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Elemental composition in the bones and gills of juvenile Atlantic Bluefin Tuna (*Thunnus thynnus*): can it be used to distinguish batches?

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ABSTRACT. Atlantic Bluefin Tuna (*Thunnus thynnus*) aquaculture has developed rapidly in recent years, making it necessary to discriminate between specimens derived from aquaculture (captive-reared) and those derived from fisheries (wild-caught). In this study, a method based on the chemical composition of discard tissues (gills and bones) in 3 batches of juvenile *T. thynnus* (tank-cultured on land, cage-reared in the sea, and wild-caught) was assessed. The concentrations of 11 macro- and micro-elements (i.e., Ca, Fe, K, Mg, Na, P, S, Cu, Mn, Zn, and Sr) were determined and evaluated using an analysis of variance (ANOVA) and 2 multivariate tests, namely a principal component analysis (PCA) and discriminant canonical analysis (DCA). The best results were obtained from the gills and from the batch corresponding to the wild-caught specimens. However, the results were not robust enough to establish any specific pattern that would facilitate the identification of the origin of *T. thynnus* specimens.

Key words: batch discrimination, bones, gills, inorganic elements, juvenile, trace elements, Atlantic Bluefin Tuna, *Thunnus thynnus.*

INTRODUCTION

In vertebrates, hard tissues are key structures that provide support for the organism and store both major and trace elements. Inorganic elements, such as Ca, P, Mg, and Mn, are important in these tissues, as they are related to certain physiological functions, such as the organic bioavailability of other elements, including Zn (Aschner and Aschner 2005, Lall and Kaushik 2021). On the other hand, the gills are a vital route for the elemental uptake of Cu (Miller et al. 1980, Grosell 2012), Na (Taylor et al. 2003), Fe (Bury et al. 2003, Bury and Grosell 2003), Mn (Rouleau et al. 1995, Baudin et al. 2000), Zn (Bury et al. 2003, Hogstrand 2012), Ca (Evans and Claiborne 2009), P (Evans and Claiborne 2009), and Mg (Dabrowska et al. 1991, Shearer and Åsgård 1992) (reviewed in Lall and Kaushik 2021). Potassium is important for maintaining osmotic balance and acid-base equilibrium (Lall 2003), while S is considered to be an indispensable nutrient for all living organisms (Komarnisky et al. 2003), given its role as an essential component of amino acids, proteins, enzymes, vitamins, and other molecules. Finally, Sr has been found to have key biological functions, including the ability to increase bone mineral density in certain species (Siccardi et al. 2010).

Although Atlantic Bluefin Tuna (*Thunnus thynnus*) (ABFT) aquaculture remains largely dependent on capture-based fattening (De La Gándara et al. 2016), notable advances in closing the full life cycle of the species in captivity have been achieved since 2016, the year in which the cycle was first successfully closed at a global level (Ortega and De la

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Gándara 2017). Currently, European regulations prohibit catching ABFT weighing less than 30 kg or with fork lengths measuring less than 115 cm (OJEU 2016). However, in the future, ABFT juveniles born in captivity might be commercialized, and smaller individuals might be sold. This would increase the likelihood of small, illegally captured individuals reaching the market (Campobasso et al. 2017) and highlights the risk of fraudulent mislabeling.

In the absence of reliable methods for distinguishing wild-caught from captive-reared ABFT, it has become necessary to explore potential methods for discriminating between batches of any age. In this regard, a natural way of identifying batches based on how culture conditions influence tissue characteristics during growth and development could be particularly useful (Jara and Chodyniecki 1999, Rooker et al. 2007, Brucka-Jastrzębska et al. 2009).

Hard tissues originating from fishery by-products have been used in many studies to extract new molecules and information (Kim and Jung 2007, Murthy et al. 2014, Talib et al. 2020, Aaenglong et al. 2022, López-Álvarez et al. 2024), given that (1) they are rich in macro- and micro-elements and (2) their chemical composition is determined by complex interactions among genetics, physiology, environmental conditions (e.g., surrounding water chemistry, temperature, and pollution), and ontogenetic changes (Walther and Limburg 2012, Sturrock et al. 2015, Limburg et al. 2018). Therefore, the characterization of a chemical profile taken from the hard tissues of a given population or batch could be employed in the future as a differential tool in the fisheries sector (Cubadda et al. 2006). Thus, the aim of the present study was to evaluate elemental concentrations in the bones and gills of ABFT juveniles from 3 batches (tank-reared on land, captive-reared in the sea, and wild-caught) and to assess their potential for use in aquaculture.

