Identification of Geographic Origin of Ictalurid Catfish and Penaeid Shrimp by Elemental Profiling

by

Li Li

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Approved by

Claude E. Boyd, Chair, Professor of Fisheries and Allied Aquacultures
David B. Rouse, Professor of Fisheries and Allied Aquacultures
Yolanda J. Brady, Associate Professor of Fisheries and Allied Aquacultures
John W. Odom, Associate Professor of Agronomy and Soils
Shuanglin Dong, Professor of Fisheries College, Ocean University of China

Abstract

Three investigations were performed related to identification of geographic origin of aquatic products by elemental profiling and multivariate statistics.

In the first study, fillets of channel catfish Ictalurus punctatus from three geographic areas, fillets of hybrid catfish (♀ I. punctatus × ♂ blue catfish I. furcatus) from one of the areas, water samples from the culture ponds, and feed samples were subjected to elemental analysis by inductively coupled plasma atomic emission spectrometry (ICP-AES). The fillets were low in concentrations of several elements, and ICP-AES could consistently detect only 11 elements (Al, Ca, Cr, Cu, Fe, K, Mg, Na, P, S, and Zn). Catfish fillets collected from the three geographic areas were different for all the 11 elements ($P<0.05$) consistently detected. In addition, composition of fillets was not greatly influenced by water chemistry or feed composition.

In the second study, elemental analyses (Al, Ca, Cr, Cu, Fe, K, Mg, Mn, Na, P, S, Se, and Zn) were made on fillets of channel catfish from ponds in which fish were fed and of channel and hybrid catfish from fertilized ponds. Hybrid and channel catfish fillets from fish in fertilized ponds did not differ for most of the elements analyzed. However, Ictalurid catfish cultured in feeding ponds and fertilized ponds differed for all elements except aluminum.

In the third study, 20 elements in Pacific white shrimp Litopenaeus vannamei from three geographic areas, water samples from the culture ponds, and feed samples were analyzed by ICP-AES. Three types of shrimp samples were prepared: head on shell on (HOSO), headless shell on (HLSO) and peeled undeveined (PUD). Sixteen elements were different among the three

types of shrimp products ($P<0.05$). Nine elements in HOSO shrimp, 13 elements in HLSO shrimp and 8 elements in PUD shrimp were different among the three geographic areas ($P<0.05$). In addition, elemental composition of shrimp showed some relationship to elemental concentrations in water, but not to elemental concentrations in feed.

Multivariate statistical methods, including principal component analysis (PCA), canonical discriminant analysis (CDA) and k-nearest-neighbor analysis, were reliable in separating Ictalurid catfish and Penaeid shrimp to their geographic origin or to the production method in all of the three investigations. Results suggested that element profiling combined with multivariate statistics has a potential for validating the geographic origin of aquatic products, but much additional research would be needed to refine the methodology.

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I. Introduction

Channel catfish Ictalurus punctatus and Pacific white shrimp Litopenaeus vannamei are important aquaculture species in the United States (US). Importations account for a large percentage of the US market sales of the two species. In 2011, imported catfish accounted for 74% of the catfish sales in the US (http: //www.aces.edu/dept/fisheries/ aquaculture/ catfish-database/catfish-2011.php), and approximately 576,000 tonnes of Pacific white shrimp worth US$ 5.1 billion were imported (http://www.usda.gov/wps/portal/usda/ usdahome).

The US and some other countries often increase the tariff on imported aquaculture products where governmental assistance to producers allow an imported product an unfair advantage over the same product produced domestically. The differential tariff has resulted in trans-shipping, i.e., a product from a high tariff country is shipped to a low tariff country, labeled as originating in the low tariff country, and shipped to the US.

In January 2009, the US Department of Agriculture (USDA) announced the final regulations for the country of origin labeling (COOL) program and method of production (MOP) program for fish and shellfish commodities. Technologies for checking, identifying, and tracing the origin of aquaculture products are necessary to enforce governmental regulations, protect consumer's rights, and ensure fair competition.

The direct incentive for this study was an incident related to possible trans-shipping of shrimp in 2009. A company in Indonesia managed a large, corporative shrimp farm that processed over 100,000 tonne of shrimp in the year 2009. Shrimp grown by the Indonesian company were sent to a trading company in Singapore from which they were shipped to the US under an Indonesian label. The shrimp were seized by the US Customs and Border Protection (CBP), who suspected that the shrimp were trans-shipped from China. CBP claimed the elemental profile of the shrimp was the same as for shrimp from China. There was no strong scientific basis to support this use of elemental profiling in shrimp, and CBP eventually released the shrimp. However, the seizure caused inconvenience to both CBP and the company. Developing a scientifically accepted method for determining the country of origin of shrimp is urgently needed to properly enforce the antidumping requirements of the US.

This investigation was initialed to assess the possibility of separating Ictalurid catfish and Pacific white shrimp of different geographic origins by using elemental profile and multivariate statistics. Water samples were collected from ponds where fish and shrimp were cultured, and the relationship between elemental composition of aquatic organisms and culture environment was investigated. A condition for elemental fingerprinting would seem to be that water chemistry affects the concentrations and relative proportions of elements in fish and shrimp. Thus, this study was designed to include sites with distinctly different water chemistries.

Classification of Ictalurid catfish from feed-based culture ponds versus fertilized ponds also was attempted. Fish from fertilized ponds rely on natural food organisms in the

same manner as wild-caught fish. Comparison of elemental composition of fillets between fed fish and fish from fertilized ponds possibly could be exploited for other species to verify method of production – aquaculture versus wild caught in the future.

II. Literature Review

Background information

Channel catfish Ictalurus punctatus is the predominant aquaculture species in the US. In 2008, catfish represented 81% of the 287,132 tonne of finfish produced in the US (FAO 2011). An estimated 95% of catfish were produced in the southeastern states of Mississippi, Texas, Arkansas, and Alabama (Hanson and Sites 2012). Meanwhile, the catfish importations accounted for 74% of US catfish sales in 2011. The major catfish exporting countries included Vietnam, China, Thailand, Malaysia, and Cambodia (Hanson and Sites 2012).

Global production of Pacific white shrimp Penaeus vannamei was over 2,720,929 tonne in 2010 (FAO 2012). The US is a major shrimp importing country, and approximately 90% of all shrimp consumed in the country are imported, farm-raised shrimp (Smith and Watts 2009). Shrimp were mainly imported from Thailand, Ecuador, India, China, and Vietnam, and frozen head-on, head-off, and peeled shrimp were the major shrimp products imported (FAO 2012).

In 2002, the US Department of Commerce (DOC) initiated an antidumping investigation into Vietnamese pangasius Pangasius bocourti, and in August 2003, the DOC announced its Antidumping Duty Order on Vietnamese pangasius. In order to standardize catfish products to a greater degree, the US Department of Agriculture (USDA) enacted mandatory the country of origin labeling (COOL) program in 2002 (Federal Register 2009). This program is

intended to provide consumers with credible information on which to base their purchasing decisions. On January 12, 2009, USDA announced the final regulations for the COOL program and fish and shellfish were covered commodities. Verifiable technology for determining the country of origin of the aquatic products will be useful to reinforce governmental supervision and protect consumer's right.

The US finilized antidumping tariffs on imported shrimp from certain countries in June 2005, and the antidumping duties varied depending on country with the highest tariff of 113 % on China (FAO 2012). Financial incentive could drive shrimp retailers to trans-ship shrimp from a high-tariff country to a low-tariff country where the shrimp would be fraudulently relabeled to evade antidumping duties.

Using element profile to determine geographical origin of agricultural products

Many studies have focused on the potential of using element profiling to determine the geographical origin of agricultural products. In most of these sudies, metal contents were analyzed by inductively coupled plasma atomic emission spectrometry (ICP –AES) or inductively coupled plasma mass spectrometry (ICP –MS). Statistical pattern recognition methods including principal component analysis (PCA), cluster analysis (CA), discriminant analysis (DA) and neural network modeling, etc. have been used to explore the data and set up classification models for country of origin.

Metal contents have been successfully used to differentiate two varieties of green coffee. Elements P, Mn and Cu were found to have the largest differences between the two varieties (Mart ń et al. 1998). By using trace metal analyses, Anderson et al. (1999) differentiated

between potatoes grown in Idaho and potatoes grown in other regions in North America. Anderson and Smith (2002, 2005) were able to determine the geographic origin of coffee and raw pistachios by using elemental analyses combined with multivariate methods. Tea samples from Africa and Asia were classified using element profiles and multi-dimensional chemomethric techniques (Moreda - Piñeiro et al. 2003). The study demonstrated the possibility to assign unknown tea samples to their country of origin based on metal profiling.

The US Customs and Border Protection (CBP) laboratories had been working on the country of origin of garlic by comparing the trace metal profile of samples to an authentic garlic database, and the trace metal data showed significant discriminatory nature (Smith 2005). Mineral content of 10 garlic cultivars were evaluated in Argentina (Camargo et al. 2010). Four variables (Br, Zn, Rb and Cr) were demonstrated to be enough to classify the cultivars. The usefulness of elemental profiling to assess the geographical origin of wines has been widely demonstrated (Coetzee et al. 2005; Fabani et al. 2010; Dutra et al. 2011).

The strategy of combining elemental analyses and statistical classification techniques to determine the geographical origin of agricultural products was favored by researchers because of its simplicity. Trace elements are more stable in agricultural products versus vitamins or organic compounds, which are susceptible to degradation from the time of harvest through storage to the time of analysis.

Element profiling to determine the grographic origin of agricultural products is facilitated by the fact that mineral composition of fruits and vegetables is influenced by the mineral composition of the soil and environment in which plant grow. However, using element profile to determine the geographic origin of raw meat is more complicated. The

elemental composition of meat is influenced by various factors including animal husbandary conditions, feed, breed, drinking water, element profile of the soil, human activities etc. (Franke et al. 2005). Moreover, animals may be grown in different environments and slaughtered at different stages of life. Nevertheless, a few studies suggest that elemental profiling can be used to determine the geographic origin of meat. Elemental concentrations of poultry and dried beef meat were determined, and the correct classification rates reported by linear discriminant analysis (LDA) were 77 and 79%, respectively (Franke et al. 2008). Sun et al. (2011) demonstrated that the multi-element fingerprint was a usefull indicator for authenticating the geographical origin of mutton in China.

Elemental composition has been widely used as an indicator of pollution of marine and aquatic organisms. Favretto et al. (1989) classified polluted and unpolluted mussels by trace metal analysis. Jung and Zauke (2008) studied the bioaccumulation of trace metals in brown shrimp to evaluate the suitability of brown shrimp as a biomonitor for certain metals. Mendil et al. (2010) compared the metal levels of fish from the polluted and unpolluted area of a local river, and found trace metal levels were high in the polluted area when compared with the unpolluted area.

A few reports also are available about using trace metal profiling to determine the geographical origin of marine and aquatic organisms. Smith and Watts (2009) determined the origin of farm-raised shrimp by trace metal profiling and multiviarate statistics using a database on the composition of shrimp from different countries that included Pacific white shrimp, black tiger shrimp Penaeus monodon, and possibly other species. Anderson et al. (2010) was able to differentiate wild and farm-raised salmon by using elemental profiles and

various modeling approaches. Sea cucumber samples from three water environments of China were seperated with 100% correct classification rate using multi-element analysis and pattern recognition techniques (Liu et al. 2012).

Mineral composition of water, fish, and feed

The essential elements for living things are classified into four categories: bulk structural elements, maroelements, trace elments and ultratrace elements (Pais and Jones 1997). Bulk structural elements are elements needed in large amounts such as cabon (C), hydrogen (H), oxygen (O), phosphorus (P), sulfur (S), and nitrogen (N). Macronutrients which are needed in moderate amounts include: calcium (Ca), chlorine (Cl), potassium (K), sodium (Na), and magnesium (Mg). The trace elements include: copper (Cu), iron (Fe), and zinc (Zn), and the ultratrace elements include: arsenic (As), boron (B), fluorine (F), iodine (I), selenium (Se), cadmium (Cd), chrominum (Cr), cobalt (Co), lead (Pb), manganese (Mn), molybdenum (Mo), nickel (Ni), tin (Sn), and vanadium (V). Seven micronutrients have been identified as essential for plants: B, Cl, Cu, Fe, Mn, Mo, and Zn. Twelve trace elements: As, Cl, Cr, Cu, F, I, Fe, Mn, Mo, Ni, Se, and V are essential for animals including man (Pais and Jones 1997).

Elements are important for the normal growth and development of the living organism. Some minerals serve as essential components of many enzymes, enzyme activators, catalysts, vitamins, hormones, and respiratory pigments, or as cofactors in metabolism, while others are structural constituents of soft tissues or skeltetal structures. Moreover, minerals play a key role in the maintenance of osmotic pressure and acid-base equilibrium of the body (FAO 1987).

Solubility of most metals in water decreases as pH increases. At the pH range of most natural waters, concentrations of free metal ions are usually rather low. However, many kinds of complexes such as iron pairs, chelates, and hydrolysis products are formed in natural waters (Boyd 2000). A good example is iron. Concentrations of ferric ion are very low even at pH 4, but because of complex formation, total iron concentration often reaches 0.25 mg/L in natural waters. Minor constituents of four kinds of water: sea water, ground water, fresh water, inland shrimp pond water are listed in Table 1.

Ground water is water located in the voids within underground gravel, sand, silt, or clay formations, in solution caverns, within limestone, and in spaces within fractured rock. The composition of ground water is variable (Table 1) and it reflects the minerals found in local geologic formations. Ground water often is more mineralized than surface water because of its longer contact time with geologic formations. As water infiltrates through soils and rocks of surface formations, it accumulates carbon dioxide and losses dissolved oxygen. Water from deep wells typically has low dissolved oxygen concentrations. Trace metals in deep waters often exist in reduced form and concentrations are frequently high. The salinity of sea water on average is 35 ppt. Seawater composition is dominated by Na and Cl (Boyd 2000). Seawater is high in B if compared with fresh water, but low in certain minor nutrients like Al and Fe (Table 1).

Table 1. Mineral composition (mg/L) of seawater, ground water, fresh water and low salinity shrimp pond water.

Element	Seawater	Ground water	Reference fresh water	Shrimp pond water
Ag	0.3×10^{-3}	9.0 - 330	0.3×10^{-3}	ND
Al	0.01	0.1-1,200	0.2	0.7
As	3×10^{-3}	0.01-2,100	0.5×10^{-3}	2.5×10^{-3}
B	4.6	ND	0.01	0.7
Ba	0.03	2.8-3.8	0.01	0.15
Cd	0.11×10^{-3}	0.01-180	0.2×10^{-3}	ND
Co	0.5×10^{-3}	0.01-0.18	0.5×10^{-3}	1.4×10^{-3}
Cr	0.5×10^{-4}	0.06-2,740	1.0×10^{-3}	2.1×10^{-3}
Cu	3.0×10^{-3}	0.01-2.8	3.0×10^{-3}	5.9×10^{-3}
Fe	0.01	0.04-6,200	0.5	0.5
Mn	2×10^{-3}	0.1-110	5.0×10^{-3}	0.02
Mo	0.01	ND	1.0×10^{-3}	2.2×10^{-3}
Ni	2×10^{-3}	0.05-0.5	0.3×10^{-3}	4.2×10^{-3}
Pb	0.3×10^{-4}	0.01-5.6	3.0×10^{-3}	2.3×10^{-3}
Se	4×10^{-3}	0.6-20	0.2×10^{-3}	5.4×10^{-3}
Ti	1×10^{-3}	ND	ND	7.2×10^{-3}
Zn	0.01	0.1-240	5.0×10^{-3}	0.02

Source: Pais and Jones 1997; Boyd 2000; Prapaiwong 2011. ND = no data.

Many factors including source water, sediment, feeding, fish activities, climate, fertilizing, and disease control could affect element level in aquaculture pond water. Ground water of 2 to 10 ppt salinity from wells was used to culture Pacific white shrimp in Alabama. Trace metal concentrations were highly variable on different sampling dates and across the inland shrimp ponds (Prapaiwong 2011). The average concentrations of Al, As, Ba, B, Cr, Co, Cu, Pb, Fe, Mn, Mo, Ni, Se, Ti, and Zn from that study are listed in Table 1. Concentrations of most metals − other than As, B, Fe, Mo, and Pb − were generally higher in saline well water than in sea water and fresh water.

Mineral concentrations are extremely variable in fish feeds. There are many reasons for this to include differences in raw ingredients used in diet formation, the addition of specific macro or trace mineral premixes, and contaminants present in conventional and un-conventional feed ingredients (Tacon and De Silva 1983). Mineral compositions of 38 commercial fish feeds available in Europe were compared and large differences were found. Ranges (g/kg) of Ca, P, K , Na, and Mg were: 7.62-22.09; 11.91-15.47; 8.89-11.52; 4.72-13.38; 1.43-2.25, respectively. The ranges (mg/kg) of Fe, Zn, Mn, Cu, Pb, Co, Ni, Cr, and Cd were: 148-247; 80-636; 39-184; 11-54; 0.9-4.9; 2.8-4.6; 1.0-4.2; 1.4-3.0; 0.18-0.34, respectively (Tacon and De Silva 1983). The major minerals, Na, S, Mg, Ca, P, and K were at concentrations from 0.01 to 1.5% of dry weight in a commercial feed for haddock. Concentrations of As, Mn, Zn, Cr, Co, Mo, Si, Cu, Al, Ba, Fe, and Sr were 0.21, 130, 314, 0.34, 2.01, 0.3, 2.04, 5.18, 4.10, 5.5, 450, and 32.2 µg/g, respectively (Roy and Lall 2006).

Wide variations have been reported in mineral concentrations in fish flesh as a result of several factors such as different species, size, age, life history, sex, sexual maturity, food

source, water chemistry, salinity, climate, contaminants, sampling procedures and analytical techniques etc. (Shearer 1994; Lall 1995; Alasalvar et al. 2002; Roy and Lall 2006; Yamashita et al. 2006).

Mineral composition of cultured and wild fish has been reported. Iron, Zn, Mn, Cr, and Ni in cultured sea bass as well as Co, Cr, Pb, and Ni in cultured sea bream were higher than those in their wild counterparts (Yildiz 2008). Wild caught salmon were higher than farm-raised salmon in As, Cu, Zn, Na, Ca, Mg, and P, while there was no difference in K between the wild and farm-raised salmon (Anderson et al. 2010). Significant differences were reported between farmed and wild rainbow trout in Ca and Fe concentrations (Fallah et al. 2011). Cultured sea bass had lower Fe and Al contents and higher Ti and V contents compared with the wild sea bass (Alasalvar et al. 2002). The differences between cultured and wild fish were thought to be related to differences in food sources.

Channel catfish are omnivorous. The main food for channel catfish in nature includes insects, algae, plant seeds, molluscs, micro-crustaceans, coleopterans, trichopterans, fish, fish remains and detritus etc. (Devaraj 1976). Small and large channel catfish have markedly different food habits. Small insects, such as dipteran larvae and pupae are important food items for catfish less than 10-cm long. As the catfish size increases, they gradually become piscivorous and small fish and plant seeds become significant items of diet (Bailey and Harrison 1948; Devaraj 1976). Channel catfish below 10-cm length were found to feed mainly on natural food items, such as dipteran larvae and pupae, rather than on supplement feed even in ponds where supplemental feed was used 6 days per week (Devaraj 1976). Catfish feed more actively during the period from sundown until about midnight and the best

temperature for feeding is between 10 and 34.5 ℃. According to a study in the Des Moines River, Iowa, the fish rarely feed in winter (Bailey and Harrison 1948).

