Dorsal fin spines as a non-invasive alternative calcified structure for microelemental studies in Atlantic bluefin tuna

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ABSTRACT

Chemical signatures in calcified structures of fishes represent natural tags of chemical and physical characteristics of the environment. The suitability of dorsal spines of Atlantic bluefin tuna (Thunnus thynnus) was investigated as an alternative non-invasive structure to otoliths. The spatial and temporal variability of trace elements was examined, for the first time, throughout the annual growth increments (i.e. translucent and opaque bands) of Bluefin tuna spines from the Bay of Biscay. Four scanning-ablation line transects were drawn and examined using LA-ICPMS to test for differences in tracer concentrations for each spine section. Firstly, results confirm the questionable spatial stability of tracers in dorsal fin spines. Secondly, most of the elements analyzed (88Sr, 137Ba, 24Mg, 55Mn, 7Li (biological essential elements), 65Zn and 65Cu (priority pollutants) were consistently found above the limit of detection (LOD). Strontium and Barium showed a similar pattern throughout annuli with concentrations significantly higher in the translucent bands in the second annulus (i.e. second winter). The concentration of Magnesium displayed an increasing pattern with annulus with no differences between translucent and opaque bands. In contrast, the concentration of Manganese showed a decreasing pattern throughout annuli, with concentrations significantly higher in the opaque bands (i.e. summer bands) regardless of annulus. Lithium, Copper and Zinc showed unclear pattern, although the concentration of Zinc seems to be in sync with the deposition of annuli currently interpreted in the ageing process. Findings indicate that the chemical signature of certain biologically essential elements is preserved stable in the dorsal fin spines strengthening its use as a non-invasive alternative structure for chemistry studies in Atlantic bluefin tuna.

1. Introduction

Over the past 20 years, chemistry of fish calcified structures (i.e. otoliths, scales, vertebrae and fin ray spines) has been applied for determining environmental histories of fish in a diverse range of aquatic environments including marine, estuarine and freshwater systems (Kerr and Campana, 2014). As such, it represents one of the most powerful tools to address fundamental questions in fish ecology and fisheries science, including stock structure, site fidelity, natal origin, and migration pathway over ecological time scale (Campana et al., 2000; Rooker et al., 2008; Smith and Whitledge, 2011; Walther and Limburg, 2012). The premise of this approach is that calcified structures in fishes generally form by the periodic deposition of daily and annual increments as the fish grows, which allow to determine ages and life history parameters (e.g. growth rate) of individual fish and fish populations (Campana and Thorrold, 2001). As these biogenic structures grow, trace amounts of elements (including heavy metals) are naturally incorporated into their mineral phase from the surrounding environment experienced by the fish (Miller et al., 2005; Lin et al., 2007). Coupling structure bio chronology with chemical record of fish's life (i.e. trace elements and/or isotopic composition) enables a retrospective description of individual fish environmental history including fish movements, life history traits, and ontogenetic development (Dufour et al., 2005; Whitledge et al., 2006; Whitledge, 2009).

The most widespread and expanding application of elemental markers in calcified structures has occurred using fish otoliths (Campana and Thorrold, 2001; Elsdon et al., 2008). Beside their chronological properties, otoliths have been the preferred bony structure to use in chemistry studies due to the fact that they are metabolically inert calcium carbonate structures (i.e. the newly deposited material is neither resorbed nor reworked after deposition) (Campana and Neilson, 1985), and hence, only ontogenetic and environmental factors should cause changes to their chemical composition (Campana, 1999). The extraction of otoliths requires...
sacrificing the fish, which is neither allowed for rare and/or endangered species, nor practical for commercially valuable fish species such as Atlantic bluefin tuna (Thunnus thynnus) as it affects the appearance of the fish diminishing its high market value. In this context, it is desirable to ascertain whether reliable microelemental data can be obtained from a non-invasive alternative calcified structure, expanding thus its use to rare, endangered and/or commercially valuable fish species. Recently, there has been an enormous growing interest in expanding the application of this method to alternative fish biogenic calcified structures including vertebrae (Smith et al., 2013), scales (Wells et al., 2003), bone (Balter and Lécuyer, 2010) and fin rays (Allen et al., 2009; Jarić et al., 2011). Particularly, teleost fin spines seem to provide an advantage over vertebrae as their extraction does not interfere with the market value of the fish.

