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Analysis of the mineral composition of Louisiana wild caught shrimp by ICP-OES and classification of geographical origin

Samantha Nichole Stein

Louisiana State University and Agricultural and Mechanical College, sstein5@tigers.lsu.edu

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ANALYSIS OF THE MINERAL COMPOSITION OF LOUISIANA WILD CAUGHT
SHRIMP BY ICP-OES AND CLASSIFICATION OF GEOGRAPHICAL ORIGIN

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The School of Nutrition and Food Sciences

by
Samantha N. Stein
B.S., Louisiana State University, 2012
May 2014

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ABSTRACT

Nationwide, seafood consumers are paying close attention to their seafood options and demanding transparency on point of origin. Recent studies have shown that shrimp can reflect the mineral content of the waters from which they are harvested. Mineral analysis was conducted using inductively coupled plasma optical emission spectrometry on the tail muscle from each coastal group and imported farmed raised samples. Analysis of variance was used to detect differences among catch locations and seasons along the Louisiana coast, as well as differences in the mineral profile of farm raised imported shrimp. Multivariate analysis of variance and descriptive analysis was used to evaluate which minerals contributed the greatest variance to the mineral profiles (Al, P, Fe, Mg, K, Na, Cu, Zn, and Ca) of Louisiana shrimp from over 100 sampling sights. The minerals Ca, and to a lesser extent Zn and Cu were identified as the most discriminating minerals (canonical correlation=0.8269, 0.3929, and 0.5547, respectively). Based on predictive discriminant analysis using cross validation of nine minerals, the catch zones of Louisiana wild caught shrimp could be predicted with an overall accuracy of 86.93% and specifically into the correct zones 1, 2, and 4 with 73.68%, 74.85%, and 95.40% accuracy, respectively.

CHAPTER 1. REVIEW OF RELATED LITERATURE

1.1 Louisiana Shrimp and Louisiana's Shrimping Communities

Americans consume more shrimp than any other type of seafood, and the amount of shrimp that Americans are consuming continues to rise. In 2011, Americans consumed an average of over of 4 pounds of shrimp per person, nearly twice the per-capita consumption in 1990 (Muncy 1984; Benfield et al. 2004; NOAA 2011; Fluech and Krimsky 2011; LDWF 2012). Although gulf shrimp fisheries are among the largest and highest valued in the United States, over 90 percent of the shrimp eaten in the United States is farmed overseas (Muncy 1984; Benfield et al. 2004; 2006; Jacquet and Pauly 2008; Grimes and Yow 2009; NOAA 2011). By value, shrimp makes up more than 30 percent of all seafood we import, mainly from Southeast Asian countries like Thailand, Indonesia, and China, followed by Ecuador and Mexico (Muncy 1984; NOAA 2011; LDWF 2012). In 2013, official import statistics indicate that the United States has imported a total of 828.6 million pounds of frozen, non-breaded shrimp compared to 817.3 million pounds through the first ten months of 2012.

In Louisiana, shrimp are the most valuable and popular seafood. Each year Louisiana shrimpers catch 90 - 120 million pounds of both brown (*Peneaus aztecus*) and white shrimp (*Litopenaeus setiferus*) and 69 percent of the domestic US shrimp are harvested from the Gulf waters. Brown and white shrimp are roughly similar in appearance and taste, and retail markets seldom distinguish between specific species (Muncy 1984; Benfield et al. 2004; Grimes and Yow 2009). These two commercially important species of penaeid shrimp comprise the majority of shrimp harvested for food in Louisiana (Figure #). They also represent the most valuable species caught off the coast of Louisiana and are widely appreciated by US consumers (Benfield et al. 2004;

NOAA 2012). Brown and white shrimp represent 95 percent of all annual landings in Louisiana, with very small quantities of other shrimp species such as seabobs, pink shrimp, rock shrimp and royal reds also being landed (Muncy 1984; Benfield et al. 2004)

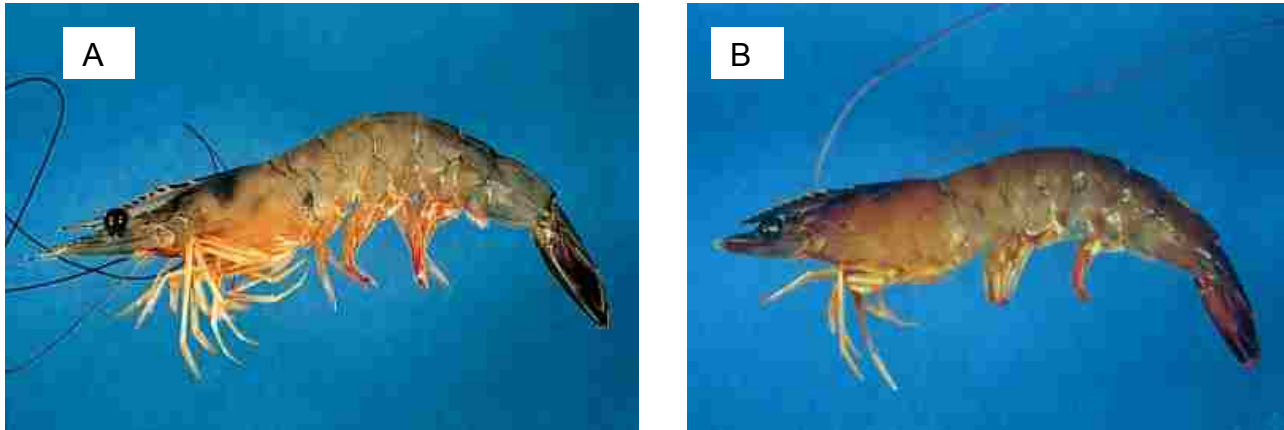


Figure 1. Louisiana's most important commercial shrimp species. A. white shrimp (*Litopenaeus setiferus*), B. brown shrimp (*Peneaus aztecus*)

In 2012, Louisiana harvested the most shrimp of any American state: 101 million pounds with a dockside value of \$146 million, accounting for 33 percent of the US shrimp catch by volume and 29 percent by value (Benfield et al. 2004; NOAA 2012). According to the National Marine Fisheries Service of the National Oceanic and Atmospheric Administration (NOAA) the total annual harvest in Louisiana (54.3 million pounds) has been larger than any year since 2009 (55.5 million pounds) and remains eleven percent higher than the average harvest during the five-year time period between 2007 and 2011 (43.7 million pounds). For the entire Gulf, the volume of shrimp landed is slightly lower (108.2 million pounds) than it was for the comparable period last year (109.8 million pounds), but slightly higher than the five-year average (106.6 million pounds).

Although supply volumes of both domestic and imported shrimp have been strong, ex-vessel prices - the price received by the captain at the point of landing - reported by NOAA continue to increase (2006; NOAA 2011; LDWF 2012). The ex-vessel price in 2013 for U15 shrimp was substantially higher at \$8.75/lb than it was in 2012 at \$6.10/lb., in 2011 (\$6.30/lb.), in 2010 (\$6.00/lb.), and 2009 (\$3.60/lb). The same trend is reported across count sizes, with the ex-vessel price for 36/40 count substantially higher in at Northern Gulf (\$3.80/lb) than they were in 2012 (\$2.65) in 2011, (\$2.30/lb) in 2010 (\$2.40/lb), and 2009 (\$1.7/lb).

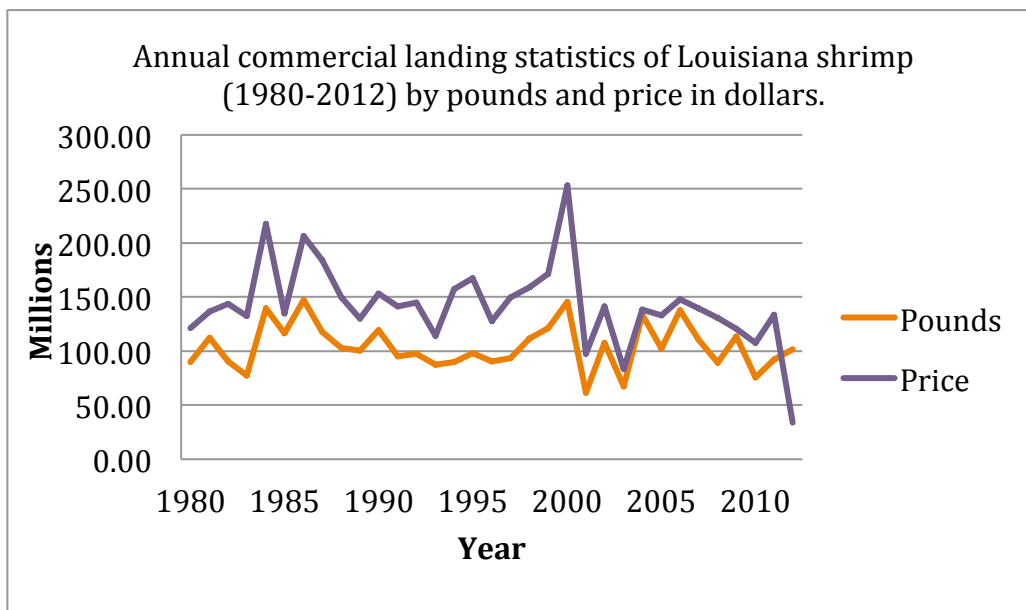


Figure 2. Annual commercial landing statistics of Louisiana Shrimp form 1980 to 2012 by pounds and price and dollars (NOAA 2012)

Louisiana’s estuaries along the Gulf of Mexico not only nourish the growth of two commercially important shrimp species but also nourish the livelihood of more than 5,000 licensed shrimpers in the state (Louisiana Sea Grant 1999 (Muncy 1984; Benfield et al. 2004; Grimes and Yow 2009; NOAA 2011; LDWF 2012). Within the domestic

shrimp fishery, there is a desire to enhance and meet consumer desire for “Wild Caught” domestic shrimp harvest. U.S. harvesters and processors are engaged in state and regional efforts to develop niche-marketing strategies for wild caught domestic shrimp. An important market strategy is the assurance of quality, the species harvested, and the harvest location (Gates and Applewhite 2013).

In 1975 the Wildlife and Fisheries commission divided the state into three shrimp management zones in order to manage shrimp on a regional rather than a state wide basis. The inside waters are divided such that, “Zone 1 extends from the Louisiana/Mississippi state line to the eastern shore of South Pass of the Mississippi River. Zone 2 extends from the eastern shore of South Pass of the Mississippi River to



Figure 3. Louisiana inshore shrimp management zones (LDWF 2014a)

the western shore of Vermilion Bay and Southwest Pass at Marsh Island. Zone 3 extends from the western shore of Vermilion Bay and Southwest Pass at Marsh Island to the Louisiana/Texas state line (Matherne 2013).” The outside waters are described as “State outside waters extending a distance of 3 nautical miles seaward of the inside/outside shrimp line from the northwest shore of Caillou Boca at -90 degrees 50 minutes 27 seconds west longitude westward to the Atchafalaya River Ship Channel at Eugene Island as delineated by the Channel red buoy line (LDWF 2014b).” The inshore season usually opens in mid-May and runs through June and ends sometime in July. Different zones may have different opening/closing dates depending upon the biological and technical data and public input (Matherne 2013).