The hybrid catfish is produced by crossing female channel catfish Ictalurus punctatus with the male blue catfish Ictalurus furcatus. Previous studies have demonstrated that the hybrid exhibits faster growth, better feed conversion, and more tolerance of low oxygen, increased resistance to many diseases such as Edwardsiella ictaluri, higher dress out percentages, and increased vulnerability to angling (Argue et al.2003; Brian et al. 2004; Wolters et al. 1996; Dunham et al. 1983, 1998).

Mineral composition of catfish was summarized by Lall (1995). The average concentrations of Ca, P, Mg, Na, and K based on wet weight were 30, 220, 45, 65, and 295 mg/100g, respectively, while concentration of Fe, Zn, Mn, Cu, Cr, and I were 15.0, 4.0, 0.6, 1.0, 0.2, and 1.0 mg/kg, respectively.

Statistical methods

Principal component analysis (PCA) is designed to reduce the number of variables to a small number of principal components which can explain most of the variance in the original variables (Manly 2005). The principal components are linear combinations of the original variables and are independent from each other. The first principal component explains the largest part of the sample variance and the second principal component explains the largest part of the variance that could not be explained by the first principal component, and so on. The number of principal components generated equals to the number of original variables; however, only the first few components providing a high percentage of variances are

considered important (Anderson et al. 1999). Samples can be plotted with respect to the first one or two principal components to show how individual samples differ from one another. PCA is good for data simplification and interpretion, and it summarizes the total variation of the data. It will not, however, highlight the between group differences.

The PRINCOMP procedure in SAS is used to run the PCA (Anderson and Smith 2002). The procedure can do PCA analysis based either on a correlation matrix or a covariance matrix. For measurements with different scales, the correlation matrix is used. SAS will standarize a data set by substracting the variable mean and then dividing the variable standard deviation to obtain an adjusted mean of zero and standard deviation of one for each variable. For the standarized data set, the total variance equals to the number of variables. Eigenvalue of a principal component indicates the variance that it accounts for out of the total variance. Generally, principal components with eigenvalue greater than one are considered important.

Canonical discriminant analysis (CDA), a variable reduction technique, generates canonical variables, which are linear combinations of the original variables. Unlike PCA, CDA highlights between group difference and minimizes within group differences to get the best possible view of group clusters. The default number of canonical variables generated is the minimum of the number of variables and the number of groups minus one (Anderson and Smith 2002, 2005). The first canonical variable reflects the most group difference, the second canonical variable captures as much as possible of the group differences not displayed by the first canonical variable, the third canonical variable displays the most group difference not reflected by the first and second canonical variables and so on. Canonical variables are not correlated with each other (Manly 2005). Each sample could obtain a score on all the

canonical variables generated. A 2-D or 3-D scatter plot can be made based on the first two or three canonical functions to gain a better visualization of the data set.

Discriminant analysis is a separative procedure using data with information about actual group membership to determine a discriminant function that can then be used in the future to predict groups for which group membership is not known. There are two purposes of doing discriminant analysis, the first is to find combinations of variables that best differentiate the groups, and the second is using the combinations to predict membership and evaluate expected error rates. For normally distributed data, linear discriminant analysis (LDA) and quadratic discriminant analysis (QDA) are often used. The LDA procedure works with data having equal within-class distribution, while QDA deals with data having unequal within-class distribution. QDA is very sensitive to the normality assumption, and when the assumption is violated, the kernel method or the k-nearest-neighbor method are often used (SAS Institute Inc. 2008). Cross-validation testing, in which each sample is removed from the dataset and tested against the discriminant function created by the remaining samples, is used to test the validity of the procedure (Anderson et al. 1999).

Literature Cited

Alasalvar, C., K. D. A. Taylor, E. Zubcov, F. Shahidi, and M. Alexis. 2002. Differentiation of cultured and wild sea bass (*Dicentrarchus labrax*): total lipid content, fatty acid and trace mineral composition. Food Chemistry 79:145-150.

Anderson, K. A., B. A. Magnuson, M. L. Tschirgi, and B. Smith. 1999. Determining the geographic origin of potatoes with trace element analysis using statistical and neural network classifiers. Journal of Agricultural and Food Chemistry 47:1568–1574.

Anderson, K. A. and B. W. Smith. 2002. Chemical profiling to differentiate geographic growing origins of coffee. Journal of Agricultural and Food Chemistry 50:2068–2075.

Anderson, K. A. and B. W. Smith. 2005. Use of chemical profiling to differentiate geographic growing origin of raw pistachios. Journal of Agricultural and Food Chemistry 53:410–418.

Anderson, K. A., K. A. Hobbie, and B. W. Smith. 2010. Chemical profiling with modeling differentiates wild and farm-raised salmon. Journal of Agricultural and Food Chemistry 58:11768-11774.

Argue, B. J., Z. J. Liu, and R. A. Dunham. 2003. Dress-out and fillet yields of channel catfish, *Ictalurus punctatus*, blue catfish, *Ictalurus furcatus*, and their F-1, F-2 and backcross hybrids. Aquaculture 228:81-90.

Bailey, R. M. and Jr. H. M. Harrison. 1948. Food habits of the southern channel catfish in the Des Moines River, Iowa. Transactions of the American Fisheries Society 75:110-138.

Boyd, C. E. 2000. Water quality: an introduction. Kluwer Academic Publishers, Boston, Massachusetts, USA.

Brian G. B., R. W. William, L. S. Juan, S. C. Roberto, and P. Sinyoung. 2004. Comparison of production, meat yield, and meat quality traits of NWAC103 line channel catfish, Norris line channel catfish, and female channel catfish × male blue catfish F1 hybrids. North American Journal of Aquaculture 66:177-183.

Camargo, A. B., S. Resnizky, E. J. Marchevsky, and J. M. Luco. 2010. Use of the Argentinean garlic (*Allium sativum* L.) germplasm mineral profile for determining geographic origin. Journal of Food Composition and Analysis 23:586-591.

Coetzee, P. P., F. E. Steffens, R. J. Eiselen, O. P. Augustyn, L. Balcaen, and F. Vanhaecke. 2005. Multi-element analysis of South African wines by ICP-MS and their classification according to geographical origin. Journal of Agricultural and Food Chemistry 53:5060-5066.

Devaraj, K. V. 1976. On the food of channel catfish stocked in farm ponds. Aquaculture 7:27-32.

Dunham, R. A., R. O. Smitherman, and W. Cliff. 1983. Relative tolerance of channel X blue hybrid and channel catfish to low oxygen concentrations. The Progressive Fish-Culturist 45:55-57.

Dunham, R. A. and B. J. Argue. 1998. Seinability of channel catfish, blue catfish, and their F(1), F(2), F(3), and backcross hybrids in earthen ponds. Progressive Fish-Culturist 60:214-220.

Dutra, S., L. Adami, A. Marcon, G. Carnieli, C. Roani, F. Spinelli, S. Leonardelli, C. Ducatti, M. Moreira, and R. Vanderlinde. 2011. Determination of the geographical origin of

Brazilian wines by isotope and mineral analysis. Analytical and Bioanalytical Chemistry 401:1575-1580.

Fabani, M. P., R. C. Arrua, F. Vazquez, M. P. Diaz, M. V. Baroni, and D. A. Wunderlin. 2010. Evaluation of elemental profile coupled to chemometrics to assess the geographical origin of Argentinean wines. Food Chemistry 119:372-379.

Fallah, A. A., S. S. Saei-Dehkordi, and A. Nematollahi. 2011. Comparative assessment of proximate composition, physicochemical parameters, fatty acid profile and mineral content in farmed and wild rainbow trout (*Oncorhynchus mykiss*). International Journal of Food Science and Technology 46:767-773.

Favretto, L., L. G. Favretto, G. P. Marletta, and M. Saitta. 1989. Principal component analysis: a chemometric aid for classification of polluted and unpolluted mussels. Analytica Chimica Acta 220:135-144.

Federal Register. 2009. Mandatory country of origin labeling of beef, pork, lamb, chicken, goat meat, wild and farm-raised fish and shellfish, perishable agricultural commodities, peanuts, pecans, ginseng, and Macadamia nuts; final rule. U.S. Fed. Reg. 74:2658-2707.

Food and Agriculture Organization of the United Nations (FAO). 1987. The nutrition and feeding of farmed fish and shrimp - A training manual 6. The essential nutrients minerals. http://www.fao.org/docrep/field/003/ab470e/AB470E06.htm.

Food and Agriculture Organization of the United Nations (FAO). 2011. National Aquaculture Sector Overview. United States of America. National Aquaculture Sector Overview Fact

Sheets. Text by Olin, P.G. In: FAO Fisheries and Aquaculture Department [online]. Rome. http://www.fao.org/fishery/countrysector/naso_usa/en.

Food and Agriculture Organization of the United Nations (FAO). 2012. Cultured Aquatic Species Information Programme. *Penaeus vannamei*. Cultured Aquatic Species Information Programme. Text by Briggs, M. In: FAO Fisheries and Aquaculture Department [online]. Rome. http://www.fao.org/fishery/culturedspecies/Litopenaeus_vannamei/en.

Franke, B. M., G. Gremaud, R. Hadorn, and M. Kreuzer. 2005. Geographic origin of meat—elements of an analytical approach to its authentication. European Food Research and Technology 221:493-503.

Franke, B. M., S. Koslitz, F. Micaux, U. Piantini, V. Maury, E. Pfammatter, S. Wunderli, G. Gremaud, J.-O. Bosset, R. Hadorn, and M. Kreuzer. 2008. Tracing the geographic origin of poultry meat and dried beef with oxygen and strontium isotope ratios. European Food Research and Technology 226:761-769.

Hanson, T. and D. Sites. 2012. Catfish database from USDA National Agricultural Statistics Service (NASS) Agricultural Statistics Board, U.S. Department of Agriculture.

Jung, K. and G.-P. Zauke. 2008. Bioaccumulation of trace metals in the brown shrimp *Crangon crangon* (Linnaeus, 1758) from the GermanWadden Sea. Aquatic Toxicology 88:243-249.

Lall, S. P. 1995. Macro and Trace Elements in Fish and Shellfish. In Fish and Fishery Products: Composition, Nutritive Properties and Stability (Ruiter, A., ed.), Wallingford: CAB International. pp. 187-214.

Liu, X., C. Xue, Y. Wang, Z. Li, Y. Xue, and J. Xu. 2012. The classification of sea cucumber (Apostichopus japonicus) according to region of origin using multi-element analysis and pattern recognition techniques. Food Control 23:522-527.

Manly, B. F. J. 2005. Multivariate statistical methods: a primer. CRC Press LLC, 2000 N.W. Corporate Blvd., Boca Raton, Florida, USA.

Martín, M. J., F. Pablos, and A. G. González. 1998. Characterization of green coffee varieties according to their metal content. Analytica Chimica Acta 358:177-183.

Mendil, D., Ö. F. Ünal, M. Tüzen, and M. Soylak. 2010. Determination of trace metals in different fish species and sediments from the River Yeşilirmak in Tokat, Turkey. Food and Chemical Toxicology 48:1383-1392.

Moreda-Piñeiro, A., A. Fisher, and S. J. Hill. 2003. The classification of tea according to region of origin using pattern recognition techniques and trace metal data. Journal of Food Composition and Analysis 16:195-211.

Pais, I. and J. B. Jones. Jr. 1997. The handbook of trace elements. CRC Press LLC, 2000 N.W. Corporate Blvd., Boca Raton, Florida, USA.

Prapaiwong, N. 2011. Water Quality in Inland Ponds for Low-Salinity Culture of Pacific White Shrimp *Litopenaeus vannamei*. Ph.D. dissertation. Auburn University, Auburn, AL, USA.

Roy, P. K. and S. P. Lall. 2006. Mineral nutrition of haddock *Melanogrammus aeglefinus*(L.): a comparison of wild and cultured stock. Journal of Fish Biology 68:1460-1472.

SAS Institute Inc. 2008. SAS/STAT 9.2 User's Guide. Cary, NC: SAS Institute Inc.

Shearer, K. D. 1994. Changes in elemental composition of hatchery-reared rainbow-trout, salmo-gairdneri, associated with growth and reproduction. Canadian Journal of Fisheries and Aquatic Sciences 41:1592-1600.

Smith, R. G. 2005. Determination of the country of origin of garlic (*Allium sativum*) using trace metal profiling. Journal of Agricultural and Food Chemistry 53:4041–4045.

Smith, R. G. and C. A. Watts. 2009. Determination of the Country of Origin of Farm-Raised Shrimp (Family Penaeide) Using Trace Metal Profiling and Multivariate Statistics. Journal of Agricultural and Food Chemistry 57:8244–8249.

Sun, S. M., B. L. Guo, Y. M. Wei, and M. T. Fan. 2011. Multi-element analysis for determining the geographical origin of mutton from different regions of China. Food Chemistry 124:1151-1156.

Tacon, A. G. J. and S. S. De Silva. 1983. Mineral composition of some commercial fish feeds available in Europe. Aquaculture 31:11-20.

Wolters W. R., D. J. Wise, and P. H. Klesius. 1996. Survival and antibody response of channel catfish, blue catfish, and channel catfish female × blue catfish male hybrids after exposure to *Edwardsiella ictaluri.* Journal of Aquatic Animal Health 8:249-254.

Yamashita, Y., Y. Omura, and E. Okazaki. 2006. Distinct regional profiles of trace element content in muscle of Japanese eel *Anguilla japonica* from Japan, Taiwan, and China. Fisheries Science 72:1109-1113.

Yildiz, M. 2008. Mineral composition in fillets of sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*): a comparison of cultured and wild fish. Journal of Applied Ichthyology 24:589-594.

III. Identification of Ictalurid Catfish Fillets to Rearing Location Using Elemental Profiling

Abstract

Fillets of channel catfish Ictalurus punctatus from three geographic areas, fillets of hybrid catfish (♀ I. punctatus × ♂ blue catfish I. furcatus) from one of the areas, water samples from the culture ponds, and feed samples were subjected to elemental analysis by inductively coupled plasma atomic emission spectrometry (ICP-AES). Fillets were low in concentrations of several elements, and ICP-AES could consistently detect only 11 elements. Although the composition of fillets was not greatly influenced by water chemistry or feed composition, both canonical discriminant analysis and k -nearest-neighbor analysis were reliable in separating fillets to geographic area of origin and in separating hybrid from channel catfish fillets based on elemental composition. This study suggests that multi-element analysis and pattern recognition techniques have potential for validating the geographic origin of catfish fillets, but much more work is needed to perfect the technique.

Introduction

About 151,552 tonne of channel catfish Ictalurus punctatus and hybrid catfish (♀ channel catfish × ♂ blue catfish I. furcatus) worth 423 million dollars were reared by aquaculture in the United States (US) in 2011 (http://www.aces.edu/dept/fisheries/aquaculture/ catfish-database/catfish-2011.php). An estimated 95% of these fish were produced in the southeastern states of Mississippi, Texas, Arkansas, and Alabama. Although catfish farming is still an important activity in the US, production has declined by about 50% since its peak in 2003 – acreage devoted to catfish culture also declined by about 50% during the same period.

Imports of frozen, boneless catfish fillets to the US from East Asia, mainly Vietnam, China, Thailand, Malaysia and Cambodia, have increased rapidly over the last decade and accounted for 74% of catfish sales in the US in 2011 (http://www.aces.edu/dept/fisheries/aquaculture/catfish-database/catfish-2011.php). American catfish farmers complain that importation of inexpensive catfish has been a major factor causing the decline in US production. The US Department of Commerce (DOC) (www.commerce.gov) has increased the tariff on catfish imported from countries where government programs afford imports an unfair market advantage over domestically-produced catfish. In January 2009, the US Department of Agriculture (USDA) announced the final regulations for country of origin labeling (COOL program) and fish and shellfish are covered commodities (Federal Register 2009). However, there are cases where exporters have intentionally mislabeled the country of origin of aquaculture products – usually to avoid increased tariffs. Technologies for checking,

identifying, and tracing the origin of aquaculture products are necessary to enforce governmental regulations, protect consumer's rights, and ensure fair competition.

Multi-element analysis and pattern recognition techniques have been used widely to determine the geographic origin of agricultural products and especially plant products (Anderson et al. 1999; Anderson and Smith 2002, 2005; Smith 2005). The method assumes that the elemental composition of an agricultural product reflects the composition of provenance soil, at least for certain elements (Anderson and Smith 2005). It is not surprising that elemental patterns or profiles can be geographic area specific, because plants often accumulate both essential and nonessential mineral elements in proportion to concentrations of these elements in the soil (Walsh and Beaton 1973; Barker and Pilbeam 2007). Plants also may accumulate essential elements in excess of physiological need (luxury consumption) when environmental concentrations are high (Devlin 1969). Moreover, in a given country, the major production of a specific crop may be restricted to areas with similar soils.

For elemental profiles of fish and other aquatic animals to be useful in determination of geographic origin, a relationship must exist among environmental and dietary elemental concentrations and tissue concentrations of elements. This relationship – if it exists – often could be obscured in aquaculture, because culture systems may be supplied water from large catchments over which pedologic characteristics vary. The ionic composition of water from such catchments may not reflect that of local soils into which ponds are constructed. Similarly, coastal ponds are supplied with marine water that tends to have a fairly similar elemental composition worldwide (Boyd 2000). Feeds are manufactured from plant and animal meals purchased from the world market, and two countries could be using aquaculture

feeds made of ingredients of identical geographical origin. Nevertheless, Smith and Watts (2009) determined the origin of farm-raised, Penaeid shrimp by elemental profiling and multivariate statistics using a database on the composition of shrimp from different countries that included Litopenaeus vannamei (Pacific white shrimp), Penaeus monodon (black tiger shrimp), and possibly other species. Sea cucumber samples from three water environments in China were separated with 100% correct classification rate using multi-element analysis and pattern recognition techniques (Liu et al. 2012).

The present study further investigates the potential of elemental profiling of aquaculture products by evaluating the ability of multi-element analysis and pattern recognition techniques to distinguish the origin of fillets from Ictalurid catfish reared in areas representing three distinctly different water chemistries.

Materials and Methods

Sample Collection and Analysis

Fifty catfish were collected in December 2010 from 10 ponds at the Delta Research and Extension Center (DREC) located in the Yazoo Basin region (usually referred to as the Delta) at Stoneville, Mississippi. Two diets each containing 32% crude proteins were used to grow the fish. Fish in five ponds received a traditional corn gluten feed containing 5% meat and bone/blood meal. In the other five ponds, fish received an alternative corn gluten feed without meat and bone/blood meal. The experiment was conducted by Menghe Li of DREC, and he collected the fish to be used for elemental analysis, packed them on ice, and shipped them by courier service to Auburn University.

Two processing plants (Harvest Select, Uniontown, Alabama and Farm Fresh, Eutaw, Alabama) collected fish samples from a total of 23 ponds on 14 catfish farms on the Blackland Prairie region (usually referred to as the blackbelt) of west-central Alabama during July and August 2010. The processors were unable to obtain hybrid catfish during this time. The samples of channel catfish were labeled to pond and farm of origin and immediately frozen. When collection was complete, samples were placed in ice chests and transported to Auburn University.

