ca result from the composite analysis. It is believed that an increased differences between the left and right valve is a function of stress related metabolic changes.

With respect to the difference observed between the valves of the Cm feeding group in the present study, metabolic stress or changes do not seem as suspect when taking into consideration experimental procedure and the comparisons of shell length and mass discussed previously. The specific diet received, however, could potentially

explain the difference. Barium is released during phytoplankton decay (Stecher and Kogut, 1999) and Ba is often linked to diatoms (Vander Putten et al., 2000) as it can substitute for Si in the frustules. A minor fraction of Ba in the water column is transformed to barite crystals but most remains labile (Ganeshram et al., 2003). It is proposed that Ba/Ca was enriched in the right valve due to exposure of one valve to settled barite or increased dissolved barium as might occur at the sediment-water interface (Tabouret et al., 2012).

Elemental shell composition differences as a function of diet

Assimilation of material from ingested food particles is directly related to the food itself, i.e., size, biochemical composition, quantity and cell wall (Bayne, 1993; Reinfelder and Fisher, 1991). Furthermore, the partitioning of an element within an algal cell has been directly related to assimilation efficiencies of specific elements studied in *Mytilus edulis* shells and soft tissues (Wang and Fischer, 1996). It follows that elements are more readily available for assimilation from certain algal cells than in others and selective feeding could potentially limit the elements internalized providing for a mechanism of diet associated elemental shell signatures. Additionally, elemental composition has been shown to differ among algal taxa and between oceanic and neritic strains (Martin and Knauer, 1973; Lee and Morel, 1995; Sunda and Huntsman, 1995; Ho et al., 2003) thus increasing potential for diet associated signatures.

With this in mind, the algal cultures used in this experiment were selected based on multiple criteria. First all genus- species pairs obtained had been isolated from as different areas and climates as possible. Second, one of the Genus species pair was

used commonly in aquaculture settings. Third, one species was isolated from inshore waters. Fourth, one pair were diatom species. The final criterion was that the algae would grow in the laboratory and in outdoor cultures. As identified by the Provasoli-Guillard National Center for Marine Algae and Microbiota of Bigelow Laboratory for Ocean Sciences, the *Pavlova specie*s (CCMP1209) was isolated from an unknown tropical site and *Pavlova pinguis* (CCMP609) the Sargasso Sea; *Isochrysis* sp. (CCMP1324) was isolated from the South Pacific off the Society Islands and Isochrysis *specie*s (CCMP1611) from Chesapeake Bay; and finally, *Chaetoceros galvestonensis* (CCMP186) was isolated from the Gulf of Mexico off St. Petersburg, FL while *Chaetoceros mulleri* (CCMP1316) from the North Pacific off Hawaii.

Of the 29 elements originally assessed, only 16 were detected in sufficient concentrations for use in the analyses. All valves collected from each experimental diet were initially assessed for differences in B/Ca, Ba/Ca, Cd/Ca, Co/Ca, Cu/Ca, Fe/Ca, K/Ca, Li/Ca, Mg/Ca, Mn/Ca, Ni/Ca, P/Ca, Pb/Ca, Si/Ca, Sr/Ca and Zn/Ca. Due to sample size limitations created for specific elements during analysis and post processing procedures, Cd/Ca, Cu/Ca, Ni/Ca, and Pb/Ca were eliminated from most of the analyses conducted.

As within the scallop experiments discussed in Chapter 2, the elemental data were highly variable both across diet groups as well as within diet groups. This, however, was similar to results previously reported with cultured bivalves (Carriker, 1996; Strasser et al., 2008), as well as, cultured foraminifera (Hintz et al., 2006). This consistent problem with cultured molluscs and other taxa further complicates analysis and, especially, evaluation of elemental trends in the shell.

I found that, in hard clams fed unique diets, the boron concentration with respect to calcium in the shell was higher in both the Cg and Mixed groups compared to the ISO and Pav609 groups and higher in the TI diet group as compared to the baseline, Cm, Control, ISO, Pav609 and Pav1209 groups. Boron has been associated with salinity (Schopf, 1980) and isotopes have been used as proxies for environmental pH (Rollion-Bard et al., 2003). Salinity was held constant among all feeding groups and therefore was considered non-influential with regard to differences revealed in my study. The growth parameters assessed also do not lend any evidence that this ratio was directly influenced by growth rates observed during the feeding trials, though, upon further consideration and examination of the right valves of the Control group, it was noted that the significant increases in B/Ca occurred in the two groups associated with the lowest level of growth. Furthermore, the increased ratio in the Cg group over two compared groups could account for influence of the clams in the specific collection (March) exhibiting poor growth. The increased ratio over the same two diets in the mixed group did contradict this association. It was previously identified in Rosenberg (1980) that effects of nutrition on boron concentrations not assessed could complicate associations between boron and environmental factors. I examined specific dietary factors in the current study with seemingly confounded results. As such, based on the deviation of trends observed, it is suggested that B/Ca is influenced by multiple factors with substitution, pre experimental conditions , and stress plausible explanations for the differences observed.

The examination of Ba/Ca suggested that Baseline values tended to be higher than or similar to those of the majority of diets in both the left and right valves.

Commonly, as in Stecher et al. (1996), Thebault et al. (2009), Putten et al. (2000), and Lazareth et al. (2007), barium is associated with phytoplankton bloom dynamics (diatoms mostly). This association was further developed by Tabouret et al. (2012) when concluding Ba enrichment was most likely due to incorporation of dissolved Ba such as might be available following an algal bloom (Stecher and Kogut 1999) or at the water-sediment interface. The conclusion made in reference to the observations in the present study is that the higher Ba/Ca ratios are due to influences prior to the start of the feeding experiment and potentially a consequence of the brown water culture methodology immediately prior to the investigation.

The analysis of Co/Ca in the left valves of the different feeding groups showed higher ratios in both the ISO and TI groups than in the Mixed diet group. Research performed by Ho et al 2003 suggested the examined Coccolithophores to have a higher Co quota than that of diatoms though this ultimately suggested being due to the origin of the isolates – the Coccolithophores being oceanic and the Diatoms being neritic. The differences in metal requirements between oceanic and neritic algal species was examined in Brand et al. 1983 where it was suggested oceanic species are associated with higher Co quotas than neritic species. Because all diet groups were seemingly similar based on the statistical analyses besides the shown difference between the Mixed diet and the two Prymesiophycea species, Co/Ca was interpreted as mostly stable during the course of this study and without experimental influence; though, sample size attributable to this result and interpretation may not be fully representative of all changes.

The results obtained from statistical analysis of Fe/Ca are interesting. Specific to the left valve comparisons, the Baseline, ISO and Pav1209 groups are all associated with higher Fe/Ca values than Cg, Cm, CTRL, and Mixed diet groups. The Pav609 group had a higher ratio value associated as compared to Cg and CTRL, while, the TI associated ratio was higher than Cg. In respect to the right valve analysis, the ISO, Baseline and Mixed diet groups were associated with a higher Fe/Ca than the control, furthermore; Baseline Fe/Ca was higher than the TI associated value. Iron has been labeled as a major component in shell deposition and otherwise linked to shell development, proteins and pigments (Almeida et al. 1996). The differences between diet groups in this study appear more attributable to the diet received versus related factors such as growth. The consistent enrichment in the Pavlovophycea and Coccolithophyceae (Prymnesiophycidae) over the Bacillariophycea (Coscinodiscophyceae) or in a diet containing the Pavlovophycea and Coccolithophyceae suggests the possibility of Fe enrichment by ingestion of particular algae. This would follow the results of Ho et al 2003 which demonstrate a slightly higher average Fe/P in Prymesiophycea species examined than in the Bacillariophycea species. Confounding factors, however, might be loss of Ca in the Pavlovophycea diets, or loss of iron in the control group giving the impression pre experimental causes were not dominant.

The findings in reference to right valve associated K/Ca are similar to those observed during the scallop research presented in Chapter 2. The K/Ca ratio value is higher in both the Baseline and Pav1209 groups as compared to Cg but all remaining diets similar. The left valve comparisons did not identify any significant differences

despite the Baseline value appearing larger than all other diet group medians and the control seemingly smaller which would support a gradual decrease in element enrichment. Carriker et al. (1996) concluded that differences in the potassium concentrations observed were possibly from ingestion of sedimentary particles containing potassium though specific examination of the algal cells being ingested was not included in the scope of the research. Conversely, Ho et al. (2003) suggested K is in higher concentration in algal cells than in seawater and that diatoms have a much higher K quota than the other taxonomic classes examined. The results of the current study do not support enrichment of potassium in clam shell by dietary intake but do suggest pre experimental factors might have influenced the observed ratios.

The analysis of Li/Ca was similar in both the left and right valve assessments. Baseline values were significantly larger as compared to the majority of the experimental diets. The ratio for TI was also significantly larger than Cg in valve assessments as well as Cm, ISO, and Pav1209 for the left valve assessment. Research conducted on Li/Ca in *Arctica islandica* concluded patterns of the elemental ratio were most likely associated with calcification rate or river inputs (Thebault et al., 2009). Thebault and Chauvaud (2011) examined Li/Ca signatures in *Pecten maximus* with the conclusion that growth, potentially temperature and increased Lithium due to diatom blooms were responsible for enrichment. In the present study, the differences observed seem more indicative of the preserved signature from pre experimental growth similar to baseline with potential loss or signature dilution due to new growth.

The left valves of each feeding group were found not to differ significantly with respect to Mg/Ca; however, the right valve comparison resulted in three significant

comparisons between the control, baseline, Cg, and mixed diet groups with the control group being associated with the highest ratio value. Magnesium has been proposed to fluctuate with temperature in certain species of bivalve (Rucker and Valentine, 1961; Dodd, 1965; Rosenburg, 1980); though, recent research suggests temperature or salinity have minor to no influence on Mg/Ca for specific bivalves (Strasser et al., 2008; Carre et al., 2006). In *Crassostrea virginica* (Carriker, 1996) and certain gastropods (Foster and Cravo, 2003) Mg has been shown to increase with size and through ontogeny. Ho et al. (2003) suggests that of the five taxonomic classes of algae examined in their study, diatoms had the largest Mg/P quota while the others were mostly similar. Assimilation of Magnesium, however, was suggested from the dissolved phase in Poigner et al. (2012). Because temperature was not a variable in the present study and enrichment was not specific to the diatom diets, growth rates, metabolic control, or calcium replacement may be plausible explanations.

The results of the diet comparisons with regard to Mn/Ca for the collected left valves established that the control group ratio was lower than all the other groups. The Cg group associated ratio was determined smaller than the ratios for Isochrysis species, the mixed diet, and both Pavlova species; and following the developing trend, the Cm group ratio was deemed smaller that of both Isochrysis groups.

The same analysis using the collected right valve ratios established that Mn/Ca was smaller in the Cg and CTRL groups than that of the baseline, Cm, ISO, Mixed, and TI groups. It was also determined the mixed diet associated ratio was larger than that of both Pavlova species.

Defining environmental associations for manganese has been problematic. Blanchard and Chasteen (1976) surmised the amount of substitution of Ca^{2+} by Mn^{2+} was correlated with tidal level, though did not consider the oxygenation of the environment (Rosenburg, 1980). Crisp (1975) attempted to correlate the Mn concentration to salinity, while Strasser et al. (2008) correlated Mn/Ca levels with that of seawater concentration, potentially confounded by biological activity. Carre et al. (2006) suggested positive Mn/Ca association with growth rates while Strasser et al. (2006) showed negative correlation. Though, Poigner et al. (2012) suggests manganese is assimilated most often through its dissolved phase, the results of the current study in conjunction with the trends visualized in Figure 3.31 seem to support the possibility that manganese enrichment was influenced by the diet received, more specifically the Prymnesiophyceae diets, which was supported in Ho et al. 2003 where it was shown average Mn/P concentrations in the Coccolithophores were greater than those in other phyla inspected, including diatom species. These results, however, do not support any differences between oceanic and neritic species (as suggested in Brand et al., 1983 and Ho et al., 2003) being translated to the shells.

The comparisons of P/Ca between diet groups only resulted in significant differences with respect to the left valve. It was determined P/Ca was increased in the ISO group over all other diets and in TI over Cg, Cm, and mixed diets. This suggests a fairly stable association with increased P/Ca in the two Isochrysis species. Ho et al. (2003) points out the major nutrients C, N, P, and S are variable in algae but the average quotas of the organic biomass being similar to that of Redfield et al. (1963). This current observation could be attributed to an increased P concentration in the shell

from feeding solely or selectively upon specific cells in conjunction with an otherwise relatively stable signature.

The Si/Ca ratio was higher in both *Pavlova* species as compared to Cg and Cm in the left collected valves, additionally; the mixed diet had an associated Si/Ca lower than the remaining diets. When the right valves of the feeding groups were compared, baseline Si/Ca was higher than in the diatom species, Pavlova species and mixed diet treatments, in addition, the Si/Ca in the control treatment higher than those associated with Cg, Mixed, Pav609 and Pav1209 feeding groups. Carriker et al. (1996) concluded that the oysters maintained in the natural environment received silica through seston not incorporated in the laboratory habitat. Considering this, the differences in Si/Ca observed are most seemingly due to pre experimental conditions with a potential secondary dietary influence.

Four comparisons with regard to Sr/Ca between feeding groups were deemed significant and established Cm group left valves were Sr enriched compared to ISO, Pav609, and Pav1209 and Mixed diet group valves enriched compared to Pav1209. When the right valves were compared, only the TI was determined enriched over the Cg group. These results do not consistently align with the description in Ho et al. (2003) where Coccolithophores were determined to have a higher Sr quota than studied diatoms and other algal phyla. As such, there is no clear association with any experimental variable nor is there an obvious indication of an artifact effect from the rearing procedure. This, however, is a common problem in determining factors influencing Sr in shells. Researchers have documented associations with temperature (Dodd, 1965), growth rates (Gilken et al., 2005; Hamer and Jenkins, 2007), and

ontogeny (Dodd, 1970; Crisp, 1975); however, there are equal accounts of opposing influence as well as results indicating no influence when considering the same factors. Poigner et al. (2012) concluded that Strontium was predominantly assimilated in particulate phase; however, the current results do not support a dietary influence on Strontium incorporation. The results do suggest multiple controlling factors with one potentially being shell growth and growth rate.

Zinc to calcium inspections revealed two significant comparisons with regard to the left shell and four with regard to the right. The baseline and Cg group were determined significantly enriched compared to the ISO group left valves. The right valve comparison established that the Cg and control were associated with higher Zn/Ca than were the Mixed and Pav1209 diet groups. As with strontium, there is not a clear indication of what contributed to the limited differences observed. Many researchers have paired zinc concentrations with environmental concentrations for purpose of contaminant surveys (Fang and Shen, 1984; Martincie et al., 1984; Puente et al., 1996; Markich, 2002; etc.). Zinc has been, however, recognized as a dietary element metabolically transformed and passed through food web interactions (Windisch 2001, Wang 2002). In the present research, the differences observed were not seemingly attributable to a contaminant source as the association was specific to diet groups. The differences observed in the current study, though, do not support, definitively, a specific algal diet contributing to relative zinc concentration.

Correlation analyses

Correlation analyses revealed associations between B, Fe, K, Mg, Mn, Si and Sr as well as Fe and Li. In relation to the current project, both Fe and Mn were determined to be potentially related to the Prymnesiophyceae diets thus a correlation between the two is logical if influenced by the same variable. Similarly, Thebault and Chauvaud (2011) suggested Li was enriched due to diatom blooms which would indicate potentially similar influences between Fe and Li though different pathways/mechanisms are suspected based on previous research discussed. Because the original assessments of B, K, Mg and Sr were uncertain as to the dominant influences surrounding enrichment, the relationship is still unclear. The correlation analyses performed with respect to Time, Length and Mass do support influence of pre experimental factors (K and Mg), size and early growth rates (Sr and Mg), or undetermined biological influences (B and Mg).

Iron, manganese and phosphorus showed no strong associations with time or growth. Because the majority of potential influences identified in the literature were held constant or consistent among trials and they seemingly acted in varied association with one another, diet still appears to be a plausible primary influence.

Silica did show moderate association with time and strontium was strongly correlated with growth. Neither Silica nor Strontium showed a clear association with the diets or assessed experimental parameters. Strontium, however, was determined to show a potential relationship with growth and pre-experimental growth rates due to the diet groups associated with the highest Sr/Ca ratios. The positive association between Si and time does not agree with the conclusions made when comparing direct dietary

influence; this however, does substantiate the possibility of two or more influences and subsequent confounded results.

The Ba, K, Li, Mg and Zn associated ratios were all negatively associated with time. The original assessments of Ba, K, Li, and Mg with regard to algal diet influence suggested the relative concentration of these elements was more strongly associated with pre experimental conditions or related factors. The determined overall decrease through time does not preclude this determination; however, exact reasoning for the decrease is seemingly element specific and dependent on the influence of the experimental feeding group. The correlation between zinc and time is potentially attributable to an experimental complication. As previously noted, Windisch (2001) and Wang (2002) demonstrated zinc to be metabolically influenced and associated with food web transfer. An association between Zn/Ca with specific algal diets was not demonstrated in this study; however, production of feeding cultures was ultimately limited by available space and laboratory equipment. Similar to the differences between valves with regard to relative zinc concentrations in the starvation control, the negative association to time is likely a consequence of limited food availability and associated metabolic influence.

The initial interpretation made concerning Co/Ca was that it appeared stable across diets and did not seem influenced by the experimental variables. Here it was strongly associated with time and moderately with mass and shell length. These associations, however, are very specific to particular diet groups and do not represent overall changes in this elements concentrations.

Conclusion

Once all results were considered, three ratios were directly associated with diet received: Fe/Ca, Mn/Ca, and P/Ca. In all three cases, the Prymnesiophycidae (the two Isochrysis diets) had a significant impact on the relative elemental concentrations, as did the Pavlovophycea diets with regard to Fe/Ca. The Mg/Ca and Sr/Ca ratios were both influenced by growth or more specifically growth rates; however, it should be noted that pre experimental growth was most attributable to this conclusion and biologic factors not assessed also seemingly contributed to differences observed. With regard to Zn/Ca and B/Ca, the results favor differences observed being most attributable to stress and assumed metabolic shifts; while the remaining elemental ratios examined, Ba/Ca, K/Ca, Li/Ca and Si/Ca, were determined to be primarily influenced by factors associated with pre experimental conditions not encompassed in the experimental scope and design.

Manganese, copper, zinc, and cadmium have been shown to be primarily associated with the organic matrix in oyster shells (Carriker et al. 1980) as was Iron (Almeida et al. 1998) and magnesium in mussel shells (Lorens and Bender 1980). This association of specific elements with the organic matrix alone suggests a high level of biologic control. In the present study, iron, manganese, and phosphorous were revealed to be influenced by diet. It has been suggested that the organic matrix is responsible for shell strength (Addadi et al. 2006) and that the organic matrix potentially influences elemental content and structure of the shell (Watabe et al. 2001, Takesue and van Green 2004, Morse et al. 2007, and Johnstone 2008). If diet associated elements are incorporated into the organic matrix and can be manipulated by dietary

changes as demonstrated, the diet of cultured bivalves could potentially be implicated in differences in shell strength and structure between wild and aquacultured *Pecten maximus* as described in Gresfrude and Strand (2006). This demonstrated diet influence also demands consideration of diet composition when conducting experiments aimed at describing influences on shell dynamics or determining cause and effect relationships.

Though diet was shown to directly affect the shell chemistry of *Mercenaria mercenaria*, resolution of the algal contribution was limited to taxonomic class with regard to only a few elements, and algal isolate origin related differences were not apparently translated to the shells as had been hypothesized. It also is evident that other contributing factors will potentially complicate detection of diet associated signals when outside a controlled environment (higher food concentrations, multiple influences on the same element, contamination, multiple algal species with similar elemental quotas, age influence, etc.). As such, applications or use of diet related elemental signatures is currently limited. It is believed that diet associated signatures can eventually be used as shell markers or in determination of food web interactions related to eutrophication, ocean acidification, or other environmental impacts capable of changing plankton composition; however, before these applications can be pursued, further research and development of alternative analytical techniques are needed.

4. Can phosphorous normalization indicate dietary influence on elemental chemistry in bivalve shells?

Introduction

The productivity and species composition of marine phytoplankton communities are controlled by a number of trace metal nutrients (iron, zinc, cobalt, manganese, copper, and cadmium) as well as major nutrients (nitrogen, phosphorus, and silicon) in biologically available forms (Sunda, 2012). Trace metals can play important roles in regulating the species composition of phytoplankton communities because of large differences in cellular trace metal concentrations and growth requirements among species (Brand et al., 1983; Sunda and Huntsman, 1995; Crawford et al., 2003; Ho et al., 2003). The species composition will ultimately affect consumers, namely bivalves as related to present research, as it defines the available food source.

As presented in Chapters 2 and 3, the types of algae ingested by marine bivalve molluscs can influence the elemental composition of the shell with differences in cellular elemental concentrations, assimilation controls, or metabolic influences being plausible mechanisms for the differences. The influences of individual algal diets, however, were not completely evident. An analytical process with more resolution to determine the contribution of an algal species appears to be necessary to allow ecological/trophic interpretations of the bivalve diets by use of shell chemistries.

Elemental composition of algae is most often analyzed by normalizing to phosphorous (referred to as element quotas) following the protocol of Redfield (1934, 1958) and facilitating the comparison of individual organisms and natural plankton samples independent of cell volume (Ho et al., 2003). These quotas can be highly variable among algal taxa (Figure 4.1), with a range of 14-57% RSE (Ho et al., 2003; Quigg et al., 2003).

In studies of shell chemistry, elemental composition is commonly normalized to calcium to offset the spatial variability within the shell. I hypothesize that normalizing element concentrations to phosphorous or phosphorous and calcium will enhance resolution to determine species-specific influence of algae on elemental shell dynamics, provided that phosphorous signatures are stable.

Although different parameters can influence phosphorous composition in algal cells versus marine bivalve molluscan shells, a finding in the previous chapter with regard to P/Ca indicated a potential opportunity, as did findings that phosphorous concentrations in the shells of freshwater bivalves were similar within the same water shed (Jurkiewicz-Karnkowska, 2002). Certain elements can be good environmental proxies based on their relative stability in shell material from different individuals until an environmental flux occurs (Tabouret et al., 2012). In my study, I observed that P/Ca in the experimental clam shells remained fairly consistent with the exception of particular diets. This chapter examines the use of (element/P)/Ca ratios and (element/Ca)/ (P/Ca) (mathematically equivalent to element/P) to test the utility for analyzing shell dynamics with respect to diet.

