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Different tools to trace geographic origin and seasonality of croaker (*Micropogonias furnieri*)

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Abstract

The aim of this study was to use proximate chemical composition, amcro and trace elements, fatty acid profile and stable isotopes as traceability tools to assess geographic origin and seasonality of croaker (*Micropogonias furnieri*). Croaker from Parnaíba contained higher ash in July and lower fat content than croaker from Santos. In contrast, croaker from Santos had statistically higher proportion of 16:1n-9+16:1n-7, 20:1n-11, 20:1n-9, MUFA and n-3/n-6 ratio than croaker from Parnaíba. Concerning seasonality, croaker caught in July had significantly higher amounts of 14:0, 15:0, 16:1n-9+16:1n-7 and saturated fatty acids than fish caught in December. Concerning elements, significant differences were also detected between seasons for Cl, Ca, Fe, Sr and S, whereas differences between geographic origins were only observed with K. $\delta^{13}C$ and $\delta^{15}N$ were statistically different between geographic origins, whereas differences between seasons were only detected in $\delta^{15}N$ ratio of croaker from Santos. Fatty acids, minerals and stable isotope are effective methods to trace geographic origin and seasonality of croaker. Nonetheless, further investigation is still required with larger samples of croaker to enable the implementation of fatty acids, elements or stable isotope as authenticity tools by food control agencies.

Keywords: *Micropogonias furnieri*; isotope stable; traceability; fatty acids; minerals

1. Introduction

Consumers are increasingly aware about the beneficial effects of fish intake to human health, which enabled the continuous increase in fish consumption worldwide (Mazzeo et al., 2008). As a result, the trade of a wide variety of fish products has increased, and consumers are increasingly concerned about the quality, origin and authenticity of the products, as well as on how they are handled, processed and stored (Herrero, 2008).

Fish adulteration can induce several consequences to consumers, such as the purchase of mislabeling or potentially harmful products and reduce the effectiveness of marine conservation (Civera, 2003). Thus, the authenticity evaluation and origin of species are important requirements to ensure quality, provide adequate security controls and develop effective regulations. Food authentication is part of traceability that includes food components identification to verify the compliance with labeling to prevent fraud. Labeling must provide information about species, origin, age and production systems (Schwagele, 2005).

The conventional fish identification is made by examination of their anatomical and morphological characteristics. However, identification becomes complicated in processed food, such as frozen fillets and precooked shellfish, where these morphological characteristics are removed (Moran, & Garcia-Vazquez, 2006). Therefore, there is an urgent need to develop methods to rapidly and accurately identify processed food that can help the authorities and fish industries to comply with the requirements for labeling and traceability, and to ensure product quality and consumer protection (Carrera, Cañas, & Gallardo, 2012).

The use of analytical techniques to determine the geographic origin of food products is the best way to prevent tampering. Gas chromatography (Busetto et al., 2008; Thomas et al., 2008), spectroscopy (Cordella, Faucon, Cabrol-Bass, & Sbirrazzuoli, 2003) and IRMS (Thomas, Jasmin, & Lees, 2005) have been proposed for food authenticity in order to identify the presence of main components in the sample or any compounds that may be characteristic of a particular food item.

Isotope ratio mass spectrometry (IRMS) is a powerful tool for the detection of adulterated and counterfeit food products (Calderone et al., 2009) and is recognized as an official method to ensure the authenticity of food products (Martin, & Martin, 1995). IRMS has been applied for assessing geographic origin of lamb (Piasentier, Valusso, Camin, & Versini, 2003) beef (Heaton, Kelly, Hoogewerff, & Woolfe, 2008) poultry meat and dried beef (Franke et al., 2007), but limited studies exist of its applicability in seafood.

It is well known that the levels of macro and trace elements in food products clearly reflect the environmental conditions at which they were produced. For this reason, the elemental content has been suggested as a good indicator of the geographic origin of food samples. Thus, techniques such as atomic absorption spectrometry (FAAS) have been successfully employed in food authentication (Gonzalvez, Armenta, & de la Guardia, 2009). Energy dispersive X-ray fluorescence (EDXRF) is another technique that can also be used in elemental determination. This technique is highly sensitive, fast, cheap and accurate to measure multi-elements.

Fatty acids profile is another useful tool to differentiating fish stocks (Joensen, Steingrund, Fjallstein, & Grahl-Nielsen, 2000), production systems (Alasalvar, Taylora, Zubcov, Shahidi, & Alexis, 2002), seasonality (Rasoarahona, Barnathan, Bianchini, & Gaydou, 2005) and geographic origin (Çelik, Diler, & Kuçukgulmez, 2005).

The city of Santos is located in the South East of Brazil in a highly industrialized area, subjected to strong anthropogenic pressure. In contrast, Parnaíba is a small town located in the North East of Brazil, where economy is based on the production of babassu oil, carnauba wax and cotton. Both cities have distinct environmental conditions, water quality and contamination levels. The croaker *Micropogonias furnieri* is considered as one of the most traditional and gastronomically important fish species captured by fisheries in Brazil, Argentina and Uruguay, being a very important resource in Santos and Parnaíba regions ,(Elsdon, & Gillanders, 2002). This species is omnivorous, showing preference for small crustaceans such as shrimp and crabs. Regarding the life cycle, young individuals migrate to estuaries, while adults migrate to coastal areas to breed. The population of croaker varies throughout the year as a result of migration and food availability (Costa, & Araujo, 2003).

