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EXAMINATION OF THE STRUCTURAL CHEMISTRY OF THE OTOLITHS OF RED PORGY, *Pagrus pagrus* BY RAMAN ANALYSIS

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Abstract

The present study focused the determine structural chemistry of the otoliths of red porgy, *Pagrus pagrus*, by using Raman analysis. The Raman analysis was conducted within the spectral scanning range of 3785-50 cm^{-1}. According to the Raman spectrum of otoliths, it becomes evident that there are two significant vibration bands that contribute to the otolith's main structure. The first is the intense vibration band at 1095 cm^{-1}, known as the symmetric (V1) band of the carbonate ($-CO_3^{-2}$) molecule. The second is the moderate in-plane bending band at 710 cm^{-1}, referred to as the in-plane (V4) band. These bands play a fundamental role in the structure of the otolith. Additionally, the Raman spectrum of the otolith reveals the presence of bands related to metal bonds forming with the carbonate ($-CO_3^{-2}$) molecule. These bands are observed at 155 and 210 cm^{-1} and exhibit a moderate intensity. However, it is important to note that the specific cations or metal bonds involved cannot be determined solely from these bands. The Raman analysis of red porgy sagittal otolith composition and structure has provided valuable insights into the chemical composition and structure of these fish ear stones. The application of Raman spectroscopy to examine otoliths seems like a highly useful technique for understanding the composition and structure of these crystals. By utilizing Raman spectroscopy, researchers can gain valuable insights into the chemical composition and structures of otoliths in the inner ear. This examination allows for the determination of the minerals and elements contained within otoliths.

Keywords: *Pagrus pagrus*, sagittal otolith, otolith chemistry, Raman spectroscopy

Introduction

It is essential to first understand the population structure within the respective region to preserve and ensure the continuity of biodiversity in seas. Parameters such as the species, feeding habits, age, and mortality rates of the organisms within the population provide valuable insights into the structure of the ecosystem (Ayyildiz, 2011). Red porgy (*Pagrus pagrus*) belongs to the class Actinopterygii (ray-finned fish), the order Perciformes (perch-like fish), and the family Sparidae. These demersal species are typically found in rocky and stony seabeds. They have a distribution range from 200 meters in the Mediterranean to 300 meters in the Atlantic, with a general depth range of 20-100 meters (Hood and Johnson, 2000; Manooch and Hassler, 1978). Red porgy, like some other Sparidae members (*Pagrus pagrus, Pagellus erythrinus, Pagrus ehrenbergi, Pagellus acarne*), exhibits hermaphroditic characteristics (Alekseev, 1982; Devlin & Nagahama, 2002). Otoliths are calcium carbonate structures found in the inner ear of fish, and they can provide important information about the fish's growth, age, and environmental conditions. In addition, the otoliths record the chemical composition of the water in which fish inhabit, serving as metabolically inert aragonite structures that can change in response to the physical and chemical properties of the environment (Campana, 1999). Consequently, they have the potential to serve as a chronicle of the environments fish have inhabited, whether temporarily or permanently (Campana, 1999; Campana & Neilson, 1985). An otolith grows by the successive addition of elements and compounds from the environment onto its growth surface. As this growth spans from the egg stage to maturity and throughout the life stages until death, it records all aspects of a fish's life history. Therefore, otoliths can provide insights into the environment in which the fish has resided. Otolith chemistry, therefore becomes a potential tool for the chronological record of the fish's habitat. Additionally, the chemical compositions in otoliths are also used to determine differences among fish stocks (Campana et al., 2000; Gillanders & Kingsford, 2000; Rooker et al., 2003). Otolith chemistry is employed for identifying the migration routes of fish, enabling the detection of fish movements (Ashford et al., 2008; Steer et al., 2010).

The main purpose of this study is to examine the structural chemistry of the otoliths (ear stones) of red porgy, *Pagrus pagrus* using Raman analysis. This study aims to determine the chemical composition and structure of the otoliths, which can provide valuable insights into the fish's growth, age, environmental conditions, and migration behavior.

