

# UC San Diego

## UC San Diego Electronic Theses and Dissertations

### Title

Applications of Fourier Transform Infrared (FT-IR) Spectroscopy for Fish Identification Using Scales and Otoliths

### Permalink

<https://escholarship.org/uc/item/4g33h6vf>

### ISBN

9798190936261

### Author

Callahan, Sarah Grace

### Publication Date

2026-09-15

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA SAN DIEGO

Applications of Fourier Transform Infrared (FT-IR) Spectroscopy for Fish Identification Using  
Scales and Otoliths

A Thesis submitted in partial satisfaction of the requirements  
for the degree Master of Science

in

Marine Biology

by

Sarah Callahan

Committee in charge:

Brice X. Semmens, Chair  
Dahiana Arcila  
Ricardo Betancur  
Rasmus Swalethorp

2026

Copyright

Sarah Callahan, 2026

All rights reserved.

The Thesis of Sarah Callahan is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

University of California San Diego

2026

## DEDICATION

This thesis is dedicated in full to the greatest support system in the world: my family, my partner, and my friends. All that I am, I have received, and everything I have received has come from you. Thank you.

## TABLE OF CONTENTS

|                                                                                  |      |
|----------------------------------------------------------------------------------|------|
| THESIS APPROVAL PAGE .....                                                       | iii  |
| DEDICATION.....                                                                  | iv   |
| TABLE OF CONTENTS .....                                                          | v    |
| LIST OF FIGURES .....                                                            | vii  |
| LIST OF TABLES.....                                                              | viii |
| ACKNOWLEDGEMENTS.....                                                            | ix   |
| ABSTRACT OF THE THESIS .....                                                     | xi   |
| INTRODUCTION .....                                                               | 1    |
| Chapter 1. MATERIALS AND METHODS.....                                            | 6    |
| <i>i. Sample Collection</i> .....                                                | 6    |
| <i>ii. Scale Preparation</i> .....                                               | 8    |
| <i>iii. Otolith Preparation</i> .....                                            | 8    |
| <i>iv. FT-IR/ATR Spectral Acquisition</i> .....                                  | 9    |
| <i>v. Statistical Analysis</i> .....                                             | 12   |
| <b><i>Acknowledgements</i></b> .....                                             | 13   |
| Chapter 2. RESULTS .....                                                         | 14   |
| <i>i. Species discrimination</i> .....                                           | 17   |
| <i>ii. Location discrimination</i> .....                                         | 20   |
| <i>iii. Comparing scales and otoliths from species with paired samples</i> ..... | 23   |
| <b><i>Acknowledgements</i></b> .....                                             | 24   |
| Chapter 3. DISCUSSION .....                                                      | 25   |
| <i>i. Species Discrimination</i> .....                                           | 25   |
| <i>ii. Location discrimination</i> .....                                         | 26   |
| <i>iii. Scales outperformed otoliths</i> .....                                   | 28   |

|                                                              |    |
|--------------------------------------------------------------|----|
| <i>iv. Regional differences within structures</i> .....      | 30 |
| <i>v. Methodological Limitations and Uncertainties</i> ..... | 31 |
| <b><i>Acknowledgments</i></b> .....                          | 32 |
| Chapter 4. CONCLUSION.....                                   | 33 |
| <b><i>Acknowledgements</i></b> .....                         | 33 |
| REFERENCES .....                                             | 34 |

## LIST OF FIGURES

|                                                                                                                      |    |
|----------------------------------------------------------------------------------------------------------------------|----|
| <b>Figure 1.</b> Sampling regions represented in the scale and otolith FT-IR datasets. ....                          | 7  |
| <b>Figure 2.</b> Representative imaging of calcified structures during FT-IR spectroscopic analysis. .               | 10 |
| <b>Figure 3.</b> Mean FT-IR spectra of scales and otoliths by anatomical region. ....                                | 11 |
| <b>Figure 4.</b> Unsupervised Principal Component Analysis (PCA) on scales and otoliths. ....                        | 17 |
| <b>Figure 5.</b> Linear Discriminant Analysis (LDA) canonical score plot (LD1 vs. LD2) for scales and otoliths. .... | 19 |
| <b>Figure 6.</b> Geographic location discrimination from scale FT-IR spectra. ....                                   | 22 |
| <b>Figure 7.</b> Geographic location discrimination from otolith FT-IR spectra.....                                  | 23 |
| <b>Figure 8.</b> Scale and otolith LD1 scores for paired fish samples. ....                                          | 24 |