In this context, this study aimed to assess the traceability of croaker (*M. furnieri*) from two distinct regions, Santos and Parnaíba and harvested in two seasons (July and December). Different traceability tools were employed to assess geographic origin and seasonality of croaker, such as proximate chemical composition, macro and trace elements, fatty acid profile and stable isotopes of carbon and nitrogen.

2. Materials and methods

2.1 Samples

Croakers were caught in two distinct regions of the Brazilian coast, namely in Santos (23º 57'17"S and 46 º 19'56"W) and Parnaíba (02 º 54'17"S and 41 º 46'36"W) in July (winter) and December (summer) of 2011. The regions have two well defined seasons: summer and winter. The specimens' morphological parameters were registered (Table 1), then all fish were eviscerated and transported on ice to the laboratory where they were separated the edible part (muscle), homogenized and frozen. A portion of each frozen sample was freeze-dried for 48 h at -40 °C (Christ, Alpha 2-4 LD Plus, Munchen, Germany) and stored at -80 °C under controlled moisture conditions until further analyses.

2.2 Proximate chemical composition

Moisture, ash, protein and lipid contents were determined according to the Association of Official Analytical Chemists methods (AOAC, 2005). All analyzes were performed in duplicate per specimen. Samples were defrozen for subsequent analyses. Analyses of moisture and ash were carried out by oven drying at 105°C (method 950.46) and muffle furnace at 550°C (method 938.08). The total level of nitrogen were determined by the Kjeldahl procedure (method 981.10), and protein levels were estimated using 6.25 conversion factor; and total lipid content was determined with the Soxhlet extraction method using ethyl ether (40–60°C; 7 h; heater plate SBS Instruments PC6L, Portugal).

2.3 Fatty acid profile

Fatty acid profile was determined in triplicate for each specimen, according to the experimental procedure of Cohen et al. (1988). Each freeze-dried sample (300 mg

dry weight) was blended in 5 mL of acetyl chloride/ methanol (1:19 v/v; Merck), shaken, and heated (80°C; 1 h). After cooling, 1 mL of Milli-Q distilled water and 2 mL of n-heptane pro analysis (Merck) were added, and samples were shaken and centrifuged (2000 g; 5 min, Sigma 2k15, Germany) until separation in two phases: an upper organic phase (composed by methyl esters) with n-heptane and a lower organic phase with methyl chloride, methanol and water. The moisture content of the upper phase was removed with anhydrous sodium sulfate (Panreac). An aliquot (2 µL) of the upper phase was then injected (split injector) on a gas chromatograph (Varian Star 3800 Cp, Walnut Creek, CA, USA) equipped with an auto sampler and fitted with a flame ionization detector at 250°C. The separation was carried out with helium as carrier gas at a flow rate of 1 mL min^{-1}, in a capillary column DB-WAX (30 m length 0.32 mm internal diameter; 0.25 µm film thickness; Hewlett–Packard) programmed at 180°C for 5 min, raised to 220 at 4°C min^{-1}, and maintained at 220°C for 25 min, with the injector at 250°C. Fatty acids were identified by comparing retention times with those of Sigma standards. Quantitative data were calculated using the peak area ratio (percent of total fatty acids) and the Varian software.

2.4 Trace elements and contaminants

Energy dispersive X-ray fluorescence (EDXRF) was used to quantify the elements S, Cl, K, Ca, Fe, Zn, As, Se, Br and Sr. The spectrometer is a self-constructed system, using a Philips X-ray generator (PW 1140/00/60 3 kV). The EDXRF technique consists of an X-ray tube equipped with a molybdenum secondary exciter. The characteristic radiations emitted by the elements in the sample were detected by lithium drifted silicon [Si (Li)] detector with 30 mm^2 active area and 8 µm beryllium window. The energy resolution was 135 eV at 5.9 keV and the acquisition system was a Nucleus PCA card. Quantitative calculations were made by the fundamental parameters method

(Custódio, Carvalho, & Nunes, 2003). The X-ray generator was operated at 50 kV, 20 mA and 1000 s acquisition time. Each freeze dried specimen sample powder (1 g) was pressed into 2 cm diameter pellets (n = 2) without any chemical treatment and glued onto Mylar films on sample holders and placed directly in the X-ray beam.

Flame atomic-absorption spectrometry (FAAS; Varian SpectrAA 55B Sydney, Australia) was used to quantify Cd and Pb in each specimen sample (n=2), according to the procedures described by Jorhem (2000). Briefly, 10 g of defrost muscle was dry-ashed at 500°C and dissolved in 15% v/v nitric acid. Concentrations of Pb and Cd were established through the external linear calibration with standard solutions: Cd $(NO_3)_2$ and Pb $(NO_3)_2$ (Merck; 1 g L^{-1} dissolved in 0.5 mol L^{-1} HNO_3).

Total Hg levels were determined by atomic absorption spectrometry using an automatic Hg analyser (LECO apparatus AMA 254, St. Joseph, MI, USA). The procedure is based on freeze dried sample decomposition (10 mg; n = 2 for each specimen) by combustion, preconcentration of mercury by amalgamation with gold and atomic absorption spectrometry. Concentrations were calculated from linear calibration with Hg standard solution absorbance (1 g L^{-1} dissolved in 0.5 mol L^{-1} HNO_3; Merck).