Material and Method

Study area

Gökçeada has an area of 290 km^2 and a coastline of 95 km. It ranks as the world's fourth-largest island in terms of abundant water resources. Gökçeada is influenced by intense current systems, boasting a unique marine ecosystem. It is also known that this current system creates surface stratification (Olson et al., 2007). Especially during the summer and winter months, waters from the Bosphorus directly reach the southern part of Gökçeada. In addition, strong north winds during the summer months lead to upwelling in the Aegean Sea. It is known that the cold waters at the bottom of the North Aegean Sea rise to the surface, causing a temperature change of 3-5°C (Zodiatis et al., 1996). This study was conducted between Gizli Harbor in the north of Gökçeada and Kefaloz Cape, at depths ranging from 40 to 120 m.

Preparation of Otoliths

The sagittal otoliths were removed by first dissecting the head with scissors, which had been separated from the body. The dissection was performed symmetrically from the neck towards the eyes on the dorsal side. Subsequently, using fine forceps, the right and left otoliths located on both sides of the head were extracted from their positions (Figure 1). Once the otoliths were removed, they were cleaned of any tissue particles on their surface. After cleaning they were placed in Eppendorf tubes for Raman analysis.

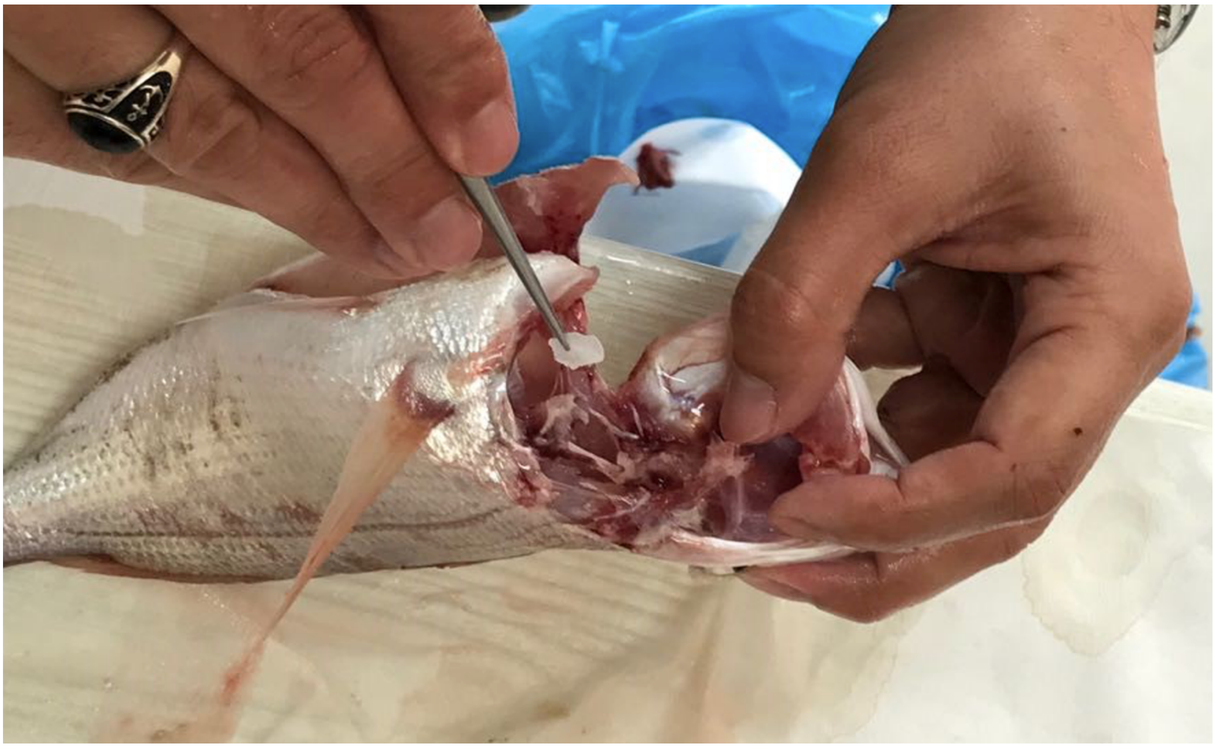

Figure 1. Image of otolith removal from fish samples

Raman Analysis

Raman spectroscopy is based on the measurement of light scattered at a specific angle when a monochromatic beam of light is directed onto the sample using a laser source. In this technique, as molecules and structures in the sample interact with the monochromatic beam of light, instead of light absorption, light scattering occurs. Non-elastic scattering during light scattering generates Raman scattering. Since laser-type sources are used to generate the light that interacts with molecules in the sample, this method is also called Laser Raman Spectroscopy. Raman analysis was conducted using the Witec Alpha 300RA confocal Raman imaging device located in the ÇOBİLTUM Center Laboratory at Çanakkale Onsekiz Mart University. The device used a 50x objective and a 532 nm green laser. The device was set with a 1800 g/mm grating, a BLZ (Blaze) of 500, and an integration time of 0.05 seconds. In the Raman spectral imaging system, a complete Raman spectrum was obtained for each image pixel. The device has the capability for manual sample positioning, planar (x-y direction), depth scanning (z direction), and automatic multi-area and multi-point measurements.

Results and Discussion

Otoliths are important structures in fish for balance and hearing (Popper & Lu, 2000). They are composed of calcium carbonate ($CaCO_3$) and protein, and otolith chemistry serves as a valuable tool for fish ecology and fisheries management. The chemical composition of otoliths can provide information about a fish's diet, habitat, and migration behavior. The chemical composition of otoliths can also reflect a fish's growth rate and diet, which can be also used to determine its age. Otolith chemistry can be used to determine whether fish of the same species belong to different stocks. The chemical composition of otoliths also reflects the chemical composition