## LIST OF TABLES

|                                                                                                                   |    |
|-------------------------------------------------------------------------------------------------------------------|----|
| <b>Table 1.</b> Overview of scale samples and FT-IR measurement points before and after quality control.....      | 15 |
| <b>Table 2.</b> Overview of otolith samples and FT-IR measurement points before and after quality control.....    | 16 |
| <b>Table 3.</b> Per-species classification accuracy for scale and otolith spectra by region input. ....           | 18 |
| <b>Table 4.</b> Species classification accuracy by tissue type and region input.....                              | 20 |
| <b>Table 5.</b> Per-species geographic classification accuracy for scale and otolith spectra by region input..... | 21 |

## ACKNOWLEDGEMENTS

I would first like to express all my gratitude to my advisor, Brice X. Semmens, and to Rasmus Swalethorp, for their unwavering support and guidance throughout this journey. I am deeply appreciative for not only the words of wisdom they have provided but also the opportunities. From volunteering on research cruises to touring NOAA facilities to getting to drive the “lab truck,” every memorable experience I’ve had was made possible through my advisors. Thank you.

I would like to thank my committee members, Dahiana Arcila and Ricardo Betancur for their feedback throughout this process. I am eternally grateful to have been supported by such accomplished and inspiring scientists, and I thank them for all the knowledge and advice they provided me during our meetings.

I also thank Toni Sleugh, Noah Ben-Aderet, and Brendan Talwar for providing the samples used in this study. This work would not have been possible without their generosity, time, and guidance.

Chapter 1, in full, is part of a paper currently being prepared for submission coauthored with Swalethorp, R., Ben-Aderet, N., Sleugh, T., Semmens, B.X. The author of this thesis was the primary investigator and author of this material.

Chapter 2, in full, is part of a paper currently being prepared for submission coauthored with Swalethorp, R., Ben-Aderet, N., Sleugh, T., Semmens, B.X. The author of this thesis was the primary investigator and author of this material.

Chapter 3, in full, is part of a paper currently being prepared for submission coauthored with Swalethorp, R., Ben-Aderet, N., Sleugh, T., Semmens, B.X. The author of this thesis was the primary investigator and author of this material.

Chapter 4, in full, is part of a paper currently being prepared for submission coauthored with Swalethorp, R., Ben-Aderet, N., Sleugh, T., Semmens, B.X. The author of this thesis was the primary investigator and author of this material.

## ABSTRACT OF THE THESIS

Applications of Fourier Transform Infrared (FT-IR) Spectroscopy for Fish Identification Using  
Scales and Otoliths

by

Sarah Callahan

Master of Science in Marine Biology

University of California San Diego, 2026

Brice X. Semmens, Chair

The ability to accurately identify fish species is crucial for fisheries management and stock assessments. Traditional techniques such as morphological or genetic analysis can be costly, time-consuming, and often destructive to the sample. This study evaluates the utility of Fourier transform infrared (FT-IR) spectroscopy as a rapid, cost-effective, and non-destructive

tool for fish identification. I collected spectral data from both otoliths and scales of several recreationally important fish in California. I then standardized the data following quality control and then used principal component analysis (PCA) to reduce dimensionality prior to performing linear discriminant analysis (LDA). The spectral data collected from the scales consistently displayed higher classification accuracy than spectral data from the otoliths. This may be due to stronger organic signatures in the scales that are associated with a greater abundance of organic material, particularly proteins such as collagen, relative to otoliths. The greater variability exhibited in the otolith spectra may reflect the heterogeneity in calcium carbonate composition across the growth regions of the samples. Overall, classification performance varied among species, with some displaying relatively high discrimination and others limited by small sample sizes and overlapping spectral features. Overall, the findings of this study support FT-IR spectroscopy as a promising and efficient tool for fish species identification with applications for fisheries monitoring and the field of ecological and paleo research.