Thirty-two samples of hybrid catfish were collected from various ponds on the E. W. Shell Fisheries Center (SFC) during fall 2010 and 2011. These ponds are situated on the Piedmont Plateau about 8 km north of Auburn, Alabama. Fish had received a 32% crude protein feed made according to the same ingredient formula. In 2010 and 2011, no channel catfish were cultured at the SFC, but eight channel catfish were collected in summer 2011 from a pond at the US Department of Agriculture Fish Diseases and Parasites Laboratory (FDPL) on the Auburn University campus. This pond is about 6 km from those at the SFC and also is built in Piedmont Plateau soils.

The geographical areas from which the fish samples were obtained will be referred to simply as Auburn, blackbelt, and Stoneville.

Fish were eviscerated, and carcasses were filleted, skinned, and trimmed manually using a ceramic knife (www.metrokitchen.com). Fillets were lyophilized to constant weight with a Labconco Lyph Lock Model 6 freeze drier (Labconco Corporation, Kansas City, MO, USA). Dried fillets were ground with the IKA Economical Analytical Mill (Cole-Parmer,

Vernon Hills, IL, USA). The steel blade in the mill was replaced with a carbide-coated blade to avoid metal contamination.

A 2.0-g aliquot of each dried and ground fish sample was weighed into a 125-mL Erlenmeyer flask, and 40 mL of a mixture of concentrated nitric and perchloric acids (7:3) were added to the flask. The flask was covered with a watch glass and held overnight at room temperature. The sample was digested on a hot plate at 190 ℃ in a perchloric acid hood. More acid mixture was added as necessary to keep the flask from going dry. When the digestion was complete (contents were light yellow), 5.0 mL of 1.0 N hydrochloric acid were added to the flask, and contents were stirred, quantitatively transferred to a 50-mL volumetric flask, and made to volume with double glass distilled water. The solution was mixed thoroughly, and filtered through an acid-washed, Whatman No. 42 filter paper into a 50-mL tube and subjected to elemental analysis by inductively coupled plasma atomic emission spectrometry (ICP-AES) (Spectro Ciros^{CCD}, SPECTRO Analytical Instruments, Inc., Mahwah, NJ, USA).

Two water samples (1 L and 100 mL) were collected from each pond from which fish samples were obtained. Water samples from ponds at DREC, SFC, and FDPL were collected on the same day as the fish samples. Water samples from blackbelt ponds were collected during a 2-d period after fish samples and farm and pond information were obtained from the processing plants. The time between fish harvest and collection of water samples ranged from 2- to 4-wk. However, water was not drained from the ponds at harvest, and the samples were collected during the summer when water seldom flows from ponds in response to rainfall. The 1-L samples were placed on ice in an insulated chest and analyzed within 48 h for pH,

conductivity, total alkalinity, total hardness, chloride, and sulfate by procedures recommended by Eaton et al. (2005). The 100-mL samples were preserved with 2.0 mL of 50% nitric acid. Preserved water samples were digested by the EPA Method 200.8 [Revised 5.4] (United States Environmental Protection Agency 1994) and analyzed by ICP-AES.

Feed samples were dried to constant weight at 80 C in a mechanical convection oven. Samples were pulverized with the IKA Economical Analytical Mill, digested the same way as the catfish fillet samples, and analyzed by ICP-AES.

Statistical Analysis

Statistical analyses were performed with SAS [Version 9.1] (SAS Institute, Cary, NC, USA). Standard deviations and relative standard deviations were used to assess the degree of variation among fish from the same pond or area. Student's t-test was used to compare elemental concentrations in fillets of channel and hybrid catfish and in channel catfish fed two diets in ponds at DREC.

Because of unequal variances, elemental composition of fish from different areas was compared by the non-parametric Kruskal-Wallis test instead of ANOVA. The Mann-Whitney U-test was used to compare among groups if significant difference were reported by the Kruskal-Wallis test.

Three methods – principle component analysis (PCA), canonical discriminant analysis(CDA), and k-nearest-neighbor method – previously used in assessing similarities in elemental profiles for determining the geographical origin of agricultural and aquacultural products (Sun et al. 2011; Liu et al. 2012) were applied to the catfish data. PCA, a variable

reduction method, was used to reduce the original, observed variables into a smaller number of artificial variables (principle components) that describe most of the variance in original variables. CDA is a variable reduction technique similar to PCA. However, sample group information is included in CDA, and this method was used to highlight between group differences and minimize the within group differences. Canonical variables that are linear combinations of the original variables were generated through this procedure. The first canonical function describes the largest amount of variation in the discriminant variables. The second canonical function describes the next largest amount of variation in discriminant variables, but it is independent from the first canonical function, etc. The k-nearest-neighbor method (Anderson et al. 1999), for which k = 5 in the present study, was used to establish a prediction model. The cross-validation procedure was used to evaluate the efficiency of the k - nearest-neighbor model.

Results and Discussion

Eleven elements – aluminum (Al), calcium (Ca), Chromium (Cr), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), sodium (Na), phosphorus (P), sulfur (S), and zinc (Zn) – were consistently above detection limits by ICP-AES and suitable for use in the statistical procedures for elemental pattern recognition. Skin, bone, and most of the blood is removed from a catfish fillet, and it consists mainly of protein and a small amount of lipid. A fillet would not be expected to contain as high a concentration of many elements – particularly metals – as would be found in analyses of whole fish, eviscerated fish, or de-headed, eviscerated fish. However, frozen fillets are the main product prepared from Ictalurid catfish,

and it was decided to use fillets rather than whole fish or other products in determining elemental profiles.

Waters from ponds in the three geographic areas were of similar pH, but markedly different with respect to several other variables (Table 1). Total alkalinity, total hardness, sulfate, chromium, and calcium for pond water samples were lowest at Auburn, intermediate in the blackbelt, and highest for Stoneville. Magnesium concentration in pond waters was similar at Auburn and in the blackbelt, but it was much higher for Stoneville. This resulted in a much higher Ca: Mg ratio in waters of ponds in the blackbelt than in waters at the other two areas. Potassium and zinc concentrations were lowest in the ponds at Auburn, but ponds in the blackbelt tended to contain higher concentrations of these two elements than did ponds at Stoneville. Iron concentration tended to be lowest in the blackbelt, intermediate at Auburn, and highest at Stoneville.

Some ponds in the blackbelt are supplied with well water from aquifers containing saline water with particularly elevated concentrations of sodium and chloride (Boyd et al. 2009). Some ponds of the present study obviously received saline well water, because sodium and chloride concentrations exceeded 1,000 mg/L in a few ponds. As a result, average sodium and chloride concentrations and conductivity were greater in waters from blackbelt ponds than in ponds at Stoneville despite Stoneville water samples having higher total alkalinity and total hardness.

Pond waters at Auburn and Stoneville did not contain as much copper as waters in blackbelt ponds. This resulted from copper sulfate applications to commercial, catfish ponds to control blue-green algae responsible for off-flavor (Tucker and Hargreaves 2004). Copper

sulfate normally is not applied to catfish ponds at the research stations from which samples from the other two areas originated – the exception would be experiments on copper as an algicide.

Ponds at both Stoneville and Auburn are located within a small area, and at each location, all ponds were supplied water from a single source – a well at Stoneville and watershed runoff captured in a reservoir at Auburn. Ponds in the blackbelt were spread over a geographic area about 50 km in diameter. Moreover, some ponds were filled only by surface runoff, while others received both surface runoff and water from wells. Thus, it is not surprising that variation – as indicated by standard deviations (Table 1) – tended to be greater for most water quality variables in the blackbelt than in the other two areas. Nevertheless, fish samples were taken from three areas of distinctly different water quality features.

Channel catfish living in the same water and receiving the same diet varied considerably in elemental composition as illustrated in Table 2 with channel catfish from the pond at FDPL and hybrid catfish from one pond at SFC. There also were some differences in elemental composition between channel catfish and hybrid catfish (Table 2) suggesting data for the two types of fish should not be pooled when using elemental profiling to ascertain geographical origin.

Pearson correlation coefficients (r) determined on pooled elemental data from fish and water samples ($n = 59$) were significant ($P < 0.05$) only for Al and S. This suggested that fish had a degree of control over their elemental composition, and the concentration of an element in the water usually did not affect the concentration of most elements in fish.

The two feeds provided fish in ponds at DREC differed in concentrations of Al, Ca, Cu, Mg, Na, P, S, and Zn (Table 3), but fillets from these fish differed only in concentration of sodium (Table 3). The observation that feed elemental composition did not have a large influence on elemental composition of catfish fillets also suggests that fish were able to exert control over concentrations of elements in their tissues. Nevertheless, when concentrations of the 11 elements in fish provided the two diets were compared by the multivariant Hotellings T^2 test, the elemental profiles differed ($T^2 = 63.52$; $P = 0.00021$). This suggests that elemental pattern recognition could be affected by the type of ration provided catfish despite only one element being different in concentration between fish provided the two rations.

Elemental Profiling

Auburn fish were separated into two categories – hybrid catfish and channel catfish – resulting in four groups of fish but only three geographic areas (Table 4). The Kruskal-Wallis test revealed that there were one or more significant differences for each of the elements among the four groups. Thus, all 11 elements were used in the comparisons of elemental profiles. Differences among elemental data were evaluated by various tests and reported in Table 4. However, it would be a daunting task to attempt to identify the origin of fish from such a complex array of data.

Each element in a fillet was standardized by subtracting from it the variable mean and then dividing by the variable standard deviation to obtain an adjusted mean of zero and standard deviation of one for each element (Manly 2005). The first four principal components with Eigenvalues greater than 1.0 accounted for 73.95% of the total variance (Table 5). The

Eigenvalue for a principal component indicates the amount of the variance that it accounts for out of a total variance of 11 in this case. Eigenvalues for the first two principal components were 2.95 and 2.40, which accounts for 26.79% and 21.80% of the total variance, respectively. Eigenvectors for each element for the first four principal components are shown (Table 5**)**. The first component was mainly correlated with Al and Ca, and Mg, P, and S had the highest weight in the second principal component. Each individual fish sample obtained a score on all the principal components generated from PCA. A 2-dimensional, scatter plot was generated using the score for the first two principal components (Fig. 1). The four groups of fish did not separate well based on the first two principal components that explained only 48.6% of the total variance.

The default number of canonical variables generated is the minimum of the number of variables and the number of groups minus one. In this case, three canonical discriminant functions were derived from the CDA. The three functions accounted for 61.15%, 25.14%, and 13.17% of the total variance, respectively. Each individual fish sample obtained a score on the three discriminant functions from CDA. The extracted canonical variable scores were used to plot a 3-dimensional scatter plot (Fig. 2). Auburn channel catfish were entirely separated from Auburn hybrid catfish. The procedure correctly separated most fillets by geographic area (water chemistries actually), but a few blackbelt channel catfish intermixed with Auburn hybrid catfish and with Stoneville channel catfish,

According to the k-nearest-neighbor method, hybrid channel catfish from Auburn and channel catfish from Stoneville could be classified to area with 100% accuracy (Table 6). Two channel catfish from the blackbelt were mixed into the space occupied by the Auburn

hybrid catfish group and one was mixed into the Stoneville group. The overall correct classification rate was 97.35%. In the cross-validation procedure, each observation was removed from the data set and tested against the discriminant function created by the remaining samples. According to the cross-validation procedure, four fish from the blackbelt group were misclassified into the Auburn hybrid catfish group – an error rate of 17.39%. One fish from the Stoneville group was misclassified into the Auburn hybrid catfish group – an error rate of 2%. The other two groups could be 100% correctly classified. The overall correct classification rate was 95.58%.

Conclusions

Despite lack of strong relationships between concentrations of elements in water and in catfish fillets, it was possible to identify the geographic origin of most catfish fillets from profiles of 11 elements that were consistently above the detection limits for ICP-AES. A more sensitive analytical technique such as inductively coupled plasma mass spectrophotometry (ICP-MS) might allow concentrations of more elements to be detected and increase the ability to separate fish according to geographic origin by elemental profiles. In this study, the CDA and k-nearest-neighbor method appeared superior to PCA for use in determining geographic origin of catfish from elemental profiles.

Although elemental profiling appears to be useful for identifying fish as to geographic areas of different water quality, it should be noted that country boundaries are geopolitical not geochemical. Catfish could possibly be cultured in different countries, but in waters of similar elemental composition. Moreover, the variation in water quality will tend to increase

with the size of the geographic area of origin. Therefore, a very large database should be used in any attempt to develop an elemental profile for a fish species in a given country. Elemental profiling has potential for determining the geographic origin of catfish and possibly other aquaculture species, but much more work will be needed to perfect the technique before it is used for checking country of origin compliance of imported aquaculture products.

Literature Cited

Anderson, K. A., B. A. Magnuson, M. L. Tschirgi, and B. Smith. 1999. Determining the geographic origin of potatoes with trace element analysis using statistical and neural network classifiers. Journal of Agricultural and Food Chemistry 47:1,568–1,574.

Anderson, K. A. and B. W. Smith. 2002. Chemical profiling to differentiate geographic growing origins of coffee. Journal of Agricultural and Food Chemistry 50:2,068–2,075.

Anderson, K. A. and B. W. Smith. 2005. Use of chemical profiling to differentiate geographic growing origin of raw pistachios. Journal of Agricultural and Food Chemistry 53:410–418.

Barker, A. V. and D. J. Pilbeam. 2007. Handbook of Plant Nutrition. CRC Press, Boca Raton, Florida, USA.

Boyd, C. A., P. L. Chaney, C. E. Boyd, and D. B. Rouse. 2009. Distribution of ground water suitable for use in saline-water aquaculture in central and west-central Alabama. Journal of Applied Aquaculture 21:228-240.

Boyd, C. E. 2000. Water Quality, An Introduction. Kluwer Academic Publishers, Boston, Massachusetts, USA.

Devlin, R. M. 1969. Plant Physiology, 2nd edition. Van Nostrand Reinhold, New York, New York, USA.

Eaton, A. D., L. S. Clesceri, E. W. Rice, and A. E. Greenberg. 2005. Standard Methods for the Examination of Water and Wastewater, 21st edition. American Public Health Association, Washington, DC, USA.

Federal Register. 2009. Mandatory country of origin labeling of beef, pork, lamb, chicken, goat meat, wild and farm-raised fish and shellfish, perishable agricultural commodities, peanuts, pecans, ginseng, and Macadamia nuts; final rule. U.S. Fed. Reg. 74:2658-2707.

Liu, X., C. Xue, Y. Wang, Z. Li, Y. Xue, and J. Xu. 2012. The classification of sea cucumber (Apostichopus japonicus) according to region of origin using multi-element analysis and pattern recognition techniques. Food Control 23:522-527.

Manly, B. F. J. 2005. Multivariate Statistical Methods: A Primer. CRC Press, Boca Raton, Florida, USA.

Smith, R. G. 2005. Determination of the country of origin of garlic (Allium sativum) using trace metal profiling. Journal of Agricultural and Food Chemistry 53:4,041–4,045.

Smith, R. G. and C. A. Watts. 2009. Determination of the country of origin of farm-raised shrimp (Family Penaeide) using trace metal profiling and multivariate statistics. Journal of Agricultural and Food Chemistry 57:8,244–8,249.

Sun, S., B. Guo, Y. Wei, and M. Fan. 2011. Multi-element analysis for determining the geographical origin of mutton from different regions of China. Food Chemistry 124: 1,151-1,156.

Tucker, C. S. and J. A. Hargreaves. 2004. Pond water quality. Pages 215-278 in C. S. Tucker and J. A. Hargreaves, editors. Biology and Culture of Channel Catfish. Elsevier Publishing, Amsterdam, The Netherlands.

United States Environmental Protection Agency. 1994. Determination of trace elements in waters and wastes by inductively coupled plasma - mass spectrometry, EPA Method 200.8 (Revision 5.4).

Walsh, L. M. and J. D. Beaton. 1973. Soil Testing and Plant Analysis. Soil Science Society of America, Madison, Wisconsin, USA.

Table 1. Average concentrations and standard deviations for water quality variables in ponds of the three, geographic (water chemistry) areas from which channel catfish samples were collected.

Variable	Auburn (n = 26)	Blackbelt (n = 23)	Stoneville (n = 10)
pH	7.70 ± 0.58	7.65 ± 0.66	7.30 ± 0.17
Conductivity (µmhos/cm)	151 ± 29	1,204 ± 1,783	803 ± 7 9
Total alkalinity (mg/L as $CaCO_3$)	59.5 ± 16.9	114.4 ± 43.8	253.8 ± 21.2
Total hardness (mg/L as $CaCO_3$)	57.2 ± 5.3	146.0 ± 85.1	278.0 ± 12.3
Chloride (mg/L)	11.1 ± 6.3	421 ± 758	131.0 ± 36.8
Sulfate (mg/L)	7.6 ± 5.3	19.5 ± 11.5	75.8 ± 14.5
Aluminum (µg/L)	2.3 ± 1.5	1.1 ± 1.0	5.3 ± 1.8
Calcium (mg/L)	9.4 ± 3.2	48.8 ± 31.4	63.8 ± 8.6
Chromium (µg/L)	1.6 ± 1.3	2.1 ± 2.2	5.0 ± 1.7
Copper (µg/L)	8.1 ± 6.8	29.4 ± 40.6	8.2 ± 2.2
Iron (mg/L)	3.2 ± 1.9	1.4 ± 1.1	5.9 ± 2.1
Potassium (mg/L)	8.8 ± 2.6	25.7 ± 8.8	13.2 ± 2.3
Magnesium (mg/L)	5.7 ± 1.7	5.8 ± 4.1	28.5 ± 2.6
Sodium (mg/L)	10.0 ± 2.2	269.9 ± 505.7	97.9 ± 17.7
Total phosphorus (mg/L)	0.34 ± 0.13	0.57 ± 0.23	0.51 ± 0.1
Zinc (µg/L)	10.7 ± 5.7	75.8 ± 51.8	28.08 ± 8.30

Table 2. Mean elemental concentrations, standard deviations (SD), and relative standard deviations (RSD) for fillets of channel catfish from one pond and hybrid catfish from another pond at Auburn, Alabama.

Element	Channel catfish		Hybrid catfish	
(mg/kg)	Mean ± SD	RSD	Mean ± SD	RSD
Aluminum	3.44 ± 2.69	78.1	3.93 ± 3.53	90.0
Calcium	1,279 ± 580	45.4	1,409 ± 1,396	99.0
Chromium	0.28 ± 0.05	20.0	0.34 ± 0.25	71.8
Copper	0.72 ± 0.08 a	11.7	0.43 ± 0.08	18.9
Iron	10.60 ± 2.41 a	22.7	5.02 ± 1.48	29.5
Potassium	6,121 ± 1,142	18.6	6,175 ± 1,137	18.4
Magnesium	1,368 ± 51	3.7	1,329 ± 119	8.9
Sodium	1,557 ± 141	9.0	1,895 ± 553	29.1
Phosphorus	10,462 ± 501 a	4.7	8,423 ± 832	9.8
Sulfur	8,230 ± 726	8.8	7,971 ± 762	9.5
Zinc	15.88 ± 2.37 a	14.9	12.67 ± 2.10	16.5

Table 3. Mean elemental concentrations and standard deviations for two diets and for fillets from channel catfish that received the diets. There were five ponds per diet, and five fish from each pond.