of the water where the fish lives. This information can be used to track fish migration patterns. Otolith chemistry is a crucial tool for fish ecology and fisheries management. This information can be used for the conservation of fish stocks and the development of sustainable fisheries (Campana & Thorrold, 2001; Ryer & Campana, 2015; Sato & Tsukamoto, 2019). When examining otolith structural components, it is generally seen that elemental contents are examined by Inductively coupled plasma atomic emission spectrometry/ Mass Spectrometer (ICP-AES/MS) (Campana et al., 2000; Francoet al., 2014; Disspain et al., 2016; Miyana et al., 2016; Gibb et al.; 2017). Inductively Coupled Plasma (ICP) is a powerful analytical technique used for elemental analysis. Despite its many advantages, there are some limitations or disadvantages associated with this method. ICP is a method to determine the elements in the samples qualitatively and quantitatively. It cannot provide chemical bonds in the sample and technical information about these bonds. Raman analysis is a powerful analytical technique that offers several advantages across various fields. Raman spectroscopy is non-destructive, meaning it doesn't damage or alter the sample being analyzed. Raman analysis provides detailed information about the chemical composition and molecular structure of a sample. By measuring the interaction between light and molecular vibrations, it reveals molecular bonds and functional groups present in the material. Compared to many other analytical techniques, Raman analysis provides relatively fast data acquisition. This rapidity allows for high-throughput analysis, particularly beneficial in industrial settings where timely decisions are essential. It was aimed to differentiate this study from other studies and to provide information on the chemistry of the otolith with a different analysis technique.

In this study, Raman analysis of the sagittal otoliths of red porgy, *Pagrus pagrus* were performed. The spectrum of these otoliths is given in Figure 2. There is no significant difference between the samples according to the Raman spectrum. Raman analysis of otoliths is a technique used to examine the chemical composition of otoliths. Raman analyses of otoliths are an important tool in otolith chemistry. These techniques enable the rapid and precise determination of the chemical composition of otoliths. Raman spectroscopy determines chemical composition by using the vibrations of molecules. Changes in the Raman spectral characteristics of otoliths can reflect a fish's growth. This information can be used to determine the fish's age. Variations in the Raman spectral characteristics of otoliths can also determine whether fish of the same species belong to different stocks. This information can be used to differentiate fish stocks. Additionally, it can reflect the chemical composition of the water in which the fish lives. This information can be used to track fish migration patterns (Campana & Thorrold, 2001; Ryer & Campana, 2015; Sato & Tsukamoto, 2019). Studies conducted using Raman spectroscopy on otoliths can be used to determine the quantity and composition of calcium carbonate ($CaCO_3$) and proteins found in the growth layers of otoliths. This information can provide insights into a fish's diet, habitat, and migration behavior.

