A ‘machine learning’ technique for discriminating captive-reared from wild Atlantic bluefin tuna, *Thunnus thynnus* (Osteichthyes: Scombridae), based on differential fin spine bone resorption

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**ABSTRACT**

The Atlantic bluefin tuna (ABFT) fishery is regulated by the International Commission for the Conservation of Atlantic Tunas (ICCAT), which establishes the allowable annual yield and the minimum capture size, and allocates capture quotas to the Contracting Parties. Despite fishery monitoring, a considerable amount of catches escapes ICCAT control. In the Mediterranean Sea, the purse seine fishery supports ABFT farming, a capture-based aquaculture activity that involves catching fish from the wild and rearing them in sea cages for a few months. The first spine of the cranial dorsal fin undergoes a continuous bone remodeling process consisting in old bone (primary bone) resorption and new bone (secondary bone) apposition. A marked increase of spine bone resorption was shown in captive-reared ABFT with respect to wild specimens. In this paper, the Random Forest (RF) and Logistic Regression (LR) techniques were applied to distinguish captive-reared from wild ABFT based on fish age, fork length, total surface of spine cross section, and surface of remodeled bone tissue in the spine cross section (sum of reabsorbed bone tissue and secondary cancellous bone). The RF system was also compared to the Logistic Regression method (LR). The percentages of properly classified animals, either wild or captive-reared, with respect to the overall number of animals, i.e. accuracy, were 95.3 ± 2.6% and 79.0 ± 5.1% for RF and LR, respectively. The percentages of the properly classified captive-reared specimens, i.e. sensitivity, were 93.5 ± 3.1% and 75.8 ± 5.3% for RF and LR, respectively. The percentages of the properly classified wild specimens were 96.7 ± 2.2% and 81.4 ± 4.9%, for RF and LR, respectively. The proposed technique appears to be a reliable investigation tool anytime the suspicion arises that illegally caught ABFT are sold as aquaculture products.

**1. Introduction**

The Atlantic bluefin tuna (ABFT), *Thunnus thynnus* (Linnaeus, 1758) (Osteichthyes: Scombridae), is a very highly priced tuna species, which is subjected to an intense fishing pressure. The fishery of ABFT is regulated by the International Commission for the Conservation of Atlantic Tunas (ICCAT). This recognizes two different ABFT stocks, the western Atlantic stock and the eastern Atlantic stock; the geographical range of the latter includes the Mediterranean Sea. The two stocks are individually managed by ICCAT, according to specific regulations (ICCAT recommendations and resolutions are available at [http://iccat.int/en/RecsRegs.asp](http://iccat.int/en/RecsRegs.asp)). The ICCAT establishes the maximum annual yield in terms of fish biomass (total allowable catches; TACs) and retains the option to suspend all the ABFT fisheries in the event that any periodical stock assessment detects a serious threat of fishery collapse. The TACs are distributed among the ICCAT Contracting Parties (Countries that adhere to ICCAT), so that each of them is assigned a fishing quota. Specific ICCAT Observer Programs aimed at monitoring the amount of catches by fishing vessels and traps are currently implemented ([https://www.iccat.int/en/ROPbft.htm](https://www.iccat.int/en/ROPbft.htm)). Since 1990s, despite more and more management measures have been introduced, illegal, unreported and unregulated tuna fishing practices have increased (Miyake et al., 2004).

In March 2010, the Convention on International Trade in
Endangered Species of Wild Fauna and Flora (CITES) discussed the proposal by the Principality of Monaco to include ABFT in the Appendix I (http://www.cites.org/eng/cop/15/prop/E-15-Prop-19.pdf), which lists threatened species whose international trade is prohibited. The proposal was not adopted by the conference because of the opposition from some nations. The inclusion of ABFT in CITES Appendix A would have seriously affected the economic sustainability of the fisheries, mainly the purse seine fishery, whose activity fuels the international ABFT market. In fact, the majority of purse seine catches is destined for the Japanese market (Mylonas et al., 2010).

In the Mediterranean Sea, the purse seine fishery supports ABFT fattening and farming aquaculture activities, which are exclusively based on fish captured from the wild. Presently no commercial complete life cycle ABFT aquaculture exists. In Mediterranean tuna farms, wild-captured fish are reared for periods ranging from 3 months to 2 years (for a review on tuna farming and fattening see Mylonas et al., 2010). Rearing of adult ABFT individuals for a few months (usually 3–7 months) is classified as ‘fattening’ and aims at increasing the fish fat mass through a diet based on small, high fat content pelagic fish. In addition, tuna fattening allows a controlled input in the market, avoiding flooding it with ABFT during its short fishing term, which is instrumental in keeping ABFT quotations high. The tuna farming activity is authorized by ICCAT in the Adriatic Sea (Croatia) only and involves the capture of immature individuals and their rearing in captivity for up to 2 years (Ticina et al., 2007).