Element	Feed		Fish	
(mg/kg)	Alternative diet	Traditional diet	Alternative diet	Traditional diet
Aluminum	462.18 ±9.56*	585.53 ±9.73	2.94 ±2.58	1.86 ±1.19
Calcium	4,329 ±94*	7,675 ±111	346.7 ±45.7	333.7 ±48.3
Chromium	1.83 ±0.23	1.88 ±0.16	0.56 ±0.29	0.49 ±0.17
Copper	11.05 ±0.09*	11.98 ±0.05	0.63 ±0.17	0.64 ±0.21
Iron	499.12 ±1.23	527.61 ±14.06	5.05 ±1.24	5.27 ±1.64
Potassium	9,322 ±3,811	11,000 ±1,473	8,634 ±2,482	8,806 ±2,441
Magnesium	3,227 ±44*	3,030 ±17	1,156 ±97	1,122 ±108
Sodium	1,063 ±17*	632 ±15	1,164 ±101*	1,034 ±113
Phosphorus	9,919 ±80*	10,544 ±196	9,910 ±818	9,730 ±773
Sulfur	3,926 ±26*	3,448 ±53	9,088 ±750	9,335 ±913
Zinc	64.07 ±1.76*	74.89 ±0.49	19.49 ±2.64	19.26 ±2.36

*Significantly different using the Student's t-test ($P < 0.05$).

Table 4. Means and standard deviations of elemental concentrations in fillets from catfish collected from three geographic (water chemistry) areas.

Element (mg/kg)	Hybrid catfish	Channel catfish			
	Auburn	Auburn	Blackbelt	Stoneville	P Value*
Aluminum	3.73 ± 2.74[(c)]	3.44 ± 2.69	7.14 ± 7.23[(f)]	2.40 ± 2.06	0.013
Calcium	870 ± 833[(a,b,c)]	1,279 ± 580[(e)]	2,852 ± 2,571[(f)]	426 ± 610	<0.0001
Chromium	0.45 ± 0.19[(a,b)]	0.28 ± 0.06[(d,e)]	0.57 ± 0.11	0.53 ± 0.24	<0.0001
Copper	0.64 ± 0.21[(b)]	0.72 ± 0.08[(d,e)]	1.04 ± 0.47[(f)]	0.64 ± 0.19	<0.0001
Iron	8.30 ± 3.04[(a,c)]	10.60 ± 2.41[(d,e)]	8.04 ± 4.08[(f)]	5.16 ± 1.44	<0.0001
Potassium	6,517 ± 1,123[(c)]	6,121 ± 1,142[(e)]	6,253 ± 737[(f)]	8,720 ± 2,438	<0.0001
Magnesium	1,093 ± 168[(a,c)]	1,368 ± 51[(d,e)]	1,090 ± 136[(f)]	1,139 ± 103	<0.0001
Sodium	1,590 ± 379[(c)]	1,557 ± 141[(e)]	1,527 ± 314[(f)]	1,099 ± 125	<0.0001
Phosphate	8,431 ± 728[(a,b,c)]	1,0462 ± 501[(d,e)]	9,088 ± 1,221[(f)]	9,820 ± 793	<0.0001
Sulfur	8,227 ± 882[(c)]	8,230 ± 726[(e)]	8,263 ± 954[(f)]	9,211 ± 836	<0.0001
Zinc	17.68 ± 3.79[(b)]	15.88 ± 2.37[(d,e)]	22.85 ± 3.89[(f)]	19.37 ± 2.48	<0.0001

*P values for Kruskal-Wallis test.

[a]Significantly different between the Auburn hybrid catfish and Auburn channel catfish using Mann-Whitney U-test ($P < 0.05$).

[b]Significantly different between the Auburn hybrid catfish and blackbelt channel catfish using Mann-Whitney U-test ($P < 0.05$).

[c]Significantly different between the Auburn hybrid catfish and Stoneville channel catfish using Mann-Whitney U-test ($P < 0.05$).

[d]Significantly different between the Auburn channel catfish and blackbelt channel catfish using Mann-Whitney U-test ($P < 0.05$).

[e]Significantly different between the Auburn channel catfish and Stoneville channel catfish using Mann-Whitney U-test ($P < 0.05$).

[f]Significantly different between the blackbelt channel catfish and Stoneville channel catfish using Mann-Whitney U-test ($P < 0.05$).

Table 5. Eigenvectors from the first, four principal component axes for 11 elements used to classify four groups of catfish fillets – three geographic areas.

Element	Principal Components			
	1	2	3	4
Aluminum	0.48	0.06	-0.02	-0.40
Calcium	0.50	0.04	-0.01	-0.40
Chromium	0.07	0.12	0.42	-0.25
Copper	0.28	-0.04	0.32	0.49
Iron	0.33	-0.07	-0.03	0.49
Potassium	-0.24	0.31	0.25	-0.13
Magnesium	0.04	0.45	-0.49	0.11
Sodium	0.38	-0.05	-0.39	0.16
Phosphate	0.11	0.59	-0.08	-0.04
Sulfur	-0.14	0.53	0.06	0.21
Zinc	0.30	0.21	0.51	0.16
Eigenvalue	2.95	2.40	1.55	1.24
% Variation	26.79	21.80	14.09	11.26
% Cumulative variation	26.79	48.60	62.69	73.95

Table 6. Classification of catfish fillet samples in four groups (three geographic areas) and percentages of observations correctly classified.

		AH[a]	AC[a]	SC[a]	BC[a]	Total
Original count	AH[a]	32	0	0	0	32
	AC[a]	0	8	0	0	8
	SC[a]	0	0	50	0	50
	BC[a]	2	0	1	20	23
% correctly classified		100	100	100	86.96	97.35[b]
Cross-validated count	AH[a]	32	0	0	0	32
	AC[a]	0	8	0	0	8
	SC[a]	1	0	49	0	50
	BC[a]	4	0	0	19	23
% correctly classified		100	100	98	82.61	95.58[c]

[a]AH, Auburn hybrid catfish; AC, Auburn channel catfish; SC, Stoneville channel catfish; BC, blackbelt channel catfish.

[b]97.35% of empirical grouped observations correctly classified.

[c]95.58% of cross-validated grouped observations correctly classified.

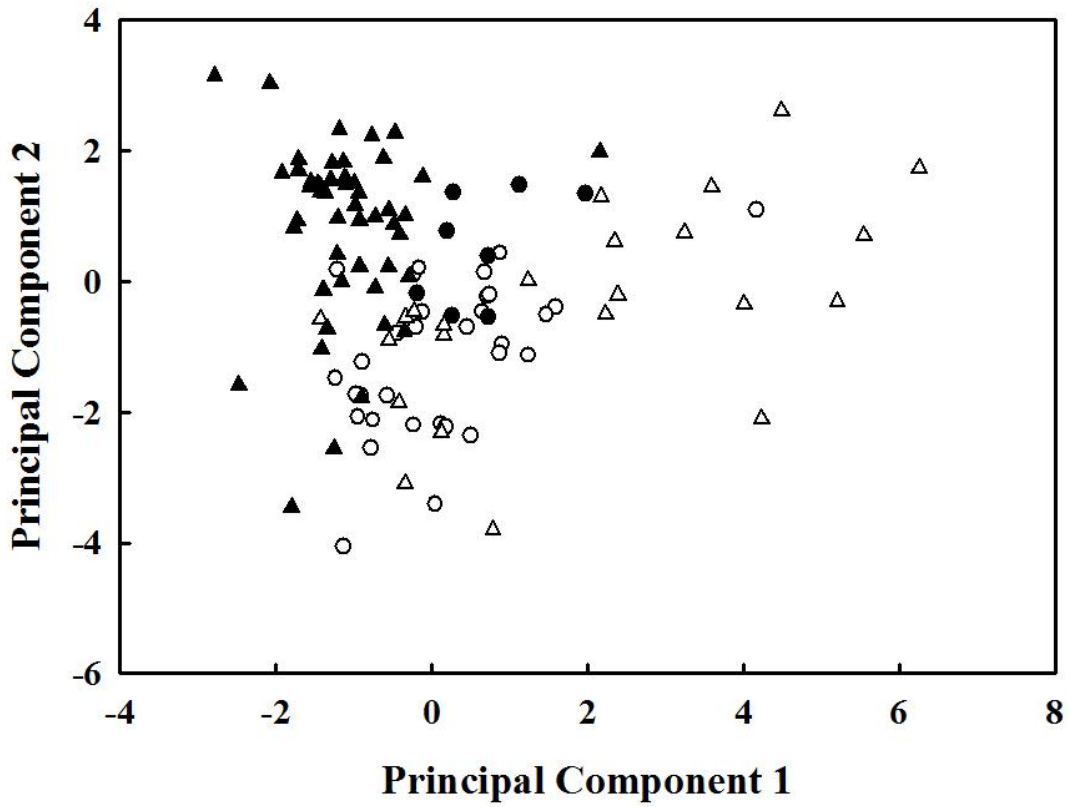

Figure1. A 2-dimensional scatter plot of individual scores for catfish fillets from fish of three geographic (water chemistry) areas based on the two, first principal components: circle = Auburn hybrid catfish; dot = Auburn channel catfish; open triangle = blackbelt channel catfish; solid triangle = Stoneville channel catfish.

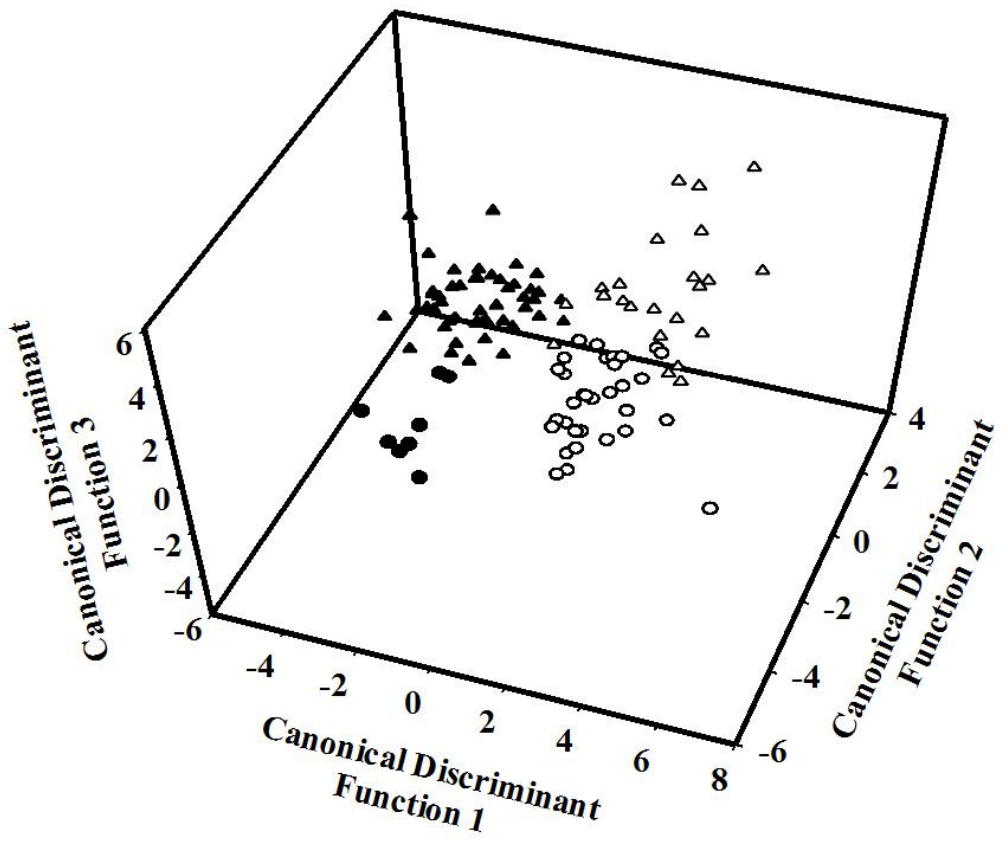

Figure 2. A 3-dimensional scatter plot of individual scores for fillets from catfish from three geographic (water chemistry) areas based on canonical, discriminant functions: circle = Auburn hybrid catfish; dot = Auburn channel catfish; open triangle = blackbelt channel catfish; solid triangle = Stoneville channel catfish.

IV. Elemental Composition of Ictalurid Catfish Produced in Fertilized Ponds and in Ponds with Feeding

Abstract

The objective of this study was to demonstrate the feasibility of combining chemical profiling with multivariate statistical methods to differentiate Ictulurid catfish produced by two culture methods - feeding and fertilization. Elemental analyses (Al, Ca, Cr, Cu, Fe, K, Mg, Mn, Na, P, S, Se, and Zn) of fillets of channel catfish Ictalurus punctatus and hybrid catfish ((♀ I. punctatus × ♂ blue catfish I. furcatus) from fertilized ponds and of hybrid catfish that received feed was performed using an inductively coupled plasma atomic emission spectrometry (ICP-AES). Fillets of samples of hybrid and channel catfish fish in fertilized ponds did not differ for most of the elements analyzed. Multivariate statistical methods including principal component analysis (PCA), canonical discriminant analysis (CDA) and k-nearest-neighbor analysis were used to separate Ictalurid catfish to different culture methods. All the three methods demonstrated that fillets from fish that received feed could be differentiated from fillets of fish cultured in fertilized ponds. Fish from fertilized ponds rely on natural food organisms is the same manner as wild- caught fish. The findings of this study demonstrated the potential of chemical profiling combined with multivariate statistical methods for verifying method of production – aquaculture versus wild caught.

Introduction

The United States and some other countries often increase the tariff on imported aquaculture products where governmental assistance to producers allows an imported product an unfair advantage over the same product produced domestically. The United States also requires both country of origin and method of production labeling of fisheries products – COOL and MOP programs, respectively (Federal Register 2009). Such labeling allows consumers – should they desire – to selectively source domestic products, to avoid products from certain countries, and to select aquaculture products in preference to wild-caught ones or *vice versa*.

Some foreign producers attempt to avoid compliance with COOL and MOP programs by falsely labeling species or by trans-shipping a high-tariff product via a low-tariff country and falsely labeling the country of origin. Multi-element analysis and pattern recognition techniques widely used for determining the geographic origin of traditional agricultural products (Anderson et al., 1999; Anderson and Smith, 2002, 2005; Smith, 2005; Sun et al., 2011) also are being considered for aquaculture products (Smith and Watts, 2009; Liu et al., 2012).

An aquaculture species may be produced in a system where its food is entirely from natural productivity or in one where its food is almost completely manufactured. However, during processing and shipping, these products may become intermingled. Differences in elemental composition between animals reared on natural food organisms and those provided feed – if they exist – could make elemental profiling less reliable for assessing geographic origin. But, if there are differences in elemental composition resulting from natural food versus feed, this fact possibly could be exploited to verify whether or not a product originated from wild-caught or aqua-cultured animals (Anderson et al. 2010).

This small study was conducted to ascertain if differences in elemental composition could be detected between fillets of Ictalurid catfish produced in either fertilized ponds or in ponds with feeding.

Materials and Methods

Sample Collection and Analysis

Ponds used in this study are located on the E. W. Shell Fisheries Center (SFC), Auburn, Alabama. The embankment-type ponds were square with water surface areas of 200 m^2 and average depths of about 0.75 m. They were supplied with water from a storage reservoir filled by runoff from a wooded watershed. This water is of low total alkalinity and total hardness (10 to 15 mg/L) and low in nutrient concentrations (Boyd, 1990).

In March, 2010, ten ponds were stocked with 25 channel catfish Ictalurus punctatus, 25 hybrid catfish ♀I. punctatus *x* ♂I. furcatus that averaged 12.5 cm in total length. Three grass carp Ctenopharyngodon idella of 20 to 25 cm in total length were stocked as an aquatic macrophyte control measure. Ponds were fertilized with 20-20-5 fertilizer (20% N, 20% P_2O_5, 5% K_2O) three times at 2-week intervals and then on a monthly basis from March to October to promote phytoplankton growth during 2010 and 2011. In late October 2011, ponds were drained and fish harvested. One or two each of channel and hybrid catfish were collected from each of nine ponds for chemical analyses – all fish were accidentally lost from one pond at harvest.

Fifteen ponds of a water quality study conducted at the SFC were the source of hybrid catfish produced with feed. The rectangular ponds were 400 m^2 with average depths of about 1 m; they were supplied water from the same source as the fertilized ponds. In March, 2011, hybrid catfish with an average individual weight of 15.2 g were stocked at 600 per pond. A

floating, pelleted feed guaranteed by the manufacturer to contain at least 32% crude protein was offered to fish on a satiation basis 6 days per week. When fish were harvested in October 2012, one hybrid catfish was collected from each pond for chemical analysis.

Length and weight of fish collected for chemical analysis were measured. Fish were eviscerated and carcasses were filleted, skinned and trimmed manually using a ceramic knife (www.metrokitchen.com). Fillets were dried to constant weight with a Labonco Lyph Lock Model 6 Freeze drier (Labconco Corporation, Kansas City, MO, USA). Dried fillets were pulverized with an IKA Economical Analytical Mill (Cole-Parmer, Vernon Hills, IL, USA). The steel blade of the mill was replaced with a carbide-coated one to avoid metal contamination.

A 2.00 g dried, pulverized sample was weighed into a 125-mL Erlenmeyer flask. A 40-mL aliquot of a solution consisting of concentrated nitric and perchloric acid (7:3) was added to the flask, and the flask was covered with a watch glass and held overnight in a conventional fume hood. The sample was digested on a hot plate at 190^{o}C in a perchloric acid hood. More of the acidic digestion solution was added as necessary to keep the flask from going dry. Digestion was considered complete when contents were light yellow, and 5.0 mL of 1.0 N hydrochloric acid were added. The contents were mixed, transferred quantitatively to a 50-mL volumetric flask, and made to volume with double glass-distilled water. The digestate was stirred thoroughly and filtered through Whatman No. 42 acid-washed, filter paper into a 50-mL tube and analyzed by inductively coupled plasma atomic emission spectrometry [CP-AES] (Spectro Ciros^{CCD}, SPECTRO Analytical Instruments, Inc., Mahwah, NJ, USA).

Feed samples were collected and dried to constant weight at 80^{o}C in a mechanical convection oven. Samples were pulverized with the IKA Economical Analytical Mill (Cole-

Parmer, Vernon Hills, IL, USA) and analyzed in triplicate following the methodology described above for catfish fillets.

A 1-L water samples was collected from each pond and analyzed for pH, conductivity, total alkalinity, chloride and sulfate (Eaton et al. 2005). A 250-ml water sample from each pond was preserved with 5 ml 1:1 nitric acid for metal analysis. The preserved samples were digested by the EPA Method 200.8 [Revised 5.4] (United States Environmental Protection Agency, 1994) and analyzed by ICP-AES.

Statistical Analysis

Data are presented as means ± standard deviations. Relative standard deviations were used to allow easy comparison of the degree of variability encountered in the various comparisons. Statistical analysis of the data was performed with SAS [Version 9.1] (SAS Institute, Cary, North Carolina, USA). Student's t-test was used to compare the element difference between channel catfish and hybrid catfish reared in the fertilized pond, the element differences between fish cultured in ponds with feeding versus fertilized ponds, the difference in water quality variables between ponds receiving feed as opposed to fertilizers. Significant difference was reported at $P \leq 0.05$.