## INTRODUCTION

Effective management of marine fisheries in California relies heavily on the ability to accurately identify species and distinguish among populations. Being able to distinguish populations is particularly useful for stock assessment and conservation efforts, as differentiating among populations is critical for understanding species aspects such as population size and age structure, recruitment, growth, habitat use, and environmental influences, all of which inform sustainable management strategies (Campana, 1999). However, in many population-monitoring cases where a full specimen would be ideal to examine, only particular biological structures, such as otoliths or scales, are available for analysis. This occurs often in studies of dietary reconstructions, sediment samples, and predator scat analyses (Campana, 2004). Similarly, fisheries monitoring programs and trawl surveys often retain and archive otoliths for age determination. This leaves these structures as the primary biological material available for subsequent analyses. The ability to reliably identify species and infer population origin from these often small, isolated structures is a key part of efforts to assess populations and improve fisheries management.

Traditional methods for identifying fish species from otoliths or scales include assessing morphological characteristics of the samples, genetic approaches, and otolith trace element analysis. Although proven to be effective, each of these approaches has unique limitations. Approaches relying heavily on morphological characteristics can be limited by similarity in shape and size among species, as well as morphological changes associated with different stages of individual maturity, which can complicate accurate species identification (Vasconcelos et al., 2021). Genetic approaches can offer higher accuracy but are often costly, time consuming, and destructive to samples (Thompson et al., 2017). Otolith trace element analysis provides

additional insight into environmental history and population structure (Campana, 1999), but typically requires specialized instrumentation and extensive sample preparation, making it less efficient and more expensive than other approaches. These limitations highlight the need for alternative methods that are rapid, cost-effective, and non-destructive while maintaining high discriminatory power.

The rising potential of Fourier transform infrared (FT-IR) and near infrared (FT-NIR) spectroscopy has extended into the field of marine biology and fisheries management, where both techniques show considerable promise for biological applications. FT-IR and FT-NIR spectroscopy detect molecular vibrations associated with chemical bonds, generating spectral signatures that represent the unique biochemical composition of a sample (Saas et al., 2024). These spectral outputs are characterized by distinct absorbance peaks and troughs at specific wavelengths, which can be analyzed using multivariate statistical approaches to distinguish among different samples. FT-NIR and FT-IR offer similar advantages such as rapid data acquisition and the ability to analyze archived materials without damaging them (Wedding et al., 2014), but FT-NIR, in particular, is commonly used for bulk sample analysis and operates within the near-infrared region of the electromagnetic spectrum (approximately 800-2,500 nm). In contrast, FT-IR spectroscopy operates within the mid-infrared region, between 4000 and 666  $\text{cm}^{-1}$  (Mohamed et al., 2017) and provides higher spatial resolution, allowing targeted analysis of specific regions within a sample at spatial scales ranging from approximately 1  $\mu\text{m}$  to several hundred  $\mu\text{m}$ . With their specific uses but complementary abilities, both FT-IR and FT-NIR are well suited for analyzing biological materials and are applicable to ecological research (Goldstein et al., 2021).

Both FT-IR and FT-NIR approaches are becoming more prevalent in marine-related applications. For example, FT-NIR spectroscopy has been used successfully to differentiate between cryptic species of *Eteline* snappers with high classification accuracy, even when using archived otoliths (Dahl et al., 2024). Thus, this technique can be applied in the analysis of current studies and has the potential to revisit historic samples from previous studies. Similar to FT-NIR, FT-IR spectroscopy has been used to identify differences in otolith mineralization and crystalline structures associated with the environment and rearing conditions of the fish, demonstrating that biochemical composition, detected using spectroscopy, can reflect underlying environmental and ecological influences (Lewis et al., 2022). These findings highlight the potential of spectroscopy-based approaches for resolving fine-scale compositional differences within biological structures, which can then be applied to species discrimination and geographic population differentiation.

Despite the recent advances in the application of spectroscopy to marine science, gaps remain in the application of spectroscopy to fisheries research specifically. Most studies have focused primarily on otoliths as the structure of interest, rather than spreading the focus to alternative calcified tissues such as scales. Additionally, while species-level discrimination has been well explored, fewer studies have examined within-species variation across geographic locations and the potential ability to differentiate between different populations of the same species. Additionally, comparing the relative utility of different biological structures, such as fish otoliths and scales, for capturing environmental signals is another open area of interest.