Material and methods

Sample collection

Samples of 75 ABFT weighing less than 1,000 g were collected from 3 different locations: inland tanks (batch 1; $n = 24$ [gills]; $n = 24$ [bones]), sea cages (batch 2; $n = 22$ [gills]; $n = 22$ [bones]), and the wild (batch 3; $n = 29$ [gills]; $n = 28$ [bones]). The tuna from batches 1 and 2 originated from naturally fertilized eggs from sea-farming facilities. These eggs were promptly collected and transferred to the inland facilities of the Spanish Institute of Oceanography (Murcia Oceanographic Centre, Spain). The larval culture was fed on rotifers and copepods in a 40-m^3 tank. Weaned fish were fed an artificial diet (Magokoro S-3; Marubeni Nissin Feed Co., Ltd., Tokyo, Japan) and maintained at 24.9 °C and at a salinity of 32.0 $g \cdot L^{-1}$ in a 20-m^3 tank.

At 41 days post-hatching, the specimens with sufficient body mass for transportation were split into 2 groups: fish from batch 1 were transferred to a 900-m^3 inland overflow system tank in the Infrastructure for Atlantic Bluefin Tuna Aquaculture facility (Cartagena, Spain) where they were fed herring (*Clupea* spp.), Brazilian Menhaden (*Brevoortia aurea*), and Atlantic Mackerel (*Scomber scombrus*). Fish from batch 2 were placed in floating sea-cages off Cartagena, Spain (37°34′39.2″N, 0°52 ′35.9″W), collected soon after their natural deaths, and sampled. Finally, the tuna from batch 3 were caught using hook-and-line fishing gear in Mazarrón Bay (Murcia, Spain) and sampled immediately after capture. Sampling of wild-caught tuna was conducted with the corresponding permits granted by the competent authorities (ICCAT REC 11-06). In accordance with European legislation (OJEU 2010), none of the procedures in the present study required ethical authorization.

Bone and gill samples were collected and washed with MilliQ water (Milli-Q Lab Water Solutions, Guyancourt, France), washed with nitric acid (2%), and washed again with MilliQ water (Milli-Q Lab Water Solutions) before being dried at room temperature. All samples were stored at –20 °C until analysis. Bone and gill samples were collected from the first branchial arch and the bony denticles of the branchial arch, respectively.

Sample preparation and analysis

Tissue samples were pre-treated as described by Salvat-Leal et al. (2023). Briefly, gill and bone samples were subjected to acid digestion using trace-metal grade nitric acid (69% Suprapure; Merck and Co, Inc., Rahaway, USA) and H_2O_2 (33% Suprapure, Merck & Co, Inc.) in Teflon reaction tubes, which were heated in a microwave digestion system (UltraClave-Microwave; Milestone SpA, Sorisole, Italy) for 20 min at 220 °C and finally diluted to 10 mL with double deionized water (Milli-Q Lab Water Solutions). Subsequently, Ca, Fe, K, Mg, Na, P, S, Cu, Mn, Zn, and Sr concentrations were determined using an inductively coupled plasma optical emission spectrophotometer (ICP-OES); 1 blank sample was analyzed for every 11 samples. Blank values were always below the detection limits for trace elements and represented less than 1–2% of the sample concentrations for major elements.

Multi-element calibration standards prepared in 4% nitric acid (AnalytiChem Canada, Inc., Baie-d'Urfé, Canada) were used. Macro- and microelement concentrations were established according to the methodology described in UNE-EN ISO 1188 (UNE 2010). The certified reference material 1577b (bovine liver; National Institute of Standards and Technology, Dicoex, Bilbao, Spain) was used for validation. Calibration curves were generated for each analytical batch using a minimum of 3 concentration points. Each analytical run began with calibration standards, followed by samples and intermediate control standards, and concluded with control standards, accepting a maximum coefficient of variation of 10%. The wavelengths (nm), recovery rates (%), and uncertainty (%) were as follows: Ca (184.0, 317.9 nm; 94.3 ± 4.3%), Fe (239.6, 238.2 nm; 103.9 ± 3.9%), K (766.5 nm; 94.6 ± 4.6%), Mg (279.5, 280.3, 285.2 nm; 94.7 ± 4.7%), Mn (260.6,

279.5 nm; 96.2 ± 6.2%), Na (589.6 nm; 95.2 ± 5.2%), P (185.9, 213.6 nm; 93.5 ± 3.5%), Cu (224.7, 324.8 nm; 96.9 ± 4.1%), S (180.7, 182.0 nm; 104.3 ± 4.3%), Zn (206.2 nm; 105.0 ± 5.0%), and Sr (421.5 nm; 97.0 ± 7.0%). All concentrations are expressed in $\mu g \cdot g^{-1}$. The detection limit was 10 $\mu g \cdot g^{-1}$ for major constituents (Ca, K, Mg, Na, P, and S) and 0.001 $\mu g \cdot g^{-1}$ for the remaining elements.