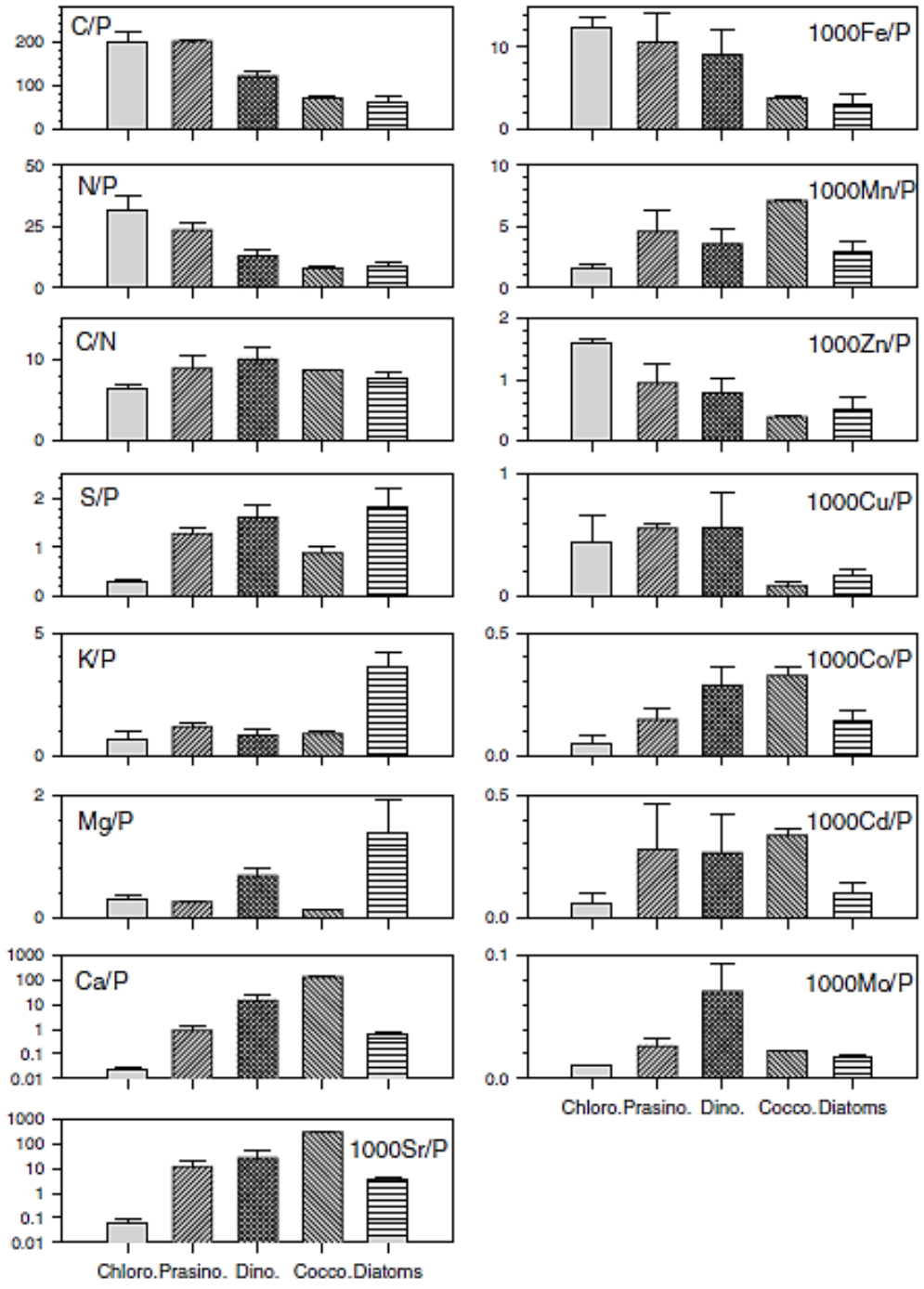


Figure 4.1: Comparison of average elemental quotas among different algal taxa from Ho et al. (2003)

Methods

The general procedures for mariculture of both the algal cultures and clams were presented in Chapter 3, as were the ICP-OES analysis methods. This chapter uses information collected during the experiments described in Chapter 3 to test the hypothesis that a modified data-analysis procedure could be used to determine the influence of algal diets and ultimately used in field diagnostics.

The elemental concentration findings from the ICP-OES analysis of *Mercenaria mercenaria* valves subsampled from different feeding experiments were transformed to new elemental ratios in the form of (element/P)/Ca and (element/Ca)/(P/Ca) [referred to herein as shell quotas]. The general trends were statistically compared using a Kruskal-Wallis analysis of ranks and subsequent post hoc analysis to determine the differences in elemental shell chemistry between feeding groups. The results were then compared to previous findings discussed in Chapter 3 to evaluate utility of the proposed alternative ratios.

Boron, barium, cobalt, iron, potassium, lithium, magnesium, manganese, silica, strontium, and zinc concentrations from left shells were analyzed using both (element/P)/Ca and element/P. No baseline comparisons could be made because ICP data for phosphorous were not usable from the baseline shell analyses.

Results

Significant differences in boron, when normalized to phosphorous and calcium were found when comparing shells from several dietary treatments (Tables 4.1 and A1). These comparisons (Figure 4.2) revealed the (B/P)/Ca ratios in shells of clams fed ISO

were lower than all other treatments except for the Pav609, while the shells from the Mixed diet treatment were higher than the Cm, Ctrl, and Pav609 diet treatments.

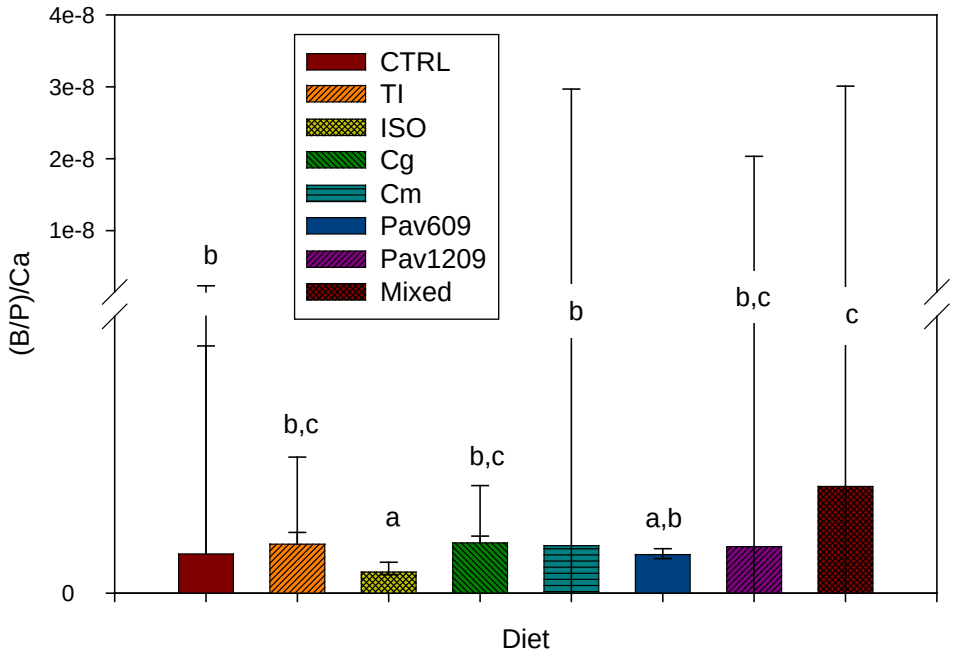


Figure 4.2: Median (B/P)/Ca ratios versus diet received. The letters indicate the similarities and differences revealed by Dunn's post hoc analyses (Table A1).

Table 4.1: Results of Kruskal-Wallis analysis for (B/P)/Ca by diet.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
CTRL	61	6	1.41E-10	8.26E-11	2.29E-09
TI	56	16	1.77E-10	1.14E-10	4.72E-10
ISO	50	7	7.62E-11	2.81E-11	1.21E-10
Cg	58	11	1.82E-10	1.32E-10	3.24E-10
Cm	62	18	1.71E-10	1.26E-10	2.21E-10
Pav609	58	18	1.39E-10	1.1E-10	1.71E-10
Pav1209	54	14	1.68E-10	1.3E-10	2.3E-10
Mixed	65	26	3.85E-10	1.93E-10	2.54E-09
H = 75.2 with 7 degrees of freedom (P = <0.001)					

When B/P data were compared among the diets (Figure 4.3), results ($P < 0.05$, Tables 4.2 and A2) were similar to those of (B/P)/Ca. The ratio for the ISO diet was lower than for all other experimental diets, while the Mixed diet ratio was significantly higher than the *Pavlova* diets, but no others.

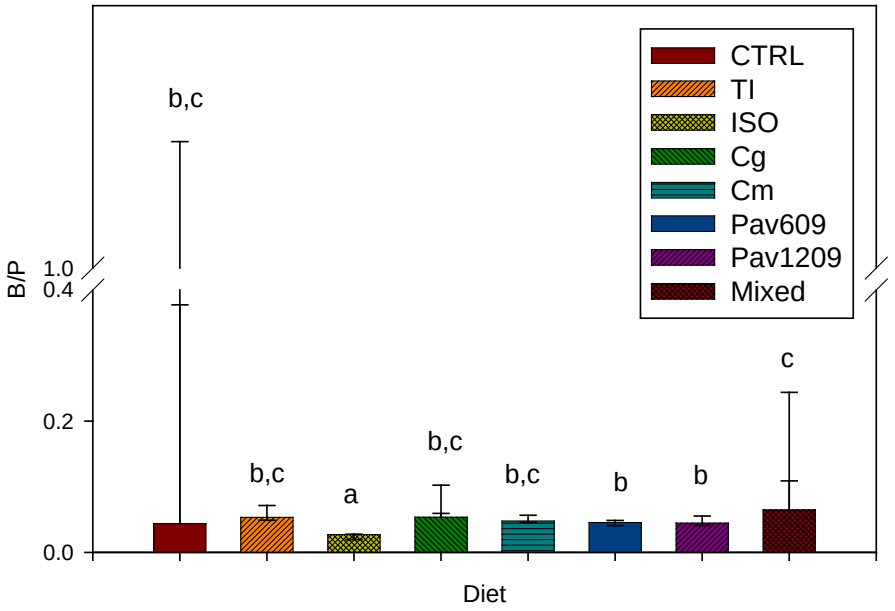


Figure 4.3: Median B/P ratios in *M. mercenaria* shells compared to diet. Letters indicate the similarities and differences revealed by Dunn's post hoc analyses (Table A2).

Table 4.2: Results of Kruskal-Wallis analysis for (B/P) by diet.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
CTRL	61	6	4.39E-02	2.38E-02	1.49E-01
TI	56	13	5.34E-02	3.38E-02	7.78E-02
ISO	50	7	2.70E-02	9.15E-03	3.37E-02
Cg	58	9	5.36E-02	3.43E-02	9.91E-02
Cm	62	18	4.79E-02	4.00E-02	5.63E-02
Pav609	58	18	4.55E-02	3.44E-02	5.39E-02
Pav1209	54	12	4.47E-02	3.16E-02	6.61E-02
Mixed	68	20	6.49E-02	4.72E-02	1.04E-01
H = 76.8 with 7 degrees of freedom (P = <0.001)					

The results for barium with respect to phosphorous and calcium followed similar trends as for boron (P< 0.05; Tables 4.3 and A3): The ratios for the ISO group were lower than for all other diets, while the ratios for the Mixed diet were higher than for all diet treatments except Cg and Cm (*Chaetoceros)* diets (Figure 4.4).

The shell Ba/P ratio differed significantly (P<0.05) between seven diets. Again, ISO was determined to have the lowest ratio as compared to the other diets (Figure 4.5, Tables 4.4 and A4). All other experimental diets were found to be similar.

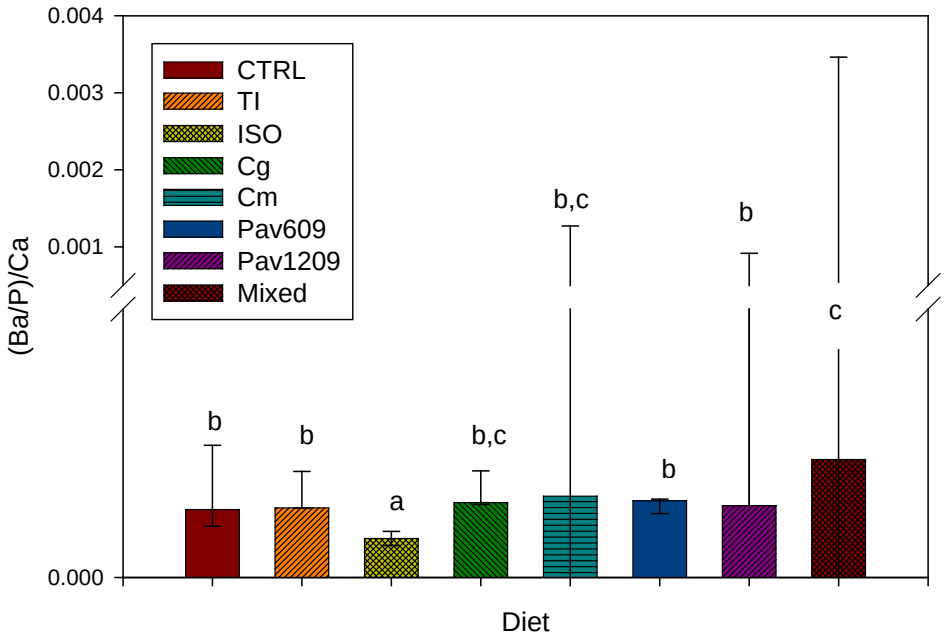


Figure 4.4 Median (Ba/P)/Ca ratios with associated 95% confidence intervals in Mercenaria shells by diet. Letters indicate the similarities and differences revealed by Dunn's post hoc analyses (Table A3).

The analysis of (Co/P)/Ca revealed somewhat different relationships among the dietary treatments, (P=0.02, Table 4.5). In this case, the ratio in the CTRL group was significantly lower than in the Pav1209 group (Figure 4.6, Table A5). The analysis of Co/P did not reveal any significant differences among diets (P=0.118).

Table 4.3: Results of Kruskal-Wallis analysis for (Ba/P)/Ca by diet.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
CTRL	61	6	5.06E-06	2.41E-06	8.35E-06
TI	56	16	5.18E-06	3.3E-06	8.71E-06
ISO	50	7	2.9E-06	1.42E-06	3.84E-06
Cg	58	11	5.58E-06	4.39E-06	7.8E-06
Cm	62	18	6.06E-06	4.82E-06	7.5E-06
Pav609	58	18	5.71E-06	4.39E-06	6.29E-06
Pav1209	54	14	5.35E-06	4.01E-06	5.92E-06
Mixed	65	24	8.79E-06	5.46E-06	1.54E-05
H = 80.3 with 7 degrees of freedom (P = <0.001)					

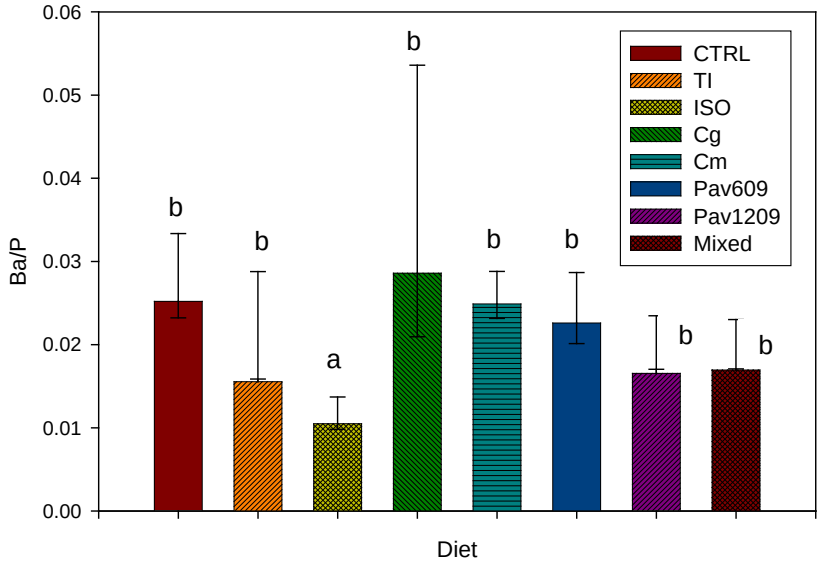


Figure 4.5: Median Ba/P ratios with associated 95% confidence intervals in *Mercenaria* shells by diet. Letters indicate the similarities and differences revealed by Dunn's post hoc analyses (Table A4).

Table 4.4: Results of Kruskal-Wallis analysis for (Ba/P) by diet.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
CTRL	61	6	2.52E-02	1.09E-02	4.16E-02
TI	56	18	1.56E-02	1.23E-02	2.52E-02

Table 4.4 (Continued)

Group	N	Missing	Median	25%	75%
ISO	50	7	1.05E-02	6.94E-03	1.47E-02
Cg	58	9	2.86E-02	1.29E-02	3.52E-02
Cm	62	18	2.49E-02	2.19E-02	3.08E-02
Pav609	58	18	2.26E-02	1.78E-02	2.61E-02
Pav1209	54	12	1.66E-02	1.45E-02	2.62E-02
Mixed	68	18	1.70E-02	1.37E-02	2.44E-02
H = 57.8 with 7 degrees of freedom (P = <0.001)					

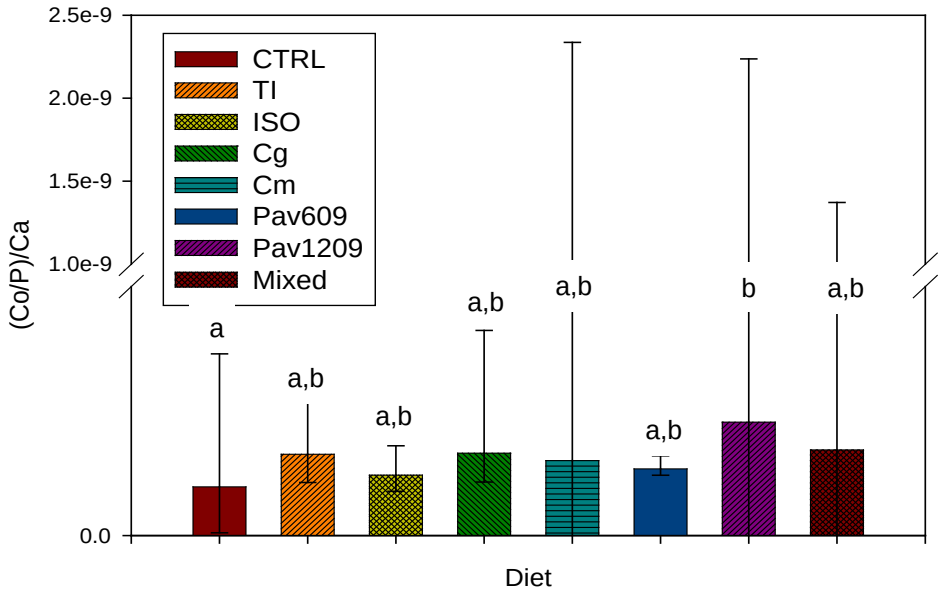


Figure 4.6: Median (Co/P)/Ca ratios with associated 95% confidence intervals in *Mercenaria* shells by diet. Letters indicate the similarities and differences revealed by Dunn's post hoc analyses (Table A5).

Table 4.5: Results of Kruskal-Wallis analysis for (Co/P)/Ca by diet.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
CTRL	54	40	3.91E-12	3.09E-12	5.01E-12
TI	56	49	6.53E-12	5.71E-12	8.92E-12
ISO	48	36	4.85E-12	3.13E-12	6.99E-12
Cg	58	46	6.61E-12	4.77E-12	1.00E-11
Cm	58	34	6.01E-12	4.49E-12	1.14E-11
Pav609	58	39	5.35E-12	4.77E-12	6.35E-12

Table 4.5 (Continued)

Group	N	Missing	Median	25%	75%
Pav1209	54	44	9.11E-12	5.64E-12	1.33E-09
Mixed	65	47	6.87E-12	5.37E-12	9.09E-12
H = 16.6 with 7 degrees of freedom (P = 0.020)					

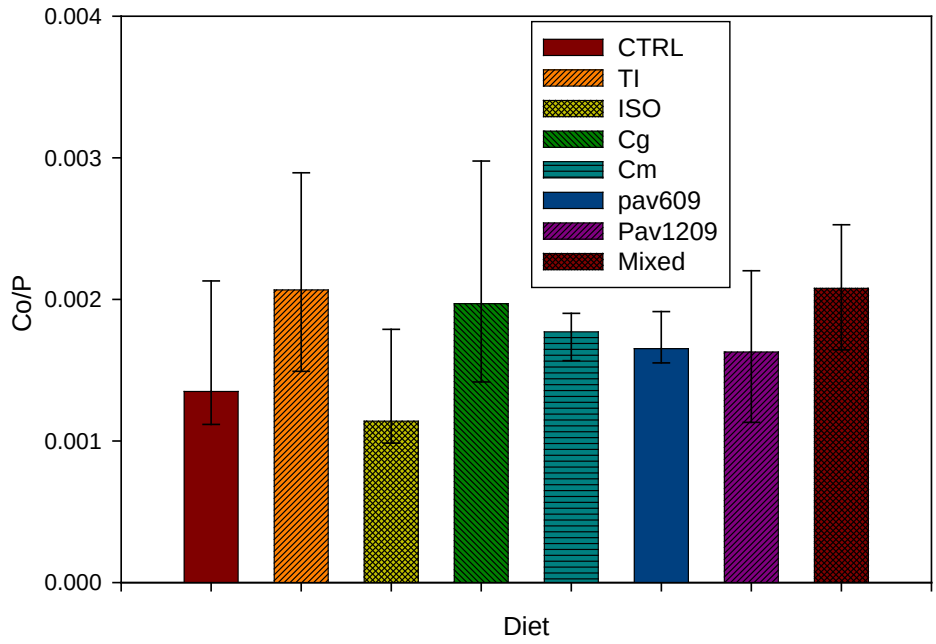


Figure 4.7: Median Co/P ratios with associated 95% confidence intervals in *Mercenaria* shells by diet. No significant differences among diets were determined.

Table 4.6: Results of Kruskal-Wallis analysis for (Co/P) by diet.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
CTRL	54	40	1.35E-03	9.23E-04	1.95E-03
TI	56	48	2.07E-03	1.55E-03	2.63E-03
ISO	48	36	1.14E-03	9.06E-04	1.94E-03
Cg	58	46	1.97E-03	1.41E-03	2.30E-03
Cm	58	34	1.77E-03	1.56E-03	1.95E-03
Pav609	58	39	1.65E-03	1.54E-03	2.05E-03
Pav1209	54	43	1.63E-03	1.06E-03	2.22E-03
Mixed	68	45	2.08E-03	1.43E-03	2.41E-03
H = 11.5 with 7 degrees of freedom (P = 0.118)					

The analyses of the iron shell quota as compared to calcium revealed the ratios for the ISO and Cg treatments to be significantly lower than for those of Cm, Mixed and Pav1209 treatments, while the ratios for the CTRL treatment to be lower than only the Mixed and Pav1209 treatments (Figure 4.8,Tables 4.7 and A6). The only significant differences in the Fe/P ratio were found between the Pav609 group and both the Cg and ISO groups (Figure 4.9, Tables 4.8 and A7).

Comparison of median (K/P)/Ca values for each experimental diet (Figure 4.10) indicated that the shells from the Mixed diet had significantly more potassium incorporated than did the shells from the ISO and CTRL groups ($P < 0.05$, Tables 4.9 and A8). The comparisons based on K/P provided similar results (Figure 4.11), with Mixed and Cm treatment ratios revealed as significantly higher than those from the ISO treatment (Tables 4.10 and A9).

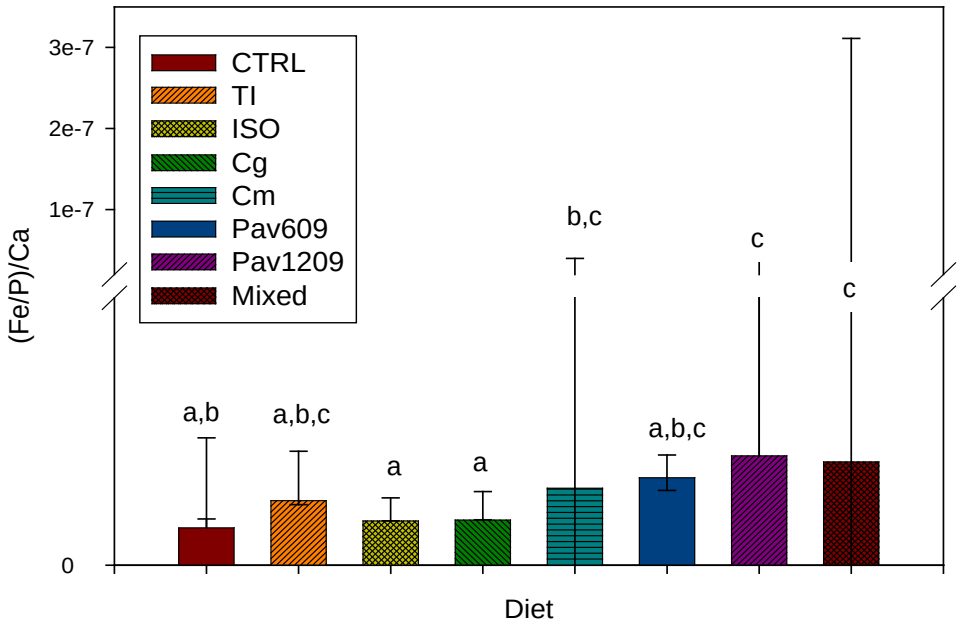


Figure 4.8: Median (Fe/P)/Ca ratios with associated 95% confidence intervals in *Mercenaria* shells by diet. Letters indicate the similarities and differences revealed by Dunn's post hoc analyses (Table A6).