Analyzing both otoliths and scales together using spectroscopy could provide new insights previously limited by focusing on just one calcified tissue, as the different tissues may have different but complementary absorbance features across specific spectral regions. Otoliths are internal calcium carbonate structures involved in balance and hearing and are widely used to

reconstruct age and life history (Neat et al., 2008). In contrast, scales are external structures that are in contact with the surrounding environment and may respond more rapidly to environmental conditions (Tzadik et al., 2017). Examining both otoliths and scales together may be useful since otoliths, as internal structures, are metabolically inert whereas scales contain softer tissues with higher metabolic turnover and generally grow more rapidly (Tray et al., 2022). As a result, scales may record more recent environmental and physiological signatures than otoliths while providing complementary information about an individual's life history (Tray et al., 2022). The spectral variation in fish scales is commonly associated with organic components such as collagen, as well as carbohydrates, lipids, and other biomolecules present within the scale and epidermal tissues (Qin et al., 2022). Many of these compounds contribute to absorbance features within the amide I and amide II regions, which are strongly linked to protein structure and composition (Aloglu & Harrington, 2018; Qin et al., 2022). Otolith spectra, alternatively, are typically more dominated by absorbance features related to calcium carbonate (Yasmeen et al., 2025). Additionally, the fingerprint region of FT-IR spectra ( $1731\text{-}650\text{ cm}^{-1}$ ) displays subtle biochemical or structural differences correlated with species or geographic locations (Movasaghi et al., 2008; Aloglu & Harrington, 2018).

To evaluate the potential of FT-IR spectroscopy for species and geographic discrimination using both otoliths and scales, this study focuses on six recreationally important species in California: Kelp Bass (*Paralabrax clathratus*), Vermilion Rockfish (*Sebastes miniatus*), Northern Anchovy (*Engraulis mordax*), Pacific Sardine (*Sardinops sagax*), Pacific Bonito (*Sarda chiliensis*), and Yellowtail Jack (*Seriola dorsalis*). By comparing spectral signatures across calcified structures and locations, this work aims to assess whether biochemical

differences captured by FT-IR can be used to distinguish among species and detect geographical variation within species.

The primary objectives of this study are to (1) evaluate the ability of FT-IR spectroscopy to distinguish among species using otolith and scale samples, (2) assess whether spectral signatures vary within species across geographic locations, and (3) compare the relative effectiveness of otoliths and scales, both independently and together, as indicators of species identity and environmental variation. I hypothesize that FT-IR spectra will differ among species and show geographic variation within species. Additionally, I expect that combining information from both tissue types will improve classification performance relative to either tissue alone.