1.2 Life Cycle

Temperature and salinity change affect the life stages (spawning, growth, habitat selection, movement, and migration) of each shrimp species in a slightly different way, causing shrimp to inhabit many niches in Louisiana estuaries and in Gulf waters (Muncy 1984; Benfield et al. 2004; Grimes and Yow 2009; NOAA 2012). Brown and White shrimp mature through the post larval and sub adult stages in Louisiana’s estuaries at slightly different times of the year, and sometimes overlap habitat use and occupy different niches in state waters (Benfield et al. 2004; NOAA 2012). White shrimp are most abundantly harvested in August, September, and October, whereas brown shrimp usually spawn earlier in the year, and are most abundantly harvested in May, June and July (Muncy 1984; Benfield et al. 2004). Though, some adults of both Brown and White species are available throughout most of the year (Benfield et al. 2004; NOAA 2012). In Louisiana waters, 60-65 percent of white shrimp are harvested in coastal or bay waters,

whereas the majority of brown shrimp landed in Louisiana are harvested in deeper, external regions. In contrast, other Gulf states shrimp harvests of all species tend to be higher offshore, outside state waters (Benfield et al. 2004).

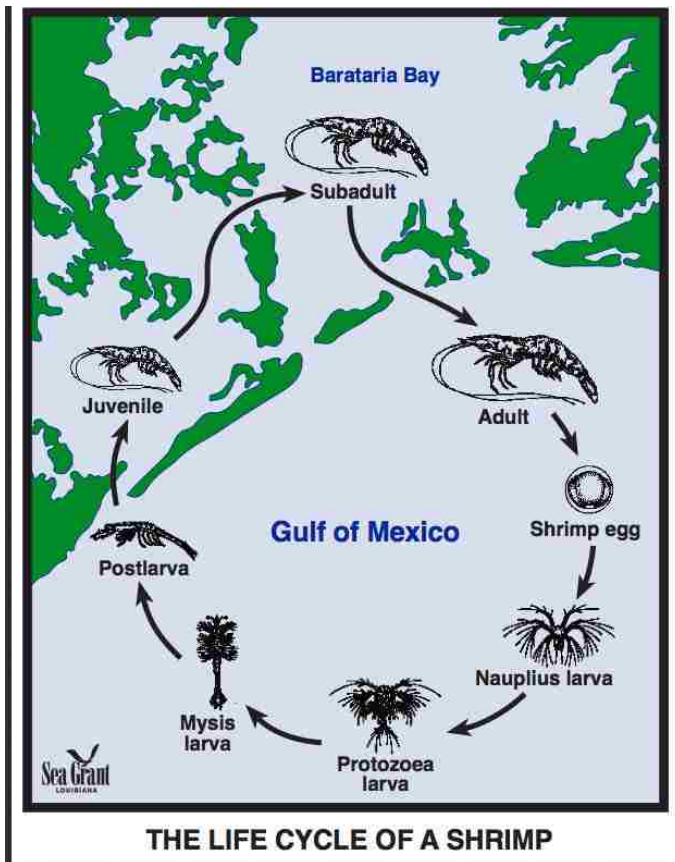


Figure 4. The life cycle of a shrimp along the coast of Louisiana (Sea Grant 2004)

While commercial shrimpers harvest almost as many brown as white shrimp in a given year, white shrimp typically grow to a larger size before they are harvested. For example, in 2004, white shrimp accounted for 55 percent of the year's landings, but nearly 70 percent of its value, according to the Louisiana Department of Wildlife and Fisheries. This size difference occurs because white shrimp remain longer in a nutrient rich environment. White shrimp spend a longer time in the estuaries and only respond

to very strong tidal changes that stimulate movement in and out of estuaries. This keeps them in a nutrient rich environment. White shrimp also tend to migrate back through the passes into estuaries over winter.

In addition to their value to commercial fisheries, Shrimp are important in estuarine and offshore food webs. The interactions of many different living, growing organisms with each other and the physical environment shape a shrimp's niche (i.e., its role in the environment, the species it interacts with, and its environmental requirements for food and shelter). The continuous but changing characteristics of a shrimp's niche can be seen by studying the major life stages of this important crustacean (Benfield et al. 2004)

1.3 Shrimp Processing and Additives

In commercial practices with marine shrimp, sulfites and phosphates are used to enhance and prolong shelf life of the shrimp. The most commonly used sulfite agents used to treat shrimp are sodium bisulfite (NaHSO_3) and sodium metabisulfite ($\text{Na}_2\text{S}_2\text{O}_5$). The dry sulfides are mixed with water and are then applied at approximately 1.25% (weight/weight) to fresh harvested whole or headless shell on shrimp. Sulfites block the process of melanosis in which enzymes cause brown melanin spots on the shrimp's shell. The FDA allows up to 100ppm (SO_2) of residue on the edible portion of the shrimp (Otwell 1992).

Phosphates, primarily sodium tripolyphosphate, are added as a blend along with approved food ingredients and can influence the pH and antimicrobial qualities of the mix. The concentrations of phosphates in prepared solutions can vary from 1 to 10%

depending on the product form and method of application. Phosphates can be added by spray, dips, soaks, or directly in packaging prior to freezing. Before adding phosphates, an addition of sodium chloride (0.25 to 1%) is typically added to increase the phosphate penetration by increasing surface solubility of phosphates (Otwell 1992). The addition of phosphates to seafood aids in moisture retention during processing, distribution, storage, and preparation. Excessive addition of phosphatases can lead to adulteration by economic fraud (Goncalves and Ribeiro 2008). Since seafood is sold by weight, increasing the phosphates will crease the water holding capacity of the treated seafood, and increase the price of the seafood. Several functional properties are associated with the addition of phosphates to seafood: retention of moisture and natural flavors, inhibiting the loss of fluids during distribution, the inhibition of the process of lipid oxidation, the stabilization of color, and the cryoprotection which increases shelf life (Goncalves and Ribeiro 2008).

1.4 Globalizing Shrimp

Increased demand for shrimp in world markets has encouraged many developing countries to engage in shrimp farming (Yanar et al. 2011). In 2012, the US imported 2,441,516 metric tons of edible seafood a value of \$16.7 billion. In 2012, the volume of imported shrimp was 533,497 tons valued at \$4.5 billion, accounting for 27% of total edible US imports (NOAA 2013). Consumers interested in food traceability and production form egg to plate are becoming more concerned about how or where animals are produced as well as nutritional differences between cultured and wild animals (Yanar et al. 2011).

As the amount of imported seafood increases, so do the problems of renaming and mislabeling. Information about seafood can be flawed and deceptive. One of the consequences of mislabeled seafood includes consumer and government economic losses. Often, this occurs when fishery products are mislabeled after they are purchased from the fisheries. In terms of ex-vessel prices, certain fish prices can be high due to resource scarcity, and instead of paying the high prices, distributors, retailers, and restaurants have been reported to buy fish of a lesser value and illegally sell these fish as their higher value relatives (Jacquet and Pauly 2008). Another consequence of mislabeled or renamed seafood includes resource losses, which have dire consequences for protected and/or illegal to sell species.

Undermining of eco-campaigns is also a consequence of renaming or mislabeling of seafood (Jacquet and Pauly 2008). For example, a wide campaign in Europe was raising awareness about the negative effects of farm raised shrimp, an industry that can destroy mangrove habitats and reduce water quality (Naylor et al. 2000). As a result, Thai shrimp, which account for nearly 30% of global production, are often exported contained the label “wild-caught” rather than “farm-raised” (Jacquet and Pauly 2008). Lastly, renaming or mislabeling of seafood increases health concerns, such as in the US, where seafood products are estimated to cause 18-20% of food born illnesses, causing 76 million illnesses annually (Butt et al. 2004; Jacquet and Pauly 2008). This is especially important in shrimp industry since high antibacterial and pesticide residues have been found in imported shellfish (Gaslund and Bengtsson 2001; Johnston and Santillo 2002; Gale 2009).

1.5 Nutritional Value of Shrimp

Shrimp are valuable natural food sources rich in protein and minerals, and contain well-balanced essential amino acids. Nutritionally, shrimp are high in protein, low in saturated fat and calories, and have a neutral flavor, which make shrimp a natural additive in salads, pastas, curry, soups, and stir-fried dishes. The nutrient profile of edible shrimp meat contains approximately 19% protein, 1% lipid, 76% water, and 89 Calories per 100g sample. The protein digestibility corrected amino acid score (PDCAAS) accounts for the amino acid content of food protein, true digestibility and its ability to supply the essential amino acids according to requirements. The PDCAAS for shrimp is 1, indicating its superior protein quality. Shrimp may contribute some cardio-protective benefits because of the lower atherogenic and thrombogenic indicators (Dayal et al. 2013). Shrimp have also been identified as a rich source of vitamin B12, selenium, ω -3 highly unsaturated fatty acids, and astaxanthin, a potent natural antioxidant (Venugopal 2009).

The human diet requires macro minerals, those found in large amounts, such as calcium (2500 mg per day), phosphorus (4mg per day), magnesium (350 mg per day), and sodium (2.3g per day) (Institute of Medicine et al.). A 100 g serving of shrimp provides >100 mg of calcium, >300 mg of phosphorus and >40 μ g of selenium. Minerals help regulate the fluid balance, enzyme production, and bone health, among other many functions. Consuming shrimp (100 g/day) would provide around ten vitamins and ten minerals. Shrimp contains key vitamins like vitamin A (180 IU), vitamin D (2 IU) and vitamin E (1.32 μ g), vitamin B12 (1.11 μ g), and vitamin B3 (1.77 mg) (USDA 2013).

1.6 Marine Shrimp Aquaculture

Shrimp aquaculture is practiced world wide, though about a dozen countries contribute to 95% of farmed shrimp. Farmed shrimp contribute to about 55% of global production (Lucas and Southgate 2012). Muir and others identified 6 key factors that differentiate the characteristics of aquaculture from fisheries outlined in Table 1 (Muir and Young 1998).

Table 1. Key discriminants between aquaculture and fisheries supply (Muir and Young 1998)

Factor	Characteristics
Management	Aquaculture is primarily a managed activity, and so can be far more definable and deterministic. It is also far more clearly specifiable in terms of location, scale and system.
Recruitment	Unlike fisheries, recruitment inputs can be known or estimated directly; with definable mortality- ties to market size, there can be some degree of correlation with outputs.
Linkages	There are broadly definable linkages between aquaculture outputs and necessary resource inputs; there are also linkages with waste outputs and other impacts, and with financial returns.
Flexibility	In terms of timing and market size; higher average values may be obtained than for the wild caught equivalent; more notably where there is a higher degree of management control.
Ownership	Ownership and rights allocation are usually more explicit; production may be a more definable determinant of local economic potential, and broadly, of national capacity; the 'live storage' potential also means that aquaculture stocks may provide a local store of food supply or wealth.
Ecology	Aquaculture systems are far more concentrated in respect of nutrients, energy and yields; their capacity and potential is linked, and ultimately constrained by the potential for collecting and applying inputs, and by local environmental capacity.

Two species, Black Tiger (*Penaeus monodon*) and Pacific White (*Litopenaeus vannamei*), represent 90-95% of commercially farmed shrimp. Before the turn of the

21st century, marine shrimp aquaculture produced more Black tiger shrimp, but currently Pacific white shrimp represent over 65% world production (Lucas and Southgate 2012).