Accuracy was checked through analysis of certified biological material (Table 2). The detection limits (DL) of each element were determined by two methods: (1) EDXRF – with the signal-to-noise approach, where the equipment compares the signal of each element with blank samples and established the minimum concentration at which the element is reliably detected; and (2) FAAS – with the residual standard deviation (RSD) of the response and the slope (S) of the calibration curve of each standard solution used [DL = (3.3×RSD)/S]. The concentration of all elements was reported as milligrams per kilogram on dry weight basis (mg kg^{-1}).

176 *3. Isotope analyses*

177 For stable isotope analysis, approximately 500 µg and 60 µg of homogeneous
178 dried material were packed in 5 x 9 mm cylindrical tin capsules for the determination of
179 nitrogen (^{15}N) and carbon (^{13}C), respectively. Samples were analyzed for stable isotope
180 ratios of carbon and nitrogen using a Delta S type isotope ratio mass spectrometer
181 (Finnigan Mat, Bremen, Germany) with an elemental analyzer CHN.

182 Isotope ratios are expressed in conventional δ notation in parts per thousand (‰)
183 relative to the universal standard:

184 $\delta_{sample} = [(R_{sample} / R_{standard}) - 1] \times 1000$

185 The results $\delta^{13}C$ and $\delta^{15}N$ isotope ratio analyses are reported on the relative δ-
186 scale and referred to the international standards V-PDB (Vienna Pee Dee Belemnite) for
187 carbon isotope ratio and atmospheric air for nitrogen isotope ratio. The analyses were
188 performed as previously described by Móri et al. (2007). Secondary standards used to
189 $\delta^{13}C$ was -28.00 per mil (eucalyptus charcoal) and 3.20 to $\delta^{15}N$ (Ammonium sulphate
190 $(NH_4)_2SO_4$). The certified values were δ13C = -39.73‰ and δ15N = -0.73‰ (working
191 standard - UREA - IVA 33802174).

192 *2.6. Statistical analysis*

193 One-way analysis of variance (ANOVA) was used to detect significant
194 differences among geographic origin and seasonality in all assessments, followed by
195 Unequal N's test to identify these differences. Whenever necessary, data were
196 transformed to satisfy normal distribution and homoscedasticity requirements, followed
197 by nonparametric analysis of variance (Kruskall–Wallis), if transformed data could not
198 meet these assumptions. Principal Component Analysis (PCA) was also employed to
199 reduce the multidimensional data sets of the several elements to lower dimensions, thus

simplifying the presentation and interpretation of data. All statistical analyses were tested at 0.05 level of probability with the software STATISTICA 8.0 © (Statsoft, Tulsa, OK, USA).

3. Results and discussion

3.1 Proximate chemical composition

The results of croaker from Santos and Parnaíba in July and December are shown in Table 3. Only ash content of croaker from Parnaíba region was statistically different than levels in Santos region. Concerning seasons, significantly higher ash content was found in croakers caught in Parnaíba during July compared to December. No significant differences in the amounts of moisture and protein were detected between geographic locations and seasons. In contrast, fat content was higher in croaker from Santos compared to Parnaíba, but no difference was found between seasons. Luzia et al. (2003) showed large variations in croaker fat among seasons (summer = 0.60%) and (winter = 3.29%). According to Stamatis and Arkoudelos (2007), variations in marine fish chemical composition are closely related to feed nutritional composition, habitat, fish size, catching season, seawater temperature, seawater salinity, animal physiological condition, maturation stage, gender and other environmental conditions.

3.2 Fatty acids profile

The fatty acids profile of croaker revealed statistical differences according to geographic origin and seasons (Table 4). Croaker from Santos had statistically lower levels of 21:0 and n-6 than croaker from Parnaíba. Concerning seasonality, Santos croaker caught in July had significantly higher amounts of 14:0, 16:1n-9+16:1n-7, 18:3n-4 than Santos croaker caught in December, whereas higher levels of 16:3n-4, 18:2n-6, 20:0, 20:2n-6 and 21:0 were observed in December. In contrast, Parnaíba

225 croaker caught in July had statistically higher amounts of 15:0, 16:1n-9+16:1n-7, 17:0,
226 16:4n-3, 18:1n-7, 19:0, 20:2n-6, 22:4n-6 than Santos croaker caught in December.
227 However, levels of 16:2n-4, 16:3n-3, 20:0, 20:4n-3, 20:5n-3, 22:5n-6, 22:5n-3, 22:6n-3,
228 PUFA, n-3, EPA+DHA were higher in Parnaíba croaker caught in December.

229 Palmitic acid was the primary saturated fatty acid (SFA) of croaker regardless of
230 season and geographic origin. No statistical differences in SFA levels were detected for
231 croaker between seasons and geographic origins. Similar results were obtained by
232 Bandarra et al. (1997) for sardines *Sardina pilchardus* as SFA were fairly constant
233 throughout the year and did not seem to be influenced by diet.

234 Oleic acid was identified as the primary MUFA in all samples. Overall, MUFA
235 was higher in croaker from Santos than from Parnaiba, though not being always
236 significant, and did not differ with season. Higher levels of C20:1n-9 were found in
237 croaker from Santos compared to Parnaíba specimens, though not significant. This fatty
238 acid has been associated with zooplankton, thus reflecting distinct zooplankton
239 consumed by croaker (Budge, Iverson, Bowen, & Ackman, 2002).