## Chapter 1. MATERIALS AND METHODS

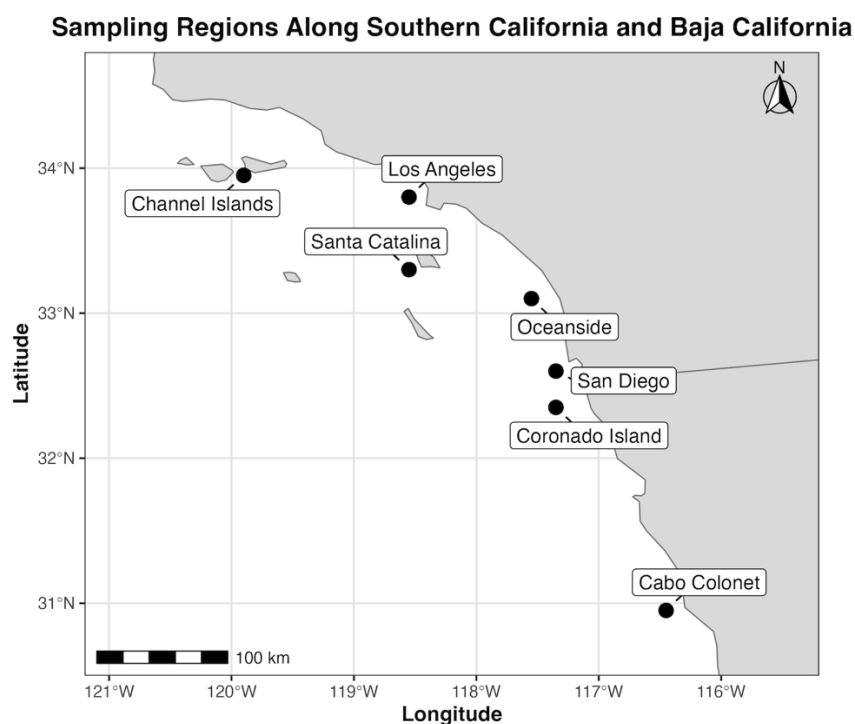
### *i. Sample Collection*

Otolith samples were obtained from various prior research projects focused on fish collected off the coast of California. Vermilion Rockfish (*Sebastes miniatus*), Kelp Bass (*Paralabrax clathratus*), and Pacific Bonito (*Sarda chiliensis*) were collected by a research team at Scripps Institution of Oceanography in collaboration with the Pflieger Institute of Environmental Research (PIER). This team obtained fish carcasses from commercial passenger fishing vessels (CPFVs) operating throughout the Southern California Bight (SCB) between July 2022 and January 2023 (Figure 1). Onboard each CPFV, each fish was given an identification number and additional metadata including capture location, total length, fork length, and weight for each fish were recorded when possible. The carcasses were then fileted and stored on ice until transferred to a -20C freezer within 48 hours (Sleugh et al., in prep). The preservation of the fish carcasses allowed for easy removal of scales to add to spectral analysis.

Otoliths from Yellowtail Jack (*Seriola dorsalis*) were obtained from a separate study that focused on describing the life history and diet behavior of the species. These fish were collected by hook and line aboard CPFVs and private fishing vessels. Sampling for this study took place between July 2014 and September 2016. Sampling was conducted at 10 different locations within the SCB as well as along the coast of Baja California (Ben-Aderet et al., 2020). Although otoliths were extracted from each individual, by the time of this study no scales were available to add to spectral analysis.

Scale samples from Pacific Sardine (*Sardinops sagax*) and Northern Anchovy (*Engraulis mordax*) that were obtained from a separate study were added to expand species coverage. Pacific Sardine and Northern Anchovy were collected between July 2023 and April 2024 from

commercial bait barges located in Dana Point, Mission Bay, Ventura, Santa Monica, and Marina del Rey, California. The fish from the bait barges were caught using purse seine methods prior to distribution. Additional Northern Anchovy samples were collected during routine sampling by the NOAA Southwest Fisheries Science Center Coastal Pelagic Fish Survey near Solana Beach, California, and Tijuana, Mexico. The fish were frozen as soon as possible after being sorted by species and capture location (Sleugh et al., in review).



**Figure 1.** Sampling regions represented in the scale and otolith FT-IR datasets.

Points represent representative regional locations rather than exact individual collection coordinates.

### *ii. Scale Preparation*

When possible, scales were collected from the same individuals used in the otolith analysis to allow for paired comparisons between calcified structures. For Vermilion Rockfish and Pacific Bonito an otolith and a scale sample were extracted from the same individual to allow for paired comparisons. For Kelp Bass, the original individuals from which the otoliths were extracted were no longer available. To enable comparison between scales and otoliths, substitute individuals were selected from the same sampling locations and with similar weights to the original specimens. These samples were used to approximate structure-based comparisons, although they do not represent true paired observations at the individual level. In addition, scale samples were collected from Pacific Sardine and Northern Anchovy to expand species coverage in the scale dataset, although no otolith samples were available for either of these species.

I removed scales from the operculum of the frozen Vermilion Rockfish, Pacific Bonito, and Kelp Bass specimens, washed with Milli-Q water, placed them in between two glass microscope slides, and allowed to dry in a fume hood for 3 days prior to spectral analysis following published protocols (Santana et al., 2015; Rumengan et al., 2017; Ravago, 2022). I selected intact and visually clean scales to ensure consistent quality and optimal contact during the FT-IR measurements. I analyzed the scales while on microscope slides.