South East Asian aquaculture is characterized by small one-hectare or smaller ponds and utilizes mechanical aeration to maintain such high densities. Ponds are lined in plastic and stocked at densities of 150 or more shrimp per square meter. In S. E. Asia, shrimp are fed relatively high protein manufactured feeds. In the Americas, larger ponds ranging from 5 to 10 ha characterize the shrimp aquaculture industry with stocking densities of 10-30 animals per square meter. Similar to S. E. Asian aquaculture, use of manufactured feeds and mechanical aeration is sometimes found in the Americas, along with the use of selected species for growth and survival against persistent pathogens (Lucas and Southgate 2012).

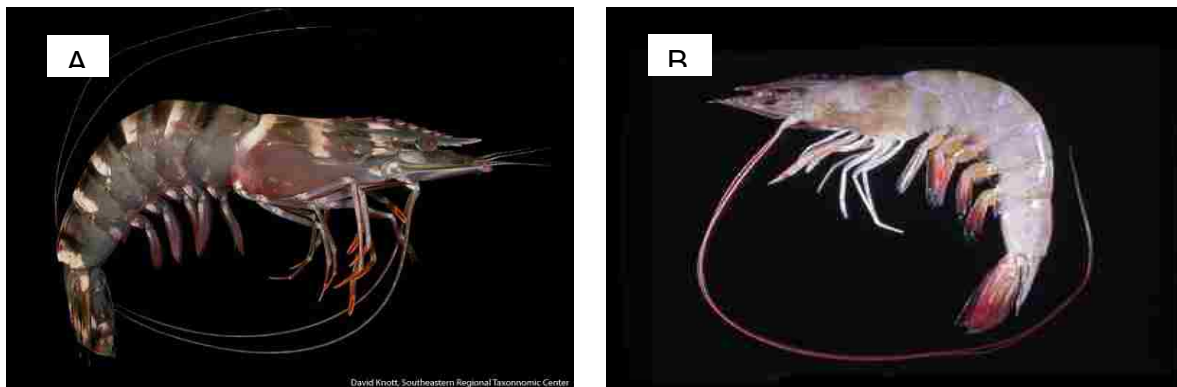


Figure 5. Marine shrimp aquaculture's most farmed species. A. Black tiger shrimp (*Penaeus monodon*), B. Pacific white (*Litopenaeus vannamei*) (PAGE 2009; Knott)

The Food and Agriculture Organization of the United Nations (FAO) reports that the expansion of shrimp farms has generated many concerns and debates of its effect on the environment and sustainability:

- Use of protective mangrove ecosystems for pond construction
- Slash and burn style use of ponds for a few years, before moving to new

areas

- Salinization of groundwater and agricultural land
- Pollution of coastal waters by pond effluents
- Overuse of marine meals leading to inefficient use of vital protein sources and disruption of marine ecosystems
- Biodiversity issues occurring from collection of wild seed and broodstock and introduction of non-native species and their associated pathogens
- Social conflicts with other resource users
- Farm discharges, causing self-pollution in shrimp growing areas

Because of the increasing awareness of the negative impacts of shrimp aquaculture practices on the environment, many countries are making efforts to comply with responsible aquaculture practices found in Article 9 of the FAO Code of Conduct for Responsible Fisheries. Aquaculture practices worldwide are increasing cost efficiency, reducing use of chemical residues, increasing traceability, and implementing hazard analysis critical control points (HACCP) to be used in processing and feed plants (FAO 2006).

The increased use of manufactured dry feed in marine shrimp aquaculture has expanded the nutrition-formulated diets and feed management industry. Feed is the highest cost associated with aquaculture production. Protein is the most expensive macronutrient and ranges in levels from 18 to 61% depending on the size, species, and feeding habits of the shrimp. Formulated shrimp feed is mainly composed of wheat flour (20-35%), soybean meal (15-45%) and fishmeal (10-25%). The remaining

ingredients include lipids from various sources, vitamins, minerals, attractants, binders, preservatives, pigments, and health additives. Feed management is crucial to a healthy stock. Inadequate feed management can promote onset of various diseases and water quality related problems(Lucas and Southgate 2012).

1.7 Mineral and Metal Contents of Shrimp

Invertebrates like shrimp are naturally rich in minerals. The main minerals in shrimp muscles (*Penaeus semisulcatus* and *Metapenaeus monoceros*) are calcium, potassium, sodium, and iron (Yasemen and Yanar, 2006). The two major sources of minerals for marine organisms are seawater and feed (Ichihashi et al. 2001). In the wild, shrimp larvae feed on plankton, while juvenile and adult shrimp are omnivorous and feed on the bottom at night on worms, algae, microscopic animals, and various types of organic debris (NOAA 2011) . Unlike terrestrial animals which are limited to mineral intake through their diet, aquatic animals may be able to take in minerals dissolved in water to meet their requirements (Davis et al. 1996).

Smith and Watts discussed potential sources for trace metal accumulation in shrimp tail meat of farm-raised shrimp starting with the pond used for shrimp production which can vary in size, shape, design and stocking density and can be completely lined with thick plastic lining or semi lined, or have no lining at all. Trace metal contents are also affected by water quality: salinity, filtration, seasonal rainfall, and aeration of highly stocked ponds. The rate at which the shrimp feed is also variable and depends on stocking density, which can range from 10 to 160 shrimp per square meter. Water quality parameters like pH, dissolved oxygen, and alkalinity vary widely within a country and more so between countries. Feed for farm-raised shrimp is likely prepared from locally

available inexpensive fish in which trace metals are transferred to shrimp from a locally specific trace metal source (Smith and Watts 2009).

Minerals serve several intra and extracellular functions; ten minerals (calcium, phosphorus, magnesium, potassium, copper, iron, zinc, manganese, selenium, and iodine) have been identified as essential in the diet of fish (Davis et al. 1996). Minerals can serve as components of structural support as bone, fins, scales, teeth, and exoskeletons (Davis et al. 1996). Minerals are also components of soft tissue: sulfur in proteins, and phosphorus in phospholipids and nucleic acids (Davis et al. 1996). Relatively soluble minerals like calcium, phosphorus, sodium, potassium, and chloride function in osmoregulation, acid base balance, and the production of membrane potentials (Davis et al. 1996).

Calcium in particular is crucial for hard tissue development, muscle contraction, nerve transmission, osmoregulation, and enzymatic activity (Davis et al. 1996), and shrimp can meet their calcium requirement directly from the water around them (Davis et al. 1996; Lovell 2002). Phosphorus is an essential mineral because of its limited availability under growth stages or rearing of shrimp. Phosphorus is directly involved in all energy-yielding reactions and has an integral role in cellular functions, as it is a key component of nucleic acids, phospholipids, phosphoproteins, ATP and several key enzymes (Lovell 2002).

Previous research suggests that mineral contents of shrimp and other marine species can vary seasonally. When mineral profiles of two species of wild caught Eastern Mediterranean shrimp (*Penaeus semisulcatus* and *Metapenaeus monoceros*) were compared during four seasons, both species showed seasonal mineral variance in

Ca, K, P, Na, and Fe but the levels of Ca remained constant (*Penaeus semisulcatus*) (Yasemen Yanar a and a 2006). Differences in mineral composition were also detected between cultured and wild green tigers shrimp (*Penaeus semisulcatus*) (Table 2). The cultured shrimp contained higher concentrations of P, K, and Zn than the wild caught, while the wild caught shrimp contained higher Ca, Mg, and Na (Yanar et al. 2011).

Table 2. Mineral content (mg/kg) in the muscle of wild and cultured green tiger shrimp (*P. semisulcatus*) (Yanar et al. 2011)

Mineral Content	Cultured	Wild
Fe	19.84±0.17 ^{a*}	20.19±0.01 ^a
Ca	89.77±0.17 ^b	107.36±0.24 ^a
Mg	579.54±03.4 ^b	691.31±0.42 ^a
Mn	1.14±0.01 ^a	1.33±0.01 ^a
Zn	25.26±0.02 ^a	23.65±0.3 ^b
Na	2949.30±4.63 ^b	3246±6.65 ^a
P	2901.6±6.77 ^a	2444.6±4.17 ^b
K	4725±6.00 ^a	3656±12.00 ^b

*Means±SE in the same row with different letter differ at significance level $p < 0.05$.

A similar study compared the seasonal mineral profiles of Turkish oysters and found seasonal variability in most of the micro- and macro-minerals with Na, Mg, and Ca highest in autumn, and K and P highest in the spring. The levels of zinc were constant throughout the year, but an increase in Cd and Cu were detected in the winter (Erkan et al. 2010).

Analysis of mineral and metals levels in foods like shrimp requires a multi element analytical technique that measures several elements simultaneously. Minerals can be found in parts per billion to percent levels, and can be complicated by naturally occurring, seasonal and varietal differences (Barnes and Debrah 1997). This multi element capability can be achieved by using inductively coupled plasma optical emissions spectroscopy (ICP-OES). ICP-OES can be used to detect the geographical

origin of food or plants using a metal fingerprint in a product and comparing it with the fingerprint from a known authentic sample of a product (Barnes and Debrah 1997).

1.8 Multivariate statistical analysis

Analysis of variance (ANOVA) is statistical technique used to test for differences in means between two or more groups and can be used to determine the impact independent variables have on dependent variables. Multivariate analysis of variance (MANOVA) is similar to ANOVA, but includes several dependent variables. ANOVA test for differences in means between two or more groups, whereas MANOVA test for the difference in two or more vectors or directions of means. MANOVA is useful for measuring several dependent variables in a single experiment and offers a better chance of identifying the most discriminating variables (French et al. 2002)

Discriminant analysis (DA) is a method used to determine which continuous variables discriminate between two or more groups and can be used to determine which variables are the best predictors of a group. DA is essentially a two-step process of testing the significance of a set of discriminant functions followed by classification. The first step of testing significance of discriminant functions is virtually identical to MANOVA in which a multivariate test is performed, and if the results are significant, variables that have significantly different means across groups are determined. These distinguishing variables become the predictor variables. Standardized coefficients for each variable are determined for each significant function and the larger the standardized coefficient, the larger or more discriminating the variable is its respective group. A canonical correlation analysis is then used to determine the successive functions and canonical roots, allowing for canonical functions to be classified. The factor structure matrix can

be used to identify which independent variable causes the most discrimination between dependent variables by comparing the correlations between the variables (Poulsen and French 2004; Prinyawiwatkul and Chompreeda 2008; Smith and Watts 2009). Wilks' lambda as used as the test for significance, and the smaller the lambda for an independent variable, the more that variable contributes to discriminating the means. Lambda values vary from 0 to 1, with 1 meaning all groups are the same. The F test form Wilks' lambda can be used to show which variables contributions are significant (Poulsen and French 2004)

Cross validation removes one of the reference samples from the data, classifies this sample against the other reference samples, and returns it to the data set until all samples have gone through this process. The accuracy of the classification is determined by the output of correctly classified samples (Picard and Cook 1984). This method provides a level of confidence in determining classification of variables (Prinyawiwatkul and Chompreeda 2008). The percent of correct classification of the removed samples is presented as percent (%) hit rate (Smith and Watts 2009).

This type of predictive discriminate analysis using mineral profiles has been used in a variety of studies to predict product origins. The mineral composition of Italian saffron was used to classify geographical origin with over 90% correct predictions (D'Archivio et al. 2014). Metal content in southern Spanish wines was used to classify their origins and their age with up to 93.6% correct predictions (Paneque et al. 2010). Multivariate statistics were also used to predict country of origin of farm-raised shrimp using greater than 90% correct classification (Smith and Watts 2009).