Statistical analysis

The results were analyzed using SPSS v. 24.0 (IBM, Armonk, USA). Geometric means and standard errors were calculated for all element concentrations. The Kolmogorov–Smirnov test was used to assess data normality, and Levene's test was used to evaluate homoscedasticity. A general linear model (GLM), with Tukey's honestly significant difference (HSD) and Scheffé post hoc tests, was used to analyze the relationships among weight, element concentration, and batch. A Kruskal–Wallis test, followed by post hoc comparisons, was used to evaluate differences among batches. The significance level for all statistical tests was set at $\alpha = 0.1$.

To identify the probable batch of a fish based on the multi-element dataset, 2 multivariate statistical analyses were performed: a principal component analysis (PCA) and discriminant canonical analysis (DCA). These methods reduce high-dimensional data into a smaller set of interpretable patterns, thereby facilitating the identification of natural groupings and the variables contributing to these patterns. In PCA, dimensionality reduction is achieved by extracting the principal components (PCs), which are linear combinations of the original variables that maximize the explained variance in the data. In contrast, DCA generates canonical discriminant functions (CDFs) that maximize group separation based on those same variables. The relative contribution of each element to the multivariate structure is assessed based on their respective loadings or coefficients.

The PCA considered a threshold-factor loading of 0.32, which corresponded to an explained average variance of 56.6% (Peterson 2000). In addition, the validity of the method was evaluated using the Kaiser–Meyer–Olkin (KMO) index, Bartlett's test of sphericity ($P < 0.05$), and the eigenvalue criterion (values > 1). The DCA employed Wilks' lambda to evaluate the significance of discrimination ($P < 0.05$), and a split-sample cross-validation procedure was performed to assess the ability of the selected variables to predict the ABFT batches. In this validation, 1 individual was removed from the original matrix. The DCA was then performed using the remaining observations to classify the omitted individual; the number of misclassified individuals indicated the degree of intermingling, whereas the proportion of correctly reclassified individuals was used as a measure of group integrity (Poulet et al. 2005, Yakubu and Okunsebor 2011).

The classification functions were used to discriminate new specimens from unknown batches. In these functions, the constants and function coefficients were obtained for each tissue, batch, and element with Eq. (1):

$$F(x) = a + (b[X]) , \quad (1)$$

where a is a constant for a combination of a tissue and batch, b is the coefficient of the classification function for the combination of an element and batch, and $[X]$ is the concentration of an element for a given tissue and batch (in a particular specimen). The result with the highest value indicated the possible batch assignment for a particular specimen.

Results

The mean weights were 592.96 ± 131.69 g (batch 1), 520.74 ± 98.06 g (batch 2), and 517.44 ± 104.19 g (batch 3). No direct influence of fish weight on element concentration was detected (GLM, $P > 0.05$). The concentrations of inorganic elements detected in the gills and bones of juvenile ABFT are shown in Table 1. The element that exhibited the highest concentration was Ca, whereas Cu exhibited the lowest concentration. In the gills, significant differences between batches were observed for the concentrations of Cu ($P = 0.001$), Mn ($P = 0.008$), S ($P < 0.001$), and Sr ($P = 0.033$). In the bones, significant differences among batches were detected for the concentrations of Cu ($P = 0.008$), Fe ($P = 0.021$), Mg ($P = 0.023$), and Zn ($P < 0.001$). No statistically significant differences were found in either tissue for Ca, K, Na, or P concentrations.

In the PCA (Fig. 1 and 2), the explained variance (EV), KMO index, Bartlett's test of sphericity, and the eigenvalue criterion were appropriate for both tissues. The KMO index exceeded 0.5 in both cases, indicating that the data were suitable for analysis (Shrestha, 2021). The 11 analyzed elements were represented by 3 PCs in both the gills and bones, which explained 88.4% and 73.7% of the total variance, respectively. The KMO indices for both tissues exceeded 0.7 (0.766 in the gills and 0.731 in the bones), although the EV was higher in the gills (>0.771) than in the bones (>0.551). For gill tissue, PC1 was composed of P, Sr, Ca, Mn, Mg, and Zn; PC2 included K, Na, and S; and PC3 was defined by Cu and Fe. No clear separation among groups was found, although batch 2 was located in the negative region of both PC2 and PC3 (Fig. 1). For bone tissue, PC1 consisted of Ca, Mg, Sr, Mn, P, Zn, and Na; PC2 included S and K; and PC3 contained Cu and Fe, with no clear differentiation among groups (Fig. 2). The PCs exhibited similar elemental composition in both tissues, except for Na, which was associated with PC2 in the gills and PC1 in the bones.