Table 4.7: Results of Kruskal-Wallis analysis for (Fe/P)/Ca by diet.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
CTRL	61	6	1.4E-10	7.41E-11	4.41E-10
TI	56	18	2.41E-10	1.58E-10	3.83E-10
ISO	50	8	1.65E-10	1.2E-10	2.56E-10
Cg	58	11	1.69E-10	1.31E-10	2.5E-10
Cm	58	15	2.88E-10	1.95E-10	5.14E-10
Pav609	58	18	3.27E-10	1.66E-10	4.74E-10
Pav1209	54	14	4.09E-10	2.28E-10	6.54E-10
Mixed	65	26	3.86E-10	2.5E-10	8.16E-10
H = 53.7 with 7 degrees of freedom. (P = <0.001)					

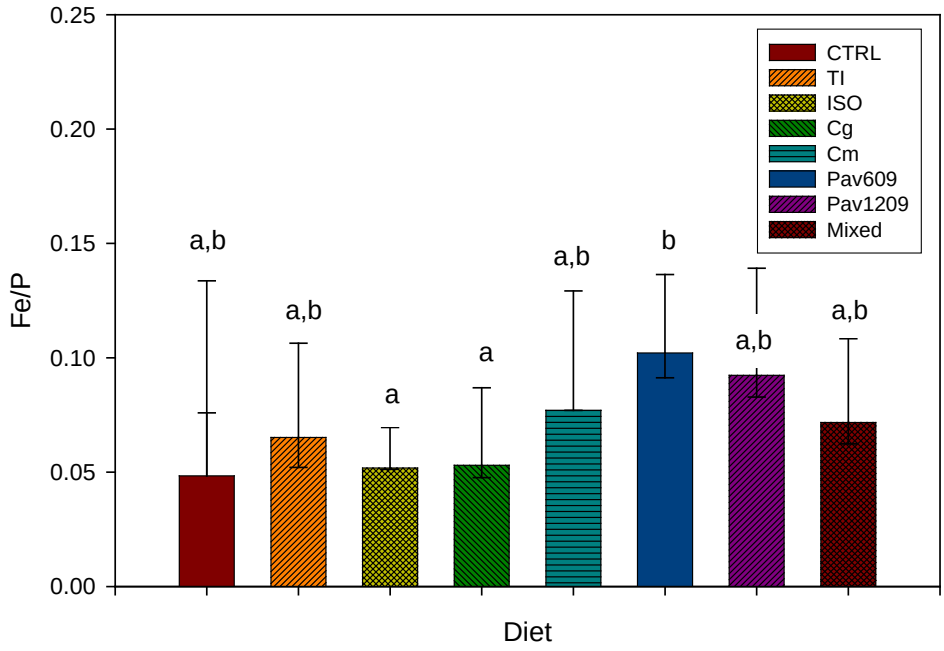


Figure 4.9: Median Fe/P ratios with associated 95% confidence intervals in *Mercenaria* shells by diet. Letters indicate the similarities and differences revealed by Dunn's post hoc analyses (Table A7).

Table 4.8: Results of Kruskal-Wallis analysis for (Fe/P) by diet.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
CTRL	61	6	4.84E-02	2.72E-02	1.89E-01
TI	56	16	6.52E-02	3.47E-02	9.29E-02
ISO	50	8	5.18E-02	3.98E-02	7.09E-02
Cg	58	10	5.30E-02	4.23E-02	7.38E-02
Cm	58	15	7.70E-02	5.04E-02	1.35E-01
Pav609	58	18	1.02E-01	5.29E-02	1.53E-01
Pav1209	54	12	9.24E-02	4.65E-02	1.32E-01
Mixed	68	20	7.17E-02	5.75E-02	9.40E-02
H = 26.4 with 7 degrees of freedom (P = <0.001)					

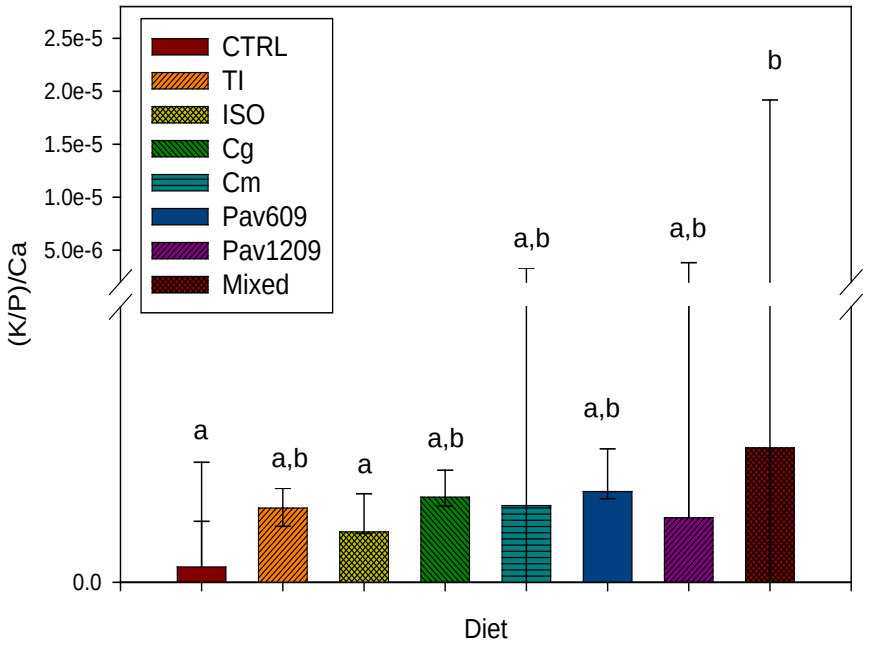


Figure 4.10: Median (K/P)/Ca ratios with associated 95% confidence intervals in *Mercenaria* shells by diet. Letters indicate the similarities and differences revealed by Dunn's post hoc analyses (Table A8).

Table 4.9: Results of Kruskal-Wallis analysis for (K/P)/Ca by diet.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
CTRL	61	6	5.55E-10	1.61E-10	5.71E-09
TI	56	25	2.7E-09	9.98E-10	4.11E-09
ISO	50	7	1.83E-09	5.38E-10	3.54E-09
Cg	58	12	3.09E-09	2.37E-09	4.35E-09
Cm	62	18	2.78E-09	1.86E-09	6.02E-09
Pav609	58	18	3.29E-09	1.48E-09	5.31E-09
Pav1209	54	16	2.34E-09	1.49E-09	7.14E-09
Mixed	65	29	4.88E-09	1.39E-09	9.66E-09
H = 23.9 with 7 degrees of freedom (P = 0.001)					

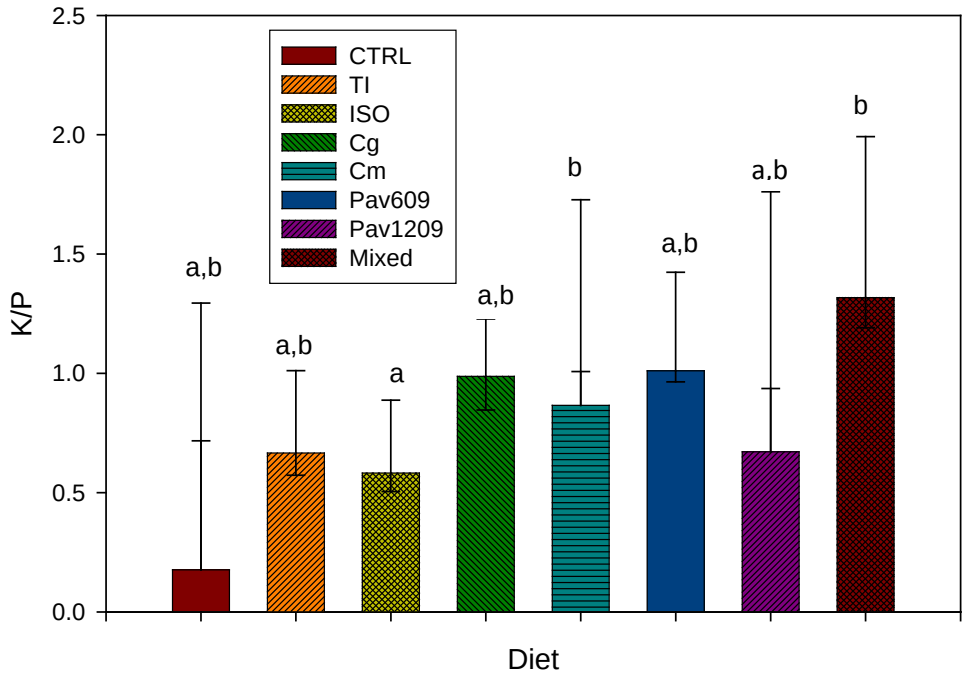


Figure 4.11: Median K/P ratios with associated 95% confidence intervals in *Mercenaria* shells by diet. Letters indicate the similarities and differences revealed by Dunn's post hoc analyses (Table A9).

Table 4.10: Results of Kruskal-Wallis analysis for (K/P) by diet.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
CTRL	61	6	1.76E-01	7.07E-02	1.98E+00
TI	56	24	6.66E-01	3.18E-01	1.11E+00
ISO	50	7	5.82E-01	1.80E-01	1.00E+00
Cg	58	10	9.87E-01	6.48E-01	1.30E+00
Cm	62	18	8.66E-01	6.56E-01	1.68E+00
Pav609	58	18	1.01E+00	5.66E-01	1.63E+00
Pav1209	54	16	6.71E-01	4.63E-01	2.17E+00
Mixed	68	24	1.32E+00	7.13E-01	2.19E+00
H = 24.9 with 7 degrees of freedom (P = <0.001)					

Comparison of median (Li/P)/Ca values between feeding groups (Figure 4.12, Tables 4.11 and A10) revealed that ratios from the ISO diet were significantly lower from all other treatments except those fed the Pav609 diet. The Mixed diet ratios were also revealed as significantly higher than those from the Pav609 diet. Comparing Li/P (Figure 4.13) only revealed that the ratios from the ISO diet group were significantly lower than those from all other diet groups (Tables 4.12 and A11).

The comparisons of magnesium concentrations as compared to phosphorous and calcium (Figure 4.14) by diet revealed that the mixed diet produced the highest ratio, being significantly higher than ratios from all diets except Cm. And again, the ISO diet produced a ratio significantly lower than most other diets, with the exception being Pav609. All other diets resulted in intermediate (Mg/P)/Ca ratios (Tables 4.13 and A12).

The shell Mg/P quota comparisons among diets (Figure 4.15, Tables 4.14 and A13) revealed once again that the ISO treatment produced the lowest shell ratios, significantly lower than all diets except the TI and the *Pav1209* diets. At the other end of the spectrum, the CTRL group had the highest Mg/P ratio, though not significantly

higher than those from the Mixed, *Chaetoceros (Cg and Cm)*, and Pav609 treatments. Overall, the ranking supported was ISO < Cg, Cm, CTRL, Mixed, Pav609; CTRL > ISO, Pav1209, TI; Cm> TI; and Mixed > ISO and TI.

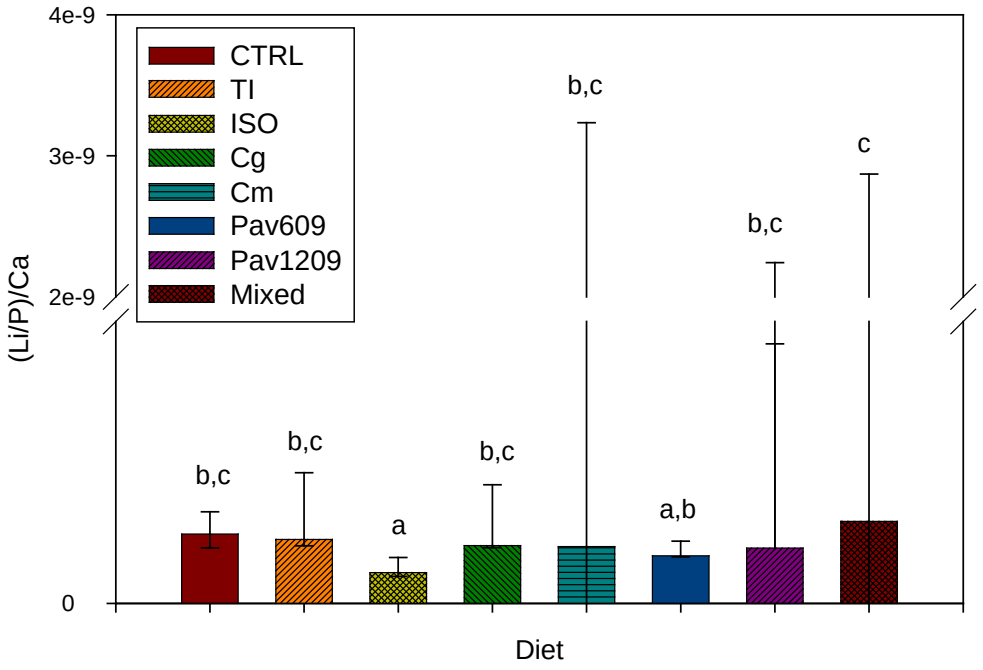


Figure 4.12: Median (Li/P)/Ca ratios with associated 95% confidence intervals in *Mercenaria* shells by diet. Letters indicate the similarities and differences revealed by Dunn's post hoc analyses (Table A10).

Table 4.11: Results of Kruskal-Wallis analysis for (Li/P)/Ca by diet.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
CTRL	61	6	1.72E-11	8.48E-12	2.43E-11
TI	56	24	1.59E-11	9.91E-12	2.02E-11
ISO	50	7	7.65E-12	2.66E-12	1.19E-11
Cg	58	13	1.43E-11	1.14E-11	1.94E-11
Cm	62	18	1.41E-11	9.82E-12	2.32E-11
Pav609	58	18	1.18E-11	9.21E-12	1.74E-11
Pav1209	54	14	1.37E-11	1.09E-11	1.78E-11
Mixed	65	28	2.04E-11	1.40E-11	6.09E-11
H = 49.0 with 7 degrees of freedom (P = <0.001)					

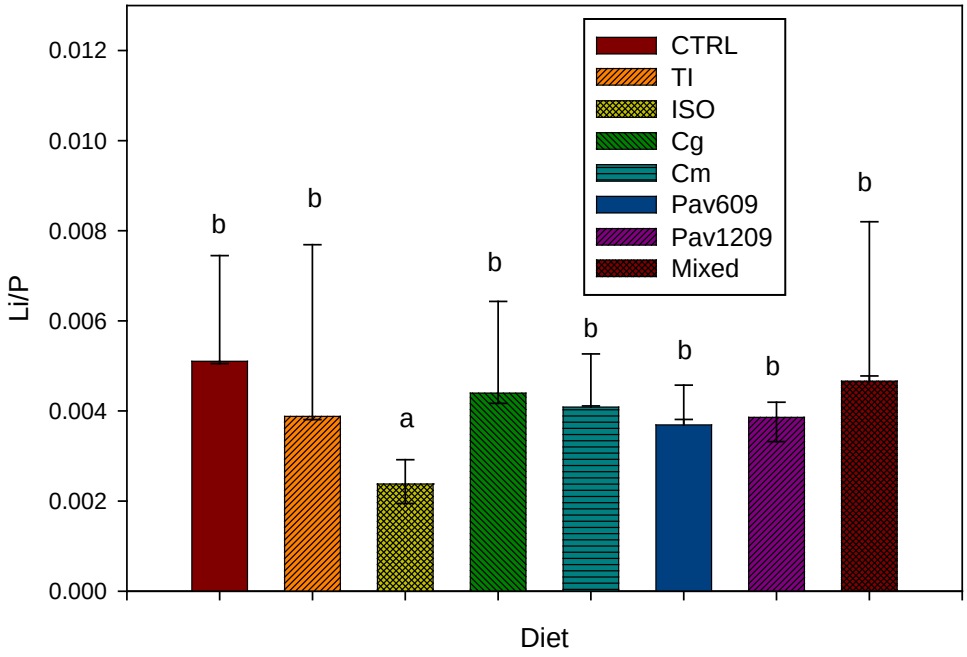


Figure 4.13: Median Li/P ratios with associated 95% confidence intervals in *Mercenaria* shells by diet. Letters indicate the similarities and differences revealed by Dunn's post hoc analyses (Table A11).

Table 4.12: Results of Kruskal-Wallis analysis for (Li/P) by diet.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
CTRL	61	6	5.10E-03	2.60E-03	9.20E-03
TI	56	23	3.88E-03	3.15E-03	5.72E-03
ISO	50	7	2.38E-03	8.71E-04	3.46E-03
Cg	58	10	4.40E-03	3.52E-03	6.14E-03
Cm	62	18	4.09E-03	3.51E-03	5.31E-03
Pav609	58	18	3.69E-03	3.50E-03	4.75E-03
Pav1209	54	15	3.86E-03	3.35E-03	4.59E-03
Mixed	68	24	4.66E-03	3.54E-03	6.53E-03
H = 54.1 with 7 degrees of freedom (P = <0.001)					

As depicted in Figure 4.16, the comparisons of manganese concentrations as compared to phosphorous and calcium among diets revealed the Mixed diet as the highest ratio, differing close to an order of magnitude from the lowest ratios in the CTRL

group (Tables 4.15 and A14). The ratios from the Cm, Ti, and Pav1209 groups were identified as statistically similar to that of the Mixed group with the remaining diet groups falling between the end values. The ratios in the CTRL treatment were significantly lower than in all other treatments.

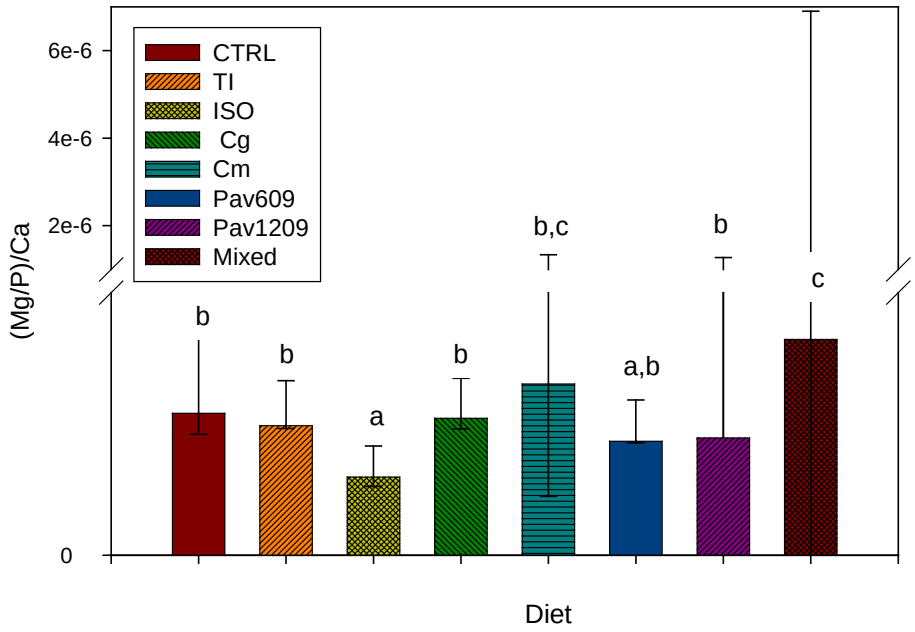


Figure 4.14: Median (Mg/P)/Ca ratios with associated 95% confidence intervals in *Mercenaria* shells by diet. Letters indicate the similarities and differences revealed by Dunn's post hoc analyses (Table A12).

Table 4.13: Results of Kruskal-Wallis analysis for (Mg/P)/Ca by diet.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
CTRL	61	6	5.4E-09	3.92E-09	7.33E-09
TI	56	18	4.93E-09	3.69E-09	7.53E-09
ISO	50	7	2.97E-09	1.11E-09	4.92E-09
Cg	58	11	5.22E-09	4.06E-09	5.98E-09
Cm	62	18	6.52E-09	4.49E-09	1.06E-08
Pav609	58	18	4.34E-09	3.66E-09	5.95E-09
Pav1209	54	14	4.47E-09	3.65E-09	6.24E-09
Mixed	65	26	8.22E-09	4.79E-09	1.69E-08
H = 58.4 with 7 degrees of freedom (P = <0.001)					

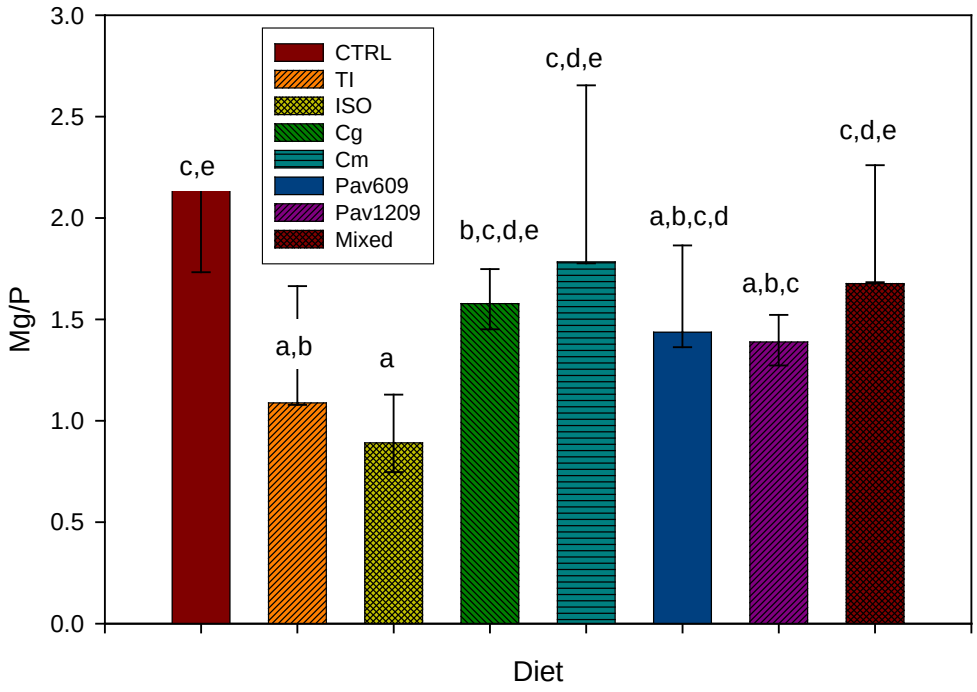


Figure 4.15: Median Mg/P ratios with associated 95% confidence intervals in *Mercenaria* shells by diet. Letters indicate the similarities and differences revealed by Dunn's post hoc analyses (Table A13).

Table 4.14: Results of Kruskal-Wallis analysis for (Mg/P) by diet.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
CTRL	61	6	2.17E+00	1.73E+00	2.40E+00
TI	56	16	1.09E+00	9.36E-01	1.34E+00
ISO	50	7	8.92E-01	3.54E-01	1.27E+00
Cg	58	9	1.58E+00	1.28E+00	1.96E+00
Cm	62	18	1.78E+00	1.37E+00	2.39E+00
Pav609	58	18	1.44E+00	1.20E+00	1.82E+00
Pav1209	54	13	1.39E+00	1.16E+00	1.53E+00
Mixed	68	21	1.68E+00	1.28E+00	2.43E+00
H = 81.0 with 7 degrees of freedom (P = <0.001)					

The comparison of Mn/P ratios between experimental diets (Figure 4.17) supported similar interpretations as those for the (Mn/P)/Ca comparisons. The ratios from CTRL group were significantly lower than all other treatments. The ratios for the ISO and Cg groups were revealed as similar, while the ratios for the Mixed, Cm, Ti, Pav609, and Pav1209 revealed to be similar (Tables 4.16 and A15).

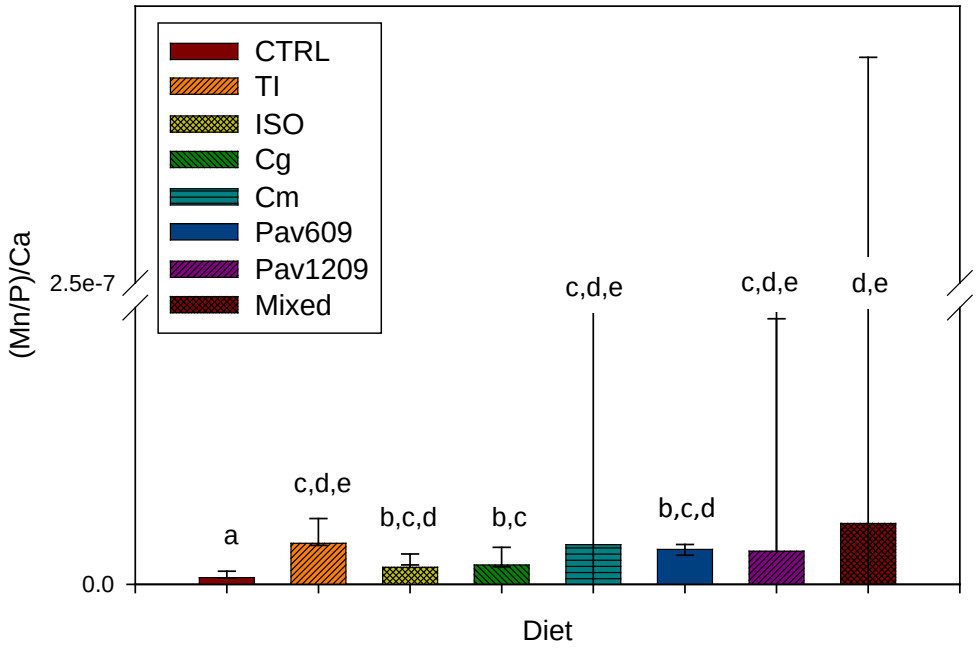


Figure 4.16: Median (Mn/P)/Ca ratios with associated 95% confidence intervals in *Mercenaria* shells by diet. Letters indicate the similarities and differences revealed by Dunn's post hoc analyses (Table A14).