1.9 Objective

Mineral profiles of wild caught Louisiana shrimp were used for determining the geographic origins or catch locations. This method can be used to identify source or origin of shrimp because of the diversity of the environment with which the shrimp were grown. Using DA and MANOVA, the minerals that discriminate between catch locations of Louisiana wild caught shrimp were determined. This provides us with the minerals that are the best predictors of catch locations. Cross validation using quadratic discriminant analysis determined the probability of a sample between catch locations. This method provides a level of confidence in determining the true catch locations of Louisiana wild caught shrimp, or the accuracy of the mineral data for the wild caught shrimp.

Indeed, diet and water in the environments of wild caught shrimp along with chemical preservatives added in the processing of farm-raised shrimp are expected to be major factors in the bioaccumulation of minerals. Providing models for bioaccumulation of minerals was not in the scope of this study.

The goal of this study is to determine if enough diversity exist in the mineral profiles to significantly validate catch locations of Louisiana shrimp. Although numerous studies have been conducted on differences in mineral profiles between cultured and wild fish or shellfish, this matter has not recently been studied on shrimp from the Gulf of Mexico. This study has been carried out to detect possible differences in mineral contents among regional domestic wild-caught shrimp species and imported pond-raised shrimp and to provide base line mineral profiles of harvest location. This profile can be used to distinguish Louisiana wild caught shrimp from farm raised shrimp and

perhaps prevent mislabeling and illegal substitution with lower cost farm raised imported shrimp. This project could also support and verify shrimp supplies for regional niche marketing strategies.

CHAPTER 2. MATERIALS AND METHODS

2.1 Sample Procurement

White and brown shrimp from the Louisiana coastline were collected at varying depths, seasons, and distances offshore by the Louisiana Department of Wildlife and Fisheries frozen and stored at -80°C until being delivered to Louisiana State University Food Science Department. The shrimp samples were thawed overnight in an 8°C cooler. After removal of heads, shells, tails, legs and intestines, the flesh was ground into a homogenous mass in an Oster Osterizer blender (Jarden Consumer Solutions, Providence, RI). The samples were stored in plastic bags at -80°C until analysis.

2.2 Mineral and Metal Analysis

Bagged shrimp samples were allowed to thaw under running water for 1 hour. Using an analytical balance (Mettler Toledo AG104, Switzerland), 3.00 g of each sample were weighed into a pre weighed crucible. Samples were dried in a drying oven (VWR, Cornelius, OR) oven at 103°C overnight. After drying, samples were charred in their crucibles using a hot plate (Thermolyne Cimarec® 3, USA) under ventilation. After charring samples were placed in a muffle furnace (Thermolyne Corp. Type 6000, Dubuque IA) at 450°C under a gradual increase ($\leq 50^\circ\text{C/h}$) in temperature for 6 hours. Once cooled in a desiccator, ash residue was dissolved in 10 mL of 10% HNO₃ solution (Sigma Aldrich, St. Louis, MO). Using a sterile 10 cc syringe (B-D® Franklin Lake, New Jersey) and a SFCA, 0.2 μm , 25 mm syringe filter (Nalgene, USA) filter dissolved sample into a 20 mL disposable scintillation vial lined with a Teflon® screw top lid.

Samples were analyzed at the W.A. Callegari Environmental Center via ICP OES, Varian Vista-MPX CCD Simultaneous OES. The instrument was calibrated

before each run with 6 solutions made from commercially purchased standards (Sigma Aldrich, St. Louis, MO). A five-point calibration curve was used ranging from 0.5 ppm to 5.0 ppm for all minerals and metals except for silicon, which ranged from 0.025 ppm to 2.5 ppm and potassium, which ranged from 1.0 ppm to 50 ppm. The calibration curve was verified with an ICV (Independent Calibration Verification) solution at 0.5 ppm immediately after calibration. The curve was verified with a dependent CCV (Continuing Calibration Verification) solution at 0.5 ppm every 10 samples and at the end of the run. An ICB (initial Calibration Blank) was run immediately after calibration. A CCB (Continuing Calibration Blank) was run after every ten samples and at the end of the run. Sample element concentrations above the curve were diluted into the curve and run again for that particular element (AOAC 2002).

2.3 Statistical Analysis

The Louisiana Department of Wildlife and Fisheries inshore management zones (Figure 3) were used to group Louisiana wild caught shrimp by catch locations. Zone 1 is bordered by the Mississippi state line and extends to the eastern shore of South Pass of the Mississippi River. Zone 2 is bordered by the South Pass of the Mississippi River to the western shore of Vermillion Bay and Southwest Pass at Marsh Island. The western shore of Vermillion Bay and Southwest Pass at Marsh Island to the Texas state line borders Zone 3. An additional zone was created to represent shrimp caught in the “outside waters”, which extend three nautical miles from the inside/outside shrimp line into the Gulf of Mexico (Figure 6) (LDWF 2014a).

All data were analyzed ($\alpha = 0.05$) using SAS, version 9.1.3 (SAS Inst., 2008). Analysis of variance (ANOVA) was performed to determine if differences existed among

catch locations, seasons, years, species, and product origin. The Tukey's studentized range test was performed to locate differences among the catch locations, seasons, years, species, and product origin. Multivariate analysis of variance (MANOVA) was performed to determine if the catch locations and seasons were different when 10 of the minerals were simultaneously considered. Descriptive discriminant analysis (DDA), along with principal component analysis (PCA) using cross validation was performed to identify nine minerals contributing to underlying group differences among catch locations and seasons.



Figure 6. Catch zones used to compare Louisiana wild caught shrimp

All data in Tables 3- 6 were expressed as mean \pm standard error. The statistical significance of any group differences was assessed using Student's t test whenever appropriate, and "*P*" value less than 0.05 was considered statistically significant. All statistical procedures were performed by Statistical Analysis Software[®] (SAS[®] 9.3).

CHAPTER 3. RESULTS AND DISCUSSION

The geographical variation in the mineral contents of Louisiana shrimp is shown in Table 3. Al was found in the highest concentration in Zone 4 (26.77 mg/kg) and Zone 3 (21.1 mg/kg) and decreased eastward to zone 1 (16.25 mg/kg). Fe was highest in Zones 2 and 3 (13.99 and 16.41 mg/kg) and was significantly lower in Zones 1 and 4 (10.61 and 12.86 mg/kg). Si was highest toward the west, Zone 3 (48.8 mg/kg) and decreased eastward to Zone 1 (32.19 mg/kg). Si in Zone 4 (35.46 mg/kg) was also significantly lower than Zones 2 and 3 but similar to Zone 1. Cu was highest in Zones 3 and 4 (6.11 and 6.06 mg/kg) and lowest in Zones 1 and 2 (4.72 and 4.95). Z was highest in the outside waters of Zone 4 (15.54 mg/kg) and was significantly lower in Zones 1, 2, and 3 (12.35, 12.81, 13.27 mg/kg). Z concentrations were significantly lower in Zone 1 compared to Zones 2 and 3.

The highest levels of S were found in Zones 1 and 3 (290.47 and 166.88 mg/kg). The S content of shrimp from Zone 2 and 4 was significantly lower in Zone 2 and 4 (179.41 and 165.46 mg/kg). P was highest in Zone 4 and 1 (4697.03 and 4026.23 mg/kg) and decreased westward to Zone 3 (2211.76 mg/kg). Mg was highest in Zones 2,3, and 4 (378.29, 397.79, 401.29 mg/kg) but was significantly higher than Zone 1 (307.69 mg/kg). K was highest in Zone 4 (3056.12 mg/kg), the outside waters. K was lower closer to the shore in Zones 1, 2, 3 (2506.82, 2747.36, and 2253.85 mg/kg) and Zone 2 was significantly higher than Zones 1 and 3. Na was highest in Zones 2, 3, and 4 (1578.84, 1511.51, and 1633.50 mg/kg) and was significantly lower in Zone 1 (1173.44 mg/kg). Ca was highest in Zone 3 (1214.01) and decreased eastward to Zone

Table 3. Geographical variation in the mineral contents (mg/kg muscle tissue)^A of Louisiana wild caught shrimp

Mineral	Zone 1	Zone 2	Zone 3	Zone 4
Al	16.25±1.2 ^{cB}	21.08±0.69 ^b	21.3±1 ^{ab}	26.77±1.85 ^a
S	290.47±45.16 ^a	179.41±15.21 ^b	166.88±44.75 ^a	165.46±16.23 ^c
P	4026.23±440.18 ^{ab}	3314.74±211.64 ^{bc}	2211.76±288.94 ^c	4697.03±283.26 ^a
Fe	10.61±0.74 ^b	13.99±0.47 ^a	16.41±1.51 ^a	12.86±0.72 ^b
Mg	307.69±13.15 ^b	378.29±14.62 ^a	397.79±17.94 ^{ab}	401.29±14.23 ^a
K	2506.82±85.67 ^c	2747.36±46.4 ^b	2235.85±80.77 ^c	3056.12±70.63 ^a
Na	1173.44±36.5 ^b	1578.84±40.83 ^a	1511.51±97.93 ^a	1633.5±47.24 ^a
Cu	4.72±0.13 ^b	4.95±0.13 ^b	6.11±0.28 ^a	6.06±0.19 ^a
Zn	12.35±0.17 ^c	12.81±0.14 ^b	13.27±0.28 ^{bc}	15.54±0.15 ^a
Ca	439.51±35.62 ^c	758.84±39.19 ^b	1214.01±115.74 ^a	405.81±20.11 ^c

^AData are expressed as mean ± standard error

^BDifferent letters for each zone within a row denote significant differences ($p < 0.05$)

1 (439.51mg/kg). Zone 4 was also significantly lower in Ca than Zone 2 and 3 (405.81 mg/kg).

The seasonal variation in the mineral contents of Louisiana shrimp is shown in Table 4. The spring months include March, April and May. The summer months include June, July, and August. The fall months include September, October, and November. The winter months include December, January, and February.