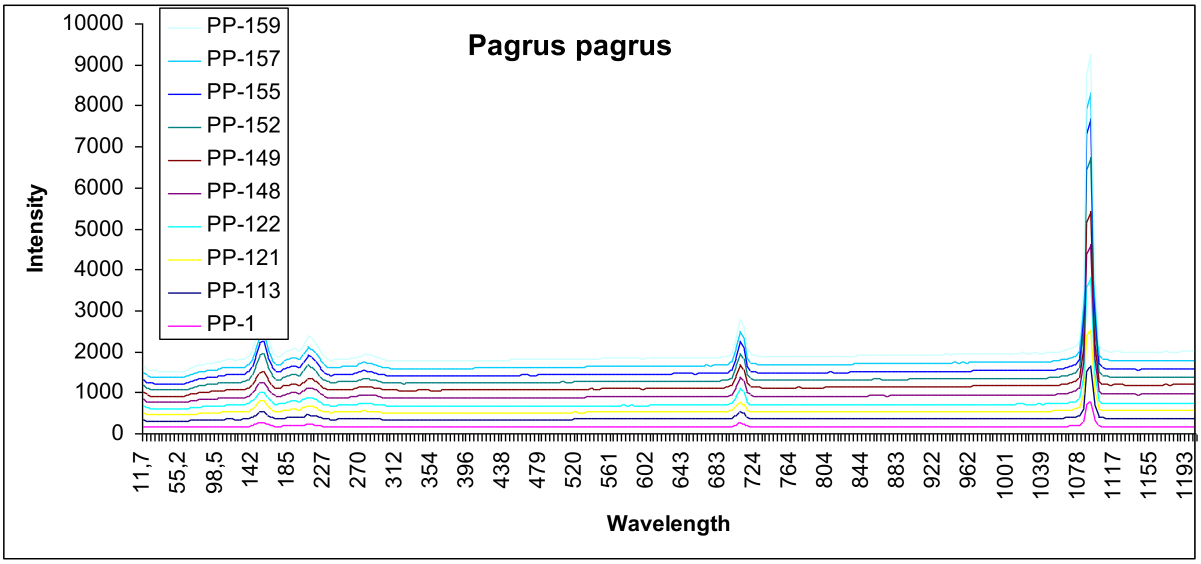

Figure 2. Raman spectrum of the sagittal otoliths of red porgy (*Pagrus pagrus*)

Raman analysis was conducted in the spectral scanning range of 3785-50 cm^{-1}. Figure 3 (B) provides Raman spectra of characterized Aragonite $Ca(CO_3)$ mineral crystals obtained from a 532 nm laser source for comparison purposes (RRUFF Project, n.d.). The Raman spectrum of the otolith from *Pagrus pagrus* (PP121) is shown in Figure 3 (A). In the otolith Raman spectrum, the symmetric (V1) 1095 cm^{-1} intense vibration band of the carbonate ($-CO_3^{-2}$) molecule, as well as an in-plane (V4) 710 cm^{-1} moderate in-plane bending band, which are fundamental to the otolith's main structure, are observed. Bands related to metal bonds forming with the carbonate ($-CO_3^{-2}$) molecule are observed at 155 and 210 cm^{-1} with moderate intensity. However, it is not possible to determine which cations or metal bonds are involved from these bands. In Figure 3 (B), spectral data from characterized minerals within the scope of the RRUFF Project (n.d.) are provided, specifically the Raman spectra of Aragonite, which is a $Ca(CO_3)$ mineral crystal (RRUFF Project, n.d.). In the Raman spectrum, it is evident that the primary Raman peaks corresponding to calcium carbonate, which is the fundamental structure of the aragonite mineral, occur at 1085, 705, and 140-280 cm^{-1}. The Raman peaks of carbonate ($-CO_3^{-2}$) fundamental to the otolith's basic structure from the Raman analysis within the RRUFF Project (n.d.) complement each other.