240 DHA (22:6n-3) was the basic polyunsaturated fatty acid in all samples. It was
241 reported that DHA constitutes the majority of PUFA in most marine fish (Alasalvar et
242 al., 2002; Orban, Nevigato, Di Lena, Casini, & Marzetti, 2003). The percentages of
243 PUFA, such as EPA and DHA, in fish muscle are mostly dependent of diet (Arts,
244 Ackman, & Holub, 2001) and the fatty acid composition might vary due to changes in
245 nutritional habits of fish (Norrobin, Olsen, & Tande, 1990). The lower PUFA content in
246 croaker from Parnaiba in July (winter) may be attributed to changes in feed availability.

247

248 *3.3 Macro and trace elements*

Elemental contents of croaker from different origins and seasons are given in Table 5. No statistical differences were detected in the concentration of Zn and Br in croaker regardless of geographic origin and season. S (Parnaíba), Cl (Santos), Ca (Parnaíba), Fe (both sites), Se (Parnaíba), Rb (Parnaíba), Sr (both sites), Pb (Santos) and As (Parnaíba) contents were significantly higher in July than in December. In contrast, the levels of Hg, Cd and Pb in Parnaíba croaker were statistically higher in December compared to July. Concerning geographic origin, K levels were always statistically higher in Santos compared to Parnaíba specimens. The metabolic function of Sr and Rb in marine organisms is still unknown and they are regarded as non-essential elements. Selenium is an essential element acting as antioxidant, anticarcinogenic, regulator of thyroid hormone metabolism and an antagonistic agent to the toxicological effects of Hg (Khan, Ali, Biaswas, & Hadi, 1987). In this study, it was possible to observe the antagonism between Hg and Se in croakers from Parnaiba captured in July, which showed significantly lower levels of Hg than the other samples, contrasting with the statistically higher levels of Se.

Mercury is one of the most well studied element due to its high toxicity (Carvalho, Pereira, & Brito, 2002). The maximum mean concentration, 0.84 mg kg^{-1} (dry weight), was measured in croaker from Parnaíba in December, whereas the lowest concentration was found in croaker from Parnaíba in July (0.09 mg kg^{-1}). It is well recognized that Hg is a mutagenic, neurotoxic and teratogenic element that can interfere with the human body functions, by damaging the renal, endocrine, gastrointestinal, cardiovascular and nervous systems (Goyer et al., 1995). The results obtained for toxic elements, such as As, Hg, Pb, and Cd, reflect an exogenous influence that may be related to environmental pollution (Carvalho, Santiago, & Nunes, 2005).

Variations in the elemental composition of marine foods are closely related to seasonal and biological differences (species, size, tissue, age, gender and maturation stage), catching areas, processing method, food source and environmental conditions (seawater chemistry, salinity, temperature and contaminant level) (Alasalvar et al., 2002).

3.4 Stable Isotope

The results of croaker isotopic analysis from different geographic origins and seasons are presented in Table 6. Carbon and nitrogen isotopic ratios were statistically different between geographic origins. Croaker from Santos has more negative values of $\delta^{13}C$ than Parnaíba specimens. Changes in $\delta^{13}C$ are due to differences in feed availability at the different regions. Indeed, in aquaculture, it has been shown that different feed sources differ in the availability of carbon for fixing (Butterworth, Li & McKinley, 2004).

In contrast, croaker from Santos had higher $\delta^{15}N$ than fish from Parnaíba. The differences in $\delta^{15}N$ values are on protein content and mostly on origin and type of protein ingested through the diets of both fish (Busetto et al., 2008). The natural diet of croaker consists mainly of benthic invertebrates and small fish (Soares, Muto, Gasparro, & Rossi-Wongtschowski, 2006) that vary according to prey availability and geographic origin of fishing areas. Croaker from Parnaiba showed $\delta^{13}C$ increase of 1‰ compared to croaker from Santos. According to Suzuki et al. (2005), changes in biochemical composition of a tissue according to the ontogeny and/or season will influence the proportion of stable isotopes, such as for $\delta^{13}C$ lipid fraction.Regarding seasonality, differences were only detected in $\delta^{15}N$ ratio in croaker from Santos, where specimens captured in July had higher $\delta^{15}N$ enrichment. Seasonal differences in the isotopic

298 composition of fish may be linked to seasonal changes in feed preferences and
299 availability (Vizzini, & Mazzola, 2003). The isotopic ratio of animals is primarily
300 determined by diet and, to some extent, also reflects their origin (De Niro, & Epstein,
301 1978). During periods of scarcity of food, fish uses the reserves accumulated in its
302 body. Consequently, more positive nitrogen values and less negative carbon values are
303 observed. Garcia et al. (2007) found similar results in croaker from Patos Lagoon ($\delta^{13}C$
304 = -17.97±1.1 and $\delta^{15}N$ = 14.39±0.3). The isotopic ratios in this study were higher than
305 those found by Corbisier et al. (2006) in croaker from Flamengo Sound, Ubatuba (-14.3
306 for $\delta^{13}C$ and 12.0 for $\delta^{15}N$). Molkentin et al. (2007) evaluated the isotopic differences in
307 $\delta\ ^{13}C$ and $\delta\ ^{18}O$ in wild and farmed salmon from different regions, and found statistical
308 differences between salmon reared in different regions, but not in wild salmon from
309 different regions. This implies that there is a considerable variation in the feed
310 composition used by fish farms, which difficult the determination of the geographical
311 origin by IRMS. Therefore, the current results with croaker indicate that $\delta^{15}N$ is a better
312 indicator for its identification.