In the DCA, 4 elements (Mg, Mn, S, and Zn) were selected for the discriminant functions in both tissues, whereas 3 elements (Ca, K, and Na) were not selected for either tissue. The CDF data are given in Table 2. Two CDFs were generated for the gills and bones, each composed of the elements identified as the most discriminant by the analytical software (Yakubu

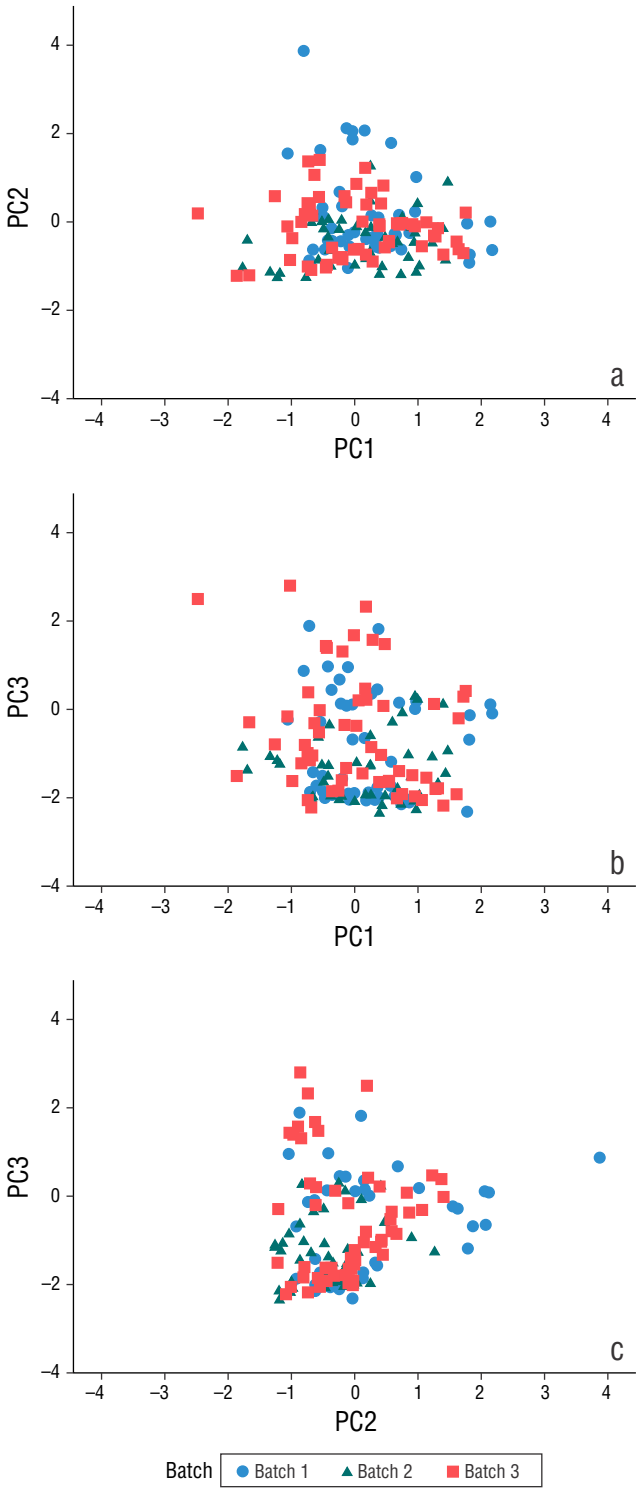

Figure 1. Spatial distribution of Atlantic Bluefin Tuna (ABFT; *Thunnus thynnus*) batches (batch 1 [tank-reared on land], batch 2 [captive-reared in the sea], and batch 3 [wild-caught]) based on principal component analysis (PCA) components for the gills: PC1/PC2 (**a**), PC1/PC3 (**b**), and PC2/PC3 (**c**). Explained variance (EV) for PCs: PC1 (EV = 48.4%; P, Ca, Mn, Sr, Mg, Zn), PC2 (EV = 24.8%; K, Na, S), and PC3 (EV = 15.1%; Cu, Fe).

