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# Chemical profiling with modeling differentiates Ictalurid catfish produced in fertilized and feeding ponds

Li Li [a, b], Claude E. Boyd [b, *], Shuanglin Dong [c]

[a] College of Fisheries and Life Science, Shanghai Ocean University, Shanghai 201306, China
[b] School of Fisheries, Aquaculture, and Aquatic Sciences, Auburn University, Auburn, AL 36849, USA
[c] The Key Laboratory of Mariculture, Ministry of Education, Ocean University of China, Qing Dao 266003, China

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ABSTRACT

Elemental analysis (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 *Ictalurus 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 in fertilized ponds did not differ in composition 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 the two culture methods based on chemical composition. The three methods each 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 in the same manner as wild-caught fish. This study demonstrated the potential of chemical profiling combined with multivariate statistical methods for verifying method of production – aquaculture versus wild-caught.


## 1. Introduction

The US often increases the tariff on imported aquaculture products from countries where governmental assistance to producers allows an imported product an unfair advantage over the same product produced domestically. The US 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 (Anderson, Hobbie, & Smith, 2010; Smith & Watts, 2009). Multi-element analysis and pattern recognition techniques are widely used for determining the geographic origin of traditional agricultural products (Anderson, Magnuson, Tschirgi, & Smith, 1999; Anderson & Smith, 2002, 2005; Smith, 2005; Sun, Guo, Wei, & Fan, 2011). These methods also are being considered for aquaculture products (Li, Boyd, & Odom, 2014; Liu et al., 2012; Smith & Watts, 2009).

An aquaculture species may be produced in a system where its food is entirely from natural productivity or in one where its food consists almost entirely of manufactured feed. During processing and shipping, aquatic animals produced by different methods may become intermingled. Differences in elemental composition between animals reared on natural food organisms and those provided feed would make elemental profiling less reliable for assessing geographic origin, but such differences could possibly be exploited to verify whether or not a product originated from wild-caught or aquacultured animals (Anderson et al., 2010).

This 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.

## 2. Materials and methods

### 2.1. Sample collection and analysis

Ponds used in this study are located on the Auburn University E. W. Shell Fisheries Center (SFC), Auburn, Alabama. The

* Corresponding author. School of Fisheries, Aquaculture, and Aquatic Sciences, Auburn University, 203 Swingle Hall, Auburn, AL 36849 USA. Tel.: +1 334 844 4078; fax: +1 334 844 0830.
*E-mail address:* boydce1@auburn.edu (C.E. Boyd).

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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 (10–15 mg $L^{-1}$) 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* ♂*Ictalurus furcatus* that averaged 12.5 cm in total length. Three grass carp *Ctenopharyngodon idella* of 20–25 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 of each 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 2011, 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, Missouri, USA). Dried fillets were pulverized with an 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.

A 2.00-g dried, pulverized sample was weighed into a 125-mL Erlenmeyer flask. The samples were digested by nitric acid and perchloric acid mixture following the same procedure used by Li et al. (2014). 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, New Jersey, USA).

Feed samples were collected and dried to constant weight at 80 °C in a mechanical convection oven. Samples were pulverized with the IKA Economical Analytical Mill (Cole-Parmer, Vernon Hills, Ilinois, USA) and analyzed in triplicate following the methodology described above for catfish fillets.

A 1-L water sample was collected from each pond and analyzed for pH, conductivity, total alkalinity, chloride and sulfate (Eaton, Clesceri, Rice, & Greenberg, 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.

### 2.2. Statistical analysis

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 (Anderson et al., 1999; Liu et al., 2012; Sun et al., 2011). 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 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 – ponds with feeding 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.

## 3. Results and discussion

### 3.1. 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

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

| Element (mg $kg^{-1}$) | Channel catfish ($n = 12$) | Hybrid catfish ($n = 12$) | *t* | *F* |
|---|---|---|---|---|
| Al | 4.04 ± 3.79 | 8.82 ± 12.35 | −1.28 | 10.62[a] |
| 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[a] |
| 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 | 6354 ± 803 | 8136 ± 1642 | −3.38* | 4.19[a] |
| Mg | 1308 ± 95 | 1340 ± 68 | −0.93 | 2.01 |
| Mn | 0.45 ± 0.13 | 0.49 ± 0.16 | −0.67 | 1.51 |
| Na | 1524 ± 208 | 1571 ± 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 are different according to Student's *t* test (*t*) at $P \leq 0.05$.
[a] Variances are not homogenous according to *F* test at $P \leq 0.105$.