The Al levels were highest in the fall, winter, and spring (18.43, 21.57, and 24.38 mg/kg) and decreased significantly in the summer (24.20 mg/kg). The S levels were highest in the winter (430.73 mg/kg) and lowest in the summer (150.43 mg/kg). P levels were highest in the summer and winter (5107.32, 4765.91 mg/kg) and decrease significantly in the spring and fall (3864.18 and 2804.09 mg/kg). Fe levels did not vary seasonally. Mg was significantly higher in the fall (394.00 mg/kg) than in the winter

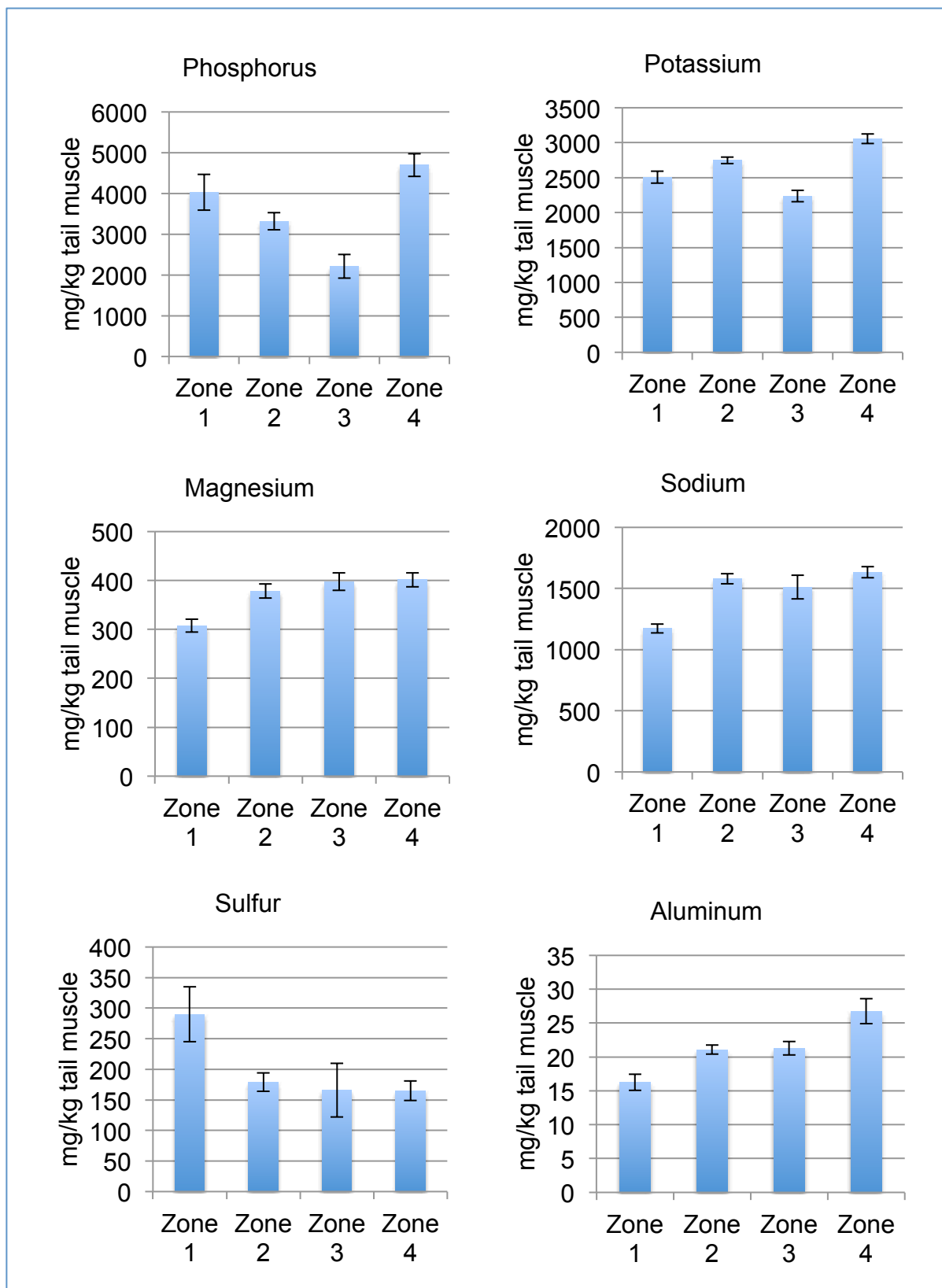


Figure 7. Variation of P, K, Mg, NA, S, and Al in Louisiana wild caught shrimp from four catch locations

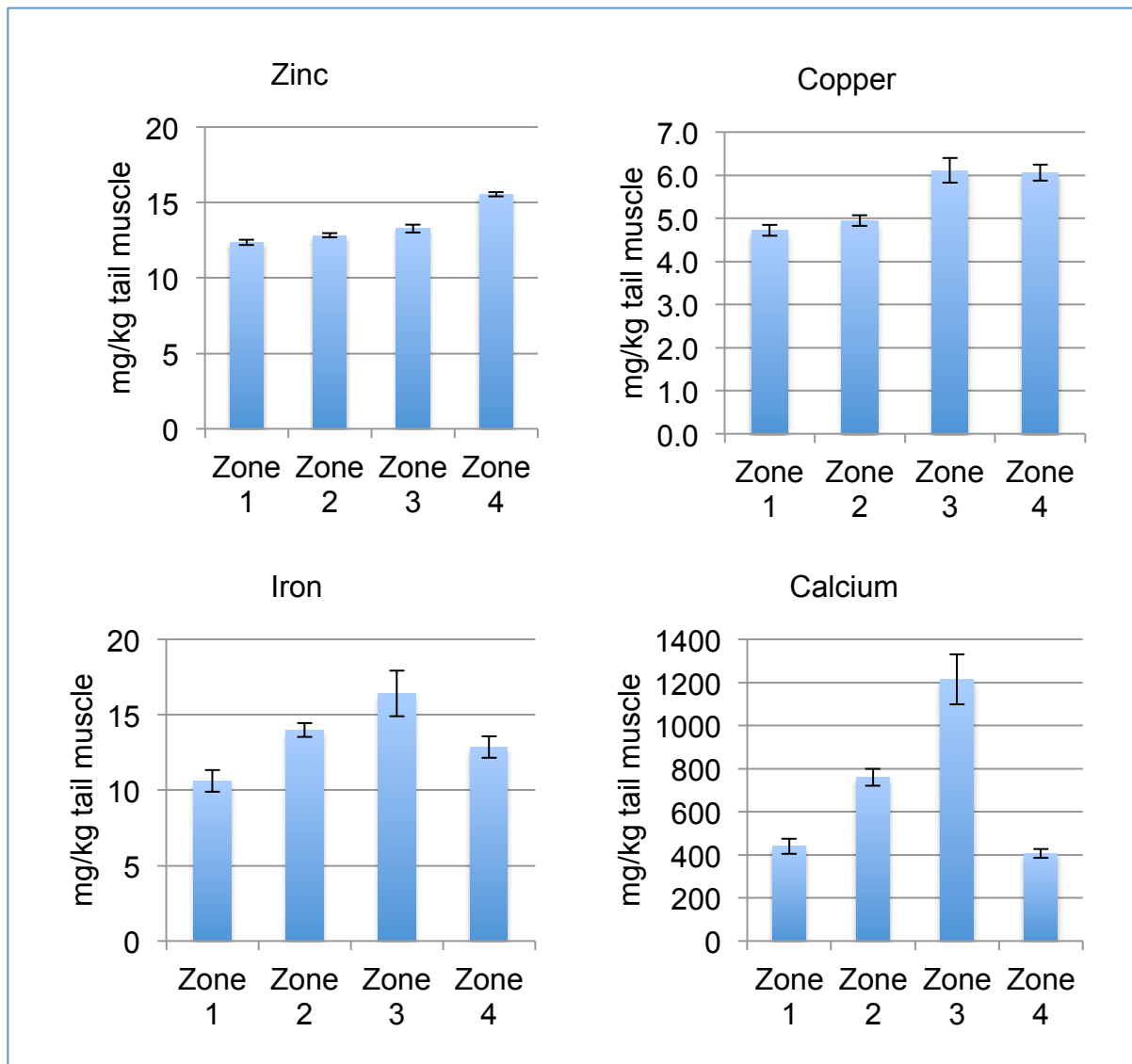


Figure 8. Variation of P, K, Mg, NA, S, and Al in Louisiana wild caught shrimp from four catch locations

(339.61mg/kg). Potassium was consistently higher in the spring, summer, and fall (2790.51, 2766.55, 2933.89mg/kg), before decreasing significantly in the winter (29.61 mg/kg). Na was highest in the summer (1440.48 mg/kg) than in the winter (1432.04 mg/kg). Cu was highest in the winter and spring months (5.71 and 4.81 mg/kg) than in the summer and fall months (5.79 and 5.13 mg/kg). Zn was the higher in the summer (14.25 mg/kg) and lowest in the spring, fall and winter (13.36, 13.33, and 13.37 mg/kg).

Ca was highest in the spring (535.28 mg/kg) and lowest in the fall (636.9 mg/kg). Figure 9 shows the seasonal variation.

Table 4. Seasonal variation in the mineral contents (mg/kg muscle tissue)^A of Louisiana wild caught shrimp

Mineral	Spring	Summer	Fall	Winter
Al	24.38±1.52 ^{ab}	24.2±2.43 ^b	18.43±0.76 ^a	21.57±1.17 ^{ab}
S	171.57±16.06 ^c	150.43±30.51 ^c	88.02±6.7 ^b	430.73±41.92 ^a
P	3864.18±337.13 ^b	5107.32±752.53 ^a	2804.09±122.54 ^c	4765.91±342.26 ^a
Fe	13.07±0.62 ^a	14.38±1.22 ^a	13.08±0.53 ^a	12.24±0.74 ^a
Mg	374.48±10.56 ^{ab}	344.92±26.64 ^{ab}	394.00±6.7 ^a	339.61±30.1 ^b
K	2790.51±58.34 ^a	2766.55±136.63 ^a	2933.89±50.77 ^a	2455.4±85.47 ^b
Na	1498.92±37.77 ^{ab}	1440.58±89.91 ^a	1571.87±37.05 ^{ab}	1432.04±73.75 ^b
Cu	4.81±0.14 ^a	5.79±0.29 ^b	5.13±0.14 ^b	5.71±0.19 ^a
Zn	13.36±0.24 ^b	14.25±0.39 ^a	13.33±0.16 ^b	13.37±0.19 ^b
Ca	525.28±32.52 ^a	742.43±93.72 ^{ab}	636.9±33.3 ^b	637.15±65.37 ^{ab}

^AData are expressed as mean ± standard error

^BDifferent letters for each zone within a row denote significant differences ($p < 0.05$)

Yanar and others (2006) reported that mineral contents (Ca, K, P, Na, and Fe) of green tiger shrimp and speckled shrimp from the Eastern Mediterranean differed seasonal in all minerals except for Ca. However, the Ca level of Louisiana wild caught shrimp varied seasonally, and the Fe content of Louisiana wild caught shrimp did not vary seasonally. Differences in the minerals that vary seasonally may be attributed to differences in species and environmental conditions.

Analysis of variance was used to detect seasonal differences of ten minerals within each catch location (Table 6). Generally, shrimp showed seasonal differences within each catch location ($p < 0.05$) with a few exceptions. In the winter months, Fe and Cu were not significantly different between catch locations. In the spring months, Al, P, Fe, and Na, did not show any significant differences between catch locations. In the