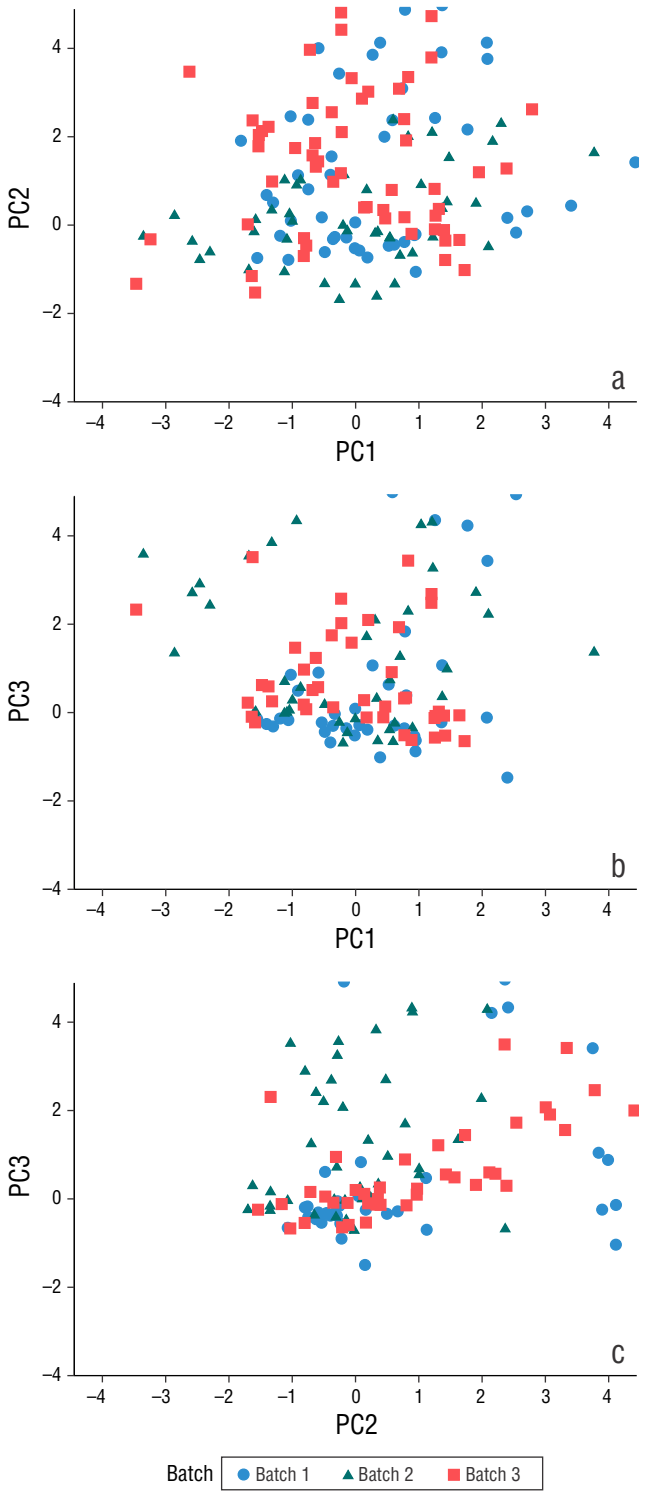

Figure 2. Spatial distribution of Atlantic Bluefin Tuna (ABFT; *Thunnus thynnus*) batches (batch 1 [tank-reared on land], batch 2 [captive-reared in the sea], and batch 3 [wild-caught]) based on principal component analysis (PCA) components for bone: PC1/PC2 (**a**), PC1/PC3 (**b**), and PC2/PC3 (**c**). Explained variance (EV) for PCs: PC1 (EV = 45.7%; Ca, Mg, Sr, Mn, P, Zn, Na), PC2 (EV = 17.2%; S, K), and PC3 (EV = 10.7%; Cu, Fe).

Table 1. Concentrations of inorganic elements in Atlantic Bluefin Tuna (ABFT; *Thunnus thynnus*) tissues by batch (batch 1 [tank-reared on land], batch 2 [captive-reared in the sea], and batch 3 [wild-caught]). Data: geometric mean ± standard error ($\mu g \cdot g^{-1}$, ww). For each element and tissue, the same superscript letter indicates statistical differences between batches.

	Gills			Bone		
Element	Batch 1	Batch 2	Batch 3	Batch 1	Batch 2	Batch 3
Ca	$(5.3 \pm 0.4) \times 10^4$	$(4.4 \pm 0.5) \times 10^4$	$(4.0 \pm 0.5) \times 10^4$	$(8.3 \pm 0.4) \times 10^4$	$(8.4 \pm 0.5) \times 10^4$	$(8.9 \pm 0.6) \times 10^4$
Cu	3.5 ± 0.2	2.6 ± 0.2^b	4.2 ± 0.4^b	0.39 ± 0.07^a	0.5 ± 0.1^b	$0.8 \pm 0.1^{a,b}$
Fe	$(6.3 \pm 1.6) \times 10^2$	$(5.2 \pm 0.7) \times 10^2$	$(5.1 \pm 2.2) \times 10^2$	29 ± 3^a	49 ± 9^a	44 ± 85
K	$(1.6 \pm 0.3) \times 10^3$	$(1.0 \pm 0.2) \times 10^3$	$(1.1 \pm 0.2) \times 10^3$	$(1.7 \pm 0.3) \times 10^3$	$(1.4 \pm 0.3) \times 10^3$	$(1.6 \pm 0.2) \times 10^3$
Mg	$(3.2 \pm 0.3) \times 10^3$	$(2.3 \pm 0.4) \times 10^3$	$(2.2 \pm 0.2) \times 10^3$	$(1.6 \pm 0.196) \times 10^3$	$(1.46 \pm 0.09) \times 10^{3b}$	$(1.9 \pm 0.1) \times 10^{3b}$
Mn	36 ± 3^a	25 ± 4	20 ± 2^a	17 ± 1	16 ± 1	16 ± 1
Na	$(3.1 \pm 0.6) \times 10^3$	$(1.4 \pm 0.3) \times 10^3$	$(1.6 \pm 0.4) \times 10^3$	$(3.2 \pm 0.3) \times 10^3$	$(2.8 \pm 0.3) \times 10^3$	$(3.5 \pm 0.2) \times 10^3$
P	$(3.6 \pm 0.3) \times 10^4$	$(2.7 \pm 0.3) \times 10^4$	$(2.6 \pm 0.2) \times 10^4$	$(4.4 \pm 0.4) \times 10^4$	$(3.8 \pm 0.2) \times 10^4$	$(4.4 \pm 0.3) \times 10^4$
S	$(5.8 \pm 0.2) \times 10^{3a}$	$(3.4 \pm 0.3) \times 10^{3a,b}$	$(4.7 \pm 0.2) \times 10^{3b}$	$(2.5 \pm 0.1) \times 10^3$	$(2.3 \pm 0.2) \times 10^3$	$(2.6 \pm 0.2) \times 10^3$
Sr	$(2.8 \pm 0.2) \times 10^{2a}$	$(2.6 \pm 0.3) \times 10^2$	$(1.8 \pm 0.2) \times 10^{2a}$	$(2.2 \pm 0.1) \times 10^2$	$(2.6 \pm 0.2) \times 10^2$	$(2.4 \pm 0.1) \times 10^2$
Zn	68 ± 6	50 ± 5	58 ± 5	37 ± 2^a	40 ± 3^b	$55 \pm 4^{a,b}$