This study stands out for the significant feature of using Raman analysis in otolith chemistry. The results obtained from Raman analysis perfectly matches with the reference $CaCO_3$ spectrum. This demonstrates that the primary fundamental structure comprising the otolith is indeed calcium carbonate. Otoliths are small calcium carbonate crystals found in the inner ear and are related to balance control. Examining otoliths using Raman spectroscopy can be used to understand the composition and structure of these crystals. Raman spectroscopy is a highly useful technique for investigating the chemical composition and structures of otoliths in the inner ear. This examination is used to determine the minerals and elements contained in otoliths. Investigating otoliths through Raman spectroscopy is a potential research area for understanding and diagnosing balance control issues or inner ear diseases (Zong et al., 2009; Thakur and Ramaswamy, 2012). Wood et al. (2022) conducted a study to investigate the impact of fish otolith mineralogy on trace element chemistry studies. The study revealed that otoliths can comprise three prevalent calcium carbonate polymorphs, namely aragonite, calcite, and vaterite. In this study, the mineral compositions of Chinook salmon otolith pairs were determined using neutron diffraction and Raman spectroscopy. Additionally, it was found that most otoliths contained at least some calcite. The study concluded that the mineral compositions of otoliths could influence otolith trace element concentrations and, therefore, affects environmental interpretations. This study emphasizes the importance of understanding otolith mineralogy for a better comprehension of otolith microchemistry studies (Wood et al., 2022).