Since early 2000, an intense research effort aimed at transforming tuna fattening and farming activities in self-sustained aquaculture has been undertaken (Berkovitch et al., 2013; Corriero et al., 2009, 2007; De Metrio et al., 2010; Micera et al., 2010; Mylonas et al., 2007; Pousis et al., 2012, 2011; Rosenfeld et al., 2012). Under the stimuli of this scientific activity, carried out in the framework of different EU, national and regional projects, a true ABFT aquaculture industry is being developed so that, in December 2014 the first ABFT individuals born and grown out in captivity in a Spanish fish farm were placed on the market (cf. newspaper La Verdad, Murcia, Spain, 06 December 2014). Moreover, in very recent times (July 2016), the closure of the ABFT life cycle with the production of F1 generation in captivity has been achieved (announcement of the Spanish Institute of Oceanography; http://www.mispecies.com/nav/actualidad/noticias/noticia-detalles/El-IEO-cierra-el-ciclo-biolgico-del-atn-rojo-Atlntico-en-cautividad/#.V53AOriLTy2). The ABFT fully produced in captivity, which are morphologically identical to wild individuals, are not managed by ICCAT and, therefore, their production is outside the quota allowance system. Hence, once the ABFT self-sustained commercial aquaculture will be eventually established, there will be the real possibility that illegally caught ABFT might be smuggled into the market as individuals born in captivity, since no tools to distinguish between wild and reared ABFT are presently available.

The ABFT is provided with median (dorsal and anal) and paired (pectoral and pelvic) fins. The dorsal cranial fin is supported by 12–15 spiny rays (spines), the caudal dorsal one is supported by a spine followed by 11–13 soft rays (rays) (Tortonese, 1975). The first spine of the cranial dorsal fin is used for age determination since its transverse sections displays well-defined growth marks, called annuli, which are interpreted as periodic events (Corriero et al., 2005; Luque et al., 2014; Santamaria et al., 2015, 2009). Growth marks are caused by the progressive apposition of bone tissue on the external surface of the spine, which becomes apparent as an ordered series of alternate opaque and translucent rings, corresponding to a faster spring-summer and a slower autumn-winter apposition, respectively, which parallels body growth (Cort, 1991; Megalofonou and De Metrio, 2000; Santamaria et al., 2009). The ABFT first dorsal spine bone tissues undergo dynamic processes: while new compact bone is added on the spine outer surface, in its inner part (the so-called core or nucleus) a physiological progressive bone resorption occurs (Cort, 1991; Megalofonou and De Metrio, 2000; Santamaria et al., 2009, 2015). In a recent paper, Santamaria et al. (2015) assessed the spine bone apposition and resorption in both wild (aged 1–13 years) and captive-reared (aged 2–11 years) Mediterranean ABFT. They reported that: a) the spine section surface grows isometrically with respect to body size; b) the fraction of spine compact bone progressively decreases with both fish size and age; c) the phenomenon of spine bone resorption is dramatically enhanced in captive-reared ABFT individuals with respect to wild animals.

The aim of the present paper was to set up a method to discriminate between wild and captive-reared ABFT by means of a Computer Aided Detection (CAD) system trained to recognize the fish origin on the basis of a number of parameters related to spine bone resorption. The present ‘machine learning technique’, the so-called Random Forest (Breiman, 2001), as applied to the spine-related data, was compared to a classical technique of supervised classification, i.e. the Logistic Regression, in order to both evaluate its strength and assess the feasibility of its adoption for practical purposes. In addition, the most discriminating variables among the tested parameters were identified, so to warrant a more effective and straightforward use of the herein proposed method.

2. Material and methods

2.1. Sampling and spine measurements

The ABFT specimens which provided the data for the present study are the same used in Santamaria et al. (2015). The sampling procedure is concisely reported below; further details are reported in Santamaria et al. (2015). In all 428 ABFT specimens (186 wild and 242 captive-reared) were sampled over the eight-year period 2003–2010 in several Mediterranean sites (Fig. 1). Wild fish were caught by commercial vessels whereas captive-reared specimens were sampled in the framework of the following research projects: EU project REPRODOTT, EU project SELFDOTT and Italian project ALLOTUNA funded by the regional government of the Apulia region. The captive-reared ABFT were in fact wild-born, that is collected from the sea and kept in rearing cages for a period ranging from a few months to three years. The fish fork length, FL, was measured to the nearest cm. The first spine of the cranial dorsal fin was removed (Fig. 2a) from the fish and processed in the laboratory. A low speed diamond saw (Buehler, Isomet) was used to cut it transversally at a distance of half the maximum spine diameter from the condyle, according to the spine sectioning standard procedure (Luque et al., 2014), and obtain a 0.7 mm thick cross-section (Fig. 2b). An age (AGE) was assigned to each fish according to Santamaria et al. (2009). The following measurements were taken on each spine section (Fig. 2c):

SD, spine diameter (=maximum transverse diameter of the spine section);
TS, total surface;
RS, reabsorbed part surface (=surface of reabsorbed bone + surface of remodeling bone);
CT, compact bone thickness (=maximum thickness of the spine compact bone layer).

TS and RS are the same raw data used in Santamaria et al. (2015); SD and CT are new measurements, i.e. previously unreported.

The measurements were taken on spine section images, using an interactive function (i.e. measurements of operator-selected surfaces by a specific image analysis software function), by means of the image analysis software Quantimet 500 W (Leica, Wetzlar, Germany).

A table reporting all measurements is available in Supplementary material.

2.2. Supervised classification methods

2.2.1. The Random Forest classifier

The raw data deriving from the measurements were used to develop a predictive system to detect the fish origin, either wild or captive-
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