and Okunsebor 2011). The percentage of cases correctly classified after the implementation of the cross-validation method in the DCA was 80.0% for the gills and 77.0% for the bones. For both tissues, batch 3 was the most clearly discriminated, followed by batch 1 and batch 2 (Table 3). The case classification functions are given in Table 4; the differences between the 3 groups are shown in Figure 3.

Discussion

Inorganic element concentrations were explored as a potential approach to discriminate batches of juvenile ABFT and to provide a simpler, more cost-effective alternative to established techniques. Artificial tagging (Block et al. 2005, Fromentin 2010), including the use of chemical markers (Warren-Myers et al. 2015, Tulli et al. 2020), molecular markers (Boustany et al. 2008, Riccioni et al. 2010), and genetic markers (Rodríguez-Ezpeleta et al. 2019), entails high logistic and economic costs that can be avoided by using natural tracers, specifically those found in waste products from aquaculture such as gill and bone tissues.

In terms of element concentrations, no differences in Ca, K, Na, and P (macro-elements) were found among batches, which contradicts our hypothesis. This was probably because these elements play key roles in physiological processes, such as maintaining osmotic balance and acid-base equilibrium, as well as in the development and maintenance of the skeletal system (Lall 2003, Zimmer et al. 2019, Lall and Kaushik 2021). Furthermore, no data on the presence of these elements in ABFT bone and gill tissues were available in the scientific literature for comparison with our findings.

Notably, Cu, Mn, S, and Sr in gill tissue were the main contributors to batch differentiation, whereas the main contributors in bone tissue were Cu, Fe, Mg, and Zn, which agrees with our hypothesis. The great variability among batches may be because these are micro-elements (except for Mg) that participate in many physiological functions, biochemical processes, and metabolic pathways, as well as being crucial components or co-factors in certain enzymatic systems (Lall 2003, National Research Council 2011, Lall and Kaushik 2021). However, only 1 element (Cu) showed statistically significant differences among batches for both tissues (batch 3 exhibited higher concentrations than either batch 1 or 2; Table 1). This agrees with a previous study of ABFT soft tissues (brain, liver, muscle, and kidney; Salvat-Leal et al. 2023), in which Cu was the only element to show statistical differences between batches for all tissue types (higher concentrations were also observed in wild-caught specimens).

Table 2. Elemental-based canonical discriminant functions (CDFs) as outcomes of the discriminant canonical analysis (DCA), their contribution to the discrimination between groups (%), and their overall accuracy (%) by tissue type (gills and bone).

	Function	Eigenvalue	Explained variance (%)	Canonical correlation	Wilks' lambda	CDF Coefficients (standardized)
Gill	1	1.45	72.2	0.77	0.26	Cu (0.93), Mg (1.9), Mn (–2.65), S (–0.87), Zn (0.81)
	2	0.56	27.8	0.60	0.64	Cu (0.13), Mg (–0.04), Mn (–0.61), S (0.91), Zn (0.38)
Bone	1	1.91	79.7	0.81	0.23	Fe (0.82), Mg (2.04), Mn (–2.20), P (0.63), S (–0.61), Sr (–1.16), Zn (0.98)
	2	0.49	20.3	0.57	0.67	Fe (0.25), Mg (–0.17), Mn (–0.69), P (–1.22), S (0.13), Sr (1.45), Zn (0.52)

Despite being present at the lowest concentration among the elements analyzed, Cu is essential for cellular function (Lall and Kaushik 2021) and is always present in fish. The higher concentrations of Cu in wild-caught specimens than in captive-reared specimens could be due to greater exposure to this element in their prey (Bustamante et al. 2002, 2004, 2006). Indeed, compared to captive-reared fish, wild-caught tuna have a more varied diet that consists of small pelagic fish, shrimp, cephalopods, and crustaceans (Sarà and Sarà 2007, Sinopoli et al. 2004, Uotani et al. 1990), all of which require Cu for their respiratory processes (Lara Jacobo et al. 2016) and general biological functioning (Rjeibi et al. 2015). In contrast, captive-reared tuna are initially fed an artificial diet, followed by small thawed pelagic fish. Therefore, Cu could accumulate in wild tuna via the trophic chain and may be a useful marker for batch discrimination.