313

314 *3.5 Principal Component analysis*

315 PCA was used to provide an overview of the capacity of macro and trace
316 elements and fatty acids to discriminate differences between croakers caught in Santos
317 and Parnaíba in different seasons (Figure 1). Factors one and two yielded 69.22% of
318 explainable results, with Fe, Pb, Sr, Cl, Ca and the fatty acids 20:0 and 20:2n-6 loading
319 heavily on the first factor, and 22:5n-6, 20:4n-6 (ARA), 16:4n-6, 17:0 isobr and 15:0
320 loading heavily in factor two (Table 7). The results illustrate clear separation between
321 geographic origin and seasons.

322 The composition of croaker differed between geographic origin and seasons.
323 Most variations are likely related to feed availability and habitat type. Croaker from
324 different geographic origins may be differentiated using total lipids, ash content, fatty
325 acids profile (e.g. 14:0, 17:0, 21:0, 16:1n-9+16:1n-7, 20:1n-11, 20:1n-9,20:4n-6, 22:4n-
326 6, MUFA, n-6 and n-3/n-6 ratio), essential elements (K), and isotopic carbon or
327 nitrogen. As far as season is concerned, its differentiation could be attained with several
328 fatty acids (14:0, 15:0, 21:0, 16:1n-9+16:1n-7, 16:2n-4 fit, 16:3n-3 and SFA), elements
329 (Cl, Ca, Fe, Sr and S), and the stable isotope $\delta^{15}N$.

330 Fatty acids, macro and trace elements and stable isotopes, combined with
331 multivariate statistical analysis are promising effective methods for authentication and
332 traceability of croakers caught in Santos and Parnaíba in different seasons. Nonetheless,
333 for the daily practice of food control, isolated tools should be used to identify fraud and
334 to reduce costs and duration of analysis. Fatty acids profile, minerals or stable isotopes
335 can indeed provide evidence of fraud in croaker from different origins and seasons.

336

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Table 1.Weight and length (mean ± standard deviation) of croakers caught in Santos and Parnaíba in different seasons

	Weight			Length		
Locality/Seasonality	**Mean**	**Max.**	**Min.**	**Mean**	**Max.**	**Min.**
CSJ (n=10)	1188.5±186.8	1580	965	39.9±2.0	42.5	37.0
CSD (n=10)	712.5±90.2	870.9	591.7	39.5±1.5	42.5	36.5
CPJ* (n=10)	244.1±142.2	497.5	96.2	27.1±4.9	34.0	21.2
CPD (n=10)	985.6±104.1	1150	840	45,.8±2.1	48.0	42.0

CSJ: Croacker Santos July; CSD: Croacker Santos December; CPJ: Croacker Parnaíba july; CPD: Croacker Parnaíba December. * weight of eviscerated fish

Table 2. Elemental concentration (mg kg^{-1} DW) and detection limits (mg kg^{-1}, DL) of certified reference material (average ± standard deviation) analyzed by FAAS and EDXRF. Abbreviations: Dry Weight (DW); Detection Limit (DL)

Element	Technique	D.L.	Certified reference material	Certified value	Present work
Hg	FAAS	0.02	Dogfish muscle (DORM-2)	4.64±0.26	4.68±0.17
Cd	FAAS	0.01	Lobster hepatopancreas (TORT-2)	27.00±1.00	27.00±0.00
Pb	FAAS	0.02	Lobster hepatopancreas (TORT-2)	0.35±0.13	0.35±0.06
As	EDXRF	0.7	Lobster hepatopancreas (TORT-2)	21.60±1.80	22.6±2.00
S	EDXRF	100	Oyster tissue (SRM 1566)	7600*	8200±500
Cl	EDXRF	100	Oyster tissue (SRM 1566)	10000*	10200±500
K	EDXRF	50	Oyster tissue (SRM 1566)	9690±50	10000±80
Ca	EDXRF	20	Oyster tissue (SRM 1566)	1500±50	1350±50
Fe	EDXRF	3	Dogfish muscle (DORM-2)	142±10	141.3±1.5
Cu	EDXRF	0.7	Oyster tissue (SRM 1566)	63.0±4.0	63.0±4.0
Zn	EDXRF	1	Dogfish muscle (DORM-2)	25.6±2.3	23.9±0.1
Se	EDXRF	1	Dogfish muscle (DORM-2)	1.4±0.09	1.2±0.1
Br	EDXRF	0.8	Freeze-dried animal blood (IAEA-A-13)	22.0±3.0	22.0±2.0
Rb	EDXRF	1.1	Orchard Leaves (SRM-1571)	11.4±0.7	12.0±1.0

* Non-certified values provided by the United States National Bureau of Standards.