Table 4.15: Results of Kruskal-Wallis analysis for (Mn/P)/Ca by diet

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
CTRL	61	6	6.87E-11	5.13E-11	1.2E-10
TI	56	17	4.23E-10	2.63E-10	7.1E-10
ISO	50	7	1.75E-10	1.31E-10	3.71E-10
Cg	58	11	2E-10	1.13E-10	3.03E-10
Cm	62	18	4.08E-10	2.44E-10	6.27E-10
Pav609	58	18	3.59E-10	1.96E-10	4.77E-10

Table 4.15 (Continued)

Group	N	Missing	Median	25%	75%
Pav1209	54	14	3.42E-10	2.61E-10	5.25E-10
Mixed	65	26	6.26E-10	4.18E-10	1.01E-09
H = 152 with 7 degrees of freedom (P = <0.001)					

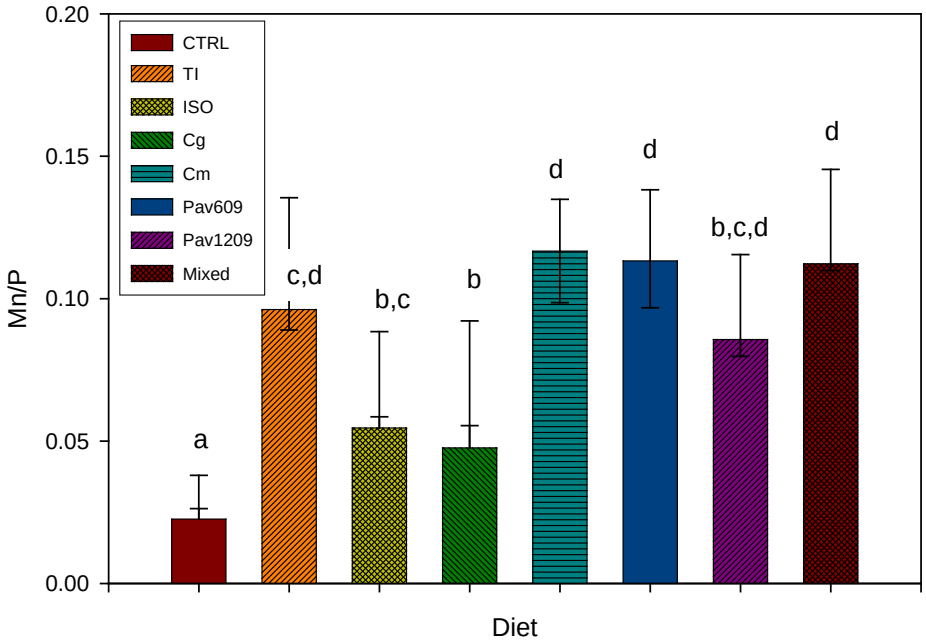


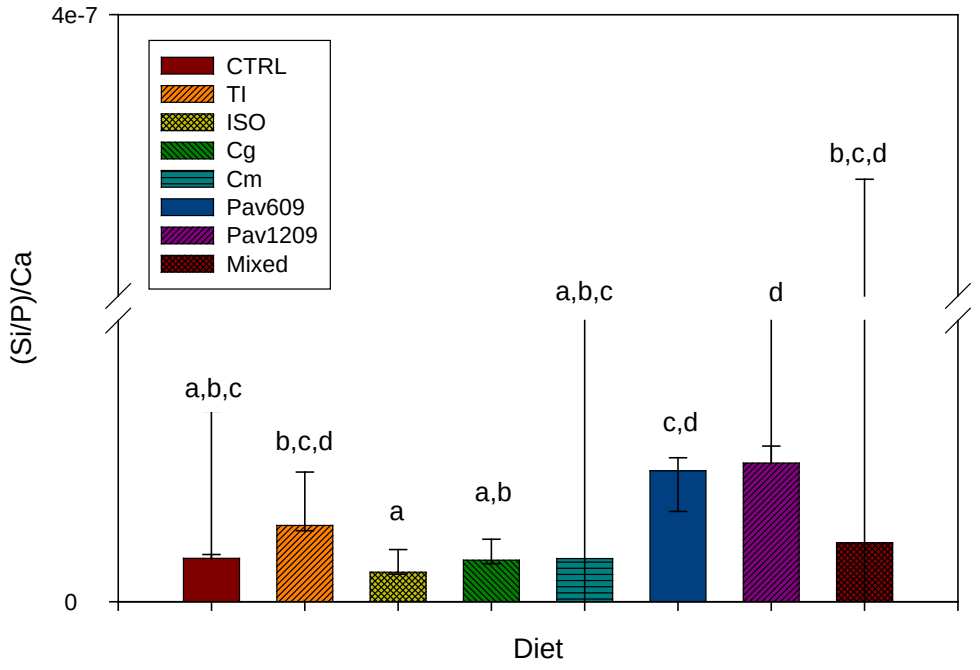
Figure 4.17: Median Mn/P ratios with associated 95% confidence intervals in *Mercenaria* shells by diet. Letters indicate the similarities and differences revealed by Dunn's post hoc analyses (Table A15).

Table 4.16: Results of Kruskal-Wallis analysis for (Mn/P) by diet.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
CTRL	61	6	2.26E-02	1.63E-02	4.66E-02
TI	56	15	9.62E-02	6.39E-02	1.46E-01
ISO	50	7	5.46E-02	4.22E-02	9.31E-02
Cg	58	9	4.76E-02	3.36E-02	1.01E-01
Cm	62	18	1.17E-01	6.43E-02	1.60E-01
Pav609	58	18	1.13E-01	6.16E-02	1.61E-01
Pav1209	54	12	8.57E-02	5.04E-02	1.31E-01
Mixed	68	20	1.12E-01	9.05E-02	1.50E-01
H = 122 with 7 degrees of freedom (P = <0.001)					

The (Si/P)/Ca ratios were highest in *Pavlova* diets with TI and Mixed diets ranking next highest and the remaining diet groups being somewhat similar (Figure 4.18). The ISO diet resulted in the lowest (Si/P)/Ca, which along with the Cg diet yielded ratios significantly lower than the *Pavlova* diets. The ratios for the Cm and CTRL were also revealed to be significantly lower than those of the Pav1209 group (Tables 4.17 and A16).

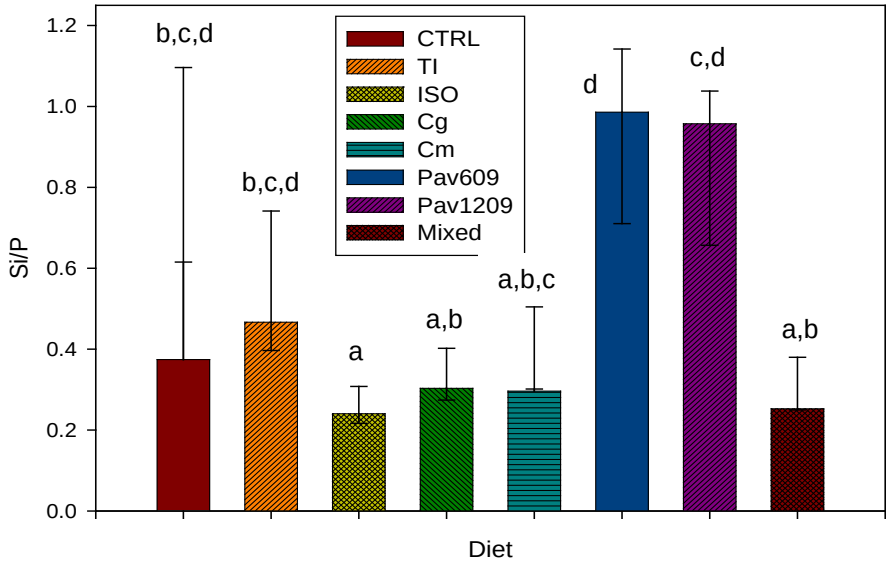
In comparing Si/P shell content between diet groups, again the highest ratios were in the two *Pavlova* diet groups, followed by TI and the CTRL, while the remaining diets were similar (Figure 4.19). The ratios from the ISO group were the lowest but not significantly different from those of the Cg, Cm, and Mixed groups (Tables 4.18 and A17).



4.18: Median (Si/P)/Ca ratios with associated 95% confidence intervals in *Mercenaria* shells by diet. Letters indicate the similarities and differences revealed by Dunn's post hoc analyses (Table A16).

4.17: Result of Kruskal-Wallis analysis for (Si/P)/Ca by diet

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
CTRL	61	6	1.08E-09	6.65E-10	3.23E-09
TI	56	18	1.9E-09	1.14E-09	3.43E-09
ISO	50	7	7.35E-10	4.27E-10	1.25E-09
Cg	58	11	1.04E-09	5.39E-10	1.67E-09
Cm	62	18	1.08E-09	6.37E-10	2.61E-09
Pav609	58	18	3.26E-09	7.75E-10	4.54E-09
Pav1209	54	14	3.45E-09	2.5E-09	4.81E-09
Mixed	65	24	1.47E-09	7.88E-10	3.13E-09
H = 49.7 with 7 degrees of freedom (P = <0.001)					



4.19: Median Si/P ratios with associated 95% confidence intervals in *Mercenaria* shells by diet. Letters indicate the similarities and differences revealed by Dunn's post hoc analyses (Table A17).

With respect to (Sr/P)/Ca, the highest concentration of Sr was found in the mixed diet group, followed by the *Chaetoceros* diet groups, then by the TI, CTRL, Pav609 and Pav1209 groups, with the ISO group significantly lowest (Figure 4.20). The statistical analyses (Tables 4.19 and A18) also revealed the ratios from the Mixed group were

significantly higher than that those of the CTRL, Pav609, and Pav1209 groups but similar to that of the *Chaetoceros* and TI groups.

4.18: Results of Kruskal-Wallis analysis for (Si/P) by diet

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
CTRL	61	6	3.74E-01	2.21E-01	1.40E+00
TI	56	16	4.67E-01	3.11E-01	7.57E-01
ISO	50	7	2.40E-01	1.41E-01	3.37E-01
Cg	58	9	3.03E-01	1.96E-01	4.83E-01
Cm	62	18	2.96E-01	1.82E-01	5.79E-01
Pav609	58	18	9.86E-01	4.16E-01	1.35E+00
Pav1209	54	12	9.57E-01	2.91E-01	1.24E+00
Mixed	68	19	2.53E-01	1.91E-01	3.92E-01
H = 48.8 with 7 degrees of freedom (P = <0.001)					

The Mixed diet group was associated with the highest concentration of strontium when Sr/P was compared among diet treatments, but not significantly different from that of the Cg, Cm, Pav609, or CTRL diet groups. The ratios from the ISO group were once again revealed as significantly lower than those from all other treatments (Figure 4.21, Tables 4.20 and A19).

Zinc as compared to phosphorus and calcium was significantly higher in Mixed diet group than in the CTRL and Pav609 groups (Figure 4.22, Tables 4.21 and A20). The ratio from the ISO group was revealed to be significantly lower than those from the Cg, Cm, Mixed, and Pav1209 groups. All other diets were revealed to be similar with respect to (Zn/P)/Ca.

Median Zn/P by diet (Figure 4.23) very closely resembled that of (Zn/P)/Ca. Again Cg group had the highest ratio, though not significantly different from the other

diet groups aside from ISO. The ratio from the ISO group was the lowest but revealed to only be significantly different from the Cg and Mixed groups (Tables 4.22 and A21).

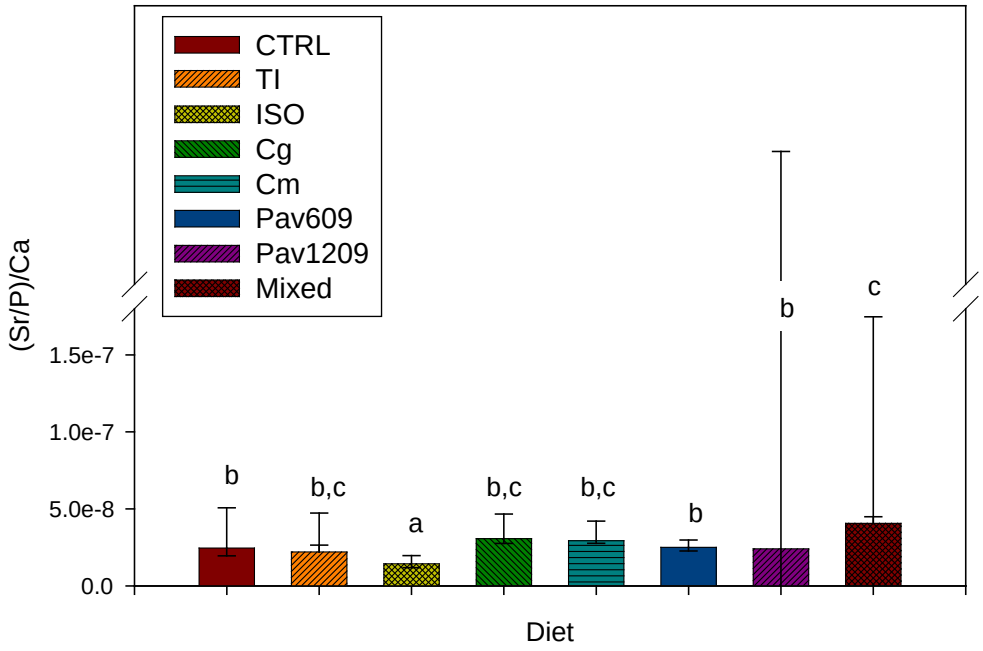


Figure 4.20: Median (Sr/P)/Ca ratios with associated 95% confidence intervals in *Mercenaria* shells by diet. Letters indicate the similarities and differences revealed by Dunn's post hoc analyses (Table A18).

Table 4.19: Results of Kruskal-Wallis analysis for (Sr/P)/Ca by diet.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
CTRL	61	6	2.46E-08	1.38E-08	4.09E-08
TI	56	19	2.2E-08	1.79E-08	4.72E-08
Table 4.36 Continued					
Group	N	Missing	Median	25%	75%
ISO	50	7	1.43E-08	6.61E-09	2.07E-08
Cg	58	11	3.07E-08	2.19E-08	3.96E-08
Cm	62	22	2.93E-08	2.13E-08	3.98E-08
Pav609	58	18	2.5E-08	1.93E-08	3.25E-08
Pav1209	54	23	2.41E-08	2.01E-08	2.67E-08
Mixed	65	28	4.07E-08	2.81E-08	7.58E-08
H = 65.3 with 7 degrees of freedom (P = <0.001)					

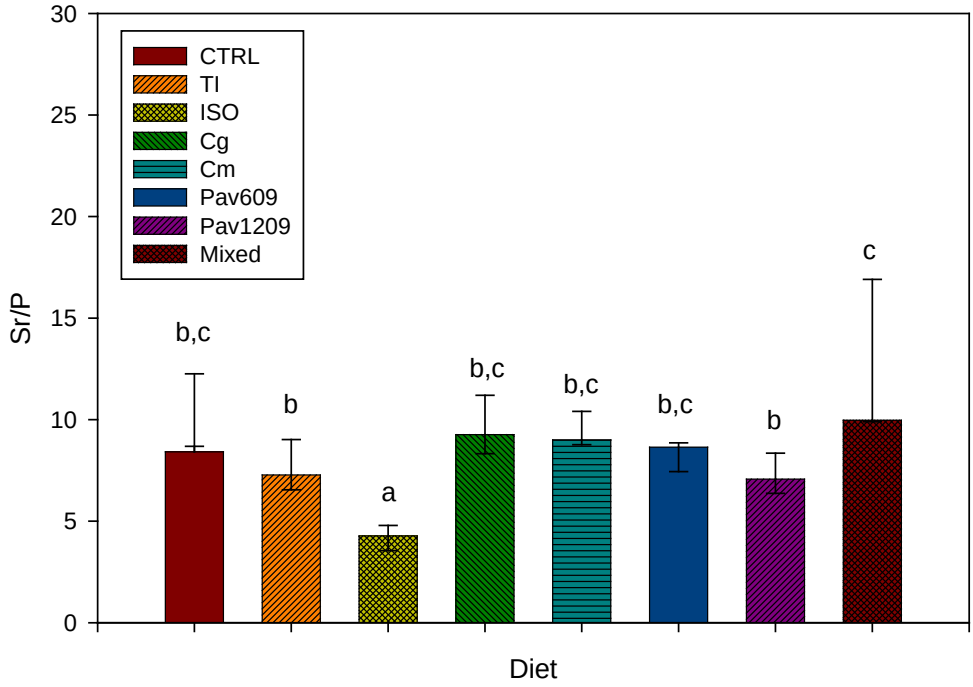


Figure 4.21: Median Sr/P ratios with associated 95% confidence intervals in *Mercenaria* shells by diet. Letters indicate the similarities and differences revealed by Dunn's post hoc analyses (Table A19).

Table 4.20: Results of Kruskal-Wallis analysis for (Sr/P) by diet

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
CTRL	61	6	8.42E+00	4.33E+00	1.66E+01
TI	56	17	7.27E+00	4.81E+00	9.22E+00
ISO	50	7	4.28E+00	2.19E+00	5.72E+00
Cg	58	9	9.26E+00	7.41E+00	1.26E+01
Cm	62	22	9.00E+00	7.90E+00	1.07E+01
Pav609	58	18	8.64E+00	6.34E+00	9.73E+00
Pav1209	54	21	7.07E+00	5.56E+00	8.47E+00
Mixed	68	22	9.97E+00	8.19E+00	1.48E+01
H = 80.3 with 7 degrees of freedom (P = <0.001)					

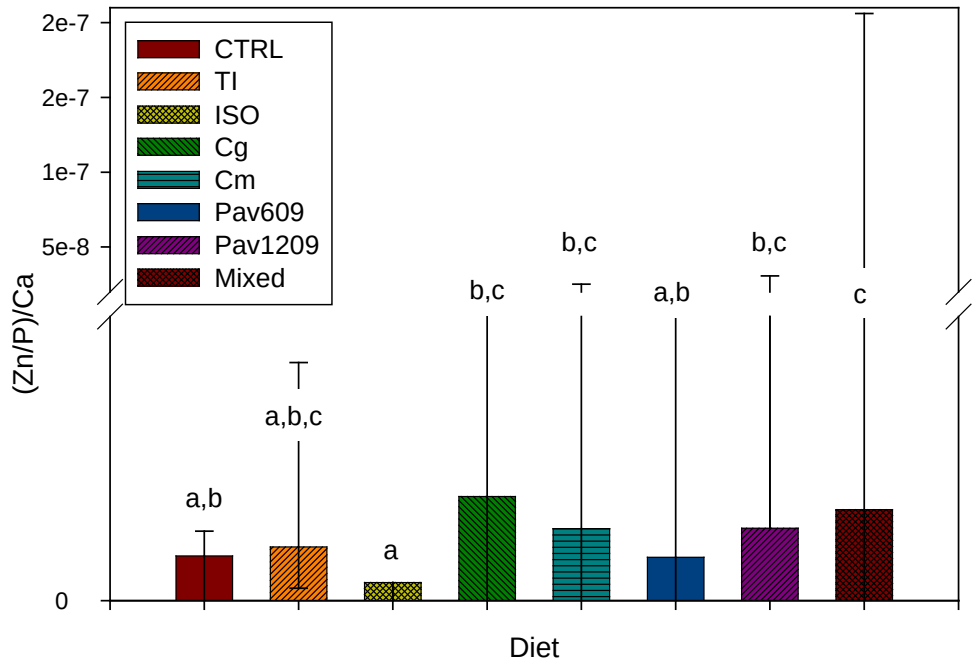


Figure 4.22: Median (Zn/P)/Ca ratios with associated 95% confidence intervals in Mercenaria shells by diet. Letters indicate the similarities and differences revealed by Dunn's post hoc analyses (Table A20).

Table 4.21: Results of Kruskal-Wallis analysis for (Zn/P)/Ca by diet.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
CTRL	53	16	4.72E-11	3.48E-11	7.70E-11
TI	37	25	5.67E-11	3.26E-11	1.75E-10
ISO	25	11	1.91E-11	-3.1E-10	4.54E-11
Cg	39	7	1.1E-10	2.76E-11	2.48E-10
Cm	45	27	7.60E-11	2.05E-11	5E-10
Pav609	39	17	4.57E-11	1.60E-11	1.06E-10
Pav1209	20	5	7.65E-11	5.31E-11	4.22E-08
Mixed	29	10	9.60E-11	7.38E-11	4E-08
H = 32.3 with 7 degrees of freedom (P = <0.001)					

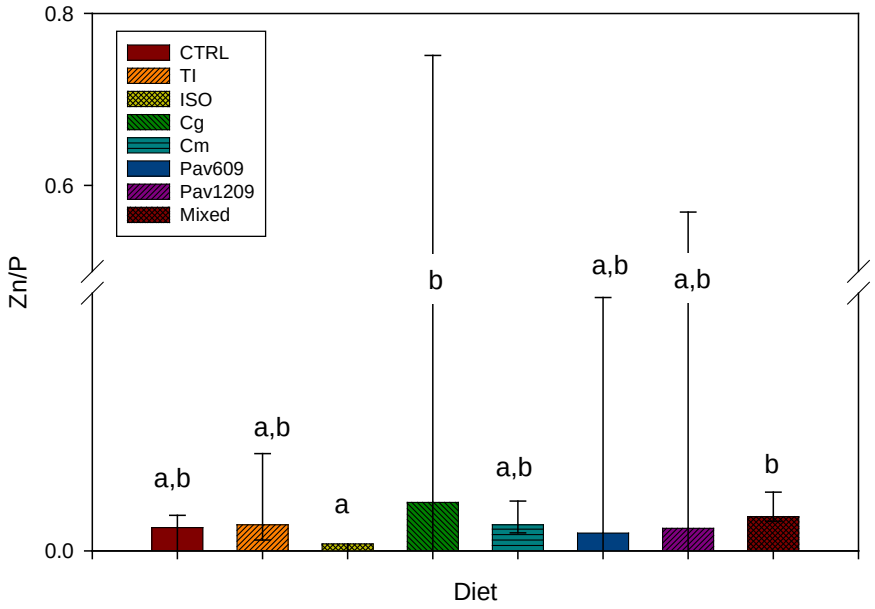


Figure 4.23: Median Zn/P ratios with associated 95% confidence intervals in *Mercenaria* shells by diet. Letters indicate the similarities and differences revealed by Dunn's post hoc analyses (Table A21).

Table 4.22: Results of Kruskal-Wallis analysis for (Zn/P) by diet

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
CTRL	53	16	1.67E-02	1.33E-02	2.52E-02
TI	37	25	1.87E-02	7.18E-03	5.94E-02
ISO	25	11	5.02E-03	-1.08E-01	1.34E-02
Cg	39	7	3.48E-02	9.85E-03	5.61E-02
Cm	45	27	1.88E-02	1.17E-02	2.43E-02
Pav609	39	17	1.28E-02	4.23E-03	3.63E-02
Pav1209	20	5	1.62E-02	1.11E-02	2.00E-02
Mixed	29	10	2.46E-02	1.97E-02	3.21E-02
H = 21.3 with 7 degrees of freedom (P = 0.003)					

Discussion

In general, problems associated with comparing the elemental concentrations to phosphorus include a) variance in P concentrations potentially not being uniform and

attributable to the same factor in each group, b) high P concentrations contributing to unrelated minimal ratios with specific diets, and c) potential confounding factors outside controlled environments as identified in previous studies. Analyses using both the (element/P)/Ca and element/P did, however, produced results considered to be of interest with regard to six elements, either due to their similarity to elemental trends observed in algae quotas described by Ho et al (2003), Sunda and Huntsman (1995), and Brand et al. (1983), or mirroring trends observed in the results described in Chapters 2 and 3. Iron, potassium, magnesium, manganese, silica, and zinc were found to most possibly be directly associated with the diet received or assumed metabolic influences. Observed relative strontium concentrations, again, support an association with growth rate. Conversely, analysis of boron, barium, cobalt, and lithium appeared to be associated with other environmental factors or factors not associated with experimental variables.