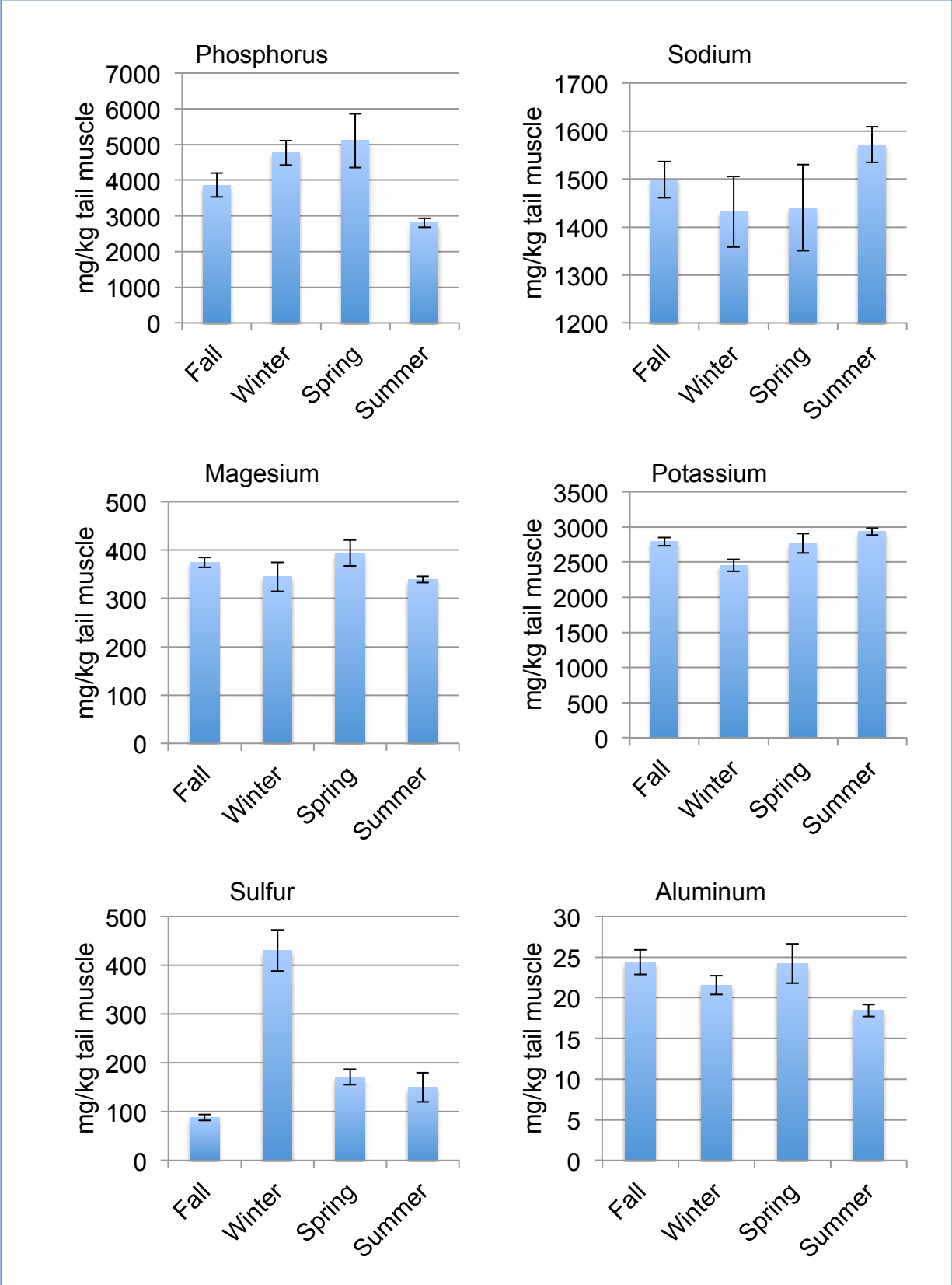


Figure 9. Seasonal variation of P, K, Mg, NA, S, and Al in Louisiana wild caught shrimp

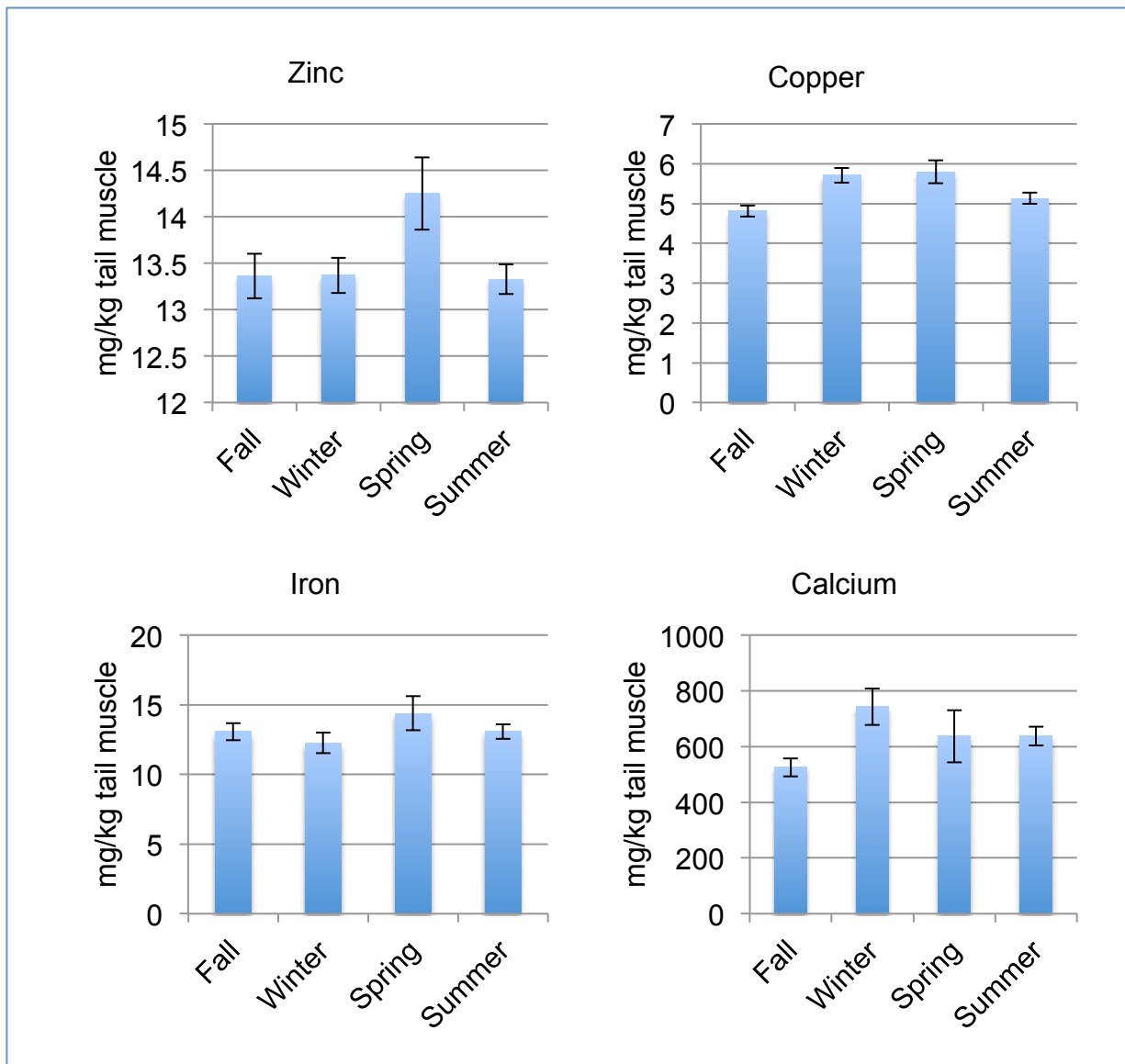


Figure 10. Seasonal variations in Zn, Cu, Fe, and Ca in Louisiana wild caught shrimp

summer and fall months, P was the only mineral that was not significantly different between catch locations.

Based on the results of Table 5, we concluded that catch locations (Zone 1, Zone 2, and Zone 4) and seasonal variation (fall, winter, spring, and summer) were significantly different when all 10 minerals were compared simultaneous. Since there were significant differences among all three-catch locations and seasons, DDA was

performed to determine which minerals were mainly responsible for group differences. S was left out of the DDA analysis and subsequent analysis due to too few data points. Catch Zone 3 was also left out of these subsequent analyses because of too few data points.

Results from DDA (Table 6) report the canonical structure r 's, which identify constructs that largely account for the group differences. Two dimensions (Can 1 and Can 2) shown in Table 6 explain the total variance. According to the pooled within group variances, the first dimension (Can 1), accounts for 85.18% explained variance and the second dimension (Can 2) accounting for 14.82% of explained variance. These pooled variances identify Ca, followed by Zn, and Cu to a lesser extent (canonical correlation= 0.852 0.441, -0.4630, respectively) as the minerals greatly contributing to the group difference among three catch locations. Based on canonical correlation value (Table 6), we conclude that the main construct that accounted for the group differences is Ca, Zn, and Cu.

Table 5. Seasonal variation in the mineral contents (mg/kg of muscle tissue)^A among the catch locations of Louisiana wild caught shrimp

Minerals	Winter		
	Zone 1	Zone 2	Zone 4
Al	22.81±3.42 ^{ab B}	19.47±1.61 ^b	22.64±1.67 ^a
S	811.36±68.57 ^a	360.88±43.81 ^b	226.53±40.58 ^c
P	6166.25±794.82 ^a	3559.84±396.07 ^b	4971.51±693.9 ^{ab}
Fe	13.99±2.08 ^a	11.71±1.13 ^a	12.02±0.96 ^a
Mg	224.69±32.85 ^b	417.92±65.9 ^a	304.2±32.26 ^{ab}
K	1957.03±139.81 ^b	2558.79±136.12 ^a	2553.59±144.8 ^a
Na	998.11±84.75 ^b	1766.62±139.07 ^a	1269.02±79.95 ^b
Cu	5.8±0.17 ^a	5.71±0.32 ^a	5.61±0.49 ^a
Zn	12.6±0.21 ^b	12.5±0.29 ^b	14.89±0.24 ^a
Ca	290.73±45.95 ^b	1098.82±120.33 ^a	326.49±41.6 ^b

(Table 5 continued)

Minerals	Spring		
	Zone 1	Zone 2	Zone 4
Al	21.6±0.25 ^a	17.34±1.19 ^a	38.53±5.94 ^a
S	53.53±2.53 ^b	371.85±25.76 ^a	88.16±10.2 ^b
P	3033.07±36.24 ^a	4889.87±1247.18 ^a	6557.59±816.21 ^a
Fe	10.55±0.27 ^a	12.62±0.98 ^a	19.64±3.22 ^a
Mg	422.23±5.9 ^a	356.58±35.65 ^{ab}	284.11±56.89 ^b
K	3434.09±46.14 ^a	2744.61±183.58 ^a	2474.46±264.25 ^b
Na	1585.66±47.29 ^a	1511.52±128.68 ^a	1233.26±174.23 ^a
Cu	4.02±0.17 ^b	6.75±0.32 ^a	4.85±0.4 ^a
Zn	11.28±0.3 ^b	14.58±0.49 ^{ab}	15.11±0.58 ^a
Ca	512.43±20.58 ^a	1027.72±130.38 ^a	315.38±66.53 ^b
Minerals	Summer		
	Zone 1	Zone 2	Zone 4
Al	15.036±1.513 ^b	20.39±1.07 ^a	14.02±1.8 ^b
S	67.527±7.365 ^a	108.99±10.19 ^a	35.43±2.67 ^b
P	2687.07±89.531 ^a	2868.98±214.36 ^a	3274.38±33.75 ^a
Fe	11.407±1.069 ^{ab}	14.28±0.74 ^a	8.37±0.59 ^b
Mg	378.388±6.076 ^b	382.14±10.63 ^b	455.94±6.14 ^a
K	2998.31±91.119 ^b	2840.96±59.42 ^b	3641.67±59.01 ^a
Na	1282.07±34.34 ^c	1553.47±49.23 ^b	2074.55±52.21 ^a
Cu	4.573±0.188 ^b	4.51±0.14 ^b	7.47±0.26 ^a
Zn	13.082±0.239 ^a	12.75±0.2 ^b	15.78±0.23 ^a
Ca	641.223±79.542 ^a	605.56±33.22 ^a	344.48±14.87 ^b
Minerals	Fall		
	Zone 1	Zone 2	Zone 4
Al	11.18±0.97 ^c	24.94±1.33 ^b	33.36±3.45 ^a
S	99.51±26.78 ^b	177.51±29.98 ^a	217.74±20.8 ^a
P	3905.56±1092.12 ^a	3155.36±348.91 ^a	4751.06±421.39 ^a
Fe	7.09±0.47 ^b	15.79±0.83 ^a	13.96±1.11 ^a
Mg	277.65±18 ^c	352.79±13.59 ^b	473.75±5.83 ^a
K	2250.06±133.51 ^c	2738.9±70.28 ^b	3254.7±29.43 ^a
Na	1118.29±60.66 ^c	1511.86±57.88 ^b	1762.04±20.23 ^a
Cu	4.14±0.22 ^b	4.32±0.21 ^b	5.94±0.17 ^a
Z	11.59±0.33 ^b	12.35±0.22 ^b	15.98±0.26 ^a
Ca	334.18±28.99 ^b	635.86±66.36 ^a	522.67±27.14 ^a

^A Data are expressed as mean ± standard error

^B Different letters for each zone within a row denote significant differences (p<0.05)

Within each catch location, seasons were also analyzed for discriminating variables. Fall and winter months were combined because fall season does not contain enough data points to allow for enough degrees of freedom. In the summer months, Ca and to a lesser extent Zn and Cu (canonical correlation=0.8269, 0.3929, and 0.5547, respectively) are the most discriminating minerals for catch locations. In the winter and fall months, Ca and to a lesser extent Zn are the most discriminating minerals (canonical correlation 0.9009, 0.4079, and 0.3823). The spring season only contains one dimension of canonical structure, because no data exist for Zone 2. Preliminary data (Table 7) shows that in the spring months Ca and to a much lesser extent Cu are the main discriminating minerals between catch locations. If more data were included in the DDA analysis of catch location in the month of spring, Zn could potentially present as a major discriminating mineral, but more data would be needed to verify. Based on these results we can conclude that Ca and to a much lesser extent Zn are the main minerals contributing to variation among catch locations and seasons, though additional data for catch location 2 in the spring months would be needed for verification.