Table 3. Proximate chemical composition (g 100 g^{-1}) of croakers caught in Santos and Parnaíba in different seasons

Locality/Seasonality	Moisture	Protein	Ash	Fat
CSJ (n=10)	78.86±0.50	18.33±0.25	1.20±0.03[b]	1.57±0.19[a]
CSD (n=10)	79.05±0.42	18.36±0.36	1.20±0.06[b]	1.68±0.29[a]
CPJ (n=10)	78.63±0.77	17.50±0.55	1,32±0.05[a]	1.18±0.15[ab]
CPD (n=10)	80.63±0,93	17.48±1.18	1.01±0.03[c]	0.79±0.10[b]

CSJ: Croaker Santos July; CSD: Croaker Santos December; CPJ: Croaker Parnaíba July; CPD: Croaker Parnaíba December. [a]Means ± S.D. with different letters in the same column are significant different at $P \leq 0.05$.

Table 4. Fatty acid content (%) of croakers caught in Santos and Parnaíba in different seasons

Fatty Acids	**CSJ** (n=10)	**CSD** (n=10)	**CPJ** (n=10)	**CPD** (n=10)
14:0*	2.11±0.50^{a}	1.01±0.31^{b}	1.09±0.25^{b}	0.61±0.19^{b}
15:0*	0.84±0.12^{a}	0.67±0.26^{ab}	0.85±0.28^{a}	0.30±0.05^{b}
16:0	21.37±2.02^{a}	20.06±4.07^{a}	25.36±2.96^{a}	21.80±0.63^{a}
16:1n-9+16:1n-7*	6.95±0.73^{a}	4.98±1.48^{b}	4.08±1.01^{b}	1.98±0.42^{c}
17:0 isobr*	0.57±0.09^{a}	0.73±0.29^{a}	0.41±0.24^{ab}	0.19±0.05^{b}
16:2n-4- Fit*	0.14±0.04^{b}	0.41±0.06^{ab}	0.17±0.08^{b}	1.19±0.28^{a}
17:0*	0.76±0.08^{b}	0.74±0.18^{b}	1.35±0.29^{a}	0.52±0.16^{b}
16:3n-4*	0.20±0.04^{b}	0.52±0.10^{a}	0.83±0.08^{a}	0.42±0.13^{ab}
16:3n-3*	0.58±0.34^{bc}	0.99±0.32^{b}	0.23±0.05^{c}	1.86±0.29^{a}
16:4n-3*	0.13±0.04^{b}	0.13±0.05^{b}	1.41±0.06^{a}	0.18±0.04^{b}
18:0**	8.61±1.05^{a}	7.84±1.42^{a}	9.52±0.33^{a}	9.78±0.64^{a}
18:1n-9*	7.81±1.47^{a}	8.26±1.76^{a}	7.06±1.60^{a}	6.75±0.55^{a}
18:1n-7*	2.70±0.37^{a}	2.34±0.72^{a}	2.48±0.42^{a}	1.51±0.29^{b}
18:2n-6***	0.80±0.07^{b}	1.53±0.36^{a}	1.75±0.32^{a}	1.12±0.08^{ab}
19:0*	0.32±0.03^{b}	0.27±0.06^{b}	0.46±0.08^{a}	0.33±0.03^{b}
18:3n-4**	0.26±0.06^{a}	0.10±0.02^{b}	0.18±0.04^{ab}	0.41±0.17^{a}
20:0**	0.00±0.00^{b}	0.22±0.04^{a}	0.00±0.00^{b}	0.29±0.13^{a}
20:1n-11**	0.78±0.28^{a}	0.65±0.22^{a}	0.44±0.15^{ab}	0.20±0.02^{b}
20:1n-9**	0.36±0.01^{ab}	0.60±0.13^{a}	0.25±0.04^{b}	0.27±0.06^{b}
20:2n-6*	0.31±0.04^{bc}	0.56±0.08^{a}	0.40±0.07^{b}	0.19±0.03^{c}
21:0*	0.15±0.08^{c}	0.29±0.02^{b}	0.55±0.07^{a}	0.60±0.10^{a}
20:4n-6*	7.11±2.00^{bc}	6.42±2.21^{c}	11.52±2.98^{ab}	12.03±1.46^{a}
20:4n-3*	0.35±0.10^{b}	0.23±0.04^{b}	0.25±0.05^{b}	0.58±0.08^{a}
20:5n-3*	8.83±1.19^{a}	6.75±1.46^{ab}	5.00±0.42^{b}	8.49±1.76^{a}
22:4n-6**	1.69±0.13^{b}	2.16±0.54^{b}	3.91±1.01^{a}	2.92±1.02^{b}
22:5n-6*	1.44±0.26^{b}	1.76±0.71^{b}	1.98±0.53^{b}	4.26±0.52^{a}
22:5n-3*	3.82±0.30^{b}	3.11±0.52^{b}	3.30±0.63^{b}	4.60±0.44^{a}
22:6n-3*	12.05±2.37^{a}	12.07±3.70^{a}	7.29±2.11^{b}	13.47±1.34^{a}
SFA**	34.98±2.89^{ab}	31.86±5.45^{b}	40.33±3.42^{a}	34.94±0.63^{ab}
MUFA*	19.75±4.02^{a}	18.40±4.86^{a}	14.51±2.11^{ab}	11.70±2.71^{b}
PUFA*	38.31±4.55^{b}	37.71±9.11^{b}	36.12±6.90^{b}	51.53±3.48^{a}
n-3*	26.19±2.79^{a}	23.48±5.81^{ab}	17.52±3.31^{b}	29.18±2.72^{a}
n-6*	11.52±2.38^{b}	13.20±3.46^{b}	19.33±4.93^{a}	20.56±2.25^{a}
w3/w6*	2.33±0.39^{a}	1.93±0.19^{ab}	1.19±0.34^{b}	1.43±0.18^{b}
EPA+DHA*	20.88±2.73^{a}	18.82±4.79^{ab}	12.29±2.36^{b}	21.96±2.65^{a}