The multivariate statistics used in this study included principal component analysis (PCA), canonical discriminant analysis (CDA) and k-nearest-neighbor analysis (Sun et al. 2011; Liu et al. 2012; Anderson et al. 1999). PCA is good for dimension reduction and data exploration. The procedure generates principal components (PCs) that are linear combinations of the original variables. The first principal component (PC) explains the highest percentage of the total variation and the second PC accounts for the second largest part of the variation remaining

unexplained by the first PC, and etc. Data can be plotted using the first two or three PCs and visual group clustering is expected. PCA measures variation in the elemental concentrations in the samples but it does not take into account of the sample group information. CDA is a variable reduction technique like PCA. The sample group information is used in the procedure. CDA minimized the within group difference and highlighted the difference between fish from the two groups – feeding ponds and fertilized ponds. The k-nearest-neighbor analysis (k =5) was used to determine a discriminant function, which could be used to classify unknown samples into predetermined classes in the future. A cross-validation procedure was used to test the validity of the k-nearest-neighbor analysis.

Results and Discussion

Chemical Analysis

Thirteen elements – aluminum (Al), calcium (Ca), chromium (Cr), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), phosphorus (P), sulfur (S), selenium (Se) and zinc (Zn) – were consistently above detection limits by ICP-AES and suitable for use in the statistical procedures for elemental pattern recognition.

There were only two differences in elemental composition between channel catfish and hybrid catfish (Table 1) – potassium was higher in hybrid catfish, and phosphorus was higher in channel catfish. Moreover, variances between channel and hybrid catfish differed for only three elements – aluminum and potassium concentrations were more variable in hybrid catfish, while the opposite was true for chromium. Aluminum concentration was highly variable with relative standard deviations of 93.8% and 140.1% in channel and hybrid catfish, respectively. Relative standard deviations for other variables were between 5.1 and 42.0%, and most were between 10

and 30%. Thus, there was a considerable degree of variation in elemental composition among the fillets of the twelve fish of each type.

Because elemental concentrations in fillets of hybrid and channel catfish from fertilized ponds did not differ greatly, data were pooled for comparison with hybrid fish which has been fed (Table 2). Fillets of catfish from fertilized ponds had higher concentrations of all but one element (Al). Aluminum concentration did not differ between the two rearing methods.

The higher concentrations of elements in fillets of fish from the fertilized ponds apparently were not related to water chemistry. The pond from which the fed catfish were collected had been heavily limed in spring 2010, received large daily inputs of feed, five had been treated weekly with copper sulfate, and five more had bags of slow-release, coated copper sulfate suspended in them. These materials all resulted in inputs of elements to the water: calcium and magnesium (hardness) and bicarbonate (alkalinity) from agricultural limestone; all measured elements from feed (Table 3); copper and sulfate from copper sulfate. The ponds that received feed had equal or greater concentrations of all elements – including phosphorus and potassium that were components of the fertilizer – than found in waters of the fertilized ponds (Table 4). Fillets of fish from fertilized ponds had higher concentrations of several elements than did fillets of fish from ponds with feeding in spite of the waters of the fertilized ponds having lower concentrations of several elements.

Enough of several fillet samples were available to allow them to be sent to the fish nutrition laboratory in the Department of Fisheries and Allied Aquacultures at Auburn University for proximate analysis (Table 5). Fish from the fertilized ponds were much smaller than those from the ponds with feeding – despite having been cultured for two growing seasons instead of one. In addition, fillets of fish from the ponds with feeding were much higher in fat content and

lower in crude protein and ash content than were fillets of fish from the fertilized ponds. The high fat content of fillets from fed fish apparently diluted elemental concentrations. The average fat content of fillets of fed fish accounted for 14.46 g/100 g more of the oven dry weight than it did in fillets of fish from fertilized ponds. Suppose a 100-g fillet contained 100 mg of element X, and that the fat content of this fillet was reduced from 19.83% to 5.37% (Table 5). After fat removal, the fillet would weigh 85.54 g but still contain 100 mg of element X. The concentration of element X would rise from 1,000 mg/L to 1,169 mg/L – an increase of 16.9%. The observed increases between elemental concentrations of fillets from fish in fertilized ponds and those in fed fish ranged from 13 to 120% with an average of 45.5%. Dilution by fat apparently was not the only reason for higher elemental concentrations in fillets from fish reared in fertilized ponds. Previous studies have demonstrated that mineral concentration in fish flesh was influenced by factors such as species, size, age, life history, sexual maturity, food source, and water chemistry (Shearer 1994; Lall 1995; Alasalvar et al. 2002; Yamashita et al. 2006).

Elemental Profiling

PCA was conducted based on 12 elements (Ca, Cr, Cu, Fe, K, Mg, Mn, Na, P, S, Se, and Zn) that were different ($P<0.05$) between fed fish and fish from fertilized ponds. To adjust for different scales of measurement between elements, data were standardized by subtracting the variable mean and then dividing the variable standard deviation to obtain an adjusted mean of 0.0 and standard deviation of 1.0 for each element (Manly 2005). The total variation of the standardized data equaled the number of variables —12 in this case. Eigenvalue for a principal component (PC) indicates the percentage of the variability that this component accounts for out of the total variation. The first three principal components (PCs) with eigenvalues greater than 1

explained 72.54% of the total variation (Table 6). Eigenvalue of the first PC was 6.46 and the first PC explained more than half percentage (53.82%) of the total variation. Eigenvector of an element for a particular PC represents the weight the element has on that PC. All elements had almost the same weight on the first PC and the eigenvectors ranged from 0.19-0.34. Copper had the strongest weight on the second PC, and the second PC explained 10.01% of the total variability. The third PC was closely positive related to Ca, and negatively related to P and S. A 2-dimensional, scatter plot was generated using the score for the first two PCs (Fig. 1). The two groups were clearly plotted in different areas based on the first PC with only two samples from the fertilized ponds intermixed with the samples of fed fish. The second PC didn't contribute appreciably to the separation.

The default number of canonical variables generated is the minimum of the number of variables and the number of groups minus one. Only one canonical discriminant variable was generated by CDA and this variable explained 100% of the total variation. Each individual fish sample obtained a score on the discriminant variable from the CDA (Fig. 2). The extracted canonical variable scores were used to plot a frequency histogram (Fig. 2). Each bar on the graph represented the number of samples that had a canonical variable score ranged of ± 0.5 of the value on the abscissa. For example, 12 fish from the fertilized ponds had the canonical variable score of 1.5 to 2.5. Canonical variable scores for fish from the ponds with feeding were all less than 0, while those for fish from the fertilized ponds were greater than 0. Fish from the two groups were clearly separated.

The k-nearest-neighbor analysis was used to set up a model to classify the samples and report expected error rates. Fed fish and samples of fish from fertilized ponds were 100% correctly classified based on 12 elements (Ca, Cr, Cu, Fe, K, Mg, Mn, Na, P, S, Se, and Zn). The

cross-validation test, in which each sample was removed from the dataset and tested against the discrimiant function created by the remaining samples, was used to test the variability of the k-nearest- neighbor analysis. According to cross-validation, the classification of fillets to culture method – feeding or fertilization – was 100% correct.

Conclusions

Fillets of Ictalurid catfish from fertilized ponds differed in elemental composition from those coming from ponds where fish were provided with manufactured feed. In the case of Ictalurid catfish, feeding is such a common practice that there is little possibility of fillets of fish from fertilized ponds becoming intermingled with those of fish from fed ponds. This is not true for some other species, and the culture method (feeding versus fertilization) could affect the resulting of elemental profiling for determining geographic area of origin.

Hybrid and channel catfish fillets from fish produced in fertilized ponds did not differ for most of the elements analyzed. This suggests that there probably would not be differences in hybrid and channel catfish from ponds with feeding, and it probably would not be necessary to have separate databases on elemental composition of hybrid and channel catfish for use in ascertaining geographical origin by elemental profiling.

Fish from fertilized ponds rely on natural food organisms is the same manner as wild-caught fish. The large difference in elemental composition of fillets between fed fish and fish from fertilized ponds could probably be exploited for other species to verify method of production – aquaculture versus wild caught.

Literature Cited

Alasalvar, C., K. D. A. Taylor, E. Zubcov, F. Shahidi, and M. Alexis. 2002. Differentiation of cultured and wild sea bass (*Dicentrarchus labrax*): total lipid content, fatty acid and trace mineral composition. Food Chemistry 79:145-150.

Anderson, K. A., B. A. Magnuson, M. L. Tschirgi, and B. Smith. 1999. Determining the geographic origin of potatoes with trace element analysis using statistical and neural network classifiers. Journal of Agricultural and Food Chemistry 47:1568-1574.

Anderson, K. A. and B. W. Smith. 2002. Chemical profiling to differentiate geographic growing origins of coffee. Journal of Agricultural and Food Chemistry 50:2068-2075.

Anderson, K. A. and B. W. Smith. 2005. Use of chemical profiling to differentiate geographic growing origin of raw pistachios. Journal of Agricultural and Food Chemistry 53: 410-418.

Anderson, K. A., K. A. Hobbie, and B. W. Smith. 2010. Chemical profiling with modeling differentiates wild and farm-raised salmon. Journal of Agricultural and Food Chemistry 58:11768-11774.

Boyd, C. E. 1990. Water quality in ponds for aquaculture. Alabama Agricultural Experiment Station, Auburn University, Alabama.

Eaton, A. D., L. S. Clesceri, E. W. Rice, and A. E. Greenberg. 2005. Standard methods for the examination of water and wastewater, 21st edition. American Public Health Association, Washington, D.C., USA.

Federal Register. 2009. Mandatory country of origin labeling of beef, pork, lamb, chicken, goat meat, wild and farm-raised fish and shellfish, perishable agricultural commodities, peanuts, pecans, ginseng, and Macadamia nuts; final rule. U.S. Fed. Reg. 74:2658-2707.

Lall, S. P. 1995. Macro and Trace Elements in Fish and Shellfish. In Fish and Fishery Products: Composition, Nutritive Properties and Stability (Ruiter, A., ed.), Wallingford: CAB International. pp.187-214.

Liu, X., C. Xue, Y. Wang, Z. Li, Y. Xue, and J. Xu. 2012. The classification of sea cucumber (*Apostichopus japonicus*) according to region of origin using multi-element analysis and pattern recognition techniques. Food Control 23:522-527.

Shearer, K.D. 1994. Changes in elemental composition of hatchery-reared rainbow-trout, salmo-gairdneri, associated with growth and reproduction. Canadian Journal of Fisheries and Aquatic Sciences 41:1592-1600.

Smith, R. G. 2005. Determination of the country of origin of garlic (*Allium sativum*) using trace metal profiling. Journal of Agricultural and Food Chemistry 53:4,041-4,045.

Smith, R. G. and C. A.Watts. 2009. Determination of the Country of Origin of Farm-Raised Shrimp (Family Penaeide) Using Trace Metal Profiling and Multivariate Statistics. Journal of Agricultural and Food Chemistry 57:8244-8249.

Sun, S.M., B. L. Guo, Y. M. Wei, and M. T. Fan. 2011. Multi-element analysis for determining the geographical origin of mutton from different regions of China. Food Chemistry 124:1151-1156.

United States Environmental Protection Agency. 1994. Determination of trace elements in waters and wastes by inductively coupled plasma – mass spectrometry, EPA Method 200.8 (Revision 5.4).

Yamashita, Y., Y. Omura, and E. Okazaki. 2006. Distinct regional profiles of trace element content in muscle of Japanese eel *Anguilla japonica* from Japan, Taiwan, and China. Fisheries Science 72:1109-1113.

Table 1. Mean elemental concentrations and standard deviations for fillets from channel catfish and hybrid catfish cultured in fertilized ponds.

Element (mg/kg)	Channel Catfish (n=12)	Hybrid Catfish (n=12)	t	F
Al	4.04 ±3.79	8.82 ±12.35	-1.28	10.62^{+}
Ca	605.8 ±254.1	722.4 ±252	-1.13	1.02
Cr	0.77 ±0.33	0.97 ±0.16	-1.89	9.18^{+}
Cu	0.79 ±0.23	0.68 ±0.13	1.46	2.07
Fe	12.81 ±3.25	14.07 ±5.09	-0.72	2.45
K	6,354 ±803	8,136 ±1,642	-3.38*	4.19^{+}
Mg	1,308 ±95	1,340 ±68	-0.93	2.01
Mn	0.45 ±0.13	0.49 ±0.16	-0.67	1.51
Na	1,524 ±208	1,571 ±166	-0.61	1.57
P	11,289 ±627	10,671 ±787	2.13*	1.57
S	10,754 ±682	10,508 ±752	0.84	1.21
Se	1.39 ±0.42	1.33 ±0.56	0.29	1.78
Zn	22.62 ±3.78	23.37 ±3.28	-0.52	1.33

Note: * Means different according to Student's t test (t) at $P \leq 0.05$.

+ Variances not homogenous according to F test at $P \leq 0.05$.

Table 2. Mean elemental concentrations and standard deviations for fillets from hybrid catfish reared in ponds with feeding and in fertilized ponds.

Element (mg/kg)	Feeding (n=15)	Fertilization (n=24)	t	F
Al	2.9 ±2.2	6.43 ±9.26	1.78	18.05^{+}
Ca	495.7 ±236.8	664.11 ±254.60	2.06*	1.16
Cr	0.52 ±0.19	0.87 ±0.27	4.42*	2.02
Cu	0.62 ±0.14	0.74 ±0.19	2.11*	1.84
Fe	9.03 ±2.85	13.44 ±4.22	3.56*	2.19
K	5,966 ±476	7,245 ±1,558	3.75*	10.70^{+}
Mg	1,009 ±86	1,324 ±83	11.45*	1.07
Mn	0.34 ±0.07	0.47 ±0.14	3.98*	4.00
Na	1,352 ±150	1,547 ±186	3.43*	1.52
P	8,270 ±631	10,980 ±764	11.49*	1.46
S	8,299 ±946	10,631 ±713	8.76*	1.76
Se	0.61 ±0.31	1.36 ±0.48	5.29*	2.40
Zn	20.35 ±2.09	22.99 ±3.48	2.66*	2.77

Note: * Means different according to Student's t test (t) at $P \leq 0.05$.

+ Variances not homogenous according to F test at $P \leq 0.05$.

Table 3. Mean elemental concentrations and standard deviations of feed.

Element (mg/kg)	Concentration (n=3)	Element (ppm)	Concentration (n=3)
Al	75 ±0.6	Mn	74.2 ±4.6
Ca	11,281 ±151	Na	783 ±30
Cr	1.2 ±0.6	P	10,932 ±986
Cu	15.7 ±0.2	S	3,046 ±89
Fe	213 ±3.1	Se	0.9 ±0.3
K	7,592 ±1,246	Zn	157 ±2.8
Mg	3,026 ±58		

Table 4. Mean elemental concentrations and standard deviations of water quality variables in ponds with feeding and in fertilized ponds.

Variable	Feeding (n=15)	Fertilization (n=9)	t
pH	8.10 ±0.39	9.04 ±1.20	-2.39*
Conductivity (µmhos/cm)	153 ±36	93 ±34	4.48*
Total alkalinity (mg L^{-1} as $CaCO_3$)	66.8 ±16.1	39.0 ±23.1	3.55*
Total Hardness (mg L^{-1} as $CaCO_3$)	53.5 ±15.6	39.5 ±21.8	1.87
Chloride (mg L^{-1})	6.20 ±0.43	5.35 ±1.32	1.95
Al (mg L^{-1})	2.45 ±1.95	0.87 ±0.81	2.79*
Ca (mg L^{-1})	10.90 ±3.21	9.80 ±5.89	0.54
Cr(µg L^{-1})	1.43 ±1.55	0.51 ±0.54	2.12*
Cu(µg L^{-1})	10.34 ±7.94	1.28 ±3.00	4.01*
Fe (mg L^{-1})	3.67 ±2.33	1.54 ±1.21	2.66*
K (mg L^{-1})	9.57 ±2.49	2.80 ±1.99	7.18*
Mg (mg L^{-1})	6.30 ±1.92	3.59 ±2.21	3.25*
Mn (mg L^{-1})	0.14 ±0.06	0.06 ±0.07	3.20*
Na (mg L^{-1})	10.54 ±1.22	6.73 ±1.59	6.79*
P (mg L^{-1})	0.36 ±0.14	0.19 ±0.17	2.84*
S(mg L^{-1} as SO_4)	6.85 ±2.57	7.69 ±3.59	-0.68
Se (µg L^{-1})	10.34 ±6.11	12.86 ±11.36	-0.64
Zn (µg L^{-1})	10.34 ±7.32	2.70 ±2.47	3.74*

Note: * Means different according to Student's t test (t) at $P \leq 0.05$.

Table 5. Means and standard deviations for weight, length, and concentrations of crude protein, fat and ash in fillets of Ictalurid catfish from ponds with feeding and fertilized ponds.

Variable	Feeding (n=5)	Fertilization (n=5)	t
Weight (g)	654.0 ±86.2	248.8 ±77.2	7.83*
Length (cm)	39.8 ±2.2	31.0 ±2.5	5.93*
Protein %	64.9 ±2.87	82.9 ±2.81	-10.06*
Fat %	19.83 ±2.47	5.37 ±0.73	9.87*
Ash%	6.37 ±0.82	7.79 ±0.57	-3.19*

Note: * means different according to Student's t test (t) at $P \leq 0.05$.

Table 6. Eigenvectors from the first, three principal component axes for 12 elements used to classify two groups of Ictaluid catfish fillets – fish from ponds with feeding and fertilized ponds.

Elements	Principal Components		
	1	2	3
Ca	0.23	-0.36	0.56
Cr	0.30	0.09	0.10
Cu	0.20	0.68	-0.25
Fe	0.32	0.25	0.23
K	0.19	-0.11	0.12
Mg	0.34	-0.26	-0.25
Na	0.32	0.22	0.10
P	0.32	-0.20	-0.44
S	0.32	-0.20	-0.39
Zn	0.30	0.28	0.25
Mn	0.29	0.10	0.22
Se	0.27	-0.22	-0.11
Eigenvalue	6.46	1.20	1.05
% Variation	53.82	10.01	8.71
Cumulative% Variation	53.82	63.83	72.54

Table 7. Classification of fillets from Ictalurid catfish into two groups (ponds with feeding and fertilized ponds) and percentages of observations correctly classified.

			Feeding	Fertilization	Total
Original	Count	Feeding	15	0	15
		Fertilization	0	24	24
	% correctly classified		100	100	100[a]
Cross-Validated	Count	Feeding	15	0	15
		Fertilization	0	24	24
	% correctly classified		100	100	100[b]

Note: [a]100% of empirical grouped observations correctly classified.

[b]100% of cross-validated grouped observations correctly classified.