In order to provide a level of confidence in determining the catch location of Louisiana wild caught shrimp, the results from the predictive discriminative analysis were used. The accuracy was estimated using quadratic discriminant analysis and cross-validation. Cross-validation removes one reference sample from the database, classifies it as an unknown sample and compares the sample against the other reference samples in the data. The sample is returned to the data set and the process repeats until all samples in the data set have been classified. The percent of correct classification of the removed samples is presented as percent (%) hit rate.

Table 6. The pooled within canonical structure (r's)^A describing variables that underlie group differences among catch locations of Louisiana wild caught shrimp

Mineral	Overall		Summer		Winter and Fall	
	Can 1 ^B	Can 2 ^B	Can 1 ^B	Can 2 ^B	Can 1 ^B	Can 2 ^B
Al	0.2732	-0.209	0.2638	0.4771	0.2105	0.1024
P	-0.1099	0.3349	0.0054	-0.2584	-0.0954	0.1385
Fe	0.0866	0.1502	0.1451	-0.2409	0.0936	0.229
Mg	0.1097	-0.3525	0.0867	0.5187	0.102	0.2907
K	0.2569	-0.1799	0.0924	0.3448	0.2494	-0.1032
Na	0.279	-0.0728	0.083	0.1661	0.3906	-0.3533
Cu	0.2946	-0.463	0.0293	0.5547	0.363	<i>0.0592</i>
Zn	0.4124	0.3102	0.3919	-0.0725	0.4079	-0.3823
Ca	0.8592	0.2654	0.8269	-0.1667	0.9009	-0.0939
Cumulative variance explained	85.18%	14.82%	73.68%	26.32%	87.28%	12.72%

^ABased on the pooled within group variances with $P < 0.001$ of Wilks' Lambda from MANOVA. Bolded and italicized values indicate attributes largely contributing to the overall differences among all shrimp samples.

^BCan 1 and Can 2 refer to the pooled within canonical structure in the first and second canonical discriminate functions, respectively

Table 7. The pooled within canonical structure (r's)^A describing variables that underlie group differences among catch locations of Louisiana wild caught shrimp in spring months

Mineral	Spring Can 1 ^B
Al	
P	0.1579
Fe	-0.092
Mg	-0.0036
K	-0.2474
Na	-0.1509
Cu	-0.2743
Zn	-0.1491
Ca	-0.4806
Cumulative variance explained	0.8518

^ABased on the pooled within group variances with $P < 0.001$ of Wilks' Lambda from MANOVA. Bolded and italicized values indicate attributes largely contributing to the overall differences among all shrimp samples.

^BCan 1 and Can 2 refer to the pooled within canonical structure in the first and second canonical discriminate functions, respectively

Based on PDA of nine minerals, catch zones of Louisiana wild caught shrimp could be predicted with an overall accuracy of 86.93%, and specifically, into the correct zones 1, 2, and 4 with 73.68%, 74.85%, and 95.40% accuracy, respectively (Table 8). In other words, when a sample, for example, belonging to Zone 1, was removed from the data set and marked as “unknown” it was correctly classified into the correct location, Zone 1, 73.68% of the time. The quadratic equation produced from this PDA can be used to classify actual unknown samples into their correct location based on their mineral profile.

A reduced model can be used to determine which mineral has the most influence on correct percent classification. By removing one mineral from the model and analyzing remaining minerals using PDA, the mineral with the lowest percent correct or percent hit range classification can be identified as important mineral for determining the percent correct classification. In the reduced model of overall catch locations (Table 8), when sodium was removed from the data set, the overall percent correct classification fell from 80.06% correct to only 67.89% correct. We can conclude that sodium is the most important mineral for determining percent correct classifications into the three zones. This conclusion remains consistent when PDA of catch locations is investigated by seasons. In the reduced models for the summer months and the combined fall and winter months, Na is also the most important mineral for determining percent correct classification into the three zones. Overall percent classification in the summer fell from 87.30% to 77.78% correct classification and from 86.93% to 79.66% correct classification in the combined fall and winter months.

Table 8. % hit rate (correct classification) for catch locations of Louisiana wild caught shrimp

Minerals	% Hit rate			
	Overall	Zone 1	Zone 2	Zone 4
A full model with 9 minerals	86.93	76.00	88.24	89.8
A reduced variable model				
without Al	85.23	72.00	86.27	89.8
without P	88.64	76.00	84.31	95.92
without Fe	84.09	76.00	82.35	91.84
without Mg	84.09	80.00	81.37	91.84
without K	84.66	68.00	87.25	87.76
without Na	79.66	76.00	74.76	91.84
without Cu	87.5	76.00	88.24	91.84
without Zn	82.95	72.00	82.35	89.8
without Ca	88.07	96.00	82.35	95.92

Note: Based on quadratic discriminant function. Hit rate (%) is the correct classification of an unknown product classified into a group (Zone 1, Zone 2, and/or Zone 3).

In both overall and reduced PDA models, Zone 4 consistently contained the highest percent correct classification for catch location, summer months, and the combined fall and winter months (Table 9 and Table 10, respectively). Zone 2 consistently contained the second highest percent classification for full model classifications for overall catch locations, summer months, and the combined fall and winter months (74.85%, 86.89%, and 88.25%, respectively). Zone 1 consistently contained the lowest percent classification for full model classification for overall catch locations, summer months, and the combined fall and winter months (73.68%, 82.93%, 76.00%, respectively).

Table 9. % hit rate (correct classification) for catch locations of Louisiana wild caught shrimp during summer months

Minerals	% Hit rate			
	Overall	Zone 1	Zone 2	Zone 4
A full model with 9 minerals	87.3	82.93	86.89	95.83
A reduced variable model				
without Al	94.44	95.12	91.8	100
without P	88.89	90.24	85.25	95.83
without Fe	85.71	82.93	86.89	87.5
without Mg	81.75	73.17	83.61	91.67
without K	82.54	70.73	86.89	91.67
without Na	77.78	58.54	83.61	95.83
without Cu	86.51	82.93	86.89	91.67
without Zn	91.27	97.56	83.61	100
without Ca	83.33	75.61	85.25	91.67

Note: Based on quadratic discriminant function. Hit rate (%) is the correct classification of an unknown product classified into a group (Zone 1, Zone 2, and/or Zone 3).

Table 10. % hit rate (correct classification) for catch locations of Louisiana wild caught shrimp during winter and fall

Minerals	% Hit rate			
	Overall	Zone 1	Zone 2	Zone 4
A full model with 9 minerals	86.93	76.00	88.24	89.8
A reduced variable model				
without Al	85.23	72.00	86.27	89.80
without P	88.64	76.00	84.31	95.92
without Fe	84.09	76.00	82.35	91.84
without Mg	84.09	80.00	81.37	91.84
without K	84.66	68.00	87.25	87.76
without Na	79.66	76.00	74.76	91.84
without Cu	87.50	76.00	88.24	91.84
without Zn	82.95	72.00	82.35	89.80
without Ca	88.07	96.00	82.35	95.92

Note: Based on quadratic discriminant function. Hit rate (%) is the correct classification of an unknown product classified into a group (Zone 1, Zone 2, and/or Zone 3).

The geographical variation in the mineral contents of Louisiana shrimp is shown in Table 11. Louisiana, Indonesia, and Vietnam all contained the highest levels of Al (21.5, 23.1, 14.7 mg/kg). China, India, and Indonesia contained the lowest concentrations of Al (4.2, 3.5, 9.2 mg/kg). Louisiana shrimp contained significantly higher levels of Fe (13.0 mg/kg) and Thailand contained significantly lower levels of Fe (1.9 mg/kg) than any other country. Mg was highest in Louisiana and Indian shrimp (370.5 and 331 and significantly lower in shrimp from Indonesia (155.4 mg/kg). Cu was the highest in shrimp from Louisiana and India (5.2 and 2.2 mg/kg) and was the lowest in shrimp from China, Indonesia, Thailand and Vietnam (1.7, 2.2, 1.5, and 1.3 mg/kg).

Zinc was also the highest in shrimp from Louisiana and India (13.4 and 13.0) and was the lowest in shrimp from China and Vietnam (9.2 and 8.4 mg/kg). No significant difference was detected in the levels of S. The average sulfur content in Louisiana shrimp was 200.5 mg/kg. Louisiana, China, and India contained the highest levels of P (3811.1, 2503.1, and 2668.6mg/kg), where as the levels of P in shrimp from Indonesia, Thailand, and Vietnam contained the lowest levels (986.5, 1678.3, 113.8mg/kg). In Louisiana shrimp, K (2761.8mg/kg) is significantly higher than any other country. Louisiana shrimp also contained the lowest level of Na than the imported samples (1504.3mg/kg), and shrimp from Thailand contained the highest level of Na (6557.9 mg/kg). Calcium was the highest in shrimp from India and Indonesia, (1233.3 and 979.4) and was the lowest in shrimp from Louisiana, China, Thailand, and Vietnam (614.0, 825.3, 645.1, and 829.2mg/kg) long with feed and environmental differences, The addition sodium bisulfite, sodium metabisulfite, and sodium tripolyphosphate likely contributed to the significant increase of Na in the imported farm raised samples. The

Table 11. Global variation in the mineral contents (mg/kg muscle tissue)^A of Louisiana wild caught shrimp and farm raised imported shrimp