Ho et al. (2003) presented analyses of the cellular content of 15 phytoplankton species with regard to C, N, P, S, K, Mg, Ca, Sr, Fe, Mn, Zn, Cu, Co, Cd and Mo (Figure 4.1). In general, they found that K concentrations are higher than in seawater and Mg lower, except in the case of diatoms where both Mg and K are relatively high. The major nutrients C, N, P, and S are variable with the average quotas of the organic biomass being similar to that of Redfield et al. (1963). The trace metals followed the general pattern of Fe>Mn>Zn>Cu>Co=Cd>Mo. The coccolithophores examined by Ho et al. (2003) had higher Mn, Co, and Cd quotas compared to the diatoms. This difference was explained by the possible difference between metal requirements of oceanic and neritic species, as the oceanic diatom examined had low Fe, Mn, and Cu quotas and higher

Co and Cd quotas compared to the coastal diatoms. Table 4.44 provides a summary of the quota comparisons by algal class as adapted from Ho et al. (2003).

Table 4.23: Interpreted ranking of algae element quotas as depicted in Ho et al. (2003). 1=highest concentration and 5 the lowest.

	Chloro	Prasino	Dino	Cocco	Diatoms
S/P	5	3	2	4	1
K/P	5	2	4	3	1
Mg/P	3	4	2	5	1
Sr/P	5	3	2	1	4
Fe/P	1	2	3	4	5
Mn/P	5	2	3	1	4
Zn/P	1	2	3	5	4
Cu/P	2	1	1	5	4
Co/P	4	3	2	1	3
Cd/P	4	2	3	1	5
Mo/P	5	2	1	3	4

In the present research, two species each representative of three taxonomic classes (Pavlovacea, Prymesiophycea, and Bacillariophycea) were used as diet variables**.** *Pavlova* sp. (CCMP1209, Pav1209) was isolated from an unknown tropical site and *Pavlova pinguis* (CCMP609) from the Sargasso Sea. *Isochrysis* sp.1 (CCMP1324, TI) was isolated from the South Pacific off the Society Islands while Isochrysis sp.*2* (CCMP1611, ISO) from Chesapeake Bay. Finally, *Chaetoceros galvestonensis* (CCMP186, Cg) was isolated from the Gulf of Mexico off St. Petersburg, FL, and *C. mulleri* (CCMP1316, Cm) from the North Pacific off Hawaii. Originally, two species of *Tetraselmis* were proposed for inclusion in the study, however, trouble maintaining these cultures ultimately lead to their removal from the feeding trials.

Iron has been suggested as a major constituent of shell deposition (Almeida et al. 1998) as well as suggested to be associated with sediment concentrations during

experiments with *Crassostrea virginica* by Carriker et al. (1996). The hypothesis that metabolic/biologic controls or ingestion of particulates could be responsible for iron concentrations observed in the shells of studied bivalves was based upon these previous findings. The Fe/P ratios appeared to suggest a link between *Pavlova* spp. and the highest concentrations of iron, though the trend was not significant. The comparisons in Ho et al. (2003), unfortunately, did not include specific Pavlovacea species, thus direct comparison is not fully possible as to the potential relationship of iron with this class of algae. However, an increased iron quota and hypothesized subsequent contribution similar to what was expected for the Coccolithophores/Prymnesiophycea is based on similarities between the classes. Of particular interest, analyses of both (Fe/P)/Ca and Fe/P, revealed the ratios from the Cg, ISO, and the CTRL groups as the lowest among the feeding groups, though again not significantly different from the majority of the remaining diet groups. Both the Cg and ISO were neritic isolates and hypothesized to be associated with iron enrichment of the shells based on findings from Sunda and Huntsman (1997), which evidently is the opposite of the current trends observed.

Potassium concentrations were expected to be higher in the shells of clams fed diatom-rich diets based on Ho et al. (2003) as compared to other classes of algae examined. Shells in the *Chaetoceros* treatments did appear to be more enriched with potassium than those from the *Isochrysis spp*. treatments, though not significantly so. The shells from the *Pavlova spp*. treatments exhibited more similar ratios to the *Chaetoceros spp*. treatments. Significant differences, however, were found only between the ratios from the Mixed group and those from the ISO and CTRL groups with

respect to (K/P)/Ca and both the Cm and Mixed groups from ISO with respect to K/P. As such, the observed trends do not support the hypothesis that trends in potassium as normalized to phosphorous can be attributable to specific classes of algae but rather indicate potential species specific influences confounded by nutritional value of the diet and potentially differences between inshore algal strains and nearshore/offshore strains.

Magnesium/phosphorous ratios presented a marginally significant trend with diet. Median ratios for both *Chaetoceros spp*. were higher than those for *Isochrysis spp*. with ratios in shells from the Cm diet significantly higher than both *Isochrysis spp*. When (Mg/P)/Ca ratios were considered, direct dietary influence was not apparent, in fact, the trends indicate growth rate as the most likely dominant influence. These findings were interpreted as suggesting multiple factors being associated with magnesium concentrations. Foster et al. (2008) demonstrated that magnesium was associated with the organic partitions of aragonitic shells and, as such, was related to compositional changes in the organic matter or extra pallial fluid contributing to changes in incorporation rate. In context of the present study, both growth rate and element availability had the potential to influence the composition of the organic matter and therefore element incorporation.

Shells of clams fed the neritic species had lower relative manganese concentrations than those fed the oceanic species, or diets composed of both, as did the CTRL group. This trend, however, was significant only when using Mn/P ratios, though the algal species of unknown origin was not significantly different from the known neritic species. These findings did support the hypothesis that oceanic algal isolates would be associated with higher manganese shell concentrations than the

neritic isolates. Of interest is the low value in the CTRL group, as this indicates potential for metabolic/stress associated changes or the influence of the pre-experimental brown-water diet composed only of neritic algal species. Though associations between manganese and biologic influences have been suggested (Strasser et al., 2008) alternative factors cannot be dismissed. Blanchard and Chasteen (1976) surmised that the amount of substitution of Ca^{2+} by Mn^{2+} was correlated with tidal level, though did not consider the oxygenation of the environment (Rosenberg, 1980). Crisp (1975) attempted to correlate the Mn concentration to salinity, while Strasser et al. (2008) correlated Mn/Ca levels with that of seawater concentration, potentially confounded by biological activity. Carre et al. (2006) suggested positive Mn/Ca association with growth rates while Strasser et al. (2006) found a negative correlation. Most researchers, however, have not considered dietary influence or stress-related influences potentially associated with their observations.

The silica ratios in the shells from clams fed *Pavlova spp*. were consistently higher than in shells from those fed diatoms. Interestingly, shells of clams fed ISO were among the least Si enriched treatments, including when compared to TI. Again, multiple factors are likely associated with these findings, however, the trends do indicate that diet can influence the Si concentration of clam shells.

Zinc-associated ratios appeared to have a similar trend to that observed by Ho et al. (2003) in that Zn concentrations in the shells from diatom treatments were higher than for the Coccolithophores/ Prymnesiophytes treatments. Furthermore, the trends for both Zn ratios were similar, with the shells from the Mixed diet, one or both the diatom diets, and Pav1209 significantly more enriched than those from the ISO group.

The Mixed diet ranked higher for both Zn ratios as compared to the control and Pav609 groups. Based on previous findings associating zinc with dietary factors and metabolic transformations (Windisch, 2001), incorporation of zinc specific to diet as well as metabolic differences amongst clams in the different feeding groups did both likely contribute to the results.

Conclusion

Because the ratios from the ISO group were consistently the lowest in the study, it is suspected that factors not assessed and potential contamination of these shells influenced the results and ultimately complicated interpretations. The results of this study, thusly, are inconclusive with respect to the influence of diet on elemental composition of the shell of *M. mercenaria*. The (Element/P)/Ca and Element/P ratios, while not necessarily capable of being a primary line of evidence, have potential utility in support of findings from standard normalized comparisons, as well as in revealing possible secondary and tertiary influences on elemental shell composition.

5. Temporal changes in the elemental shell chemistry of *Mercenaria mercenaria* during starvation-induced stress and death

Introduction

The composition of fossil shells is generally determined by some combination of the physiochemical environment inhabited by the organism, biologic controls of skeletal growth, and diagenetic alterations (Gomez-Alday and Elorza, 2001). Shell alteration begins while the organism is living and factors that cause shell weathering are among the same factors associated with changes in the shell-deposition metabolism (Rosenberg, 1980). Thus, knowledge of initial shell chemistry is vital in making interpretations as to chemical alterations and subsequent reconstruction of environments (Rosenberg, 1980).

Shell dissolution can occur during periods of anaerobiosis in bivalve molluscs (Crenshaw, 1980) which are characterized by decreased pH and increased succinic acid (Crenshaw and Neff, 1969). Environmental stresses can also cause shell dissolution and ultrastructural changes (Davies and Sayre, 1970; Wilbur, 1972). As discussed in chapters 2 and 3, environmental stresses can ultimately lead to changes in organismal metabolism and changes in chemical composition.

The focus of this chapter is the description of changes in elemental chemistry observed in the shells of starved individuals and that of the dead individuals. The goal is to provide a chronological view of changes potentially occurring in bivalve populations with regard to shell chemistry during events leading to death of the organism and shortly after death.

Methods

The shells analyzed during this research were originally cultured as part of the control group for the feeding experiments described in Chapter 3. A subsample of valves was collected at the start of the starvation period to provide a baseline to compare elemental changes through time with subsequent collections in March, May, July and October. The collected clams were examined during valve separation and notes taken to document the visible changes in health of the animals by use of mantle retraction, tissue occupancy of the shell, gaping, tissue deterioration, and the lack of tissue.

The shells were treated identically as described in Chapter 3 and analyzed for elemental composition using the ICP-OES. Each elemental concentration was again transformed to a ratio as compared to calcium for statistical analysis.

The element/Ca levels were initially graphed by collection month to determine the general trends associated with each element. The data were then examined by collection month using analysis of variance or Kruskall-Wallis one-way analysis of variance on ranks followed by an appropriate post hoc analysis as in the previous chapters.

Results

No dead clams were observed until July though mantle retraction, small tissue volumes and some intermediate gaping was observed during March with increased signs of stress noted in both May and July. The July collection was the first time empty valves and deteriorating tissue were observed. All the animals in the October collection were dead, with the majority of shells void of tissue and only a few with small remnants of decomposing tissue apparent.

Due to unsuccessful analysis of specific elements for some of the collection groups, the analyses were confined to B, Ba, Co, Fe, K, Li, Mg, Mn, Si, and Zn. The results herein thus focus on these elements as compared to Ca and changes in those ratios over the course of the experiment.

The B/Ca ratios by collection month (Figure 5.1, Tables 5.1 and B1) revealed a higher relative boron concentration in July compared to all other collections. The only other significant difference was observed between the March and October collections.

The Ba/Ca ratios (Figure 5.2, Tables 5.2 and B2) indicate significant loss of Ba from the shells between January and March. The ratios thereafter, however, did not significantly change. Of note was the apparent decrease in deviation from the median values through the course of the experiment.

Cobalt concentrations as compared to calcium again were associated with a general downward trend to the July collection with a significant increase observed in October (Figure 5.3). The Co/Ca ratios in the shells from July were significantly lower than those from the baseline (January) and March collections (Tables 5.3 and B3).

There is a large degree of uncertainty as to the amount of change between January and July as the May median is based upon of a single clam.

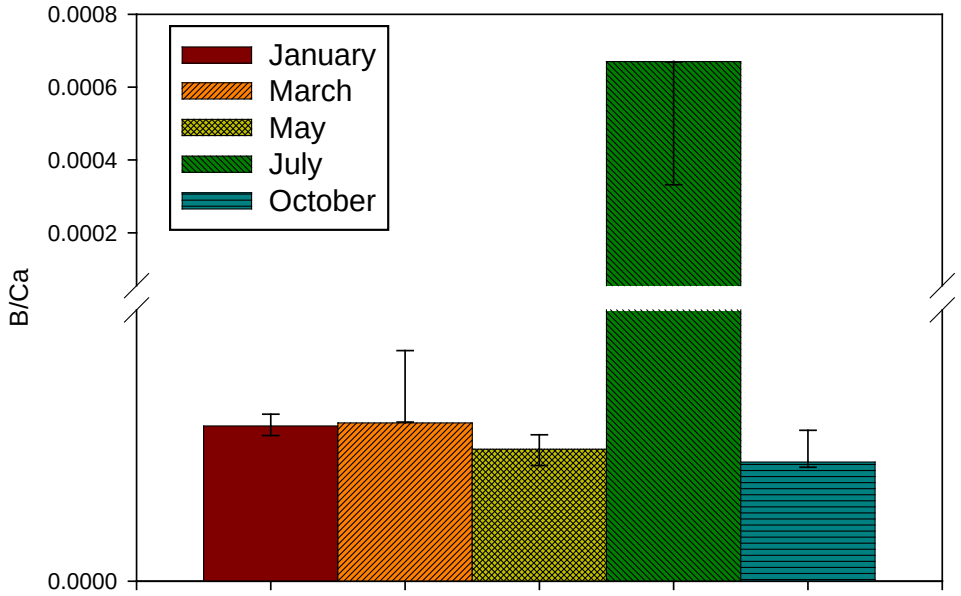


Figure 5.1: Median B/Ca compared to month collected with associated 95% confidence intervals.

Table 5.1: Summary of analysis of variance on ranks for B/Ca.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
January	14	1	2.86E-05	2.71E-05	3.17E-05
March	16	1	2.92E-05	2.78E-05	4.87E-05
May	11	1	2.43E-05	2.19E-05	2.66E-05
July	21	1	6.70E-04	3.80E-05	7.91E-04
October	16	1	2.20E-05	2.14E-05	2.60E-05
H = 46.0 with 4 degrees of freedom (P = <0.001)					

The Fe/Ca ratios exhibited a significant decrease between the baseline (January) and both the March and May collections followed by a significant increase between the May and October collections (Figure 5.4, Tables 5.4 and B4). The ratios of the shells from the March and May collections were not significantly different, nor were the ratios

of the shells from the July and October collections. Furthermore, the ratios from the baseline collection and those from the July and October collections were not significantly different.

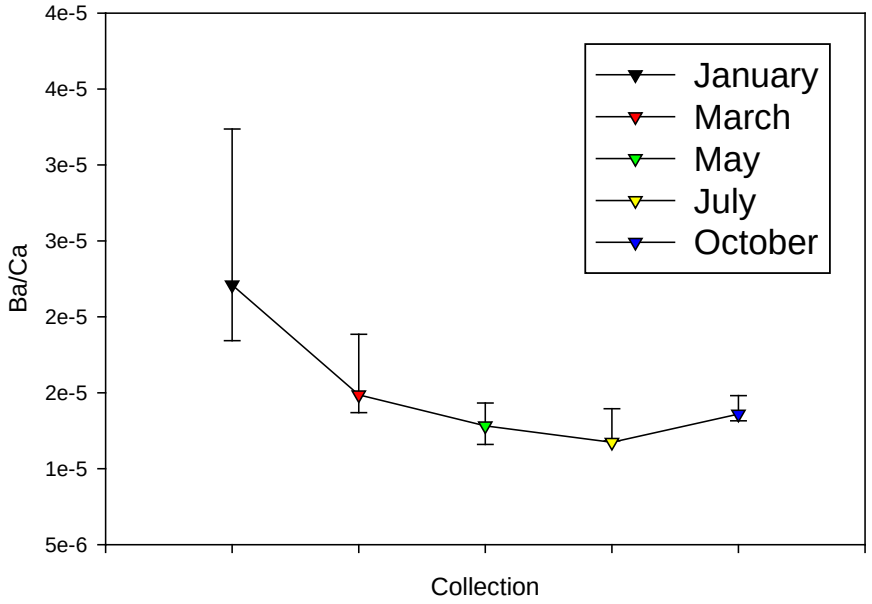


Figure 5.2: Median Ba/Ca compared to month collected with associated 95% confidence intervals.

Table 5.2: Summary of analysis of variance on ranks for Ba/Ca.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
January	14	1	2.21E-05	1.83E-05	2.66E-05
March	16	1	1.49E-05	1.29E-05	2.02E-05
May	11	1	1.28E-05	1.14E-05	1.49E-05
July	21	1	1.17E-05	1.13E-05	1.41E-05
October	16	1	1.36E-05	1.29E-05	1.51E-05
H = 33.1 with 4 degrees of freedom (P = <0.001)					

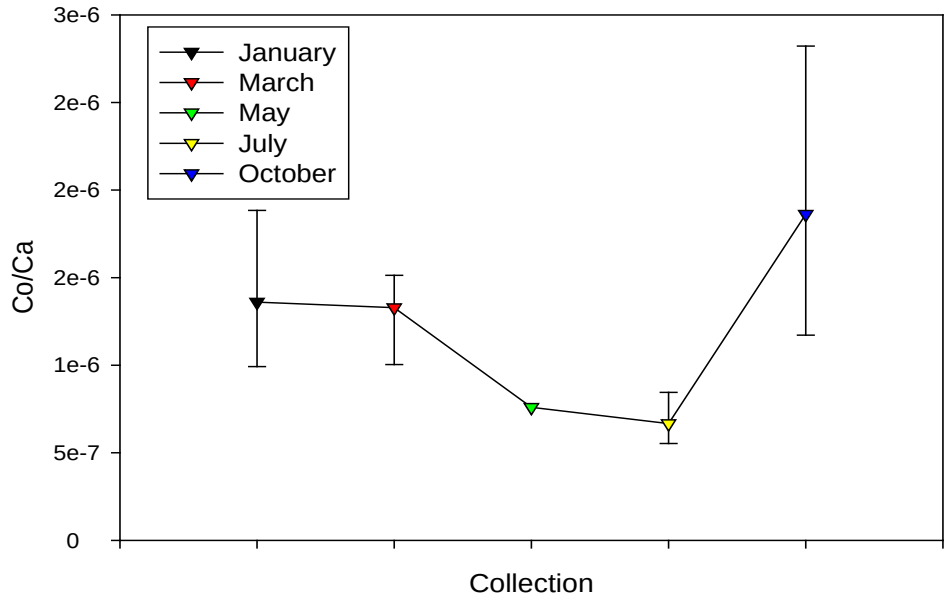


Figure 5.3: Median Co/Ca compared to month collected with associated 95% confidence intervals.

Table 5.3: Summary of analysis of variance for Co/Ca.

One Way Analysis of Variance					
Normality Test:	Passed	(P = 0.742)			
Equal Variance Test:	Passed	(P = 0.182)			
Group Name	N	Missing	Mean	Std Dev	SEM
January	12	7	1.44E-06	3.59E-07	1.61E-07
March	13	8	1.26E-06	2.05E-07	9.18E-08
May	11	10	7.60E-07	0.00E+00	0.00E+00
July	20	14	6.99E-07	1.39E-07	5.68E-08
October	14	11	2.00E-06	3.33E-07	1.92E-07
Source of Variation	DF	SS	MS	F	P
Between Groups	3	3.67E-12	1.22E-12	18.32	<0.001
Residual	15	1.00E-12	6.68E-14		
Total	18	4.68E-12			
Power of performed test with alpha = 0.050: 1.000					

The median K/Ca ratios over time (Figure 5.5) revealed a significant decrease between both the baseline (January) and March collections, and between the March and July collections (Tables 5.5 and B5). A further decrease was revealed between the

May and July collections. Again, the variability observed decreased through the experiment.

The temporal comparison of Li/Ca (Figure 5.6) revealed a gradual decrease in relative Li concentrations of the shells. The Li/Ca ratios of the clam shells from the January collection were higher than those from the July and October collections (Tables 5.6 and B6). The ratios of the shells from the March and May collections were also revealed to be higher than those from October. The variability of Li/Ca ratios by collection again decreased through the experiment.

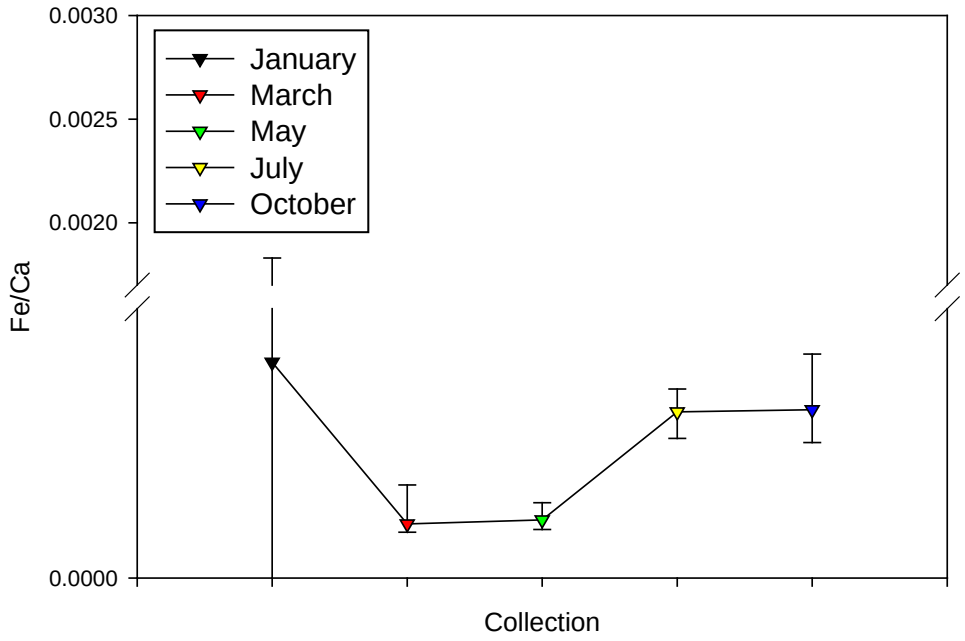


Figure 5.4: Median Fe/Ca compared to month collected with associated 95% confidence intervals.

Table 5.4: Summary of analysis of variance on ranks for Fe/Ca.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
January	14	1	8.01E-05	5.73E-05	7.70E-04
March	16	1	2.01E-05	1.28E-05	4.12E-05

Table 5.4 (Continued)

Group	N	Missing	Median	25%	75%
May	11	1	2.16E-05	1.90E-05	2.39E-05
July	21	1	6.17E-05	4.61E-05	7.70E-05
October	16	1	6.25E-05	4.53E-05	7.00E-05
H = 41.4 with 4 degrees of freedom (P = <0.001)					

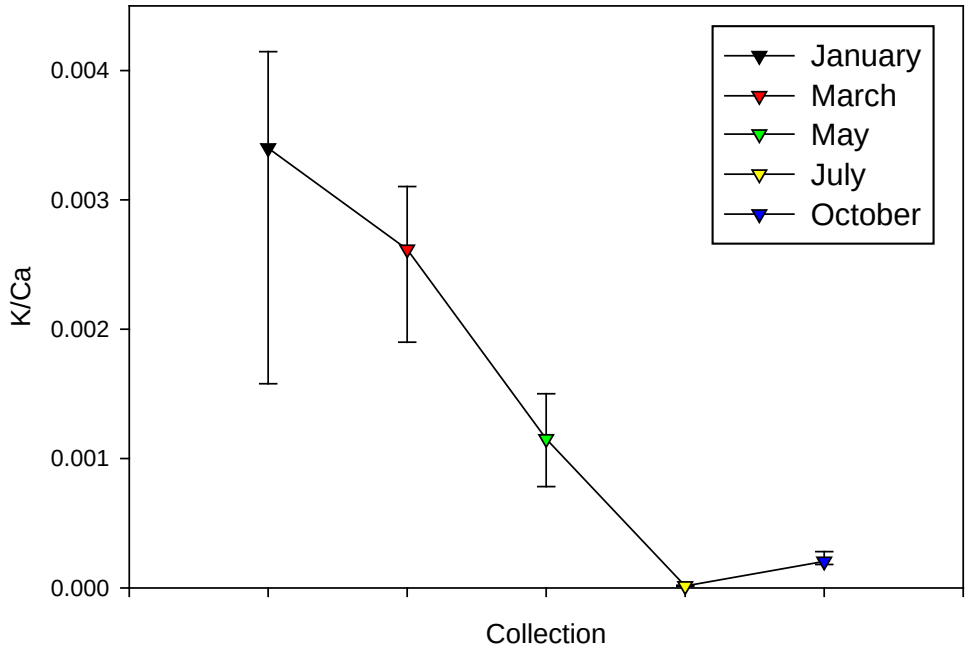


Figure 5.5: Median K/Ca compared to month collected with associated 95% confidence intervals.