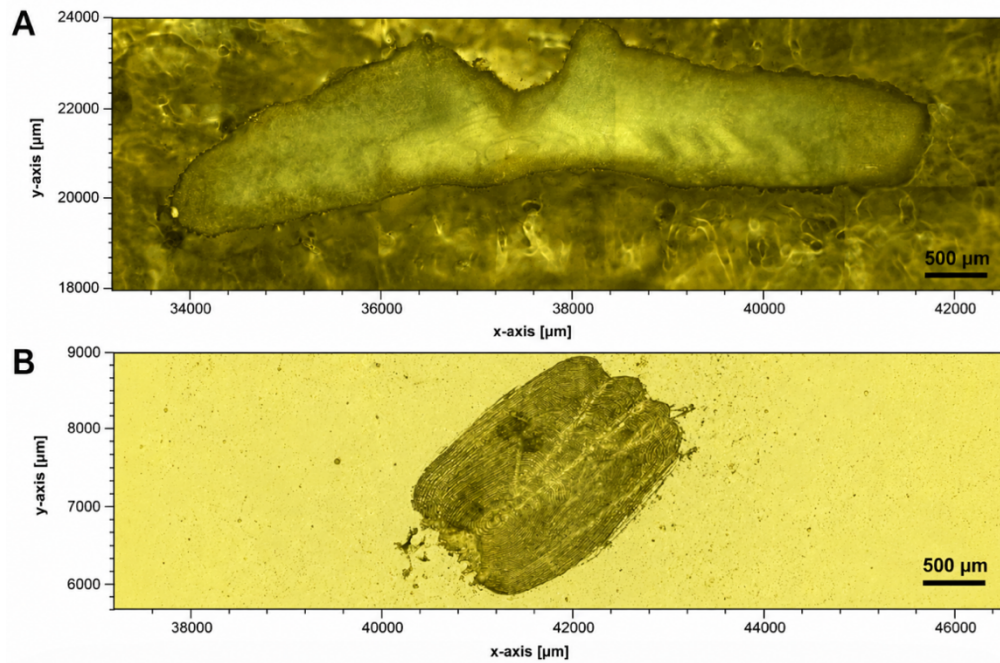
### *iii. Otolith Preparation*

All otoliths used for this study had been previously dissected from fish specimens, prepared for age determination, and mounted on glass slides prior to this study. The species that otoliths were taken from include Vermilion Rockfish, Kelp Bass, Pacific Bonito, and Yellowtail Jack. Each otolith was cross-sectioned through the core using grinding techniques and secured on microscope slides with mounting resin. Before spectral analysis, I removed a thin layer of

mounting resin by hand using fine 600-grit sandpaper to re-expose the polished otolith surface and ensure that the spectrometer was taking a clear reading of the otolith itself. During this sanding process, I only removed the surface layer of resin to ensure the otoliths remained securely attached to the microscope slide and took care to avoid altering the structure of the sample and leave as minimal scratch marks from the sandpaper as possible. To verify that residual mounting resin did not influence spectral data, I performed quality control checks by comparing spectra collected from pure resin with those from exposed otolith surfaces. Characteristic resin peaks were identified and confirmed to be absent from all otolith spectra used in subsequent analysis.

#### *iv. FT-IR/ATR Spectral Acquisition*

I collected all spectral measurements using a Bruker LUMOS II FT-IR spectrometer. Samples were positioned on the instrument stage, and spectra were acquired from the otolith and scale surfaces using a diamond attenuated total reflectance (ATR) crystal coupled with a liquid nitrogen-cooled mercury cadmium telluride (LN-MCT) detector (Figure 2). The detector operates across the mid-infrared spectral range ( $\sim 4000\text{-}400\text{ cm}^{-1}$ ), and ATR measurements were collected at microscale spatial resolution ( $\sim 1.25\mu\text{m}$ ). In ATR mode, infrared radiation is internally reflected within a crystal and interacts with a sample in direct contact with its surface to generate a spectrum based on molecular and elemental composition (*Attenuated Total Reflectance (ATR)* | Bruker, n.d.). I selected this mode because transmission-based FT-IR analysis would have required sample thickness of less than  $\sim 20\mu\text{m}$ , which could not be achieved for these calcified structures, as preparing the otoliths and scales to this thickness would have required decalcification and other processing steps that would alter their biochemical composition.