CSJ: Croaker Santos July; CSD: Croaker Santos December; CPJ: Croaker Parnaíba July; CPD: Croaker Parnaíba December. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, n-3: fatty acids omega 3, n-6: fatty acids omega 6, n-3/n-6 fatty acids ratio, EPA: Eicosapentaenoic acid, DHA: Docosahexaenoic acid. Different superscript letters in each row indicate significant differences. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$.

Table 5. Essential and non-essential elements content (mg kg^{-1}) of dried croaker caught in Santos and Parnaíba in different seasons.

Minerals	**CSJ** (n=10)	**CSD** (n=10)	**CPJ** (n=10)	**CPD** (n=10)
S*	11748.79±537.25^{a}	11299.06±891.39^{a}	11895.54±1478.63^{a}	8693.01±334.37^{b}
Cl*	8073.50±765.36^{a}	4857.83±1148.07^{b}	6475.96±635.00^{ab}	4503.41±521.02^{b}
K***	18886.00±472.10^{a}	17856.86±1539.46^{a}	11399.29±240.66^{b}	12431.58±664.64^{b}
Ca**	891.94±109.72^{ab}	715.28±12.13^{b}	1437.75±240.66^{a}	536.63±27.47^{b}
Fe***	24.41±0.80^{a}	15.41±0.74^{b}	27.53±1.32^{a}	15.61±1.37^{b}
Cu*	2.92±0.06^{ab}	2.56±0.26^{b}	5.53±1.12^{a}	3.42±0.18^{ab}
Zn	19.87±1.08^{a}	18.16±1.15^{a}	18.49±0.79^{a}	18.72±0.79^{a}
Se**	2.67±0.32^{b}	3.18±0.77^{b}	8.02±1.85^{a}	3.94±1.04^{b}
Br*	26.72±1.64^{a}	22.54±0.71^{a}	25.38±1.67^{a}	26.54±2.66^{a}
Rb*	2.42±0.17^{b}	2.32±0.17^{b}	3.46±0.52^{a}	2.60±0.20^{b}
Sr*	3.19±0.12^{b}	1.69±0.51^{c}	5.60±0.47^{a}	3.64±0.23^{b}
Hg***	0.60±0.10^{a}	0.68±0.01^{a}	0.09±0.00^{b}	0.84±0.10^{a}
Cd**	0.00±0.00^{b}	0.00±0.00^{b}	0.01±0.01^{b}	0.02±0.00^{a}
Pb***	0.16±0.03^{b}	0.00±0.00^{c}	0.15±0.04^{b}	0.27±0.02^{a}
As***	13.59±1.48^{b}	11.69±0.18^{b}	24.15±3.85^{a}	10.69±0.39^{b}

CSJ: Croaker Santos July; CSD: Croaker Santos December; CPJ: Croaker Parnaíba July; CPD: Croaker Parnaíba December. Different superscript letters in each row indicate significant differences. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$.

Table 6. Stable isotope ratios of Carbon ($\delta^{13}C$) and Nitrogen ($\delta^{15}N$) of croakers caught in Santos and Parnaíba in different seasons

Locality/Seasonality	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
CSJ (n=10)	14.02±0.55[a]	-17.78±0.35[a]
CSD (n=10)	13.12±0.74[b]	-17.84±0.48[a]
CPJ (n=10)	11.52±0.22[c]	-16.72±0.67[b]
CPD (n=10)	11.45±0.66[c]	-16.10±0.39[b]

CSJ: Croaker Santos July; CSD: Croaker Santos December; CPJ: Croaker Parnaíba July; CPD: Croaker Parnaíba December. [a]Means ± S.D. with different letters in the same column are significant different at $P \leq 0.05$.

Table 7. Factor loadings of explorative PCA for croaker standard data set [a]

Variable	Factor 1	Factor 2
Ash	0,006	-0,190
Fat	**0,723**	0,006
S	-0,615	0,259
Cl	**-0,963**	-0,001
K	**-0,756**	0,040
Ca	**-0,959**	-0,035
Fe	**-0,992**	-0,033
Cu	**-0,884**	-0,014
As	**-0,928**	-0,053
Se	0,642	-0,221
Rb	-0,356	-0,014
Sr	**-0,965**	-0,106
Hg	**0,783**	-0,011
Cd	**-0,909**	-0,027
Pb	**-0,988**	-0,040
14:00	**-0,860**	-0,381
15:00	-0,544	**-0,716**
16:1w7+9	**-0,804**	-0,319
16:2w4	**0,922**	0,200
17:00	-0,222	-0,682
17:iso	0,302	**-0,798**
16:3w4	**0,912**	-0,153
16:3w3	0,657	0,222
16:4w3	0,072	**0,785**
18:1w7	-0,500	-0,682
18:2w6	**0,902**	-0,040
19:00	-0,619	0,456
18:3w4	**-0,911**	-0,152
20:00	**0,971**	-0,054
20:1w11	-0,324	-0,572
20:1w9	**0,832**	-0,417
20:2w6	**0,936**	-0,287
21:00	**0,808**	0,239
20:4w6	-0,200	**0,841**
20:4w3	-0,742	0,420
20:5w3	-0,691	0,576
22:4w6	0,531	0,544
22:5w6	0,331	**0,870**
22:5w3	**-0,705**	0,558
22:6w3	0,107	**0,864**
SFA	-0,529	-0,259
MUFA	-0,243	-0,738
PUFA	-0,098	**0,964**
W3	-0,267	**0,926**
W6	0,118	**0,818**
W3/W6	-0,449	0,000
EPA+DHA	-0,228	**0,926**
15N	-0,644	0,307
13C	-0,110	0,254