The first spiniform ray of the first dorsal fin (fin spines hereafter) has proven useful for direct ageing purposes of Atlantic bluefin tuna, at least for small and medium sized specimens (Luque et al., 2014), and comparative analysis has recently shown that spine ages are in close agreement with otolith ages (Rodriguez-Marín et al., 2013). To assess the fin spines as an alternative structure for chemistry studies is particularly important for the eastern Atlantic bluefin tuna population since its current growth curve is based on estimates using this structure (JCCAT, 2013). Unlike otoliths, spines are calcium phosphate structures (commonly in forms of hydroxypatite) metabolically active and subject to elemental mobilization and resorption (often associated with periods of protracted nutritional stress), destroying early growth increments (Prince and Pulos, 1983; Hill et al., 1989). Consequently, this may limit their utility as records of the physiochemical environment used during fish’s lifetime. The degree of discrimination in the absorption and incorporation of elements into the spine and whether resorption occurs for this structure is still poorly understood (Gillanders, 2001). Besides, evidence suggests that chemical signatures are not transported across growth bands, so if there is metabolic reworking, it is likely to be minimal (Hale et al., 2006; Tillett et al., 2011). Despite this limitation, several studies have successfully used trace element analyses of fin rays to reconstruct the environmental history of several freshwater and anadromous fish species (Clarke et al., 2007; Smith and Whitledge, 2010; Jarić et al., 2011; Phelps et al., 2012), indicating that at least some components of spines are metabolically stable, allowing reconstructing individual fish environmental history as well as to examine fish movements and stock structure.

The main goal of this study was to explore the first dorsal fin spine of Atlantic bluefin tuna in terms of its chemistry to evaluate whether the structure preserves chemical signature that can potentially be used as natural markers of individual fish environmental history. Specific objectives include the examination of the variability of elemental composition spatially and temporally across the spine section. We also tested for differences in the element concentrations between the opaque and translucent bands either within and among transects (Fig. 1). Spines were ablated at several spots, equally spaced, ensuring that either the translucent and opaque bands were targeted along each laser transect. Ablation transects were marked out prior to analysis within the laser setting program. The number of ablation spots in each spine section was constrained by the ablation crater diameter and the width of the translucent and opaque bands for each individual spine (Fig. 1).

Spine sections were analyzed by laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS). The system consists of an ultraviolet laser ablation with high-resolution digital camera (NWR 213, New Wave Research, USA) and quadrupole inductively coupled plasma mass spectrometer with a Xs cone (ICPMS, Xseries II, Thermo Scientific, USA). Typical operating conditions of LA-ICPMS are given in Table 1.

NIST glass 614 from the National Institute of Standards and Technology (Gaithersburg, MD, USA) was used as the external standard to create calibration curves for elements of interest. The same parameters were applied to the standard except that the dwell time of ablation was set as 20 s. Calcium was assumed to be evenly contributed across the whole spine at a concentration of 16% (Davies et al., 2011), and used as the internal standard to correct variations in ablation yield and counting efficiencies of ICPMS. Pre-ablation (10 s) was written into the ACl script of Thermo Plasma Lab software and performed for each sample and standard before ablation. To avoid potential contamination, integration was only applied to the stable fragment of the spectrum, usually 5 s after ablation.

Standard analyses were performed at the beginning and every 10 runs. Eleven isotopes, corresponding to eleven elements (11 analytes and calcium) were quantified in the spines of Atlantic bluefin tuna, including biologically essential elements ($^{88}$Sr, $^{137}$Ba, $^{24}$Mg, $^{55}$Mn, $^{7}$Li, $^{59}$Co), priority pollutants ($^{208}$Pb, $^{60}$Ni, $^{60}$Cu, $^{60}$Zn), and emerging environmental pollutants ($^{51}$V). Normalization is essential in LA-ICPMS to compensate for variations in ablation yield because of energy drift and sample density. The intensity of the isotopes of interest was systematically normalized against the $^{48}$Ca signal after subtraction of the mean background signal. The ablation crater for all runs was 80 μm in diameter, and the estimated limit of detection (LOD) (μg/g) for examined elements was estimated as the quantity of analytes required to produce a signal equivalent to three times standard deviation of corresponding elements in the Nist 614 (n = 47). These LODs were estimated as: $^{88}$Sr: 3.77, $^{137}$Ba: 0.54, $^{24}$Mg: 6.46, $^{7}$Li: 0.45, $^{55}$Mn: 0.16, $^{51}$V: 0.27, $^{59}$Co: 0.12, $^{60}$Ni: 0.31, $^{60}$Cu: 0.32, $^{60}$Zn: 1.17, $^{208}$Pb:
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