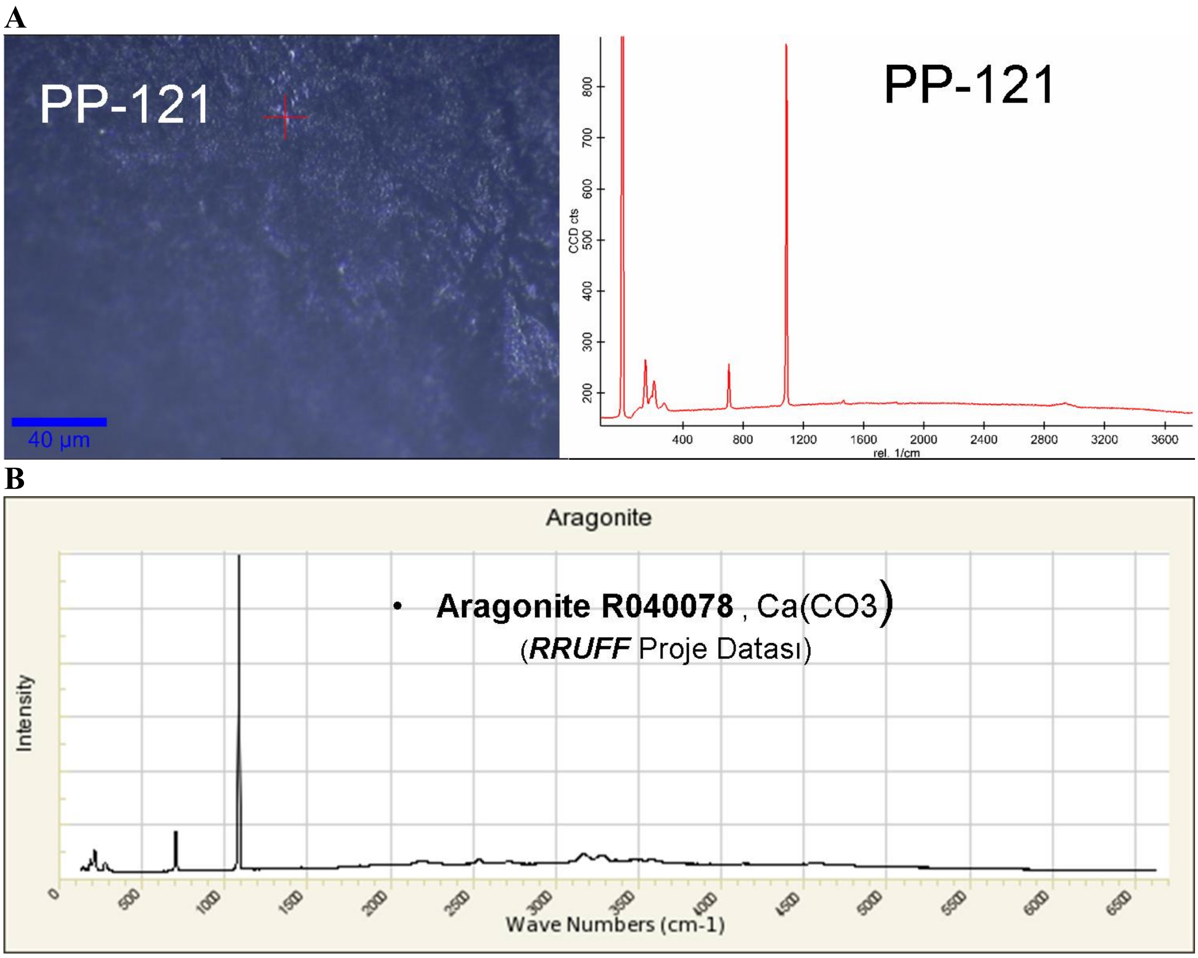

Figure 3. Raman spectrum of A) Red porgy (*Pagrus pagrus*, PP121) otolith and B) Aragonite Ca(CO_3) mineral crystal (RRUFF Project, n.d)

Conclusion

Raman spectroscopy utilizes molecular vibrations to determine the chemical composition of a substance. Analyzing otoliths using Raman spectroscopy is a valuable technique for examining their chemical composition. It serves as an important tool in otolith chemistry, allowing for rapid and precise determination of their composition. The Raman spectrum of the sagittal otoliths of the red porgy, *Pagrus pagrus* exhibits several significant features. First, the symmetric (V1) 1095 cm^{-1} intense vibration band of the carbonate ($-CO_3^{-2}$) molecule, which is fundamental to the otolith's main structure, is clearly observed. Additionally, an in-plane (V4) 710 cm^{-1} moderate in-plane bending band, also essential to the otolith's structure, is determined. These two bands provide valuable insights into the composition and structure of the otolith. Furthermore, the Raman spectrum of the otolith reveals the presence of bands related to metal bonds forming with the carbonate ($-CO_3^{-2}$) molecule. Specifically, bands at 155 and 210 cm^{-1} with moderate intensity suggest the involvement of cations or metal bonds. However, determining the specific cations or metal bonds solely based on these bands is challenging. In conclusion, the Raman analyses of otoliths conducted in this study revealed no significant differences between the samples based on the Raman spectrum. Raman analysis of the sagittal otoliths of red porgy, *Pagrus pagrus* has provided valuable insights into its composition and structure.

Ethical approval

The animal study protocol was approved by the Institutional Review Board (or Ethics Committee) of Canakkale Onsekiz Mart University, Animal rights local ethics committee (protocol code 2017/39650 and 27.11.2017).

Informed consent

Not available.

Data availability statement

The authors declare that data can be provided by corresponding author upon reasonable request.

Conflicts of interest

There is no conflict of interests for publishing this study.
The corresponding author is responsible on behalf of all authors' declaration.

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Contribution of authors

All authors of the study made contributions in various aspects of the research. They were involved in the preparation of the samples, conduction of experiments, evaluation of results, and writing of the article. Each author likely had specific roles and responsibilities within these areas, but the exact details of their individual contributions are provided below.
Bayram Kızılkaya: Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing original draft, Review and editing
Hakan Ayyıldız: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing original draft, Review and editing
Aytaç Altın: Conceptualization, Investigation, Methodology, Resources, Review and editing

"All authors have read and agreed to the published version of the manuscript."

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