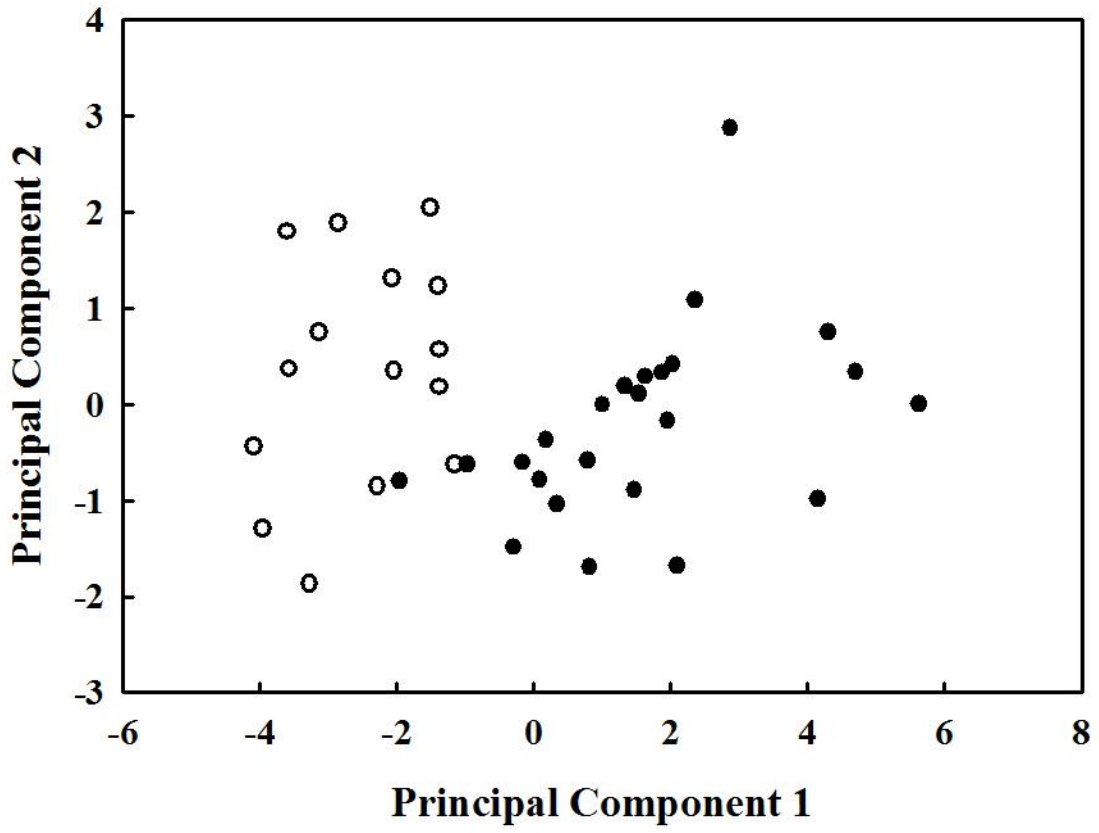

Figure 1. A 2-dimensional scatter plot of individual scores for fillets from Ictalurid catfish cultured in fertilized pond and in ponds with feeding based on the two, first principal components: Circle = ponds with feeding; Dot = fertilized ponds.

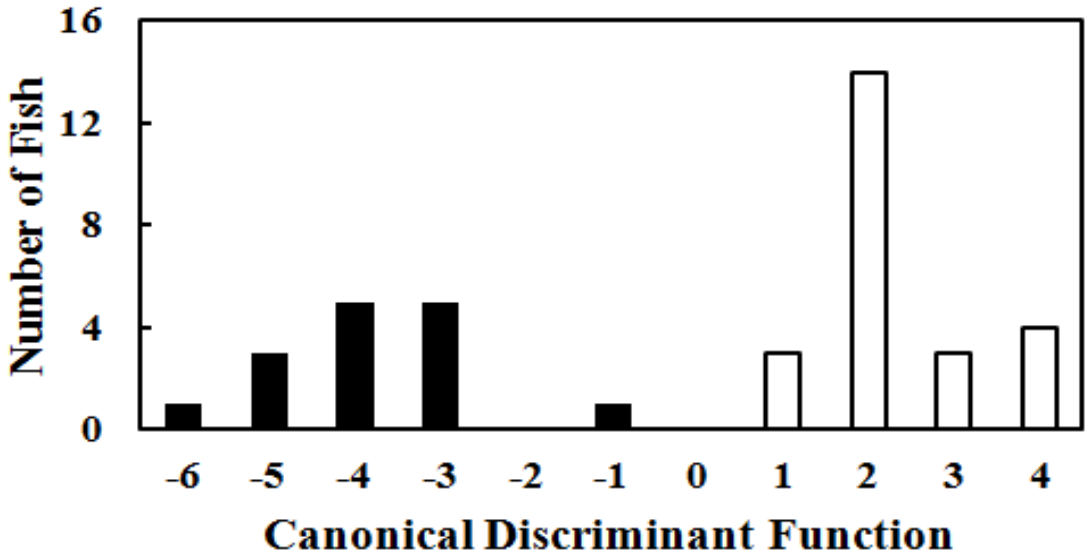

Figure 2. Canonical discriminant analysis (CDA) frequency histogram using canonical discriminant variable representing of fillets from Ictalurid catfish reared in ponds with feeding (black bars) or in fertilized ponds (white bars).

V. Identification of Pacific White Shrimp (Litopenaeus vannamei) to Rearing Location Using Elemental Profiling

Abstract

Twenty elements in Pacific white shrimp Litopenaeus vannamei from three geographic areas, water samples from culture ponds, and feed samples were analyzed by inductively coupled plasma atomic emission spectrometry (ICP-AES). Three types of shrimp samples prepared were: head on shell on (HOSO), headless shell on (HLSO) and peeled undeveined (PUD). Sixteen elements were different among the three shrimp products ($P<0.05$). In addition, elemental composition of shrimp showed some relationship to elemental concentration in water, but not to elemental composition of feed. Multivariate statistical analysis including principal component analysis (PCA), canonical discriminant analysis (CDA) and k-nearest-neighbor analysis were used to separate shrimp products to geographic origins. Shrimp samples from three geographic areas could be differentiated by PCA and CDA. The k-nearest-neighbor analysis gave an overall correct classification rate of 100.0% and cross-validation rate of 100.0%. The study demonstrated the potential usefulness of elemental profiling combined with multivariate statistics in validating the geographical origin of shrimp.

Introduction

The United States (US) is a major shrimp importing country that imported approximately 576,000 tonne worth USD 5.1 billion in 2011 (USDA 2012). Frozen head on shell on (HOSO), headless shell on (HLSO) and peeled undeveined (PUD) shrimp are the major products imported by the US (FAO 2012). The US Department of Commerce (DOC) started implementing antidumping duties on cultured shrimp imported into the country in 2004 (Smith and Watts 2009). The antidumping tariffs vary among countries (FAO 2012), and differential tariffs have resulted in trans-shipping, i.e., a product from a high tariff country is shipped to a low tariff country, labeled as originating in the low tariff country, and shipped to the US. Techniques for determining the true country of origin of shrimp is required to help the US Customs and Border Protection (CBP) properly enforce antidumping duties on shrimp.

Shrimp is a good protein source of low fat content. That is the most popular seafood product in the US. Production of farm-raised Pacific white shrimp Litopenaeus vannamei increased steadily to over 2,720,929 tonnes in 2010 (FAO 2012). It is by far the most popular shrimp species. As the shrimp consumption continues to increase in the US, food safety concerns about shrimp have increased substantially. A label of geographical origin of the shrimp is required in the US to inform consumers. Technologies for checking, identifying and tracing the origin of shrimp also are necessary for enforcing this regulation.

Trace metal profiling has been widely used in agriculture to determine the geographic origin of agricultural products. Anderson et al. (1999) differentiated potatoes grown in Idaho from the other potato-growing regions in North America through trace metal analysis. Anderson and Smith (2002; 2005) were able to determine the geographic growing origin of coffee and raw pistachios by using elemental analysis combined with multivariate statistical methods. The CBP

laboratories have been working on the country of origin of garlic by comparing the trace metal profile of sample to an authentic garlic database (Smith 2005). Sun et al. (2011) demonstrated that the multi-element fingerprint was a useful indicator for authenticating the geographical origin of mutton in China. However, only a few reports are available about using element profile to determine the geographical origin of aquatic food materials (Smith and Watts 2009; Liu et al. 2012).

In the present study, the possibility of separating shrimp will be assessed from three regions in the US by use of elemental profiles and multivariate statistics.

Materials and Methods

Sample Collection and Analysis

Pacific white shrimp were obtained from eight ponds at the Claude Peteet Mariculture Center, Alabama Marine Resources Division, Gulf Shores, Alabama (site C); five ponds at the Harlingen Shrimp Farm, Bayview, Texas (site H); and five ponds at the Greene Prairie Aquafarm located about 6 km north of Forkland, Alabama, on Alabama Highway 43 (site G).

The shrimp were put on ice and transported to Auburn University for analysis. For each pond, three types of shrimp samples were prepared – HOSO, HLSO and PUD. Samples were washed with distilled water and the lengths and weights of shrimp were recorded. Shrimp were dried to a constant weight at 80℃ in a mechanical convection oven. Dried shrimp were ground with the IKA Economical Analytical Mill (Cole-Parmer, Vernon Hills, Illinois, USA). The steel blade of the mill was replaced with a carbide-coated one to avoid metal contamination. Four to six shrimp of each of the three products from each pond were mixed homogenously to make one sample of each shrimp product. Ten HOSO shrimp samples from one pond at site G were

collected to allow an estimate of the variation in elemental composition among shrimp in a pond.

A 2.0-g aliquot of previously dried and ground shrimp sample was weighed into a 125-mL Erelenmeyer flask, and 40 mL of a mixture of concentrated nitric and perchloric acid (7:3) were added. The flask was covered with a watch glass and kept overnight at room temperature in a conventional fume hood before being digested on a hot plate at 190 ℃ in a perchloric acid hood. More acid mixture was added as need to keep the flask from going dry. Digestion was considered complete when the contents were light yellow. The residue was dissolved in 5.0 ml of 1.0 N hydrochloric acid, transferred to a 50-mL volumetric flask, and made to volume. The contents were mixed thoroughly, filtered into a 50-mL tube and analyzed by inductively coupled plasma atomic emission spectrometry [ICP-AES] (Spectro Ciros^{CCD}, SPECTRO Analytical Instruments, Inc., Mahwah, NJ, USA).

Samples of feed used for growing shrimp were collected and dried to constant weight at 80 ℃ in a mechanical convection oven. Samples were ground with the IKA Economical Analytical Mill (Cole-Parmer, Vernon Hills, Illinois, USA). Feeds were digested in triplicate and analyzed by ICP-AES in the same way as the shrimp sample. Water from the shrimp ponds was collected and analyzed for salinity, conductivity, total alkalinity, chloride and sulfate. Another 250-ml water sample was preserved with 5 ml of 1:1 nitric acid for metal analysis. The preserved sample was digested by EPA Method 200.8 [Revised 5.4] (United States Environmental Protection Agency 1994) and analyzed by ICP-AES.

Statistical Analysis

Statistical analysis was performed with SAS [version 9.1] (SAS Institute, Cary, NC, USA). Pearson correlation coefficients between elemental concentrations in HOSO, HLSO, and PUD

shrimp and in water were determined. Mean concentration of elements of the two diets and in shrimp cultured by the two diets were compared by Student's t test. Mean concentrations of elements in HOSO, HLSO, and PUD shrimp, the HOSO, HLSO, PUD shrimp from three locations, and water from the three locations were compared by one-way analysis of variance (ANOVA). Duncan's multiple comparison was used to compare among groups if a significant difference was reported by ANOVA.

Multivariate statistics used in this study included principal component analysis (PCA), canonical discriminant analysis (CDA) and k-nearest-neighbor analysis, all of which have be used previously in assessing similarities in elemental profiles for determining the geographical origin of agricultural and aquacultural products(Sun et al. 2011; Liu et al. 2012; Anderson et al. 1999). PCA is a dimension reduction technique. The purpose of PCA is to reduce dimension and interpret the data. CDA is a dimension reduction technique similar to PCA. However, CDA highlights between group differences and minimizes the within group differences to give best group clusters. The k-nearest-neighbor analysis is used to create a classification model and report the correct classification rate. A cross-validation procedure is used to test the validity of the k-nearest-neighbor analysis.

Results and Discussion

The ICP-AES instrument used in this study was capable of measuring 25 elements. Concentrations of three elements – Ag, Cd, and Pb – were often below detection limits, and two others – Si and B – could not be measured reliably in shrimp or feed because of the presence of these elements in the digestion mixture. Thus, presentation of elemental data will be limited to 20 elements that could be reliably and consistently measured in all samples.

Among ten HOSO shrimp samples taken from the same pond at site G, relative standard deviations (RSD) for means were less than 10% for Ca, K, Mg, Na, P, S, and Zn, but over 30% for Ba, Mo, and Ti (Table 1). The range in RSD was 3.56 to 41.4% with an average of 15.6%. Mean elemental data for the 10 samples of shrimp from the same pond were compared with the mean elemental data for single samples of HOSO shrimp from five other ponds at site G. Differences ($P < 0.05$) were found only for concentrations of K, Mg, and P. The RSD values for shrimp from different ponds ranged from 3.7 to 49.0% with an average of 22.6%. Thus, some elements had a fairly high variation both among shrimp from the same pond and among shrimp from different ponds at the same location. Nevertheless, the elemental composition of shrimp from the same farm did not appear to differ much. This is an encouraging finding with respect to elemental profiling for it suggests that shrimp produced under similar environmental conditions have similar elemental composition.

Shrimp came from ponds at locations representing three distinctly different water chemistries (Table 2). Ponds at site G were filled with low-salinity water from an aquifer and had average salinity of 2.7 ppt. Ponds at the two other locations were in coastal areas and supplied with brackish water – average salinities were 12.0 ppt at site C and 20.3 ppt at site H. Concentrations of several variables – conductivity, chloride, sulfate, Al, Ca, Cu, K, Mg, Ni, Na, and Se – tended to increase as salinity increased, but in some cases differences were not significant. Other elements did not have a clear relationship to salinity – Ba, Co, Cr, Ti, and Zn concentrations were highest at site G despite this site having the lowest salinity. Thus, elemental proportions in waters of the three sites were strongly influenced by salinity, but there also were site-specific differences in these elemental proportions as well.

Pearson's correlation coefficient was calculated for the relationship between concentrations of each element in water and in the three shrimp products (Table 3). There were several significant correlations – six for HOSO, seven for HLSO, and six for PUD shrimp. This suggests that elemental composition of shrimp should show some relationship to elemental concentrations in the culture environment – a positive observation with respect to elemental profiling to determine geographic origin.

Elemental data for the three shrimp products were pooled for all ponds and farms. Four elements (As, Cr, K, and Mo) did not differ among HOSO, HLSO, and PUD shrimp (Table 4). Five elements (Ca, Cu, Mg, Mn, and Zn) were different for each shrimp product. HOSO shrimp were higher than HLSO and PUD shrimp in concentration of Al, Ca, Co, Cu, Fe, Mg, Mn, Na, Ti, and Zn. The HLSO shrimp were higher than PUD shrimp in Ca, Cu, Mg, Mn, P, and Zr. The PUD shrimp were higher than the two other shrimp products in Se and S, but lower in Ba, Cu, Mg, Mn, P and Zr.

The shrimp head contains many important organs: heart, gills, stomach, and hepatopancreas. The hepatopancreas had higher concentrations of Co, Cu, and Zn as compared to exoskeleton and muscle, but the exoskeleton was higher in Mn and Ba (Tu et al. 2008). Hepatopancreas is the accumulation site for Cu (Wu and Yang 2001), and this organ also contains greater Fe concentrations than found in muscle (Wu and Yang 2011). Shrimp shell is made of protein (39.6%), chitin (22.6%), and ash (28.1%) (Ferrer et al. 1996). Calcium concentration is high in shrimp shell. Manganese and Ba are chemically similar to Ca, and Mn can substitute for Ca in shell (Tu et al. 2008). Muscle is comprised mostly of protein, and Zn and Fe accumulate to a greater degree in muscle than do other trace elements (Wu and Yang 2011; Paez-Osuna and Ruiz-Fernandez 1995 a,b). Thus, it is not surprising that HOSO shrimp tended

to be highest in most elements, and that there were considerable differences in concentrations of several elements among the three shrimp products. Certainly, an effort to identify shrimp to geographic origin using elemental analysis and pattern recognition techniques should take into account differences in elemental composition of the three main shrimp products.

The two diets used for shrimp from site C did not differ greatly in feed ingredient composition. Menhaden fish oil used in diet 1 was replaced by soybean oil in diet 2. Nevertheless, six elements – Al, Cu, Fe, Mo, Na, and Ni – were significantly different between the two diets. Elemental content of shrimp cultured with these two diets, however, did not exhibit a difference for any of the 20 elements. The fact that there were no differences in shrimp elemental composition related to diet also is a positive finding with respect to the possible utility of elemental profiling for determining geographic origin. However, it should be noted that the two diets did not differ greatly in their ingredient formulas – only soybean oil versus fish oil. A wide variety of feed formulations are used in shrimp aquaculture worldwide, and much greater variation in elemental composition of shrimp feed than observed here can no doubt be found.

Elemental Profiling

HOSO Shrimp

According to one- way ANOVA, 11 elements (Al, Ca, Co, Cr, Fe, K, Mn, Mo, Ni, S, and Zr) out of 20 showed no difference among three locations ($P>0.05$) for HOSO shrimp (Table 6). Shrimp from sites C and H had higher As and Mg concentrations than shrimp from site G, and site G shrimp had the highest Ba concentration when compared with shrimp form sites C and H. Copper, P and Zn concentration of shrimp from sites G and C were significantly higher than for shrimp from site H. Shrimp from site H has the highest Na concentration, while P, Se and Ti

concentrations were highest in the shrimp from site C. One apparent characteristic of shrimp from site G was low As and Mg concentrations and high Ba content; thus shrimp from site G could be separated from the other two sites based on As, Mg, and Ba concentrations.

PCA was conducted based on nine elements that were different ($P<0.05$) among the three sites: As, Ba, Cu, Mg, Na, P, Se, Ti, and Zn (Table 6). Data were standardized by subtracting the variable mean and then dividing the variable standard deviation to obtain an adjusted mean of 0.0 and standard deviation of 1.0 for each element (Manly 2005). Total variance of the standardized data equaled the number of variables - nine in this case. The eigenvalue for a principal component (PC) indicates the percentage of the variance that it accounts for out of the total variance. The first three principal components (PCs) with eigenvalues greater than 1.0 explained 82.7 % of the total variability (Table 7). The eigenvalue for the first PC was 3.85 and the first PC explained 42.7% of the total variability. Variables Ti, Se, P, Mg, Cu, and As had the highest positive weight, and the variable Ba had the highest negative weight on the first PC. The second PC explained 27.8% of total variability; it was strongly and positively correlated with variables P, Zn, Cu, and Ba, negatively correlated with variables As, Mg, and Na. Each individual shrimp sample was scored on all the PCs generated from PCA. A 2-dimensional, scatter plot was generated using the score for the first two PCs (Fig. 1). Shrimp from the three sites could be separated based on the first two PCs, which explained 70.49% of the total variation.

In order to optimize between group variations and minimize within group differences, CDA was used with the nine elements that were different among the three sites. The procedure generated two discriminant dimensions (Can 1 and Can 2), both of which were significant. The canonical correlations for dimension Can 1 and Can 2 are 0.99 and 0.97, respectively. Can 1 explained 72.53% of total variation, while Can 2 explained the remaining 27.47% of the

variation. Each individual shrimp sample obtained a score for the two discriminant functions from CDA. The extracted canonical variable scores were used to plot a scatter plot (Fig. 2). The graph clearly shows that samples from three different sites could be separated based on Can 2. Shrimp from sites H and C could be separated from shrimp from site G based on Can 1.

The k-nearest-neighbor analysis was used to establish a model to classify samples. The cross-validation test, in which each sample is removed from the dataset and tested against the discriminant function created by the remaining samples, was used to test the validity of K-nearest-neighbor analysis. Shrimp from the three sites could be 100% accurately classified based on the nine variables: As, Ba, Cu, Mg, Na, P, Se, Ti, and Zn (Table 8). Cross-validation gave the same results.

