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Mini Review

Physicochemical Analysis Techniques: An Alternative for the Study of Bone Deformations in Salmonids

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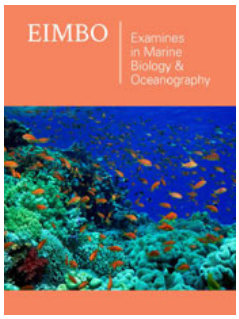

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Abstract

During salmonid development, some disturbances in the bone mineralization processes can occur, which, consequently, affect the microstructure of the bone. The objective of this work was to evaluate the composition of the bone and to perform an elemental quantification (Ca/P) of it, using physicochemical analysis tech-niques, such as: i) Scanning Electron Microscopy coupled to EDX (SEM-EDX), ii) Total Reflection X-ray Fluo-rescence (TXRF), and iii) BET Surface Area Analysis in rainbow trout (Oncorhynchus mykiss) with bone de-formation. Ten rainbow trout specimens weighing 400 grams and measuring from 25 to 35cms in length were analyzed, of which five were normal specimens and five had a mandibular deformation. The results show an increase in bone porosity and microfractures, associated with a moderate decrease in the bone mineral content.

Keywords: Deformation; Rainbow trout; Elemental physicochemical analysis

Introduction

During the process of bone formation, maturation and remodelling, farmed fish often suffer alterations in bone structure, which later manifests themselves in deformations [1-3]. For many years, the analysis of such deformations was carried out through histological studies of the mineralized tissue, which meant the decalcification of the said tissue and the obtention of histological sections of variable thickness, later visualized by light microscopy or transmission electron microscopy, in time-consuming processes prior to obtaining any results [4]. Although these histological techniques offer an analysis field of tissue architecture, they provide little quantifiable chemical information regarding the loss of mineralization. As a consequence, X-ray images have generally been used as complementary studies [4]. Although these protocols provide a panoramic view that allows us to observe the bone condition of the fish, this approach does not provide quantitative data on the loss of mineralization and does not allow detailed visualization of possible superficial damage to the bone structure, such as the presence of micro-fractures and porosity [5]. In this regard, the incorporation of physicochemical analysis techniques offers a quantifiable alternative, without the need to decalcify the bone tissue, making use of the same sample through a complete microstructural analysis by Variable Pressure Scanning Electron Microscopy coupled to an Energy Detector Dispersive X-ray (VP SEM-EDX). Subsequently, the sample is pulverized for surface area analysis (porosimetry) and elemental quantification through a Total Reflection X-ray Fluorescence (TXRF) technique.

Discussion

The SEM-EDX analysis allowed the demonstration of the presence of microfractures and increased porosity in the bone structure of fish with mandibular deformation. The differences in mineralization observed are directly related to the microstructural alteration of the bone (Table1). The elemental analysis obtained by TXRF (Table 2) differs comparatively to that of the SEM-EDX; the latter also shows the presence of carbon, a factor that constitutes an indicator of the organic component of bone (Table 1). This indicator has been reported to reflect a structural compensation mechanism [5,6] and in normal fish its value is lower than that observed in deformed fish. Regarding the presence and quantification of minerals, the percentages exhibited by both techniques showed higher values, given the homogenization by reduction by the pulverization process. In addition, with both techniques it was possible to detect the presence of other metals such as iron and aluminum [7]. The zones most affected by the mineralization corresponded to the areas of interest with greater porosity or presence of microfractures [5]. The porosity and surface area analysis showed a slightly larger pore size, while the available surface area was found to be much smaller (Table 3), indicating that the control fish were less likely to develop microfractures [5].

Table 1: Elemental microanalysis in rainbow trout from farmed (Scanning Electron Microscopy SU3500, Hitachi, Japan coupled to X-Flash Detector, Bruker, Germany).

Elemental Microanalysis (SEM-EDX)			
Elements	**Units (Mass percentage %)**	**Normal Fish**	**Deformed Fish**
Aluminium	%	**ND	0,90
Phosphorous	%	7,99	5,74
Oxygen	%	19,01	17,00
Potassium	%	1,20	2,24
Calcium	%	15.39	11,06
Iron	%	0,46	0,98
Carbon	%	54,05	61,19

Source: **ND: Non detected.

Table 2: X-ray Fluorescence analysis in rainbow trout from farmed (Total reflection X-ray fluorescence spectrometer S4 T-STAR, Bruker, Germany).

Total Reflection X-Ray Fluorescence Spectrometry (TXRF)				
Element Number	**Name**	**Units (Mass percentage %)**	**Normal Fish**	**Deformed Fish**
13	Aluminium	%	1,15	1,65
15	Phosphorous	%	16,03	16,26
16	Sulphurus	%	3,42	1,98
19	Potassium	%	15,76	21,65
20	Calcium	%	58,97	58,00
26	Iron	%	0,77	0,45

Table 3: Superficial area and porosity analysis in rainbow trout from farmed (Nova Win-Quantachrome, Boynton Beach, Florida, USA).

Superficial Area and Porosity Analysis (BET)			
Parameters	**Units**	**Normal Fish**	**Deformed Fish**
Porosity	nm	4,51	5,20
Superficial Area	m^2/g	66,39	44,63

Conclusion

Physicochemical analysis techniques offer a quantifiable result of bone mineralization in salmonids, mainly focused on the percentages of calcium, phosphorus and carbon. These techniques also allow detection of the presence of metals such as aluminum and iron, a fact that could be due to the characteristics of freshwater in volcanic areas. The complementary analyzes of SEM-EDX and TXRF provide details of the bone microstructure and its alterations in mineralization (SEM-EDX) and they provide more precise results (TXRF) of the different elements present in the bone. The present work contributes in an important way towards a more precise multi-factor analysis of the existing mandibular bone deformations in farmed salmonids.

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