For the remaining elements with significant statistical differences, the bone tissues of batch 1 specimens showed higher concentrations of Mg than those of batch 2 and higher concentrations of Zn than those of batch 2 or batch 3 (Table 1). In addition, intermediate Fe concentrations were found in batch 1 specimens. Interestingly, the Mn and S concentrations in gill tissues were highest in batch 1 specimens. To the best of our knowledge, no data regarding these elements in juvenile ABFT gill or bone tissues have ever been published, although several studies of soft tissues have reported significantly higher concentrations in wild tuna than in farmed specimens (Vizzini et al. 2010, Percın et al. 2011, Sogut and Percin 2011, Milatou et al. 2015, Salvat-Leal et al. 2023).

This finding agrees with our Cu concentrations in bone tissue but not in gill tissue. Several hypotheses could explain this difference, including (1) the nature and function of this tissue (i.e., the gills are in direct contact with the environment and filter the surrounding water); (2) fluctuations in the conditions affecting our batches (e.g., diet, water composition, levels of physical activity, and interactions between elements within the organism); (3) the influence of physical factors (e.g., temperature, currents, and dissolved oxygen); and (4) the presence of food residues, feces, and urine (see review in Lall and Kaushik 2021).

Based on these observations, the distribution patterns revealed by multivariate analyses were examined. The PCA revealed no clear differences in the spatial distribution of individuals among the 3 batches, although the gills were the only tissue in which PC3 (Cu and Fe) showed a slight distinction for batch 2 specimens (Fig. 1), which agrees with the aforementioned significance of Cu concentrations in this tissue. By comparison, the introduction of PC3 (Cu and Fe) in the PCA for bone tissue did not improve batch separation, even though both elements showed statistically significant differences between batches 1 and 2. When comparing these results with studies of soft tissues (Salvat-Leal et al. 2023), superior discrimination has been reported in some soft tissues (kidney and muscle). Contrary to our hypothesis, it seems that this particular statistical test cannot be used with these hard tissues to distinguish ABFT batches.

Table 3. Discriminant canonical analysis (DCA) classification accuracy (*) and misclassification (%) by batch (batch 1 [tank-reared on land], batch 2 [captive-reared in the sea], batch 3 [wild-caught]) and tissue type (gills and bone).

	Function	1	2	3
Gill	1	79.2*	8.3	12.5
	2	13.6	77.3*	9.1
	3	0.0	17.2	82.8*
Bone	1	75.0*	12.5	12.5
	2	31.8	63.6*	4.5
	3	7.1	3.6	89.3*

In terms of the DCA, 2 functions whose performance indices agree with those reported by Balzarini et al. (2015) were created to maximize differences between populations. Significant discrimination was observed for all tissues (Wilks' lambda, $P < 0.05$), and the correlation coefficients indicated that the derived functions were useful, especially CDF1, which explained 59.1% of the total variance in gill tissue and 65.6% in bone tissue (when values higher than 45% were expected) (Torrado-Fonseca and Berlanga-Silvente 2013). For both tissues, Ca, K, and Na were not included in the DCA functions, which coincides with the previously noted absence of statistically significant differences for those elements. For both tissues, 4 elements were selected for the CDFs (Mg, Mn, S, and Zn); Cu was also selected for gill tissue and Fe and Sr for bone tissue. Surprisingly, Cu did not appear in the DCA selection of elements for bone tissue. Thus, there were elements in the DCA functions with and without statistical differences between batches (Table 1), indicating that 1 specific pattern could not be identified for discriminating among batches based on these elements. Finally, the gills showed the highest DCA success rate, with the lowest percentage of confusion between batches 1 and 3. This percentage was higher than those reported for ABFT liver and muscle tissues but lower than those reported for kidney and brain tissues (Salvat-Leal et al. 2023).