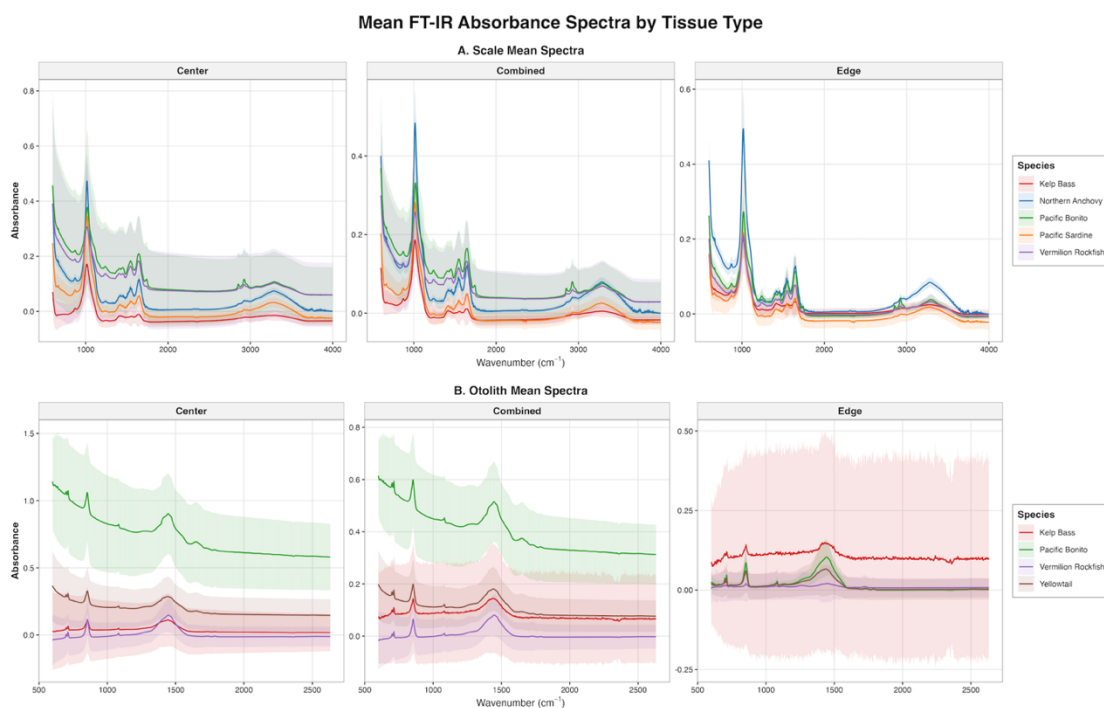


**Figure 2.** Representative imaging of calcified structures during FT-IR spectroscopic analysis.

(A) Sectioned otolith positioned for FT-IR spectral collection, following mounting and polishing. (B) Fish scale positioned for FT-IR spectral collection, showing the intact scale surface and measurement region. Axes are displayed in micrometers ( $\mu\text{m}$ ), and scale bars represent 500  $\mu\text{m}$ .

I took spectral readings from both the centers and edges of the otolith and scales for each sample (Figure 3). The otolith center was defined as the visible core, and the scale center was defined as the focus. I acquired the center measurements using a manually positioned grid of measurement points that I adjusted to the size of each sample so that it fully covered the core or focus. I collected edge measurements along the outermost margin of each otolith and the outermost growth ring of each scale and manually selected measurement locations to provide representative coverage around the perimeter. I collected ten spectral measurements from the center and ten that I had dispersed around the outer edge of the otolith. For scales, I took twenty measurements from the center, and I spread twenty measurements around the outermost ring of each scale. I selected these anatomical regions because they represent material deposited during

different periods of the fish's life, with the center reflecting the earliest growth and the edge representing the most recently deposited material. Comparing these different anatomical regions allowed me to evaluate whether spectral signatures differed between earlier and more recently deposited growth and whether these differences improved species or location discrimination. Both the otolith and scale centers were clearly visible during spectral acquisition, allowing for consistent placement of measurements. I collected the multiple spectra per sample to assess repeatability and reduce noise.



**Figure 3.** Mean FT-IR spectra of scales and otoliths by anatomical region.

(A) Mean scale FT-IR absorbance spectra ( $\pm 1$  SD) by species for center-only (left), combined (center), and edge-only (right) fish-averaged datasets following quality control. (B) Mean otolith FT-IR absorbance spectra ( $\pm 1$  SD) by species for center-only (left), combined (center), and edge-only (right) fish-averaged datasets following quality control.