Mineral	Louisiana	China	India	Indonesia	Thailand	Vietnam
Al	21.5±0.6 ^A	4.2±0.7 ^B	3.5±0.1 ^b	23.1±2.5 ^a	9.2±2.7 ^b	14.7±3.5 ^{ab}
S	200.5±13.9 ^a	83.3±1.8 ^a	-	-	169.3±9.0 ^a	239.5±15.2 ^a
P	3811.1±161.7 ^a	2503.1±183.9 ^{ab}	2668.6±477.6 ^{ab}	986.5±22.0 ^b	1678.3±129.5 ^b	1113.8±32.4 ^b
Fe	13.0±0.3 ^a	3.7±0.56 ^b	3.3±0.1 ^b	3.1±0.3 ^b	1.9±0.2 ^c	3.0±0.6 ^b
Mg	370.5±8.6 ^a	200.17±6.0 ^{bc}	331.8±60.4 ^{ab}	155.4±3.7 ^c	179.7±11.4 ^{bc}	179.7±1.6 ^{bc}
K	2761.8±36.4 ^a	916.16± 102.5 ^c	1870.2±441.8 ^b	787.6±74.1 ^c	589.5±84.4 ^c	647.0±110.7 ^c
Na	1504.3±26.3 ^e	3841.2±291.2 ^d	4408.3±800.4 ^{cd}	5706.2±319.6 ^b	6557.9±492.8 ^a	4511.8±73.7 ^c
Cu	5.2±0.1 ^a	1.7±0.2 ^b	4.0±0.1 ^a	2.2±0.3 ^b	1.5±0.1 ^b	1.3±0.2 ^b
Zn	13.4±0.1 ^a	9.2±0.5 ^{bc}	13.0±0.5 ^a	10.1±0.3 ^b	7.5±0.3 ^d	8.4±0.5 ^{cd}
Ca	614.0±23.9 ^b	825.3±52.4 ^b	1233.3±180.7 ^a	979.4±60.5 ^a	645.1±29.0 ^b	829.2±23.2 ^a

^AData are expressed as mean ± standard error

^BDifferent letters for each zone within a row denote significant differences (p<0.05)

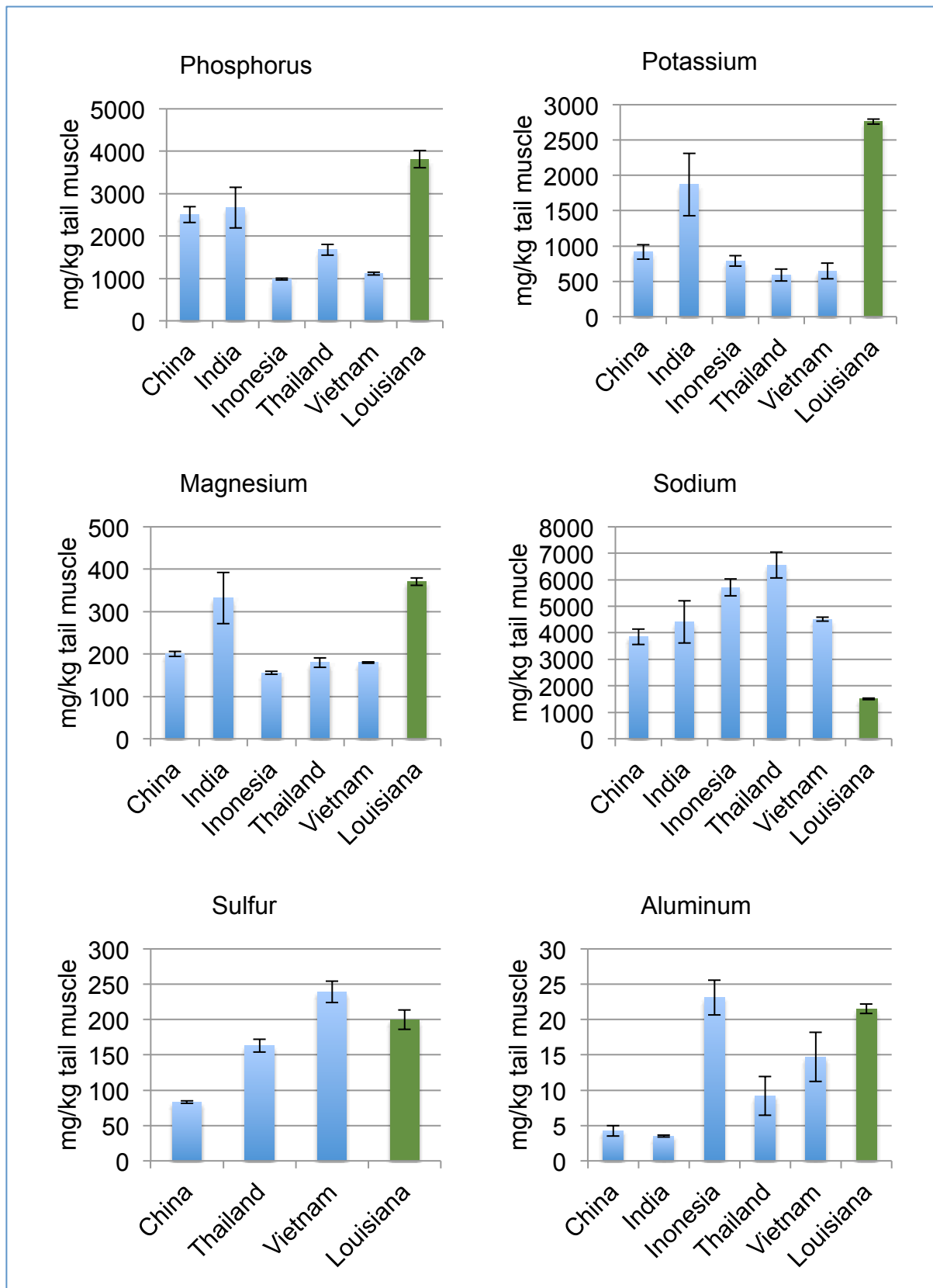


Figure 11. Variation in P, K, Mg, Na, S, and Al in farm raised imports and Louisiana

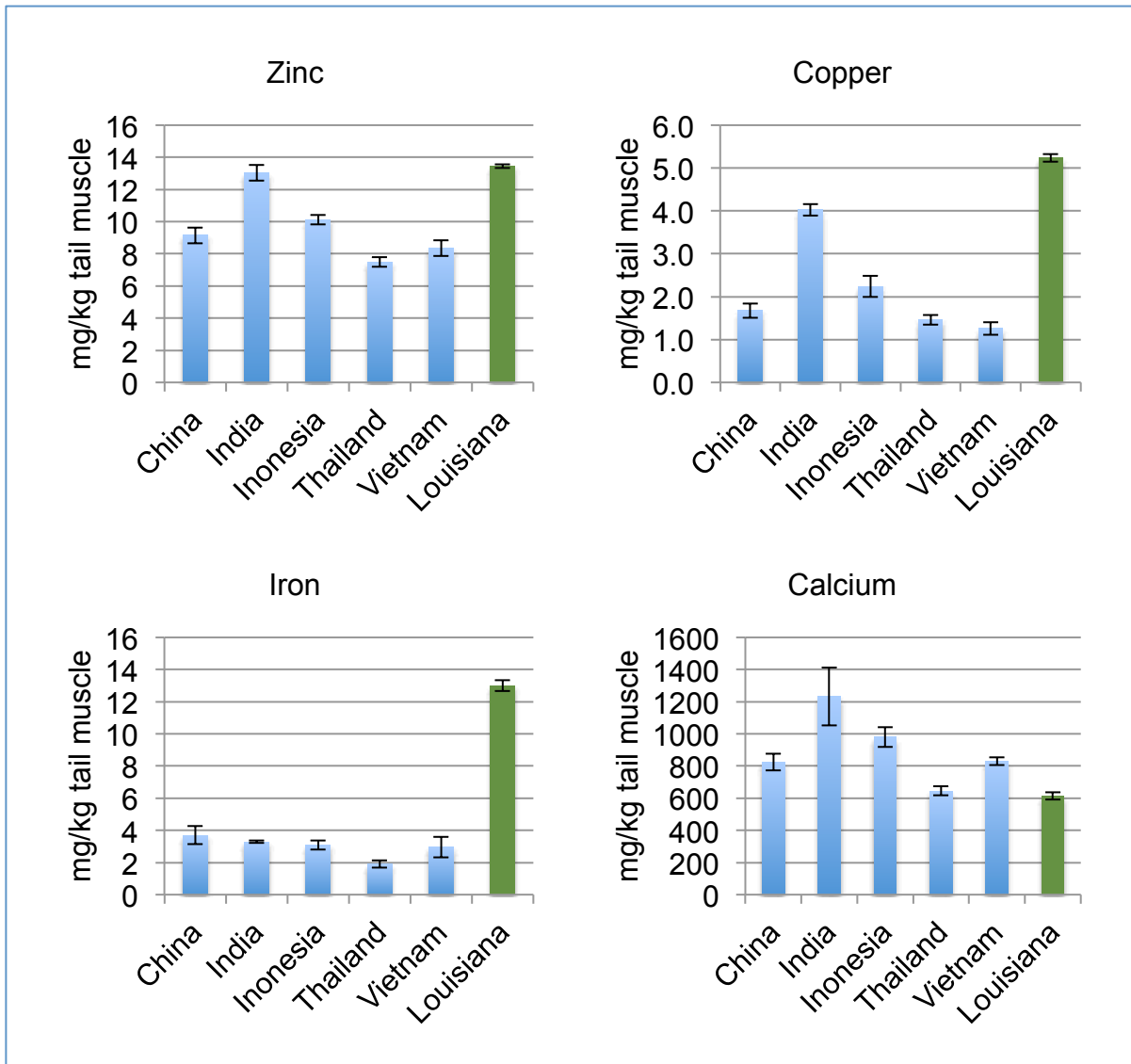


Figure 12. Variation in Zn, Cu, Fe, and Ca in farm raised imports and Louisiana

Louisiana wild caught shrimp were not treated with any chemical preservatives.

However, there was no difference in S levels.

Mineral contents of seafood are influenced by their diet and water quality. These differences are most likely derived from mineral passed from soil in earthen ponds to water or feeding artificial diet or live feed (Yanar 2005).

Based on the large variation in mineral content of farm raised imported shrimp and Louisiana wild caught shrimp, farm raised imported shrimp would not likely be predicted into one of the analyzed catch location of Louisiana shrimp. Therefore, using predictive discriminate analysis we can expect that farm raised imported shrimp could be differentiated from wild caught Louisiana shrimp based on their mineral profile. This can be used to identify and regulate shrimp that have been fraudulently mislabeled as Louisiana wild caught shrimp.

CHAPTER 4. CONCLUSIONS

The mineral contents of Louisiana wild caught shrimp vary along the coastline of Louisiana and seasonally. Using multivariate statistical analysis, Ca and to a much lesser extent Zn, and Cu were determined to contribute the most variance among sample locations overall and seasonally, though additional data from the spring months could further validate this observation. Na is the most important mineral to provide the correct percent classification into the Louisiana catch locations. Unknown classifications or unknown sources of Louisiana wild caught shrimp can be predicted using predictive discriminant analysis. Louisiana wild caught shrimp show significant differences when compared to the farm raised imported shrimp. Imported seafood illegally mislabeled, as Louisiana wild caught shrimp would likely be detected using predictive discriminate analysis form existing database of Louisiana wild caught shrimp. These predictions can be used as a type of regulation test for labeling seafood. Potentially, these results can also be used to develop regional niche marketing strategies for Louisiana wild caught shrimp.

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VITA

Samantha Nichole Stein, a native of Vacherie, Louisiana, received her bachelors' degrees in Food Science and Biological Science from Louisiana State University in 2007. She will receive her master's degree from the School of Nutrition and Food Sciences in May 2014.