Table 5.5: Summary of analysis of variance on ranks for K/Ca.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
January	14	1	3.40E-03	3.72E-06	4.72E-03
March	16	1	2.62E-03	1.57E-03	3.23E-03
May	11	1	1.15E-03	9.58E-04	1.60E-03
July	21	1	1.51E-05	9.83E-06	2.25E-05
October	16	1	2.05E-04	1.58E-04	2.46E-04
H = 41.2 with 4 degrees of freedom (P = <0.001)					

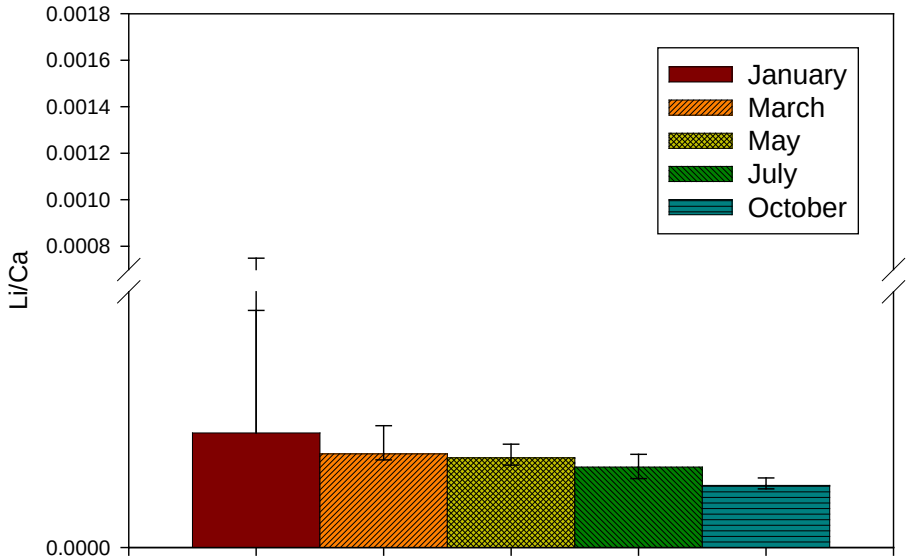


Figure 5.6: Median Li/Ca compared to month collected with associated 95% confidence intervals.

Table 5.6: Summary of analysis of variance on ranks for Li/Ca.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
January	14	1	4.03E-06	3.86E-06	9.14E-04
March	16	1	3.29E-06	2.91E-06	4.29E-06
May	11	1	3.16E-06	2.92E-06	3.60E-06
July	21	1	2.83E-06	2.16E-06	3.32E-06
October	16	1	2.19E-06	2.01E-06	2.27E-06
H = 37.5 with 4 degrees of freedom (P = <0.001)					

The Mg/Ca trend revealed an increase in relative Mg concentration between the baseline and March collections followed by a decrease in Mg concentration (Figure 5.7). The Mg/Ca ratios were significantly higher in the shells from the March collection compared to those of the January, July and October collections; while Mg/Ca ratios of the shells from the May collection were significantly higher than those of the July and

October collections (Tables 5.7 and B7). Of note, the only significant differences from baseline ratios were those from the March collection.

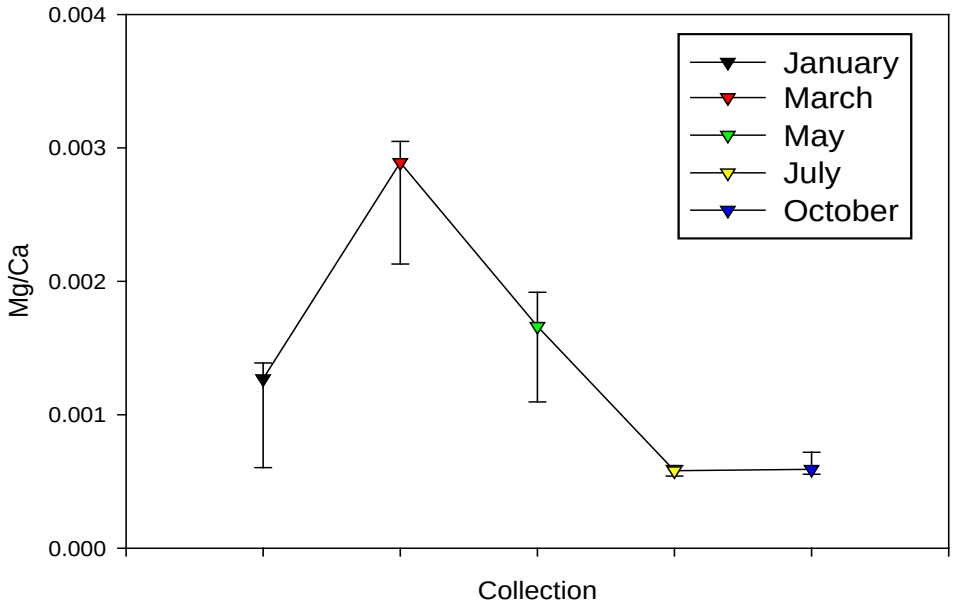


Figure 5.7: Median Mg/Ca compared to month collected with associated 95% confidence intervals.

The Mn/Ca ratios revealed a decrease in relative Mn concentration of the shells followed by a small increase in the shells of the October collection (Figure 5.8), though all changes appeared to be within the deviation of the baseline (January) collection. Statistical analyses (Tables 5.8 and B8) revealed that Mn/Ca ratios were higher in the shells from the baseline collection than those from the shells in the May, July, and October collections. In addition, March Mn/Ca ratios were higher than May and July.

Table 5.7: Summary of analysis of variance on ranks for Mg/Ca.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
January	14	2	1.27E-03	5.35E-04	1.41E-03

Table 5.7 (Continued)

Group	N	Missing	Median	25%	75%
March	16	1	2.89E-03	1.87E-03	3.35E-03
May	11	1	1.66E-03	1.30E-03	1.89E-03
July	21	1	5.80E-04	5.19E-04	6.51E-04
October	16	1	5.91E-04	5.13E-04	7.31E-04
H = 43.8 with 4 degrees of freedom (P = <0.001)					

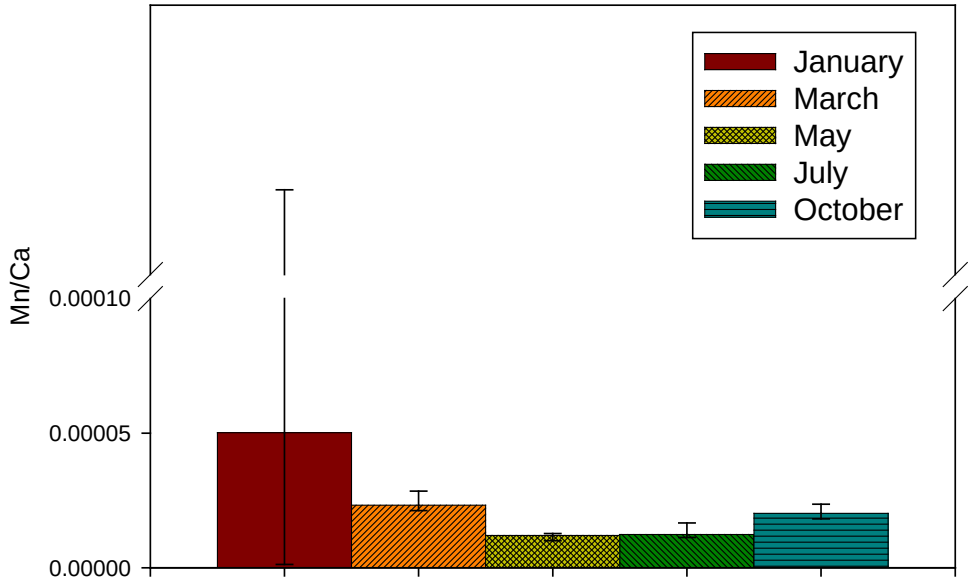


Figure 5.8: Median Mn/Ca compared to month collected with associated 95% confidence intervals.

Table 5.8: Summary of analysis of variance on ranks for Mn/Ca.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
January	14	2	5.01E-05	3.18E-05	2.00E-04
March	16	1	2.32E-05	2.17E-05	3.01E-05
May	11	1	1.20E-05	9.44E-06	1.27E-05
July	21	1	1.23E-05	1.02E-05	1.80E-05
October	16	1	2.02E-05	1.77E-05	2.21E-05
H = 47.3 with 4 degrees of freedom (P = <0.001)					

The Si/Ca ratios over time were more variable than the elements previously discussed (Figure 5.9). The statistical analyses (Tables 5.9 and B9) revealed a significant decrease of Si/Ca ratios in the shells collected between January and May and that relative concentrations of silica were higher in shells collected in both July and October as compared to those collected in March and May.

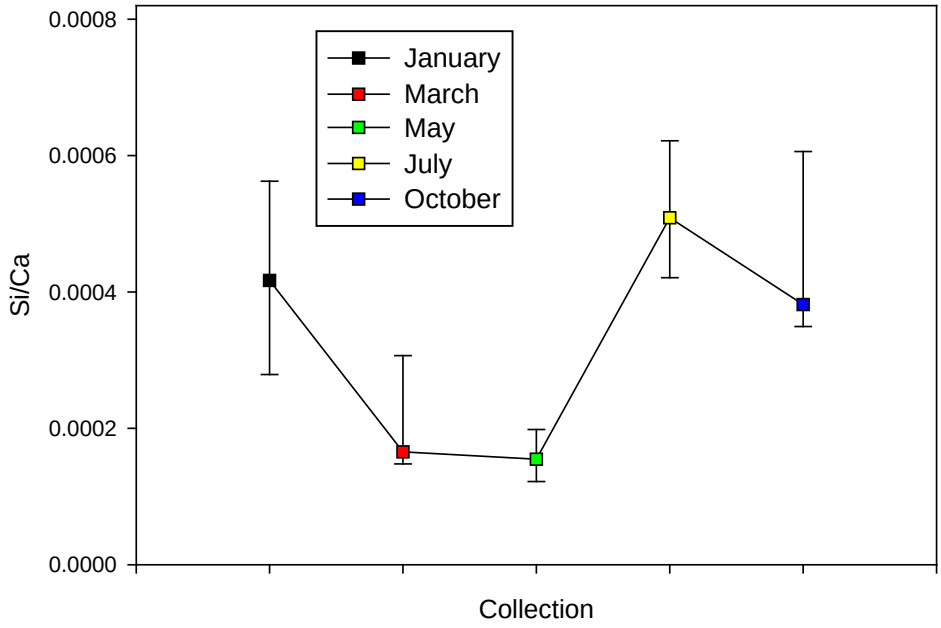


Figure 5.9: Median Si/Ca compared to month collected with associated 95% confidence intervals.

Table 5.9: Summary of analysis of variance on ranks for Si/Ca.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
January	10	1	4.17E-04	2.93E-04	5.26E-04
March	16	1	1.65E-04	1.45E-04	2.64E-04
May	11	1	1.55E-04	1.26E-04	1.69E-04
July	21	1	5.09E-04	3.70E-04	6.31E-04
October	16	1	3.82E-04	3.52E-04	5.24E-04
H = 35.7 with 4 degrees of freedom (P = <0.001)					

The trend in Zn/Ca by collection month (Figure 5.10) was a small increase in median Zn/Ca ratios of the shells collected between January and March followed by a gradual decrease through July followed by a more dramatic decrease between July and October. The Zn/Ca ratios of the shells from both January and March collections were significantly higher than those from the July and October collections (Tables 5.10 and B10). Thusly, a significantly decreased concentration of zinc was observed from baseline to the final experimental collection.

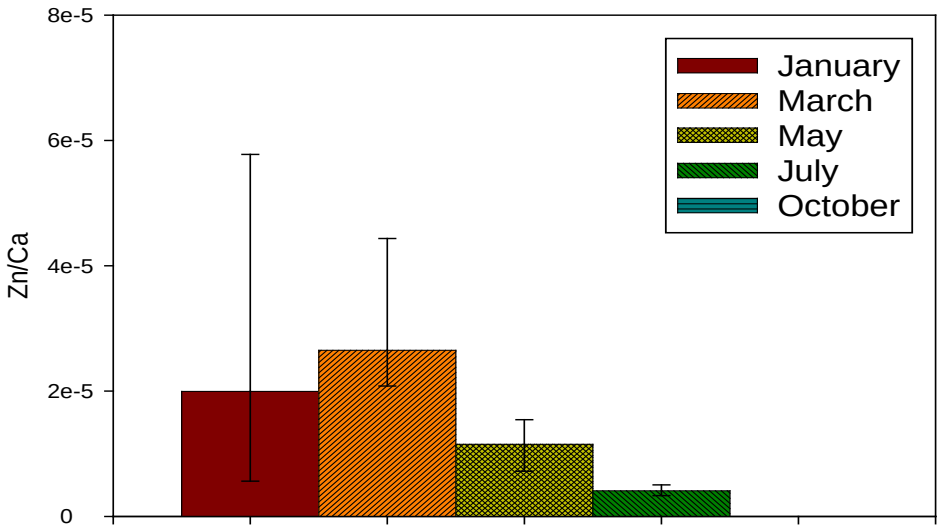


Figure 5.10: Median Zn/Ca compared to month collected with associated 95% confidence intervals. The October measurements were below the concentration of the standard.

Table 5.10: Summary of analysis of variance on ranks for Zn/Ca.

One Way Analysis of Variance					
Normality Test:	Failed	(P < 0.050)			
Kruskal-Wallis One Way Analysis of Variance on Ranks					
Group	N	Missing	Median	25%	75%
January	14	2	2.00E-05	1.61E-05	2.80E-05
March	16	2	2.65E-05	1.87E-05	4.07E-05
May	11	1	1.15E-05	8.27E-06	1.45E-05
July	21	10	4.09E-06	3.06E-06	4.91E-06
October	13	7	-2.22E-04	-2.75E-04	-1.45E-04
H = 39.7 with 4 degrees of freedom (P = <0.001)					

Discussion

The current study demonstrates the changes in 10 element/calcium ratios through time for starved *Mercenaria mercenaria*. These results illustrate both changes due to assumed metabolic stress and alterations occurring post mortem which provides valuable insight into the mechanisms of shell dissolution and utility of specific elements in paleontological analyses aimed at environment reconstruction. The importance of knowledge pertaining to incorporation pathways, organic versus inorganic partitioning, and shell dissolution mechanisms is emphasized.

Boron concentrations as compared to calcium of the collected shells were not found to be significantly lower than the baseline concentrations; instead, there was a dramatic increase in the Ba/Ca of the shells from the July collection. Studies of mollusc shells by Furst et al. (1976) suggested that boron was associated with the inorganic shell fraction and that it appeared to substitute for carbon. The substitution of carbon by boron was later supported during analysis performed by Cook (1977). It was noted, though, that effects of other factors such as temperature and nutrition were not considered (Rosenberg 1980). Additionally, the results of Hemming and Hanson (1992) demonstrated an association between pH and boron incorporation in carbonates, however, the findings were more specific to isotopic ratios. The results of the current study are not ultimately explained by incorporation of boron into the shell void of adequate carbon levels. However, association between the changes in relative concentration of boron in the collected shells and assumed metabolic changes due to starvation stresses cannot be dismissed.

Barium concentrations as compared to calcium were associated with a general decrease over time as compared to the baseline concentration, though a small increase associated with dead shells was observed. The factors associated with incorporation of barium into bivalve shells are still in debate, but recent evidence in Tabouret et al. (2012) with regard to *Pecten maximus* suggests Ba to be directly incorporated into the shell via dissolved phases and in proportion to the concentrations observed in the surrounding waters. This conclusion supported that given in Gilliken (2006) which determined the Ba concentrations observed in the shells of *Mytillus edulis* to be directly associated with $[Ba/Ca]_{Water}$ and not determined by diet or solely/primarily influenced by phytoplankton blooms, thus salinity extrapolations could be made using Ba/Ca from archival shells. Seemingly missing from the discussions of these studies is the potential for change when the target bivalve species is under stress. The present study identified a gradual decrease in Ba/Ca in the shells of starved *M. mercenaria* with a significant decrease from baseline. The gradual loss observed in this study was potentially related to shell dissolution and lost metabolic controls due to starvation/stress, as such, these results suggest that Ba/Ca may not be consistently preserved nor dependable for environmental interpretations. The partitioning of Ba between the mineral and organic shell fractions, however, is an important consideration in determining the effectiveness of Ba as an environmental proxy.

Cobalt concentrations as compared to calcium were associated with a general downward trend to the third experimental collection with a sharp increase observed for the last collection. Cobalt has been a focus of research generally specific to environmental contamination studies (for example, Byron et al., 1978; Szefer et al.,

1990; Hedouin et al., 2006; Rainbow et al., 1993). In a study conducted by Babukutty and Chacko (1992), cobalt was determined to be mostly associated with the shells of *Villorita cyprinoides*; however, Cravo et al. 2004 suggested Co was partitioned in the soft tissues of *Patella aspera*. In the current study, with recognition of potential differences between phyla and mineralogical differences, the observed trend in Co as compared to calcium is seemingly attributable to shell dissolution and organic matter decomposition mechanisms, as well as indicating a possible higher concentration in the organic matter.

Iron concentrations exhibited an initial decrease from baseline levels but were determined to be within the range of baseline values during the last two collections. Iron has been described as an essential component of shell deposition often associated with the organic matrix (Almeida, 1998; Wang et al. 2003; Zhang et al., 2003), and has been suggested to more easily incorporated into soft tissues than in shells (Cravo et al., 2004). Conversely, iron has been associated with the shell and soft tissues of bivalves due to metal contamination (Pitts et al., 1994; Bryan et al., 1978). The trends observed in the current study were probably influenced by ratio alteration due to shell dissolution and preservation of organic fractions.

The relative concentrations of potassium were observed to follow a downward trend with a small, but insignificant, increase between collections four and five. Potassium has been suggested be incorporated through ingestion of particulates (Carriker et al., 1996). The downward trend was potentially associated with shell dissolution and the increase potentially signifying organic content being retained (Lutz and Rhoads, 1977).

An overall decrease in Li/Ca was observed in the present study. Though incorporation dynamics have been associated with plankton and water concentration dynamics (Thebault et al., 2009; Thebault, 2012), the decreased concentrations were determined to be associated with loss due to shell dissolution.

The trend observed regarding Mg/Ca as compared to collection suggested an initial increase in relative concentration followed by a downward trend. Magnesium has been shown to substitute for calcium though primarily into calcite rather than in aragonite (Clarke and Wheeler, 1922; Dodd, 1965). Overall, influences attributable to magnesium incorporation have the potential to be highly variable (Rosenberg, 1980). As such the initial increase in magnesium in the current study could be due to replacement of calcium followed by effects of shell dissolution.

The trend in Mn/Ca over time showed a decreased relative concentration followed by a small increase in the last collection. Manganese has been described as being partitioned in both organic and mineral phases of the bivalve shell (Comfort, 1951; Fox, 1966) as well as capable of substituting for calcium in biogenic carbonates (White et al., 1977; Blanchard and Chasteen, 1976). Overall, the decrease seems to have suggested loss through shell dissolution followed by a potential ratio shift due to retained organic-phase associations.

Silica concentrations relative to calcium decreased initially then rebounded to within baseline ranges. Silica has been shown to be involved with early diagenetic transformations in association with microbial activity and decay of organic matter (Holdaway et al., 1982). These described transformations, however, were aligned with specific sediment environments. The present results were not influenced by diagenetic

effects isolated during burial but were equivalent to changes occurring prior to burial if occurring in the natural environment. The Si/Ca associated trends are most likely due to shell dissolution, with potential influence from microbial activity.

Zinc showed a general decrease over time in the present study, though the concentrations determined for collection four were below the ICP standard concentrations, thus the actual degree of change is not known. As discussed in previous chapters, zinc is often discussed in terms of environmental contamination, though Windisch (2001) did specify zinc as important biologically and associated with metabolic and food web interactions. As shown in Chapter 3, zinc appeared to be associated with metabolic change. As such, the decrease in zinc observed in the current study is likely attributable to both metabolic shifts and shell dissolution alterations.

Conclusion

In summary, Ba, Co, Fe and Si were higher relative to Ca in shells at the start of the experiment then declined as the animals starved, with some increase following the death of the clams. Potassium, Li, Mg, and Mn relative to Ca were high in the baseline shell group then declined at somewhat different rates through the course of the experiment. Zinc to Ca ratios remained high in the live or recently dead shells (through July), but then dropped, with high variability, in the dead shells analyzed from the final collection. Only B, which as described above, tends to substitute for C, appeared to behave very differently relative to Ca, compared to the trends seen in the other trace

elements analyzed. If the major factor in the elemental changes was dissolution, boron could be expected to behave somewhat differently.

6. Conclusions

Recent research specific to the bivalve shell has been predominantly focused on associating changes in elemental concentrations with environmental parameters such as temperature, salinity, seasonal variability in water concentrations, pollution, and effluent river waters. The influences of phytoplankton dynamics (Stecher et al., 1996) or their elemental composition (e.g. Gilliken, 2005) have only recently been suggested as potential factors in the variability of bivalve shell chemistry. Barium and lithium have been the primary elements of interest with regard to phytoplankton associations, though the majority of the studies allude to phytoplankton bloom dynamics as being attributed to elemental signals observed. Barium correlated with phytoplankton blooms (diatoms mostly) in several studies (Stecher et al., 1996; Putten et al., 2000; Thebault et al., 2009; and Lazareth et al., 2007), which was further developed in Tabouret et al. (2012) where Ba enrichment was determined to be most likely due to incorporation of dissolved Ba, which as might be available following an algal bloom (Stecher and Kogut, 1999). Research presented in Gilliken et al. (2006) demonstrated an influence of $[Ba/Ca]_{water}$ on $[Ba/Ca]_{shell}$ of *Mytillus edulis* and they concluded that there was no direct association between Ba/Ca enrichment and dietary uptake. The influence of phytoplankton blooms on $[Ba/Ca]_{water}$, however, were later suggested as being a possible explanation for Ba peaks observed and that feeding on algal species with high Ba concentrations is

another plausible influence based on examination of Ba concentration in the gut, though the degree of dietary Ba incorporation was not discussed. With respect to Li/Ca, Thebault and Chauvaud (2011) examined signatures in *Pecten maximus* with the conclusion that growth, potentially temperature and increased lithium due to diatom blooms were responsible for enrichment. Interestingly, though it has been suggested that algal elemental compositions are a plausible influence on the elemental composition of bivalve shells, confirming information remains scarce.

The goal of this dissertation was to assess the influence of diet, specifically consumption of algal taxa, on the elemental chemistries of the shells of two marine bivalve mollusks. Overall, differences were observed between the two bivalve species, as well as among treatments within species. However, experimental design and logistical challenges are suspected to have influenced the results and thereby limited interpretations. Because whole shell samples were examined, including the organic matrix, results were influenced by both the mineral phase and organic phase. Additionally, ontogenetic influences cannot be compared because the ages and sizes of the experimental scallops and clams were different. Algal cultures produced insufficient concentrations for adequate feeding during the last months of the *Mercenaria* experiments. And finally, complications during elemental analysis restricted sample sizes and the elements reported. Nevertheless, the dissertation yielded the following conclusions:

1. Chapter 2 experiments with *Argopecten irradians concentricus* revealed:
    a. highest growth rates and survivorship in treatments fed *Pavlova pinguis* or mixed algal diets,

b. no differences in shell chemistry between the left and right valves,
c. significant differences in shell chemistries for only two elements (Mg and K) between feeding groups, and
d. only K in the shells appeared to be directly influenced by diet.