Concentrations of Co, P, Mg, Mn, Na, and Ba in shrimp were correlated with concentrations of these elements in water ($P<0.05$) (Table 3). Pond water from site G (Table 2) was high in Ba concentration (92.9 μg/L), and shrimp cultured in this water (Table 6) also had a high Ba concentration (63.64 ppm) (Table 6) as compared with HOSO shrimp from sites C and H. Barium is chemically similar to calcium (Tu et al. 2008), and concentrations of Ba and Ca in water often are correlated, because Ba often is a minor component of calcium carbonate (Hem 1970); thus the high Ba concentration in waters at site G probably results from the soil in the ponds containing a high concentration of limestone (Pine and Boyd 2010). Concentrations of Mg in water and in shrimp also were closely correlated. Well water used to fill the shrimp pond in site G was low in Mg concentration (Pine and Boyd 2011), and low Mg concentration was detected in the shrimp from site G ($P<0.05$).

HLSO Shrimp

The one way ANOVA showed that 13 elements: As, Ba, Ca, Cu, Fe, K, Mg, Mn, Mo, Na, P, S, and Ti were different ($P<0.05$) among the three locations (Table 6). Shrimp from site G contained the highest Ba, Cu, and S concentrations and the lowest As and Mg levels. Concentrations of Fe, P, and Ti were highest in shrimp from site C compared with shrimp from sites H and G, while Ba and Mn concentrations were lowest in shrimp from site C. High Na, low K and P concentrations were detected in shrimp from site H.

In order to obtain an easy visualization of information contained in the dataset, PCA was conducted on the basis of the 13 elements that were significantly different among locations - As, Ba, Ca, Cu, Fe, K, Mg, Mn, Mo, Na, P, S, and Ti. The first five PCs with eigenvalues greater than 1.0 represented 89.35% of total variability (Table 7). The eigenvalue for the first PC was 4.03 and the first PC explained 30.97% of total variability. The first PC was strongly and negatively correlated with As, Mg and Ti, but positively correlated with Ba, Ca, and S. HLSO shrimp from three locations could be separated based on the first PC (Fig. 1). Sodium had the highest negative weight on the second PC, while Fe, K, P, and Ti had the highest positive weight.

CDA generated two new variables (Can 1 and Can 2), and the canonical correlations for the two variables were 0.996 and 0.979, respectively. The first variable (Can 1) explained 85.5 % of the variance and the second variable (Can 2) explained 14.45 % of the variance. A scatter plot was generated based on Can 1 and Can 2 (Fig. 2). Shrimp from different sites were ploted in different spaces showing that they could be successfully differentiated based on either Can 1 or Can 2.

The k-nearest-neighbor analysis was used to establish a model to classify the samples. The cross-validation procedure was used to evaluate this model. As shown in Table 8, shrimp from the three sites could be accurately classified based on the 13 variables, As, Ba, Ca, Cu, Fe, K, Mg, Mn, Mo, Na, P, S, and Ti. Cross-validation gave the same results.

Concentrations of Ba, Mg, P, S, K, Zr, and Na in the HLSO shrimp were significantly correlated with concentrations of these elements in water ($P<0.05$). The Pearson correlation coefficient was more than 0.7 for Ba, Mg, and P (Table 3).

PUD Shrimp

Concentrations of As, Ba, K, Mg, Na, P, Ti, and Zr were different among the three sites for PUD shrimp (Table 6). The PUD shrimp from site G could be easily separated from shrimp from the other two sites based on low As, Mg concentrations and high Ba concentration. The PUD shrimp from site H were lower in K, P, and Ti and high in Na and Zr compared with shrimp from site C ($P<0.05$). Water in site H has the highest salinity (Table 2). The high Na and low P characteristic of site H shrimp was related to the high Na and low P levels in water ($P<0.05$). Copper was significantly different among locations for HOSO and HLSO shrimp, but it did not exhibit a difference for PUD shrimp. This could be explained by the low Cu level in the PUD shrimp compared with that of the HOSO and HLSO shrimp (Table 4).

Principal component analysis was conducted based on elements As, Ba, K, Mg, Na, P, Ti, and Zr. The first three PCs explained 77.88% of total variability. Eigenvalue for the first PC was 2.85, and the first PC explained 35.63% of the total variability (Table 7). The first PC was strongly and positively correlated with As, Mg, and Zr but negatively correlated with Ba and K.

The second PC with eigenvalue equal to 2.44 explained 30.47% of total variability. Variables K, P, and Ti showed the highest positive weight, and Na, Ba, and Zr showed the highest negative weight on the second PC. A 2-dimensional, scatter plot was generated using the score for the first two PCs (Fig. 1). Shrimp from site H was plotted in different area from shrimp obtained from sites G and C. Shrimp from sites G and C could be separated, but the cluster was not very clear.

Canonical discriminant analysis was conducted using elements that were different among the three sites (Table 6). The two canonical discriminant functions (Can 1, Can 2) generated by the CDA procedure explained 66.35% and 33.65% of total variance, respectively. The 2-D scatter plot generated by Can 1 and Can 2 clearly showed that PUD shrimp collected from the three different sites could be successfully separated (Fig. 2). According to Can 1, shrimp from site C could be separated from shrimp from the two other sites. The PUD shrimp from site H could be distinguished from the remainder based on Can 2.

For PUD shrimp, k-nearest-neighbor analysis gave the same result as for HOSO and HLSO shrimp (Table 8). The PUD shrimp from the three sites could be 100% correctly classified based on eight variables: As, Ba, K, Mg, Na, P, Ti, and Zr. Cross-validation gave the same results.

Concentrations of Ba, Co, Cu, Mg, K, and Na in PUD shrimp were correlated with concentrations of these elements in water ($P<0.05$). Pearson correlation coefficients were more than 0.6 for Ba, Mg, and Na (Table 3). Concentrations of Ba, Mg, and Na in HOSO, HLSO and PUD shrimp were significantly correlated with concentrations of the elements in water (Table 3). Sodium plays an important role in osmotic regulation by crustaceans (Roy et al. 2010), and shrimp are capable of exchanging magnesium ions in saline water. A study of *Penaeus indicus* showed that absorption of Mg ions from the environment could meet the Mg requirement of this

shrimp species (Ahamad Ali 1999). After being exposed to barite media, grass shrimp (Palaemonetes Pugio) accumulated higher levels of Ba in their hepatopancreas, muscle, and exoskeleton (Brannon and Rao 1979). Previous findings mentioned above might explain the high correlation between shrimp and water for Na, Mg, and Ba. The Pearson correlation coefficient for P was 0.72 and 0.71 in the HOSO and HLSO shrimp, respectively (Table 3). However, P in the PUD shrimp was not significantly correlated with P concentrations in water (Table 3). Phosphorus is a component of alkaline phosphatase, which is important in osmotic regulation by crustacean (Pinonia and López Mañanes 2004).

Conclusions

Elemental composition of shrimp showed some relationship to elemental concentrations in the water, but not to elemental concentrations in feed. This might be because water chemistries for the three sites where shrimp were collected were distinctly different. However, the two diets did not differ greatly in their ingredient formulas.

Elemental profiling combined with multivariate statistical method was successfully used to identify the shrimp products to site of origin. As the HOSO, HLSO and PUD shrimp were different for most of the elements analyzed, separate databases will be necessary for different shrimp products for use in ascertaining geographical origin of shrimp. In addition, when using elemental profiling to determine the country origin of shrimp, it should be noted that country boundaries are geopolitical not geochemical. Shrimp from different countries might be cultured in waters of similar elemental composition. Moreover, the variation in water quality will tend to increase with the size of the geographic area of origin. Therefore, the method should be used

with great caution and a very large database should be used in any attempt to develop an elemental profile for a shrimp species in a given country.

Literature Cited

Ahamad Aii, S. 1999. Calcium, phosphorus and magnesium requirements in the diet of shrimp *Penaeus indicus*. Asian Fisheries Science 12:41-49.

Anderson, K. A., B. A. Magnuson, M. L. Tschirgi, and B. Smith. 1999. Determining the geographic origin of potatoes with trace element analysis using statistical and neural network classifiers. Journal of Agricultural and Food Chemistry 47:1568-1574.

Anderson, K. A. and B. W. Smith. 2002. Chemical profiling to differentiate geographic growing origins of coffee. Journal of Agricultural and Food Chemistry 50:2068-2075.

Anderson, K. A. and B. W. Smith. 2005. Use of chemical profiling to differentiate geographic growing origin of raw pistachios. Journal of Agricultural and Food Chemistry 53:410-418.

Brannon, A.C. and K. R. Rao. 1979. Barium, strontium and calcium levels in the exoskeleton, hepatopancreas and abdominal muscle of the grass shrimp, Palaemonetes pugio: Relation to molting and exposure to barite. Comparative Biochemistry and Physiology Part A: Physiology 63:261-274.

Food and Agriculture Organization of the United Nations (FAO). 2012. Cultured Aquatic Species Information Programme. *Penaeus vannamei*. Cultured Aquatic Species Information Programme. Text by Briggs, M. In: *FAO* Fisheries and Aquaculture Department [online]. Rome. http://www.fao.org/fishery/culturedspecies/Litopenaeus_vannamei/en.

Ferrer, J., G. Paez, Z. Marmol, E. Ramones, H. Garcia, and C. F. Forster. 1996. Acid hydrolysis of shrimp-shell wastes and the production of single cell protein from the hydrolysate. Bioresource Technology 57:55-60.

Hem, J. D. 1970. Study and Interpretation of the Chemical Characteristics of Natural Water. U.S. Geol. Survey, Water-Supply Paper 1473. U.S. Gov. Printing Office, Washington, D. C. 363 pp.

Liu, X. F., C. H. Xue, Y. M. Wang, Z. J. Li, Y. Xue, and J. Xu. 2012. The classification of sea cucumber (*Apostichopus japonicus*) according to region of origin using multi-element analysis and pattern recognition techniques. Food Control 23:522-527.

Manly, Bryan F. J. 2005. Multivariate statistical methods: a primer. CRC Press LLC, 2000 N.W. Corporate Blvd., Boca Raton, Florida, USA.

Pȧez-Osuna, F. and C. Ruiz-Fernȧndez. 1995a. Comparative bioaccumulation of trace metals in *Penaeus stylirostris* in estuarine and coastal environments. Estuarine, Coastal and Shelf Science 40:35-44.

Pȧez-Osuna, F. and C. Ruiz-Fernȧndez. 1995b. Trace metals in the Mexican shrimp *Penaeus vannamei* from estuarine and marine environments. Environmental Pollution 87:243-247.

Pine, H. J. and C. E. Boyd. 2010. Adsorption of magnesium by bottom soils in inland brackish water shrimp ponds in Alabama. Journal of the World Aquaculture Society 41(4):603-609.

Pine, H. J. and C. E. Boyd. 2011. Magnesium budget for inland low-salinity water shrimp ponds in Alabama. Journal of the World Aquaculture Society 42:705-713.

Pinoni, S. A. and A. A. López Maȵanes. 2004. Alkaline phosphatase activity sensitive to environmental salinity and dopamine in muscle of the euryhaline crab *Cyrtograpsus angulatus*. Journal of Experimental Marine Biology and Ecology 307, 35-46.

Roy, L.A., D. A. Davis, I. P. Saoud, C. A. Boyd, H. J. Pine, and C. E. Boyd. 2010. Shrimp culture in inland low salinity waters. Reviews in Aquaculture 2:191-208.

Smith, R. G. 2005. Determination of the country of origin of garlic (*Allium sativum*) using trace metal profiling. Journal of Agricultural and Food Chemistry 53:4041-4045.

Smith, R. G. and C. A. Watts. 2009. Determination of the country of origin of farm-raised shrimp (Family Penaeide) using trace metal profiling and multivariate statistics. Journal of Agricultural and Food Chemistry 57:8244-8249.

Sun, S. M., B. L. Guo, Y. M. Wei, and M. T. Fan. 2011. Multi-element analysis for determining the geographical origin of mutton from different regions of China. Food Chemistry 124:1151-1156.

Tu, N. P. C., N. N. Ha, T. Ikemoto, B. C. Tuyen, S. Tanabe, and I. Takeuchi. 2008. Regional variations in trace element concentrations in tissues of black tiger shrimp *Penaeus monodon* (Decapoda: Penaeidae) from South Vietnam. Marine Pollution Bulletin 57:858-866.

United States Department of Agriculture (USDA). 2012. Economic Research Service. Data Sets. Aquaculture Data. http://www.ers.usda.gov/data/aquaculture/ShrimpImportsVolume.htm; http://www.ers.usda.gov/data/aquaculture/ShrimpImportsValue.htm.

United States Environmental Protection Agency (USEPA). 1994. Determination of trace elements in waters and wastes by inductively coupled plasma – mass spectrometry. EPA Method 200.8 (Revision 5.4).

Wu, X.Y. and Y. F. Yang. 2011. Heavy metal (Pb, Co, Cd, Cr, Cu, Fe, Mn and Zn) concentrations in harvest-size white shrimp *Litopenaeus vannamei* tissues from aquaculture and wild source. Journal of Food Composition and Analysis 24:62-65.

Table 1. Mean elemental concentrations, standard deviations, relative standard deviations and range of HOSO shrimp from a pond in site G.

Element (mg/kg)	Average	SD	RSD	Range
Al	147	22.1	15.1	119 – 189
As	1.28	0.22	17.09	1.08 - 1.73
Ba	84.1	27.3	32.5	55.9 – 120
Ca	29,794	2,005	6.73	26,371-33,131
Co	0.12	0.02	19.1	0.10-0.15
Cr	1.09	0.16	15.1	0.83-1.28
Cu	82.3	9.50	11.5	65.1-100
Fe	91.4	18.6	20.3	59.0-125
K	11,673	854	7.32	10,571-13,326
Mg	1,268	37.4	2.95	1,216-1,340
Mn	3.04	0.41	13.6	2.38-3.63
Mo	0.12	0.05	41.42	0.08-0.23
Na	6,194	277	4.47	5,763-6,579
Ni	0.30	0.07	22.2	0.2-0.4
P	11,732	418	3.56	11,290-12,401
S	6,989	242	3.46	6,477-7,280
Se	1.67	0.32	19.0	1.28-2.00
Ti	4.75	1.53	32.3	2.28-7.40
Zn	51.6	2.02	3.93	48.5-54.1
Zr	0.12	0.03	21.5	0.08-0.15

Table 2. Mean elemental concentrations and standard deviations of water quality variables in ponds from three regions.

Variable	G (n=6)	C (n=8)	H (n=5)
Salinity(ppt)	2.70 ±0.52[a]	12.0 ±2.07[b]	20.3 ±3.42[c]
Conductivity(µs/cm)	3,821 ±750[a]	17,936 ±2,715[b]	24,150 ±3,862[c]
Chloride(mg/L)	1,644 ±303[a]	7,060 ±1,055[b]	12,009 ±2,092[c]
Alkalinity(mg/L as $CaCO_3$)	163 ±8.91[a]	50.9 ±16.6[b]	181 ±51.4[a]
Al(µg/L)	571 ±246[a]	625 ±166[a]	1,017 ±386[b]
As	Undetectable		
Ba(µg/L)	92.9 ±22.6[a]	8.93 ±1.57[b]	52.3 ±5.07[c]
Ca(mg/L)	44.8 ±11.9[a]	109 ±21.4[b]	231 ±23.1[c]
Co(µg/L)	0.94 ±0.38[a]	0.32 ±0.38[b]	0.51 ±0[b]
Cr(µg/L)	2.38 ±1.10[a]	1.08 ±0.57[b]	0.82 ±0.28[b]
Cu(µg/L)	2.89 ±1.43[a]	2.93 ±1.21[a]	9.59 ±17.8[a]
Fe(µg/L)	622 ±212[a]	590 ±194[a]	519 ±195[a]
K(mg/L)	69.9 ±11.3[a]	162 ±29.3[b]	242 ±41.7[c]
Mg(mg/L)	14.0 ±2.08[a]	436 ±80.0[b]	764 ±133[c]
Mn(µg/L)	29.8 ±17.5[a]	13.3 ±10.6[b]	54.4 ±11.6[c]
Mo(µg/L)	2.13 ±1.23[a]	1.66 ±0.85[a]	2.76 ±1.38[a]
Na(mg/L)	1,123 ±264[a]	4,207 ±708[b]	6,649 ±1,105[c]
Ni(µg/L)	2.89 ±0.53[a]	3.57 ±2.15[a]	3.98 ±1.11[a]
P(µg/L)	583 ±70.2[a]	802 ±231[a]	247 ±92.1[b]
S(mg/L as SO_4)	45.3 ±5.82[a]	897 ±153[b]	1,714 ±285[c]
Se(µg/L)	6.63 ±8.51[a]	8.48 ±6.03[a]	9.29 ±16.9[a]
Ti(µg/L)	4.59 ±2.52[a]	0 ±0[b]	0.61 ±1.37[b]
Zn(µg/L)	31.6 ±28.3[a]	17.1 ±2.96[a]	10.1 ±2.94[b]
Zr(µg/L)	0.77 ±0.84[a]	0.64 ±0.45[a]	1.02 ±0.36[a]

Note: Values followed by the same lowercase letters were not different ($p \leq 0.05$) according to ANOVA and Duncan's Multiple Comparison test.

G: Greene Prairie Aquafarm; C: Claude Peteet Mariculture Center; H: Harlingen Shrimp Farm.

Table 3. Pearson correlations of elements in HOSO, HLSO, and PUD shrimps and elements in water (n=18).

Elements	Pearson Correlation Coefficient		
	HOSO	HLSO	PUD
Al	0.18	0.2	0.13
As	As could not been detected in water		
Ba	0.85*	0.83*	0.72*
Ca	-0.29	-0.13	0.2
Co	0.48*	0.44	0.66*
Cr	0.22	0.04	0.09
Cu	-0.39	-0.4	-0.52*
Fe	0.05	0.09	0.04
K	-0.23	-0.55*	-0.48*
Mg	0.71*	0.79*	0.74*
Mn	0.47*	0.31	0.05
Mo	-0.06	-0.07	0.23
Na	0.68*	0.55*	0.60*
Ni	0.17	-0.43	-0.26
P	0.72*	0.71*	0.42
S	-0.2	-0.50*	0.14
Se	0.06	-0.45	0.004
Ti	-0.36	-0.39	-0.39
Zn	-0.09	0.29	-0.01
Zr	0.15	0.48*	0.28

Note: * Elements in shrimp and water were correlated ($P \leq 0.05$).

Table 4. Mean elemental concentrations and standard deviations for 20 elements in the HOSO, HLSO and PUD shrimp.