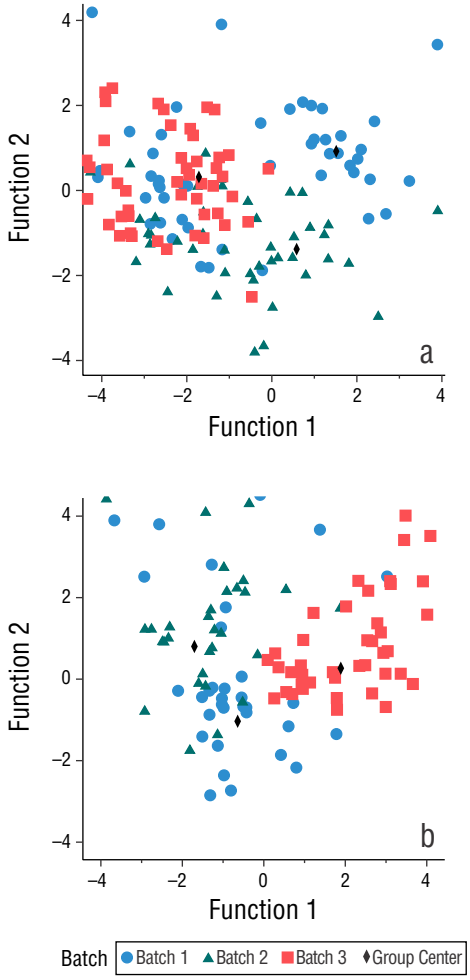

Figure 3. Spatial distribution of Atlantic Bluefin Tuna (ABFT; *Thunnus thynnus*) batches (batch 1 [tank-reared on land], batch 2 [captive-reared in the sea], and batch 3 [wild-caught]) based on functions from the discriminant canonical analysis (DCA) and group separation by tissue type: gills (function 1; explained variance [EV] = 27.8%; Cu = 0.933; Mg = 1.917; Zn = 0.809; S = –0.872; Mn = –2.647 | function 2; EV = 72.2%; S = 0.908; Zn = 0.378; Cu = 0.129; Mg = –0.042; Mn = –0.610) (**a**) and bone (function 1; EV = 20.3%; Mg = 2.042; Zn = 0.978; Fe = 0.816; P = 0.634; S = –0.611; Sr = –1.164; Mn = –2.197 | function 2; EV = 79.7%; Sr = 1.451; Zn = 0.522; Fe = 0.246; S = 0.133; Mg = 0.172; Mn = –0.688; P = –1.216) (**b**).

Table 4. Classification equations by batch (batch 1 [tank-reared on land], batch 2 [captive-reared in the sea], and batch 3 [wild-caught]) and tissue type (gills and bone).

	Batch	Equation
Gill	1	(–14.2) + (–0.607 × [Cu]) + (–22.8 × [Mg]) + (0.333 × [Mn]) + (44.0 × [S]) + (–0.037 × [Zn])
	2	(–6.44) + (0.059 × [Cu]) + (–5.04 × [Mg]) + (0.154 × [Mn]) + (21.86 × [S]) + (–0.019 × [Zn])
	3	(–10.2) + (1.00 × [Cu]) + (12.3 × [Mg]) + (–0.162 × [Mn]) + (23.3 × [S]) + (0.042 × [Zn])
Bone	1	(–12.0) + (–0.007 × [Fe]) + (–29.8 × [Mg]) + (0.592 × [Mn]) + (–0.322 × [P]) + (48.9 × [S]) + (0.033 × [Sr]) + (–0.054 × [Zn])
	2	(–14.2) + (–0.008 × [Fe]) + (–69.7 × [Mg]) + (0.701 × [Mn]) + (–2.11 × [P]) + (58.6 × [S]) + (0.079 × [Sr]) + (–0.051 × [Zn])
	3	(–14.1) + (0.001 × [Fe]) + (58.2 × [Mg]) + (–0.293 × [Mn]) + (–0.286 × [P]) + (32.8 × [S]) + (0.019 × [Sr]) + (0.129 × [Zn])

Conclusions

In conclusion, the results of the element concentration analysis and multivariate tests used in the present study (PCA and DCA) indicated that some degree of discrimination among ABFT batches can be achieved using the macro- and micro-elemental composition of soft gill tissues and hard bone tissues. However, while the results are promising, differences between batches were not always sufficiently consistent to establish a definitive pattern that would allow infallible batch identification based solely on some of the analyzed elements or on a single differentiation method.

Declarations

Supplementary material

This work includes no supplementary material.

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Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

Conceptualization: AO, DR; Data curation: ISL, DR, EB; Formal analysis: ISL, DR; Funding acquisition: AO; Investigation: ISL, EB; Methodology: AO, DR; Project administration: AO; Resources: AO, DR; Supervision: AO, DR, EB; Validation: EB; Visualization: ISL; Writing–original draft: ISL; Writing–review and editing: AO, DR, EB.

Data availability

The data for this study are available from the corresponding author by reasonable request.

Ethical approvals and permits for studies involving animals

Sampling of wild-caught tuna was conducted with the corresponding permits granted by the competent authorities (ICCAT REC 11-06). In accordance with European legislation (OJEU 2010), none of the procedures in the present study required ethical authorization.

Use of AI tools

The authors did not employ any AI tools in this work.

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