2. Chapter 3 experiments with *Mercenaria mercenaria* revealed that:
    a. axial growth and shell thickening were variable among treatments, with only the Mixed and Cm treatments exhibiting significant growth, though experimental procedure prevented true growth determinations;
    b. significant differences in shell chemistry between the left and right valves, with the higher ratios in the right valve;
    c. several elemental ratios were significantly different between feeding groups, including Fe, Mn, and P;
    d. Mg/Ca and Sr/Ca ratios appeared to be primarily influenced by growth rates, though pre-experimental growth and biologic factors not assessed also probably contributed to differences observed;
    e. Zn/Ca and B/Ca were likely influenced by stress and metabolic changes;
    f. Ba/Ca, K/Ca, Li/Ca and Si/Ca were likely influenced by factors not addressed in the experimental design; and
    g. in both scallop and clam shells, Mg/Ca was directly related to growth rate, with diet as a contributing factor as nutritional value of the algal species or algal mix was directly associated with growth rate.

3. Normalizing elemental ratios to phosphorus did not enhance resolution of trends in ratios among dietary treatments.
4. Temporal assessment of shell chemistry in clams starved for the duration of the experiment indicated changes associated with shell dissolution.

The development of inter-valve differences in the clam experiment was primarily attributed to assumed metabolic changes associated with stress/starvation of the animals, potentially coupled with genetic controls. This association was further supported during diet group comparisons, where relative Zn and B concentrations were determined likely related to stress as exemplified in the starved clams. A starvation control, however, was not established during the scallop trials, thus a comparison between species cannot be made. Interestingly, the differences between clam left and right valves revealed a higher ratio for the right valve, which follows the observations of Carriker et al. (1996) in that right valves of *Crassostrea virginica* were elementally enriched compared to the left valve. This suggests a common genetic control which is emphasized in equivalved bivalves during times of stress.

In both scallop and clam shells, Mg/Ca was most directly attributable to growth rate, but seemingly was confounded by other biologic controls. This association supports previous findings associated with multiple bivalve species (e.g., Carre et al., 2006; Gilliken et al., 2006; and Strasser et al., 2008). The results of this study, however, include Mg in the organic fraction as well as potential influences of shell dissolution. Diet, none-the-less, is a contributing factor as nutritional value of the algal species or algal mix has been demonstrated to be directly associated with growth.

The identification of different elements being attributable to algal diet for the scallops (K/Ca) and clams (Fe/Ca, Mn/Ca, and P/Ca) indicates a potential species specific effect. A difference between the experiments, however, may also have contributed to differences in interpretation. Prior to the experiment, the clams were moved to a brown water system, which increased chances for influence of sediment on K enrichment as proposed by Carriker et al. (1996). This complication was eliminated from the scallop trials. The potential for this or alternative pre-experimental factors influencing the differences revealed in the K/Ca of the clam shell is more plausible than algal diet. Both possibilities, however, suggest K incorporation by ingestion of enriched particles. Partitioning of K in diatoms, the algal class having the highest average K/P (Ho et al., 2003), as well as the digestibility of the cells, may also be inhibiting translation of the algal signal to the shell.

Manganese, copper, zinc, and cadmium have been shown to be primarily associated with the organic matrix in oyster shells (Carriker et al., 1980), as was iron (Almeida et al., 1998) and magnesium in mussel shells (Lorens and Bender, 1980). This association of specific elements with the organic matrix suggests a high level of biologic control. In the present clam study, iron, manganese, and phosphorous were demonstrated to be directly influenced by diet. Because the organic matrix is responsible for shell strength (Addadi et al., 2006), the organic matrix potentially influences elemental content and structure of the shell (Watabe et al., 2001; Takesue and van Green, 2004; and Johnstone, 2008). If diet associated elements are incorporated into the organic matrix and can be manipulated by dietary changes as demonstrated, diet of cultured bivalves could potentially be implicated in differences in

shell strength and structure between wild and aquacultured animals such as those described in Gresfrude and Strand (2006) regarding *Pecten maximus.* At the very least, the demonstrated dietary influence of specific algal classes on shell chemistry demands consideration of diet composition when conducting experiments aimed at describing influences on shell dynamics or determining cause and effect relationships. The elements influenced and subsequent effects of the alteration, however, are most probably species specific, as are the pathways of element incorporation.

The variations in Ba/Ca and Li/Ca in the clam experiments cannot be attributed to the experimental diets. Instead, both ratios indicate pre-experimental factors as the dominant influence of the differences revealed. Though partitioning of Ba in diatoms and digestibility again comes into question, the revealed difference between the right and left valves from the Cm diet seemingly supports direct incorporation as might occur at the sediment-water interface as previously suggested by Tabouret et al. (2012).

Though diet was shown to directly affect the shell chemistry of *Mercenaria mercenaria*, resolution of the algal contribution was limited to taxonomic class with regard to only a few elements, and differences related to the origin of the algal isolate were not apparently translated to the shells as had been hypothesized. The lack of resolution between clams fed different algal diets may, in part, be due to the taxonomic classes of algae used and similarity in element quotas with respect to specific elements. Other explanations include, a. partitioning of the elements in the algal cell or digestibility of the cell inhibiting transfer of elements in concentrations high enough to produce an associated signal, b. incorporation pathway in clams does not support dietary uptake as a major contributor, and c. multiple influences are associated with the elemental

dynamics with any dietary influence being masked. In an attempt to increase the ability to discern the contribution of specific species of algae to shell chemistry and gain insight as to potential cofactors influencing elemental signatures, experimental analyses using Element/P and (Element/P)/Ca were employed .

Several problems were identified with comparing the elemental concentrations to phosphorus, which include variance in P concentrations potentially not being uniform and attributable to the same factor in each group, high P concentrations contributing to unrelated minimal ratios with specific diets, and potential confounding factors outside controlled environments as identified in previous studies. Though there was a greater degree of separation apparent between species in the same taxonomic class, most differences observed were not significant; furthermore, the differences between the neritic and oceanic diets were not translated to the shells, in fact, the results were opposite of those hypothesized. Additionally, because the ratios from the ISO group were consistently revealed as the lowest in the study, factors not assessed and potential contamination of these shells influenced the results, ultimately complicating interpretations. In short, the results of this study are inconclusive. These ratios might be used in succession with currently used methods to provide multiple lines of evidence ultimately helping to further describe element incorporation and associations between controlling influences. However, more research is required and measures must be taken to ensure P concentrations are relatively stable and influenced by a single factor.

The inspection of shell chemistry through time of clams starved for the duration of the experiment showed that, in general, elemental concentration as compared to calcium decreases as the animal approaches death or is under stress. The main

factors are seemingly due to the mechanics of shell dissolution. The results of this study appear to support the retention of original shell elemental chemistry for some elements. However, elements easily absorbed to the shell surface, and those associated with metabolic influences or with the organic fractions of shells, will deviate from initial shell chemistries when compared with calcium as a function of shell dissolution mechanics thus are unreliable as environmental proxies. Continued analysis of incorporation pathways and determination of the degree to which an element is partitioned between the mineral and organic portions of the shell for individual bivalve species is again recommended before environmental factors are associated with elemental composition of archival shell.

The mechanisms of shell deposition and factors controlling elemental incorporation are complex and influenced by many factors. The results of this dissertation demonstrate that specific algal diets can influence elemental shell chemistry both directly and indirectly. Thus, diet should be considered when interpreting shell chemistry during both laboratory and field experiments. Though further research is needed, algal diet may influence shell chemistry in ways that hold promise for developing proxies of ecological shifts associated with changes in phytoplankton composition.

Recommendations for future work

- Laboratory work similar to Ho et al. (2003) is needed to address element quotas on a species-specific basis for algal species commonly used in aquaculture and commonly ingested by bivalves. Associations between shell elemental

composition and diet cannot be definitively made unless the differences between algal species are known.

- Further examination of dietary influence on shell dynamics for multiple species of bivalves exhibiting different shell microstructural properties and life histories is necessary to determine species-specific influences and commonalities among similar species. Experimental design should allow for uniform growth and analysis of shell fractions separately in conjunction with ontogenetic examinations.
- Fracture analysis on subsamples of shells with known diet is necessary to determine the effects diet can have on the strength of the shell.
- Microstructural analyses on fractured shells are needed to determine differences based on elemental chemistry and dietary influences. This will further increase knowledge as to the influence organic matter has on the microstructure and elemental composition of the mineral partition.
- Determine algae combinations for specific study areas to allow differentiation between the cultured animals and wild populations, then test length of retention of the signature. For a marker to be useful, it must be retained long enough to provide the resolution needed as well be distinguishable from environmental variability.
- Perform feeding experiments with controlled environmental changes to further determine influences potentially confounding dietary influences or that are dominant over dietary influence.

- Increased experimentation with local species of bivalve and algae to catalogue potential signatures that can then be examined in the field. Upon these determinations, the use of shell for ecological association will be possible given ability to differentiate between the signatures and natural variability.
- Use information obtained from feeding experiments and microstructural analysis to determine capability of manipulating shell structure via diet. This will potentially allow shell strength engineering or pearl composition engineering by a low cost mean.

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Appendix A:

Chapter 4 Post Hoc analysis results

Table A1: Results summary of the post hoc analyses (Dunn's Method) for (B/P)/Ca differences between diets

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Cg vs. ISO	114.869	5.411	Yes
Cm vs. ISO	107.152	4.967	Yes
CTRL vs. ISO	93.427	4.562	Yes
Mixed vs. Cm	73.134	3.305	Yes
Mixed vs. CTRL	86.859	4.124	Yes
Mixed vs. ISO	180.286	8.104	Yes
Mixed vs. Pav609	112.777	4.981	Yes
Pav1209 vs. ISO	117.822	5.331	Yes
TI vs. ISO	116.359	5.265	Yes
Cg vs. Cm	7.716	0.366	No
Cg vs. CTRL	21.441	1.073	No
Cg vs. Pav609	47.36	2.188	No
Cm vs. CTRL	13.725	0.675	No
Cm vs. Pav609	39.643	1.804	No
CTRL vs. Pav609	25.918	1.24	No
Mixed vs. Cg	65.417	3.002	No
Mixed vs. Pav1209	62.464	2.759	No
Mixed vs. TI	63.927	2.824	No
Pav1209 vs. Cg	2.953	0.136	No
Pav1209 vs. Cm	10.669	0.485	No
Pav1209 vs. CTRL	24.394	1.167	No
Pav1209 vs. Pav609	50.313	2.237	No
Pav1209 vs. TI	1.463	0.065	No
Pav609 vs. ISO	67.509	3.055	No

Table A1 (Continued)

Comparison	Diff of Ranks	Q	P<0.05
TI vs. Cg	1.49	0.0689	No
TI vs. Cm	9.207	0.419	No
TI vs. CTRL	22.932	1.097	No
TI vs. Pav609	48.85	2.172	No

Table A2: Results summary of the post hoc analyses (Dunn's Method) for (B/P) differences between diets

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Cg vs. ISO	141.186	6.421	Yes
Cm vs. ISO	126.495	5.606	Yes
CTRL vs. ISO	129.922	6.066	Yes
Mixed vs. ISO	181.038	8.194	Yes
Mixed vs. Pav1209	77.854	3.502	Yes
Mixed vs. Pav609	78.621	3.49	Yes
Pav1209 vs. ISO	103.184	4.52	Yes
Pav609 vs. ISO	102.417	4.431	Yes
TI vs. ISO	129.57	5.71	Yes
Cg vs. Cm	14.691	0.672	No
Cg vs. CTRL	11.264	0.545	No
Cg vs. Pav1209	38.002	1.718	No
Cg vs. Pav609	38.768	1.729	No
Cg vs. TI	11.616	0.528	No
Cm vs. Pav1209	23.311	1.027	No
Cm vs. Pav609	24.077	1.047	No
CTRL vs. Cm	3.427	0.161	No
CTRL vs. Pav1209	26.738	1.24	No
CTRL vs. Pav609	27.505	1.258	No
CTRL vs. TI	0.352	0.0164	No
Mixed vs. Cg	39.852	1.865	No
Mixed vs. Cm	54.544	2.484	No
Mixed vs. CTRL	51.116	2.459	No
Mixed vs. TI	51.469	2.33	No
Pav1209 vs. Pav609	0.767	0.033	No
TI vs. Cm	3.075	0.136	No
TI vs. Pav1209	26.386	1.156	No
TI vs. Pav609	27.152	1.175	No

Table A3: Results summary of the post hoc analyses (Dunn's Method) for (Ba/P)/Ca differences between diets

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Cg vs. ISO	122.247	5.725	Yes
Cm vs. ISO	138.969	6.405	Yes
CTRL vs. ISO	95.778	4.65	Yes
Mixed vs. CTRL	91.824	4.398	Yes
Mixed vs. ISO	187.602	8.494	Yes
Mixed vs. Pav1209	77.426	3.443	Yes
Mixed vs. Pav609	81.901	3.642	Yes
Mixed vs. TI	77.651	3.453	Yes
Pav1209 vs. ISO	110.176	4.957	Yes
Pav609 vs. ISO	105.701	4.756	Yes
TI vs. ISO	109.951	4.947	Yes
Cg vs. CTRL	26.468	1.317	No
Cg vs. Pav1209	12.071	0.555	No
Cg vs. Pav609	16.546	0.76	No
Cg vs. TI	12.296	0.565	No
Cm vs. Cg	16.722	0.788	No
Cm vs. CTRL	43.191	2.111	No
Cm vs. Pav1209	28.793	1.303	No
Cm vs. Pav609	33.268	1.505	No
Cm vs. TI	29.018	1.313	No
Mixed vs. Cg	65.355	3.023	No
Mixed vs. Cm	48.633	2.214	No
Pav1209 vs. CTRL	14.398	0.685	No
Pav1209 vs. Pav609	4.475	0.198	No
Pav1209 vs. TI	0.225	0.00994	No
Pav609 vs. CTRL	9.923	0.472	No
TI vs. CTRL	14.173	0.674	No
TI vs. Pav609	4.25	0.188	No

Table A4: Results summary of the post hoc analyses (Dunn's Method) for (Ba/P) differences between diets

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Cg vs. ISO	128.058	5.873	Yes
Cm vs. ISO	144.482	6.457	Yes
CTRL vs. ISO	122.712	5.777	Yes

Table A4 (Continued)

Comparison	Diff of Ranks	Q	P<0.05
Mixed vs. ISO	88.741	4.089	Yes
Pav1209 vs. ISO	91.709	4.051	Yes
Pav609 vs. ISO	121.183	5.286	Yes
TI vs. ISO	77.379	3.33	Yes
Cg vs. CTRL	5.346	0.261	No
Cg vs. Mixed	39.317	1.874	No
Cg vs. Pav1209	36.349	1.656	No
Cg vs. Pav609	6.874	0.309	No
Cg vs. TI	50.679	2.247	No
Cm vs. Cg	16.425	0.758	No
Cm vs. CTRL	21.77	1.031	No
Cm vs. Mixed	55.741	2.584	No
Cm vs. Pav1209	52.773	2.344	No
Cm vs. Pav609	23.299	1.022	No
Cm vs. TI	67.103	2.904	No
CTRL vs. Mixed	33.971	1.666	No
CTRL vs. Pav1209	31.003	1.45	No
CTRL vs. Pav609	1.528	0.0705	No
CTRL vs. TI	45.333	2.059	No
Mixed vs. TI	11.362	0.506	No
Pav1209 vs. Mixed	2.968	0.136	No
Pav1209 vs. TI	14.33	0.613	No
Pav609 vs. Mixed	32.442	1.466	No
Pav609 vs. Pav1209	29.474	1.278	No
Pav609 vs. TI	43.805	1.853	No

Table A5: Results summary of the post hoc analyses (Dunn's Method) for (Co/P)/Ca differences between diets

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Pav1209 vs. CTRL	44.029	3.162	Yes
Mixed vs. CTRL	34.595	2.887	No
Pav1209 vs. ISO	34.267	2.38	No
Cg vs. Cm	2.458	0.207	No
Cg vs. CTRL	29.512	2.231	No
Cg vs. ISO	19.75	1.439	No
Cg vs. Pav609	13.32	1.074	No
Cm vs. CTRL	27.054	2.392	No

Table A5 (Continued)

Comparison	Diff of Ranks	Q	P<0.05
Cm vs. ISO	17.292	1.454	No
Cm vs. Pav609	10.862	1.052	No
ISO vs. CTRL	9.762	0.738	No
Mixed vs. Cg	5.083	0.406	No
Mixed vs. Cm	7.542	0.719	No
Mixed vs. ISO	24.833	1.981	No
Mixed vs. Pav609	18.404	1.664	No
Mixed vs. TI	2.238	0.149	No
Pav1209 vs. Cg	14.517	1.008	No
Pav1209 vs. Cm	16.975	1.341	No
Pav1209 vs. Mixed	9.433	0.711	No
Pav1209 vs. Pav609	27.837	2.119	No
Pav1209 vs. TI	11.671	0.704	No
Pav609 vs. CTRL	16.192	1.367	No
Pav609 vs. ISO	6.43	0.519	No
TI vs. Cg	2.845	0.178	No
TI vs. Cm	5.304	0.367	No
TI vs. CTRL	32.357	2.078	No
TI vs. ISO	22.595	1.413	No
TI vs. Pav609	16.165	1.087	No

Table A6: Results summary of the post hoc analyses (Dunn's Method) for (Fe/P)/Ca differences between diets

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Cm vs. Cg	65.925	3.141	Yes
Cm vs. ISO	68.015	3.153	Yes
Mixed vs. Cg	102.604	4.763	Yes
Mixed vs. CTRL	97.942	4.705	Yes
Mixed vs. ISO	104.694	4.734	Yes
Pav1209 vs. Cg	93.503	4.371	Yes
Pav1209 vs. CTRL	88.841	4.299	Yes
Pav1209 vs. ISO	95.593	4.351	Yes
Cm vs. CTRL	61.263	3.026	No
Mixed vs. TI	61.183	2.699	No
Pav609 vs. ISO	64.368	2.93	No
Cg vs. ISO	2.09	0.099	No
Cm vs. Pav609	3.647	0.167	No

Table A6 (Contiunued)

Comparison	Diff of Ranks	Q	P<0.05
Cm vs. TI	24.504	1.107	No
CTRL vs. Cg	4.662	0.236	No
CTRL vs. ISO	6.752	0.331	No
Mixed vs. Cm	36.679	1.668	No
Mixed vs. Pav1209	9.101	0.407	No
Mixed vs. Pav609	40.326	1.802	No
Pav1209 vs. Cm	27.578	1.262	No
Pav1209 vs. Pav609	31.225	1.404	No
Pav1209 vs. TI	52.082	2.312	No
Pav609 vs. Cg	62.278	2.911	No
Pav609 vs. CTRL	57.616	2.788	No
Pav609 vs. TI	20.857	0.926	No
TI vs. Cg	41.422	1.909	No
TI vs. CTRL	36.759	1.752	No
TI vs. ISO	43.511	1.954	No

Table A7: Results summary of the post hoc analyses (Dunn's Method) for (Fe/P) differences between diets

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Pav609 vs. Cg	81.171	3.664	Yes
Pav609 vs. ISO	80.629	3.526	Yes
Cm vs. Cg	58.412	2.688	No
Cm vs. CTRL	33.763	1.603	No
Cm vs. ISO	57.87	2.578	No
Cm vs. Mixed	9.256	0.426	No
Cm vs. TI	41.728	1.836	No
CTRL vs. Cg	24.649	1.206	No
CTRL vs. ISO	24.108	1.137	No
CTRL vs. TI	7.966	0.37	No
ISO vs. Cg	0.542	0.0248	No
Mixed vs. Cg	49.156	2.327	No
Mixed vs. CTRL	24.507	1.199	No
Mixed vs. ISO	48.615	2.223	No
Mixed vs. TI	32.473	1.466	No
Pav1209 vs. Cg	64.506	2.95	No
Pav1209 vs. Cm	6.094	0.271	No
Pav1209 vs. CTRL	39.857	1.879	No

Table A7 (Continued)

Comparison	Diff of Ranks	Q	P<0.05
Pav1209 vs. ISO	63.964	2.832	No
Pav1209 vs. Mixed	15.35	0.702	No
Pav1209 vs. TI	47.823	2.092	No
Pav609 vs. Cm	22.759	1.001	No
Pav609 vs. CTRL	56.522	2.628	No
Pav609 vs. Mixed	32.015	1.445	No
Pav609 vs. Pav1209	16.665	0.729	No
Pav609 vs. TI	64.488	2.787	No
TI vs. Cg	16.683	0.753	No
TI vs. ISO	16.142	0.706	No

Table A8: Results summary of the post hoc analyses (Dunn's Method) for (K/P)/Ca differences between diets

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Mixed vs. CTRL	73.426	3.558	Yes
Mixed vs. ISO	75.382	3.466	Yes
Cg vs. CTRL	42.074	2.187	No
Cg vs. ISO	44.03	2.156	No
Cg vs. TI	25.694	1.149	No
Cm vs. Cg	11.571	0.57	No
Cm vs. CTRL	53.645	2.755	No
Cm vs. ISO	55.601	2.693	No
Cm vs. Pav1209	7.136	0.335	No
Cm vs. Pav609	2.936	0.14	No
Cm vs. TI	37.265	1.651	No
CTRL vs. ISO	1.956	0.0998	No
Mixed vs. Cg	31.351	1.463	No
Mixed vs. Cm	19.78	0.914	No
Mixed vs. Pav1209	26.917	1.202	No
Mixed vs. Pav609	22.717	1.027	No
Mixed vs. TI	57.046	2.418	No
Pav1209 vs. Cg	4.435	0.21	No
Pav1209 vs. CTRL	46.509	2.29	No
Pav1209 vs. ISO	48.465	2.261	No
Pav1209 vs. TI	30.129	1.293	No
Pav609 vs. Cg	8.635	0.415	No
Pav609 vs. CTRL	50.709	2.535	No

Table A8 (Continued)

Comparison	Diff of Ranks	Q	P<0.05
Pav609 vs. ISO	52.665	2.49	No
Pav609 vs. Pav1209	4.2	0.193	No
Pav609 vs. TI	34.329	1.49	No
TI vs. CTRL	16.38	0.758	No
TI vs. ISO	18.336	0.808	No

Table A9: Results summary of the post hoc analyses (Dunn's Method) for (K/P) differences between diets

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Cm vs. ISO	67.17	3.15	Yes
Mixed vs. ISO	77.864	3.651	Yes
Cg vs. CTRL	32.346	1.647	No
Cg vs. ISO	50.219	2.405	No
Cg vs. TI	34.625	1.526	No
Cm vs. Cg	16.952	0.817	No
Cm vs. CTRL	49.298	2.451	No
Cm vs. Pav1209	13.644	0.62	No
Cm vs. Pav609	0.77	0.0355	No
Cm vs. TI	51.577	2.232	No
CTRL vs. ISO	17.873	0.883	No
CTRL vs. TI	2.279	0.103	No
Mixed vs. Cg	27.645	1.332	No
Mixed vs. Cm	10.693	0.504	No
Mixed vs. CTRL	59.991	2.982	No
Mixed vs. Pav1209	24.337	1.105	No
Mixed vs. Pav609	11.464	0.528	No
Mixed vs. TI	62.27	2.695	No
Pav1209 vs. Cg	3.308	0.153	No
Pav1209 vs. CTRL	35.654	1.7	No
Pav1209 vs. ISO	53.526	2.417	No
Pav1209 vs. TI	37.933	1.59	No
Pav609 vs. Cg	16.181	0.76	No
Pav609 vs. CTRL	48.527	2.348	No
Pav609 vs. ISO	66.4	3.039	No
Pav609 vs. Pav1209	12.874	0.571	No
Pav609 vs. TI	50.806	2.154	No
TI vs. ISO	15.594	0.672	No

Table A10: Results summary of the post hoc analyses (Dunn's Method) for (Li/P)/Ca differences between diets

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Cg vs. ISO	86.382	4.17	Yes
Cm vs. ISO	93.122	4.471	Yes
CTRL vs. ISO	86.858	4.393	Yes
Mixed vs. ISO	142.2	6.528	Yes
Mixed vs. Pav609	87.826	3.964	Yes
Pav1209 vs. ISO	82.399	3.861	Yes
TI vs. ISO	93.724	4.133	Yes
Cg vs. Pav1209	3.983	0.189	No
Cg vs. Pav609	32.008	1.516	No
Cm vs. Cg	6.739	0.327	No
Cm vs. CTRL	6.264	0.319	No
Cm vs. Pav1209	10.723	0.505	No
Cm vs. Pav609	38.748	1.826	No
CTRL vs. Cg	0.476	0.0244	No
CTRL vs. Pav1209	4.459	0.221	No
CTRL vs. Pav609	32.484	1.609	No
Mixed vs. Cg	55.818	2.589	No
Mixed vs. Cm	49.079	2.265	No
Mixed vs. CTRL	55.342	2.679	No
Mixed vs. Pav1209	59.801	2.699	No
Mixed vs. TI	48.476	2.067	No
Pav1209 vs. Pav609	28.025	1.29	No
Pav609 vs. ISO	54.374	2.548	No
TI vs. Cg	7.342	0.327	No
TI vs. Cm	0.602	0.0267	No
TI vs. CTRL	6.866	0.318	No
TI vs. Pav1209	11.325	0.492	No
TI vs. Pav609	39.35	1.708	No