[a] All of the variables are reported; factor weights >0.7 and <-0.7 are shown in bold type.

Figure 1

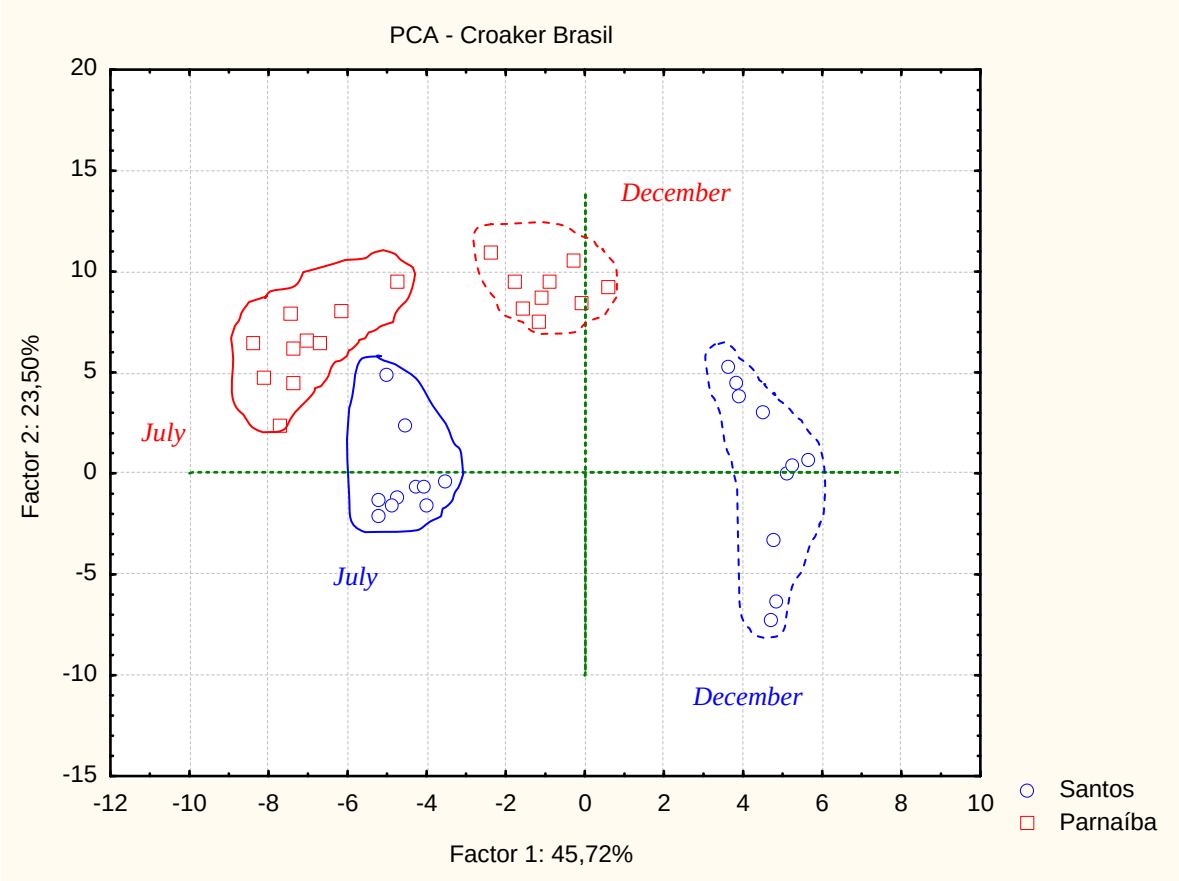

Figure 1. Principal Component Analysis of croakers caught in Santos and Parnaíba in different seasons.

Highlights

- Croaker geographical origin could be distinguished by proximate chemical composition (lipids and ash), fatty acids profile (14:0, 17:0, 21:0, 16:1n-9+16:1n-7, 20:1n-11, 20:1n-9, 20:4n-6, 22:4n-6, MUFA, n-6 and n-3/n-6 ratio), essential elements (K), or stable isotopes (C and N).
- Croaker seasonality could be distinguished by fatty acids (14:0, 15:0, 21:0, 16:1n-9+16:1n-7, 16:2n-4 fit, 16:3n-3 and SFA), elements (Cl, Ca, Fe, Sr and S), and stable isotopes ($\delta^{15}N$).
- Fatty acids profile, minerals and stable isotopes are promising methods for authentication and traceability of croakers caught in Santos and Parnaíba in different seasons.