Element (ppm)	HOSO (n=18)	HLSO (n=18)	PUD (n=18)
Al	210.5 ± 94.9[a]	130.7 ± 50.3[b]	98.0 ± 71.4[b]
As	2.29 ±0.90[a]	1.80 ±0.70[a]	1.94 ±0.92[a]
Ba	23.2 ±28.2[a]	15.5 ±21.5[a]	1.00 ±1.21[b]
Ca	27,650 ±2,252[a]	17,134 ±2,273[b]	2,264 ±692.5[c]
Co	0.11 ±0.05[a]	0.04 ±0.03[b]	0.04 ±0.03[b]
Cr	0.95 ±0.30[a]	0.90 ±0.33[a]	1.14 ±0.58[a]
Cu	87.3 ±15.0[a]	42.2 ±5.3[b]	26.6 ±3.57[c]
Fe	123.9 ±66.9[a]	67.5 ±36.1[b]	65.9 ±49.1[b]
K	10,064 ±1,403[a]	10,114 ±1,579[a]	10,267 ±2,014[a]
Mg	2,223 ±640[a]	1,808 ±358.5[b]	1,387 ±225.1[c]
Mn	5.11 ±2.16[a]	2.55 ±1.04[b]	1.33 ±0.69[c]
Mo	0.16 ±0.21[a]	0.06 ±0.09[a]	0.07 ±0.08[a]
Na	6,606 ±1,549[a]	5,547 ±1,193[b]	5,208 ±1,162[b]
Ni	0.30 ±0.12[a]	0.11 ±0.075[b]	0.30 ±0.37[a]
P	10,902 ±1,480[a]	10,952 ±1,374[a]	9,234 ±896.1[b]
S	6,800 ± 568.6[a]	7,060 ± 428.5[a]	7,490 ± 397.4[b]
Se	1.58 ±0.38[a]	1.48 ±0.39[a]	1.85 ±0.42[b]
Ti	8.16 ±5.23[a]	4.61 ±3.08[b]	4.91 ±3.91[b]
Zn	52.7 ±3.50[a]	45.6 ±3.15[b]	49.8 ±3.96[c]
Zr	0.19 ±0.13[a]	0.15 ±0.08[a]	0.07 ±0.08[b]

Note: Values followed by the same lowercase letters were not different ($p \leq 0.05$) according to ANOVA and Duncan's Multiple Comparison test

Table 5. Mean elemental concentrations and standard deviations for 20 elements in two diets and HOSO shrimp fed by the two diets.

Element (mg/kg)	Site C Diet		Site C Shrimp	Site C Shrimp
	1	2	1	2
Al	965.1 ±26.2*	1,122 ±24.9	278.8 ±132.7	247.5 ±68.9
As	0.43 ±0.05	0.30 ±0.10	3.01 ±1.07	2.44 ±0.56
Ba	13.9 ±0.51	13.5 ±0.33	4.82 ±0.48	4.33 ±0.81
Ca	13,942 ±704.8	14,327 ±297.6	27,470 ±638	28,363 ±3,080
Co	0.53 ±0	0.63 ±0.05	0.08 ±0.05	0.08 ±0.07
Cr	3.39 ±0.46	4.33 ±0.62	0.96 ±0.20	1.2 ±0.33
Cu	92.9 ±0.78*	105.3 ±1.45	94.7 ±15.0	99.9 ±11.1
Fe	802.7 ±16.0*	889.6 ±27.0	176.3 ±95.7	153.0 ±54.4
K	6,764 ±1,796	6,046 ±1,149	10,848 ±1,044	10,325 ±952.1
Mg	2,533 ±71.4	2,482 ±45.2	2,624 ±182.3	2,728 ±182.6
Mn	150.0 ±7.15	159.9 ±26.8	3.92 ±1.05	3.68 ±0.70
Mo	2.6 ±0.13*	2.96 ±0.08	0.04 ±0.04	0.08 ±0.03
Na	782.7 ±21.5*	626.8 ±9.46	5,824 ±422.7	6,240 ±456.6
Ni	4.33 ±0.11*	4.83 ±0.08	0.33 ±0.08	0.26 ±0.15
P	14,762 ±791.1	15,421 ±788.0	11,869 ±714.8	12,357 ±1,076
S	4,208 ±299.7	4,249 ±133.8	6,617 ±730.8	6,718 ±714.3
Se	0.82 ±0.17	1.05 ±0.50	1.78 ±0.13	1.94 ±0.35
Ti	49.9 ±23.2	64.6 ±25.3	13.0 ±7.05	10.7 ±3.79
Zn	153.1 ±4.29	169.2 ±9.89	55.1 ±2.11	53.8 ±2.25
Zr	0.09 ±0.04	0.14 ±0.08	0.15 ±0.08	0.14 ±0.04

Note: * different between the two diets and shrimp fed by the two diets according to the Student's t test at $P \leq 0.05$.

Site C: Claude Peteet Mariculture Center.

Table 6. Mean elemental concentrations and standard deviations for 20 elements in HOSO, HLSO and PUD shrimp from three different regions.

Elements	HOSO			HLSO			PUD		
(ppm)	G	C	H	G	C	H	G	C	H
Al	144 ±47.1[a]	263 ±99.3[a]	193 ±85.6[a]	110 ±37.2[a]	158 ±51.2[a]	108 ±45.3[a]	65.2 ±38.8[a]	134 ±81.0[a]	73.0 ±62.8[a]
As	1.18 ±0.24[a]	2.73 ±0.85[b]	2.71 ±0.12[b]	0.89 ±0.21[a]	2.09 ±0.55[b]	2.26 ±0.18[b]	0.89 ±0.20[a]	2.45 ±0.94[b]	2.2 ±0.22[b]
Ba	63.64 ±22.07[a]	4.57 ±0.67[b]	12.49 ±2.16[c]	43.0 ±25.1[a]	2.99 ±0.64[b]	8.04 ±0.41[c]	2.32 ±1.67[a]	0.32 ±0.22[b]	0.76 ±0.35[b]
Ca	28,150 ±2,889[a]	27,916 ±2,114[a]	26,723 ±1,965[a]	19,193 ±1,002[a]	15,740 ±2,272[b]	17,304 ±1,645[ab]	2,351 ±730[a]	2,039 ±423[a]	2,538 ±993[a]
Co	0.14 ±0.04[a]	0.08 ±0.06[a]	0.13 ±0.04[a]	0.05 ±0.04[a]	0.02 ±0.03[a]	0.05 ±0.03[a]	0.05 ±0.03[a]	0.03 ±0.02[a]	0.05 ±0.03[a]
Cr	0.95 ±0.24[a]	1.08 ±0.28[a]	0.76 ±0.33[a]	0.91 ±0.33[a]	1.05 ±0.31[a]	0.66 ±0.26[a]	1.31 ±0.87[a]	1.23 ±0.53[a]	0.83 ±0.12[a]
Cu	88.9 ±6.61[a]	97.3 ±12.6[a]	69.5 ±6.44[b]	47.0 ±5.06[a]	40.9 ±3.92[b]	39.4 ±5.14[b]	28.8 ±2.82[a]	26.3 ±2.45[a]	24.8 ±5.07[a]
Fe	81.8 ±24.8[a]	165 ±73.1[a]	101 ±53.1[a]	47.9 ±19.8[a]	92.9 ±34.1[b]	46.7 ±28.5[a]	43.3 ±29.9[a]	95.4 ±53.4[a]	41.1 ±34.8[a]
K	10,373 ±1,144[a]	10,587 ±966[a]	8,919 ±1,766[a]	10,748 ±1,217[a]	10,727 ±1,181[a]	8,499 ±1,471[b]	10,874 ±1,546[a]	11,344 ±1,383[a]	7,938 ±1,393[b]
Mg	1,320 ±49.2[a]	2,676 ±178[b]	2,403 ±460[b]	1,307 ±41.5[a]	2,015 ±92.7[b]	1,978 ±307[b]	1,119 ±50.0[a]	1,474 ±108[b]	1,516 ±259[b]
Mn	5.92 ±2.37[a]	3.80 ±0.84[a]	6.40 ±2.58[a]	3.09 ±1.15[a]	1.89 ±0.48[b]	3.08 ±1.13[a]	1.18 ±0.56[a]	1.18 ±0.67[a]	1.71 ±0.81[a]
Mo	0.13 ±0.03[a]	0.06 ±0.04[a]	0.34 ±0.36[a]	0.03 ±0.02[ab]	0.03 ±0.04[a]	0.15 ±0.15[b]	0.08 ±0.09[a]	0.03 ±0.05[a]	0.12 ±0.10[a]
Na	5,945 ±520[a]	6,032 ±464[a]	8,186 ±2,285[b]	5,298 ±438[a]	4,989 ±327[a]	6,688 ±1,830[b]	4,892 ±320[a]	4,606 ±283[a]	6,488 ±1,613[b]
Ni	0.35 ±0.13[a]	0.29 ±0.12[a]	0.26 ±0.13[a]	0.09 ±0.06[a]	0.15 ±0.07[a]	0.09 ±0.09[a]	0.42 ±0.61[a]	0.35 ±0.26[a]	0.1 ±0.13[a]
P	10,852 ±399[a]	12,113 ±885[b]	9,016 ±625[c]	10,942 ±685[a]	12,020 ±852[b]	9,252 ±621[c]	8,989 ±624[ab]	9,845 ±557[a]	8,503 ±995[b]
S	7,080 ±400[a]	6,668 ±671[a]	6,732 ±536[a]	7,463 ±285[a]	6,906 ±367[b]	6,904 ±426[b]	7,657 ±308[a]	7,391 ±402[a]	7,481 ±489[a]
Se	1.41 ±0.42[a]	1.86 ±0.26[b]	1.3 ±0.13[a]	1.38 ±0.20[a]	1.58 ±0.44[a]	1.42 ±0.49[a]	1.62 ±0.54[a]	2.00 ±0.32[a]	1.84 ±0.38[a]
Ti	4.20 ±2.06[a]	11.8 ±5.38[b]	6.25 ±3.14[a]	2.44 ±1.38[a]	6.97 ±2.78[b]	2.99 ±2.15[a]	3.09 ±2.25[a]	7.48 ±4.06[b]	2.62 ±2.65[a]
Zn	53.5 ±3.63[a]	54.5 ±2.14[a]	49.0 ±2.68[b]	47.7 ±2.85[a]	45.6 ±1.90[a]	43.3 ±3.98[a]	49.8 ±2.03[a]	50.5 ±4.18[a]	48.7 ±5.4[a]
Zr	0.17 ±0.07[a]	0.14 ±0.06[a]	0.3 ±0.21[a]	0.14 ±0.05[a]	0.11 ±0.061[a]	0.21 ±0.10[a]	0.03 ±0.03[a]	0.04 ±0.04[a]	0.15 ±0.10[b]

Note: The HOSO, HLSO, PUD shrimp from three regions were compared by ANOVA and Duncan's Multiple Comparison test, respectively. Values followed by the same lowercase letters were not significantly different among the three regions ($p \leq 0.05$). G: Greene Prairie Aquafarm; C: Claude Peteet Mariculture Center; H: Harlingen Shrimp Farm.

Table 7. Eigenvectors from the first three principal component axes for nine elements used to classify the HOSO shrimp from three regions; the first five principal component axes for 13 elements used to classify the HLSO shrimp from three regions; the first three component axes for eight elements used to classify the PUD shrimp from three regions.

Elements	Principal Components (HOSO)			Principal Components (HLSO)					Principal Components (PUD)		
	1	2	3	1	2	3	4	5	1	2	3
As	0.31	-0.38	-0.19	-0.37	-0.14	0.24	-0.10	0.10	0.43	0.17	-0.28
Ba	-0.34	0.38	0.14	0.43	0.10	0.02	-0.18	-0.007	-0.28	-0.25	0.68
Ca	NA	NA	NA	0.32	0.08	0.39	0.15	-0.18	NA	NA	NA
Cu	0.35	0.38	0.09	0.28	0.20	0.17	-0.55	0.29	NA	NA	NA
Fe	NA	NA	NA	-0.24	0.33	0.38	-0.13	-0.21	NA	NA	NA
K	NA	NA	NA	-0.003	0.42	-0.14	0.35	0.20	-0.17	0.51	0.30
Mg	0.39	-0.32	0.21	-0.39	-0.17	0.26	-0.06	0.28	0.55	0.05	0.10
Mn	NA	NA	NA	0.26	-0.17	0.41	0.05	-0.46	NA	NA	NA
Mo	NA	NA	NA	0.03	-0.28	0.33	0.59	0.22	NA	NA	NA
Na	-0.03	-0.39	0.63	0.07	-0.38	0.25	-0.29	0.40	0.35	-0.35	0.40
P	0.32	0.39	-0.03	-0.13	0.48	0.09	0.06	0.26	0.19	0.49	0.40
S	NA	NA	NA	0.32	0.20	0.27	0.21	0.40	NA	NA	NA
Se	0.39	0.09	-0.42	NA	NA	NA	NA	NA	NA	NA	NA
Ti	0.45	-0.04	0.14	-0.30	0.30	0.35	-0.07	-0.23	0.25	0.39	0.13

Zn	0.24	0.38	0.54	NA	NA	NA	NA	NA	NA	NA	NA
Zr	NA	NA	NA	NA	NA	NA	NA	NA	0.42	-0.37	0.13
Eigenvalue	3.85	2.50	1.10	4.03	3.29	2.11	1.14	1.05	2.85	2.44	0.94
% Variation	42.73	27.76	12.21	30.97	25.27	16.24	8.80	8.07	35.63	30.47	11.77
Cumulative% Variation	42.73	70.49	82.70	30.97	56.24	72.48	81.28	89.35	35.63	66.11	77.88

Note: NA: Not Applicable.

Table 8. Classification of the HOSO, HLSO and PUD shrimp into three locations and percentage of observations correctly classified.

		C	H	G	Total
Original	C	8	0	0	8
	H	0	5	0	5
	G	0	0	5	5
	Total (count)	8	5	5	18
	% Correctly Classified	100	100	100	100[a]
Cross-Validated	C	8	0	0	8
	H	0	5	0	5
	G	0	0	5	5
	Total (count)	8	5	5	18
	% Correctly Classified	100	100	100	100[b]

Note: [a]100% of empirical grouped observations correctly classified.

[b]100% of cross-validated grouped observations correctly classified.

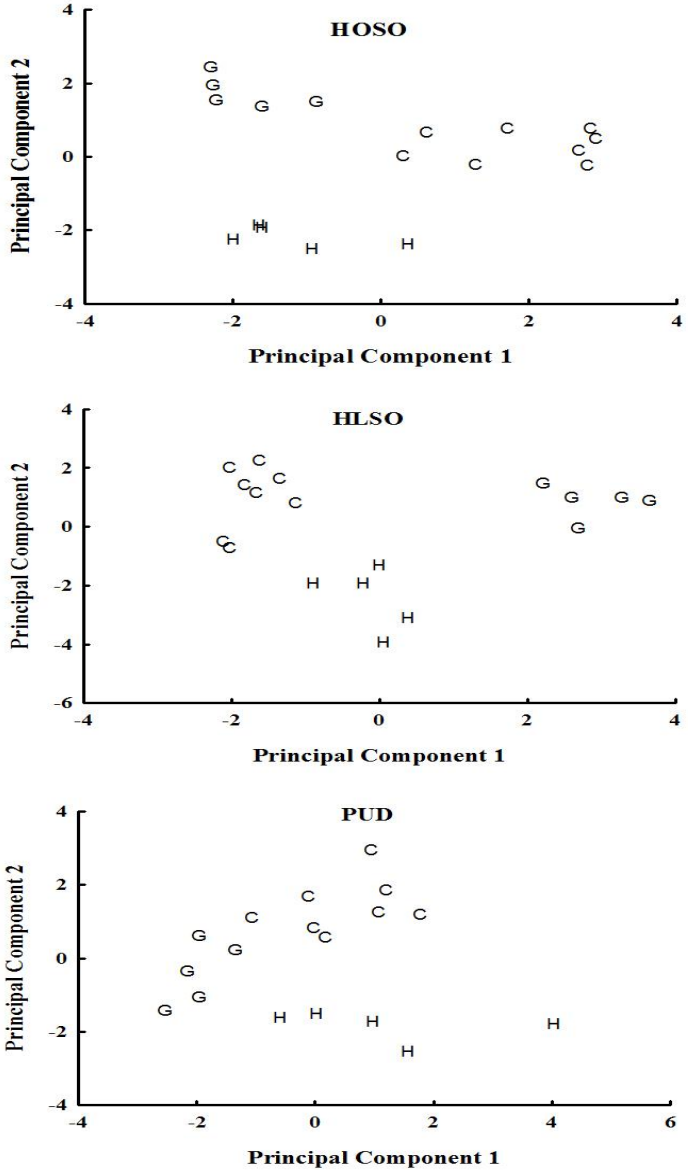

Figure 1. A 2-dimensional scatter plot of individual scores for HOSO, HLSO and PUD shrimp from three locations based on the two, first principal components: G, Greene Prairie Aquafarm; C, Claude Peteet Mariculture Center; H, Harlingen Shrimp Farm.

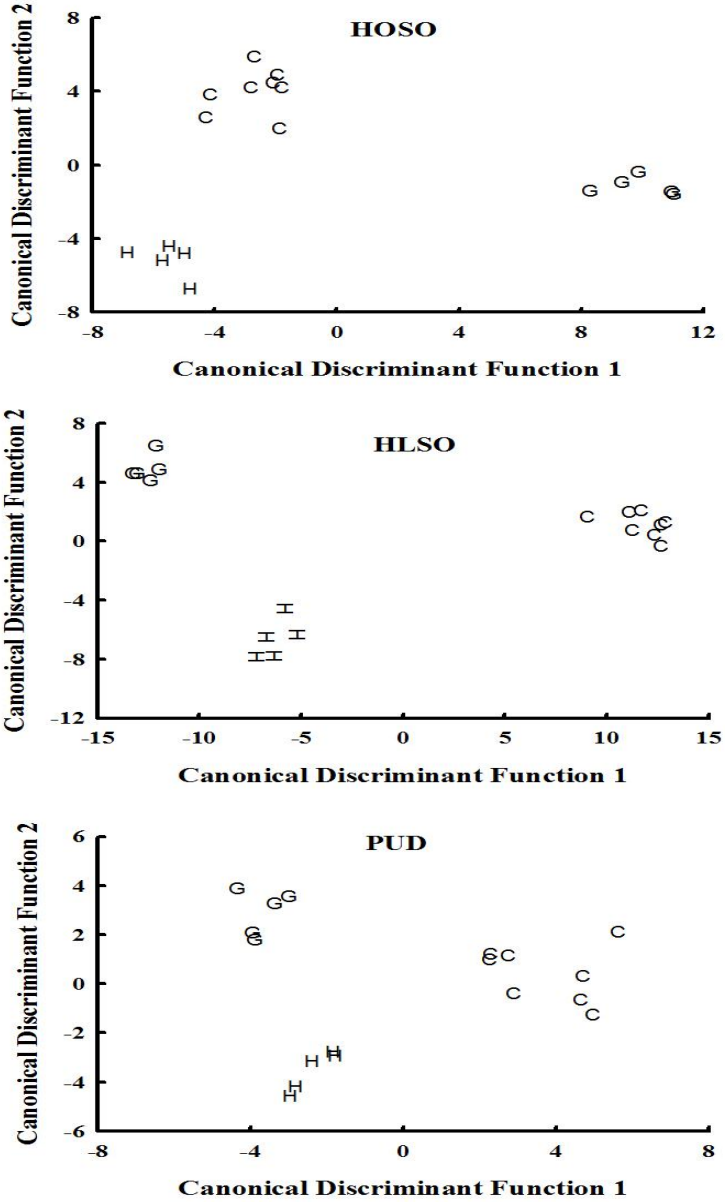

Figure 2. A 2-Dimensional scatter plot of CDA results for the HOSO, HLSO and PUD shrimp samples by the two canonical discriminant functions: G, Greene Prairie Aquafarm; C, Claude Peteet Mariculture Center; H, Harlingen Shrimp Farm.