Table A11: Results summary of the post hoc analyses (Dunn's Method) for (Li/P) differences between diets

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Cg vs. ISO	115.098	5.48	Yes
Cm vs. ISO	110.215	5.138	Yes
CTRL vs. ISO	125.724	6.175	Yes

Table A11 (Continued)

Comparison	Diff of Ranks	Q	P<0.05
Mixed vs. ISO	130.555	6.087	Yes
Pav1209 vs. ISO	75.455	3.411	Yes
Pav609 vs. ISO	90.054	4.098	Yes
TI vs. ISO	99.139	4.283	Yes
Cg vs. Cm	4.884	0.234	No
Cg vs. Pav1209	39.643	1.838	No
Cg vs. Pav609	25.044	1.169	No
Cg vs. TI	15.959	0.706	No
Cm vs. Pav1209	34.76	1.58	No
Cm vs. Pav609	20.16	0.923	No
Cm vs. TI	11.076	0.481	No
CTRL vs. Cg	10.626	0.538	No
CTRL vs. Cm	15.509	0.767	No
CTRL vs. Pav1209	50.269	2.401	No
CTRL vs. Pav609	35.669	1.716	No
CTRL vs. TI	26.585	1.207	No
Mixed vs. Cg	15.457	0.74	No
Mixed vs. Cm	20.341	0.954	No
Mixed vs. CTRL	4.832	0.239	No
Mixed vs. Pav1209	55.101	2.505	No
Mixed vs. Pav609	40.501	1.853	No
Mixed vs. TI	31.417	1.364	No
Pav609 vs. Pav1209	14.6	0.649	No
TI vs. Pav1209	23.684	1.001	No
TI vs. Pav609	9.084	0.386	No

Table A12: Results summary of the post hoc analyses (Dunn's Method) for (Mg/P)/Ca differences between diets

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Cg vs. ISO	78.114	3.701	Yes
Cm vs. ISO	121.729	5.675	Yes
CTRL vs. ISO	84.475	4.149	Yes
Mixed vs. Cg	74.517	3.439	Yes
Mixed vs. CTRL	68.157	3.255	Yes
Mixed vs. ISO	152.631	6.901	Yes
Mixed vs. Pav1209	82.413	3.661	Yes
Mixed vs. Pav609	95.513	4.243	Yes

Table A12 (Continued)

Comparison	Diff of Ranks	Q	P<0.05
Mixed vs. TI	78.433	3.44	Yes
Pav1209 vs. ISO	70.218	3.196	Yes
TI vs. ISO	74.198	3.332	Yes
Cg vs. Pav1209	7.896	0.367	No
Cg vs. Pav609	20.996	0.976	No
Cg vs. TI	3.916	0.179	No
Cm vs. Cg	43.615	2.079	No
Cm vs. CTRL	37.255	1.841	No
Cm vs. Pav1209	51.511	2.357	No
Cm vs. Pav609	64.611	2.957	No
Cm vs. TI	47.531	2.146	No
CTRL vs. Cg	6.361	0.32	No
CTRL vs. Pav1209	14.257	0.686	No
CTRL vs. Pav609	27.357	1.316	No
CTRL vs. TI	10.277	0.487	No
Mixed vs. Cm	30.902	1.405	No
Pav1209 vs. Pav609	13.1	0.586	No
Pav609 vs. ISO	57.118	2.599	No
TI vs. Pav1209	3.98	0.176	No
TI vs. Pav609	17.08	0.754	No

Table A13: Results summary of the post hoc analyses (Dunn's Method) for (Mg/P) differences between diets

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Cg vs. ISO	101.91	4.699	Yes
Cm vs. ISO	137.851	6.194	Yes
Cm vs. TI	103.77	4.577	Yes
CTRL vs. ISO	151.067	7.151	Yes
CTRL vs. Pav1209	83.319	3.891	Yes
CTRL vs. TI	116.986	5.425	Yes
Mixed vs. ISO	127.771	5.834	Yes
Mixed vs. TI	93.69	4.197	Yes
Pav609 vs. ISO	87.23	3.826	Yes
Cg vs. Pav1209	34.163	1.555	No
Cg vs. Pav609	14.68	0.664	No
Cg vs. TI	67.83	3.067	No
Cm vs. Cg	35.941	1.667	No

Table A13 (Continued)

Comparison	Diff of Ranks	Q	P<0.05
Cm vs. Mixed	10.08	0.463	No
Cm vs. Pav1209	70.103	3.112	No
Cm vs. Pav609	50.62	2.233	No
CTRL vs. Cg	49.157	2.411	No
CTRL vs. Cm	13.216	0.63	No
CTRL vs. Mixed	23.296	1.13	No
CTRL vs. Pav609	63.836	2.96	No
Mixed vs. Cg	25.861	1.221	No
Mixed vs. Pav1209	60.023	2.707	No
Mixed vs. Pav609	40.54	1.816	No
Pav1209 vs. ISO	67.747	2.991	No
Pav1209 vs. TI	33.667	1.46	No
Pav609 vs. Pav1209	19.483	0.845	No
Pav609 vs. TI	53.15	2.29	No
TI vs. ISO	34.08	1.495	No

Table A14: Results summary of the post hoc analyses (Dunn's Method) for (Mn/P)/Ca differences between diets

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Cg vs. CTRL	81.388	4.084	Yes
Cm vs. Cg	85.662	4.071	Yes
Cm vs. CTRL	167.05	8.233	Yes
Cm vs. ISO	75.309	3.501	Yes
ISO vs. CTRL	91.741	4.493	Yes
Mixed vs. Cg	135.393	6.231	Yes
Mixed vs. CTRL	216.781	10.323	Yes
Mixed vs. ISO	125.041	5.637	Yes
Mixed vs. Pav609	76.036	3.368	Yes
Pav1209 vs. Cg	76.557	3.548	Yes
Pav1209 vs. CTRL	157.945	7.577	Yes
Pav609 vs. CTRL	140.745	6.752	Yes
TI vs. Cg	87.445	4.024	Yes
TI vs. CTRL	168.833	8.04	Yes
TI vs. ISO	77.092	3.475	Yes
Cm vs. Pav1209	9.105	0.415	No
Cm vs. Pav609	26.305	1.2	No
ISO vs. Cg	10.353	0.489	No

Table A14 (Continued)

Comparison	Diff of Ranks	Q	P<0.05
Mixed vs. Cm	49.731	2.254	No
Mixed vs. Pav1209	58.836	2.606	No
Mixed vs. TI	47.949	2.111	No
Pav1209 vs. ISO	66.205	3.004	No
Pav1209 vs. Pav609	17.2	0.767	No
Pav609 vs. Cg	59.357	2.751	No
Pav609 vs. ISO	49.005	2.224	No
TI vs. Cm	1.783	0.0808	No
TI vs. Pav1209	10.887	0.482	No
TI vs. Pav609	28.087	1.244	No

Table A15: Results summary of the post hoc analyses (Dunn's Method) for (Mn/P) differences between diets

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Cg vs. CTRL	81.535	3.966	Yes
Cm vs. Cg	85.192	3.92	Yes
Cm vs. CTRL	166.727	7.877	Yes
Cm vs. ISO	74.519	3.321	Yes
ISO vs. CTRL	92.208	4.329	Yes
Mixed vs. Cg	104.541	4.919	Yes
Mixed vs. CTRL	186.077	9.002	Yes
Mixed vs. ISO	93.868	4.272	Yes
Pav1209 vs. CTRL	135.415	6.315	Yes
Pav609 vs. Cg	83.873	3.761	Yes
Pav609 vs. CTRL	165.408	7.607	Yes
Pav609 vs. ISO	73.2	3.184	Yes
TI vs. Cg	69.705	3.147	Yes
TI vs. CTRL	151.241	7.005	Yes
Cm vs. Pav1209	31.313	1.387	No
Cm vs. Pav609	1.319	0.0577	No
Cm vs. TI	15.487	0.682	No
ISO vs. Cg	10.673	0.488	No
Mixed vs. Cm	19.349	0.886	No
Mixed vs. Pav1209	50.662	2.291	No
Mixed vs. Pav609	20.669	0.923	No
Mixed vs. TI	34.836	1.565	No
Pav1209 vs. Cg	53.879	2.449	No

Table A15 (Continued)

Comparison	Diff of Ranks	Q	P<0.05
Pav1209 vs. ISO	43.206	1.903	No
Pav609 vs. Pav1209	29.993	1.297	No
Pav609 vs. TI	14.167	0.609	No
TI vs. ISO	59.032	2.584	No
TI vs. Pav1209	15.826	0.689	No

Table A16: Results summary of the post hoc analyses (Dunn's Method) for (Si/P)/Ca differences between diets

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Mixed vs. ISO	69.106	3.147	Yes
Pav1209 vs. Cg	110.926	5.126	Yes
Pav1209 vs. Cm	77.984	3.548	Yes
Pav1209 vs. CTRL	75.02	3.589	Yes
Pav1209 vs. ISO	132.912	6.014	Yes
Pav609 vs. Cg	69.801	3.225	Yes
Pav609 vs. ISO	91.787	4.153	Yes
TI vs. ISO	85.206	3.804	Yes
Cg vs. ISO	21.986	1.036	No
Cm vs. Cg	32.942	1.561	No
Cm vs. ISO	54.928	2.546	No
CTRL vs. Cg	35.906	1.797	No
CTRL vs. Cm	2.964	0.146	No
CTRL vs. ISO	57.892	2.827	No
Mixed vs. Cg	47.119	2.192	No
Mixed vs. Cm	14.177	0.649	No
Mixed vs. CTRL	11.214	0.54	No
Pav1209 vs. Mixed	63.807	2.854	No
Pav1209 vs. Pav609	41.125	1.828	No
Pav1209 vs. TI	47.707	2.093	No
Pav609 vs. Cm	36.859	1.677	No
Pav609 vs. CTRL	33.895	1.621	No
Pav609 vs. Mixed	22.682	1.014	No
Pav609 vs. TI	6.582	0.289	No
TI vs. Cg	63.219	2.881	No
TI vs. Cm	30.278	1.359	No
TI vs. CTRL	27.314	1.287	No
TI vs. Mixed	16.1	0.711	No

Table A17: Results summary of the post hoc analyses (Dunn's Method) for (Si/P) differences between diets

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
CTRL vs. ISO	80.815	3.794	Yes
Pav1209 vs. Cg	75.645	3.438	Yes
Pav1209 vs. ISO	103.239	4.548	Yes
Pav1209 vs. Mixed	85.777	3.898	Yes
Pav609 vs. Cg	84.059	3.77	Yes
Pav609 vs. Cm	76.338	3.339	Yes
Pav609 vs. ISO	111.654	4.857	Yes
Pav609 vs. Mixed	94.192	4.224	Yes
TI vs. ISO	76.529	3.329	Yes
Cg vs. ISO	27.595	1.262	No
Cg vs. Mixed	10.133	0.479	No
Cm vs. Cg	7.722	0.355	No
Cm vs. ISO	35.317	1.574	No
Cm vs. Mixed	17.855	0.822	No
CTRL vs. Cg	53.22	2.589	No
CTRL vs. Cm	45.498	2.15	No
CTRL vs. Mixed	63.352	3.082	No
CTRL vs. TI	4.285	0.197	No
Mixed vs. ISO	17.462	0.799	No
Pav1209 vs. Cm	67.923	3.009	No
Pav1209 vs. CTRL	22.425	1.046	No
Pav1209 vs. TI	26.71	1.155	No
Pav609 vs. CTRL	30.84	1.418	No
Pav609 vs. Pav1209	8.415	0.364	No
Pav609 vs. TI	35.125	1.501	No
TI vs. Cg	48.934	2.194	No
TI vs. Cm	41.213	1.803	No
TI vs. Mixed	59.067	2.649	No

Table A18: Results summary of the post hoc analyses (Dunn's Method) for (Sr/P)/Ca differences between diets

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Cg vs. ISO	107.723	5.35	Yes
Cm vs. ISO	110.844	5.289	Yes
CTRL vs. ISO	82.581	4.252	Yes

Table A18 (Continued)

Comparison	Diff of Ranks	Q	P<0.05
Mixed vs. CTRL	80.596	3.973	Yes
Mixed vs. ISO	163.177	7.627	Yes
Mixed vs. Pav1209	89.303	3.844	Yes
Mixed vs. Pav609	83.332	3.829	Yes
Pav1209 vs. ISO	73.873	3.286	Yes
Pav609 vs. ISO	79.844	3.81	Yes
TI vs. ISO	96.231	4.498	Yes
Cg vs. CTRL	25.142	1.327	No
Cg vs. Pav1209	33.85	1.533	No
Cg vs. Pav609	27.879	1.358	No
Cg vs. TI	11.492	0.548	No
Cm vs. Cg	3.121	0.152	No
Cm vs. CTRL	28.264	1.426	No
Cm vs. Pav1209	36.971	1.619	No
Cm vs. Pav609	31	1.453	No
Cm vs. TI	14.614	0.672	No
CTRL vs. Pav1209	8.707	0.406	No
CTRL vs. Pav609	2.736	0.138	No
Mixed vs. Cg	55.454	2.645	No
Mixed vs. Cm	52.332	2.405	No
Mixed vs. TI	66.946	3.018	No
Pav609 vs. Pav1209	5.971	0.262	No
TI vs. CTRL	13.65	0.673	No
TI vs. Pav1209	22.357	0.962	No
TI vs. Pav609	16.386	0.753	No

Table A19: Results summary of the post hoc analyses (Dunn's Method) for (Sr/P) differences between diets

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Cg vs. ISO	137.971	6.62	Yes
Cm vs. ISO	149.743	6.835	Yes
CTRL vs. ISO	125.375	6.175	Yes
Mixed vs. ISO	162.158	7.665	Yes
Mixed vs. Pav1209	78.338	3.443	Yes
Mixed vs. TI	71.975	3.315	Yes
Pav1209 vs. ISO	83.82	3.631	Yes
Pav609 vs. ISO	114.843	5.242	Yes

Table A19 (Continued)

Comparison	Diff of Ranks	Q	P<0.05
TI vs. ISO	90.183	4.089	Yes
Cg vs. CTRL	12.596	0.643	No
Cg vs. Pav1209	54.15	2.411	No
Cg vs. Pav609	23.128	1.088	No
Cg vs. TI	47.788	2.233	No
Cm vs. Cg	11.772	0.554	No
Cm vs. CTRL	24.368	1.176	No
Cm vs. Pav1209	65.923	2.811	No
Cm vs. Pav609	34.9	1.565	No
Cm vs. TI	59.56	2.654	No
CTRL vs. Pav1209	41.555	1.892	No
CTRL vs. Pav609	10.532	0.508	No
CTRL vs. TI	35.192	1.686	No
Mixed vs. Cg	24.188	1.181	No
Mixed vs. Cm	12.415	0.576	No
Mixed vs. CTRL	36.783	1.846	No
Mixed vs. Pav609	47.315	2.194	No
Pav609 vs. Pav1209	31.023	1.323	No
Pav609 vs. TI	24.66	1.099	No
TI vs. Pav1209	6.362	0.27	No

Table A20: Results summary of the post hoc analyses (Dunn's Method) for (Zn/P)/Ca differences between diets

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Cg vs. ISO	58.313	3.719	Yes
Cm vs. ISO	56	3.212	Yes
Mixed vs. CTRL	45.979	3.329	Yes
Mixed vs. ISO	84.039	4.876	Yes
Mixed vs. Pav609	48.971	3.196	Yes
Pav1209 vs. ISO	72.25	3.973	Yes
Cg vs. Cm	2.313	0.16	No
Cg vs. CTRL	20.252	1.714	No
Cg vs. Pav609	23.244	1.715	No
Cg vs. TI	9.229	0.557	No
Cm vs. CTRL	17.939	1.276	No
Cm vs. Pav609	20.932	1.346	No
Cm vs. TI	6.917	0.379	No

Table A20 (Continued)

Comparison	Diff of Ranks	Q	P<0.05
CTRL vs. ISO	38.061	2.479	No
CTRL vs. Pav609	2.993	0.227	No
Mixed vs. Cg	25.727	1.815	No
Mixed vs. Cm	28.039	1.742	No
Mixed vs. Pav1209	11.789	0.698	No
Mixed vs. TI	34.956	1.937	No
Pav1209 vs. Cg	13.938	0.91	No
Pav1209 vs. Cm	16.25	0.95	No
Pav1209 vs. CTRL	34.189	2.283	No
Pav1209 vs. Pav609	37.182	2.269	No
Pav1209 vs. TI	23.167	1.222	No
Pav609 vs. ISO	35.068	2.096	No
TI vs. CTRL	11.023	0.678	No
TI vs. ISO	49.083	2.55	No
TI vs. Pav609	14.015	0.798	No

Table A21: Results summary of the post hoc analyses (Dunn's Method) for (Zn/P) differences between diets

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
Cg vs. ISO	62.538	3.989	Yes
Mixed vs. ISO	68.692	3.986	Yes
Cg vs. Cm	19.387	1.345	No
Cg vs. CTRL	17.988	1.523	No
Cg vs. Pav1209	22.743	1.485	No
Cg vs. Pav609	26.178	1.932	No
Cg vs. TI	16.776	1.013	No
Cm vs. ISO	43.151	2.475	No
Cm vs. Pav1209	3.356	0.196	No
Cm vs. Pav609	6.79	0.437	No
CTRL vs. Cm	1.399	0.0995	No
CTRL vs. ISO	44.55	2.902	No
CTRL vs. Pav1209	4.755	0.317	No
CTRL vs. Pav609	8.19	0.622	No
Mixed vs. Cg	6.154	0.434	No
Mixed vs. Cm	25.541	1.587	No
Mixed vs. CTRL	24.142	1.748	No
Mixed vs. Pav1209	28.896	1.71	No

Table A21 (Continued)

Comparison	Diff of Ranks	Q	P<0.05
Mixed vs. Pav609	32.331	2.11	No
Mixed vs. TI	22.93	1.271	No
Pav1209 vs. ISO	39.795	2.189	No
Pav1209 vs. Pav609	3.435	0.21	No
Pav609 vs. ISO	36.36	2.174	No
TI vs. Cm	2.611	0.143	No
TI vs. CTRL	1.212	0.0745	No
TI vs. ISO	45.762	2.377	No
TI vs. Pav1209	5.967	0.315	No
TI vs. Pav609	9.402	0.535	No

Appendix B:

Chapter 5 post hoc analysis summaries

Table B1: Statistical analysis results summary – Dunn's pairwise comparison procedure – for B/Ca

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
July vs. January	28.077	3.714	Yes
July vs. March	21.133	2.916	Yes
July vs. May	42.4	5.16	Yes
July vs. October	43.067	5.943	Yes
March vs. October	21.933	2.831	Yes
January vs. May	14.323	1.605	No
January vs. October	14.99	1.864	No
March vs. January	6.944	0.864	No
March vs. May	21.267	2.455	No
May vs. October	0.667	0.077	No

Table B2: Statistical analysis results summary – Dunn's pairwise comparison procedure – for Ba/Ca

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
January vs. July	40.848	5.404	Yes
January vs. March	23.356	2.905	Yes
January vs. May	38.223	4.283	Yes
January vs. October	30.156	3.751	Yes
March vs. July	17.492	2.414	No
March vs. May	14.867	1.716	No
March vs. October	6.8	0.878	No
May vs. July	2.625	0.319	No
October vs. July	10.692	1.475	No
October vs. May	8.067	0.931	No

Table B3: Statistical analysis results summary – pairwise comparison procedure – for Co/Ca

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):					
Overall significance level = 0.05					
Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
January vs. July	7.39E-07	4.723	0.000272	0.006	Yes
March vs. July	5.60E-07	3.575	0.00276	0.009	Yes
October vs. January	5.59E-07	2.96	0.00974	0.01	Yes
October vs. July	1.30E-06	7.101	0.00000362	0.005	Yes
October vs. March	7.38E-07	3.911	0.00139	0.007	Yes
October vs. May	1.24E-06	4.145	0.000864	0.006	Yes
January vs. March	1.80E-07	1.099	0.289	0.025	No
January vs. May	6.78E-07	2.396	0.0301	0.013	No
March vs. May	4.99E-07	1.761	0.0985	0.017	No
May vs. July	6.08E-08	0.218	0.831	0.05	No

Table B4: Statistical analysis results summary – Dunn's pairwise comparison procedure – for Fe/Ca

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
January vs. March	39.056	4.858	Yes
January vs. May	40.323	4.518	Yes
July vs. March	29.283	4.041	Yes
July vs. May	30.55	3.718	Yes
October vs. March	30.8	3.976	Yes
October vs. May	32.067	3.702	Yes
January vs. July	9.773	1.293	No
January vs. October	8.256	1.027	No
March vs. May	1.267	0.146	No
October vs. July	1.517	0.209	No

Table B5: Statistical analysis results summary – Dunn's pairwise comparison procedure – for K/Ca

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
January vs. July	32.808	4.34	Yes
March vs. July	42.3	5.837	Yes
March vs. October	24.533	3.167	Yes
May vs. July	31.5	3.833	Yes
January vs. May	1.308	0.147	No
January vs. October	15.041	1.871	No
March vs. January	9.492	1.181	No

Table B5 (Continued)

Comparison	Diff of Ranks	Q	P<0.05
March vs. May	10.8	1.247	No
May vs. October	13.733	1.585	No
October vs. July	17.767	2.452	No

Table B6: Statistical analysis results summary – Dunn's pairwise comparison procedure – for Li/Ca

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
January vs. July	30.815	4.077	Yes
January vs. October	45.749	5.69	Yes
March vs. October	30.6	3.95	Yes
May vs. October	26.333	3.04	Yes
January vs. March	15.149	1.884	No
January vs. May	19.415	2.176	No
July vs. October	14.933	2.061	No
March vs. July	15.667	2.162	No
March vs. May	4.267	0.493	No
May vs. July	11.4	1.387	No

Table B7: Statistical analysis results summary – Dunn's pairwise comparison procedure – for Mg/Ca

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
March vs. January	26.833	3.31	Yes
March vs. July	41.667	5.829	Yes
March vs. October	39.333	5.147	Yes
May vs. July	27.8	3.43	Yes
May vs. October	25.467	2.981	Yes
January vs. July	14.833	1.941	No
January vs. October	12.5	1.542	No
March vs. May	13.867	1.623	No
May vs. January	12.967	1.447	No
October vs. July	2.333	0.326	No

Table B8: Statistical analysis results summary – Dunn's pairwise comparison procedure – for Mn/Ca

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
January vs. July	42.233	5.526	Yes
January vs. May	49.283	5.5	Yes
January vs. October	23.55	2.905	Yes
March vs. July	27.35	3.826	Yes
March vs. May	34.4	4.026	Yes
October vs. May	25.733	3.012	Yes
January vs. March	14.883	1.836	No
July vs. May	7.05	0.87	No
March vs. October	8.667	1.134	No
October vs. July	18.683	2.614	No

Table B9: Statistical analysis results summary – Dunn's pairwise comparison procedure – for Si/Ca

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
January vs. May	28.9	3.135	Yes
July vs. March	29.233	4.266	Yes
July vs. May	36.2	4.659	Yes
October vs. March	25.8	3.522	Yes
October vs. May	32.767	4.001	Yes
January vs. March	21.933	2.593	No
July vs. January	7.3	0.907	No
July vs. October	3.433	0.501	No
March vs. May	6.967	0.851	No
October vs. January	3.867	0.457	No

Table B10: Statistical analysis results summary – Dunn's pairwise comparison procedure – for Zn/Ca

All Pairwise Multiple Comparison Procedures (Dunn's Method) :			
Comparison	Diff of Ranks	Q	P<0.05
January vs. July	23.917	3.71	Yes
January vs. October	33.333	4.317	Yes
March vs. July	28.143	4.523	Yes
March vs. October	37.56	4.984	Yes
January vs. May	12.167	1.84	No
July vs. October	9.417	1.201	No
March vs. January	4.226	0.696	No
March vs. May	16.393	2.564	No

Table B10 (Continued)

Comparison	Diff of Ranks	Q	P<0.05
May vs. July	11.75	1.741	No
May vs. October	21.167	2.654	No