#### *v. Statistical Analysis*

I used a standardization multivariate pipeline to evaluate species- and location-level differences from the scale and otolith spectral data. I first aggregated individual measurements to the fish level to avoid pseudo replication. Thus, for each fish, I averaged spectra within anatomical regions (center and edge) following point-level quality control, resulting in one representative spectrum per fish per anatomical region. From this, I constructed three datasets for each tissue type: center-only, edge-only, and a combined set, which represents the average of both regions. Before averaging the data, I applied point-level quality control using an iterative Pearson correlation approach to remove outlier spectra within each individual fish and region. In evaluating the scale spectra, I used a correlation threshold of  $r < 0.90$  to remove measurements that may have unintentionally recorded surface scratches or had poor contact with the sample. For the otolith spectra, I used a threshold of  $r < 0.50$  to account for the greater natural heterogeneity of the otolith composition. After filtering, any fish-region combination that had fewer than 5 points remaining after outlier removal was flagged as unreliable.

After quality control steps were performed, I standardized the spectral data such that each wavenumber had a mean of zero and a variance of one and I then analyzed using principal component analysis (PCA). PCA was used to visualize the variation patterns and assess natural clustering among samples. Linear discriminant analysis (LDA) was used to conduct supervised classification to assess the ability of spectral data to discriminate among species and geographic locations. I applied PCA prior to LDA, with the first 20 principal components retained as input features to reduce the high dimensionality of spectral data.

I evaluated model performance using leave-one-out cross-validation (LOO-CV) and I calculated classification accuracy for each dataset. To assess statistical reliability and compare

performance across region inputs, I computed 95% Wilson confidence intervals for all accuracy estimates. I applied regularized LDA to improve model stability for location discrimination analyses with small sample sizes. I conducted all analyses in a parallel framework for scale and otolith datasets, allowing direct comparison of species discrimination, geographical discrimination, and regional effects across tissue types.

### ***Acknowledgements***

Chapter 1, in full, is part of a paper currently being prepared for submission coauthored with Swalethorp, R., Ben-Aderet, N., Sleugh, T., Semmens, B.X. The author of this thesis was the primary investigator and author of this material.

## Chapter 2. RESULTS

The scale dataset included 140 fish across five species, yielding 5,600 spectral measurements, while the otolith dataset included 177 fish across four species, generating a total of 3,540 measurements. I conducted analyses using three datasets for each tissue type: measurements from the center-only, measurements from the edge-only, and a combined dataset averaging measurements of both the anatomical regions. Species with individuals that had both otolith and scale samples available included Kelp Bass, Pacific Bonito, and Vermilion Rockfish, while Northern Anchovy and Pacific Sardine were unique to the scale dataset and Yellowtail Jack was unique to the otolith dataset. Point-level quality control resulted in the removal of 12.7% of scale spectra and 16.1% of otolith spectra. After filtering, two scale fish-region combinations were excluded due to insufficient valid measurements. In contrast, 27 otolith fish-region combinations were excluded. All of the otolith-region combinations that were excluded were measurements taken from the edge region of the otolith. As a result of quality filtering, the otolith edge-only and combined datasets were reduced from 177 to 150 fish (Table 1; Table 2).

**Table 1.** Overview of scale samples and FT-IR measurement points before and after quality control.

Fish sample counts and total FT-IR measurement points by species and geographic region before and after outlier removal. Measurement points from center and edge anatomical regions are combined.

| <b>Table 1. Overview of scale samples and FT-IR measurement points before and after quality control</b> |                       |                      |                                     |                                    |
|---------------------------------------------------------------------------------------------------------|-----------------------|----------------------|-------------------------------------|------------------------------------|
| <b>Geographic region</b>                                                                                | <b>Fish before QC</b> | <b>Fish after QC</b> | <b>Measurement points before QC</b> | <b>Measurement points after QC</b> |
| <b>Kelp Bass</b>                                                                                        |                       |                      |                                     |                                    |
| Los Angeles                                                                                             | 1                     | 1                    | 40                                  | 26                                 |
| Oceanside                                                                                               | 16                    | 16                   | 640                                 | 510                                |
| San Diego                                                                                               | 8                     | 8                    | 320                                 | 265                                |
| Santa Catalina                                                                                          | 11                    | 11                   | 440                                 | 346                                |
| Ventura/channel islands                                                                                 | 2                     | 2                    | 80                                  | 68                                 |
| <b>Northern Anchovy</b>                                                                                 |                       |                      |                                     |                                    |
| Los Angeles                                                                                             | 5                     | 5                    | 200                                 | 196                                |
| NaN                                                                                                     | 14                    | 14                   | 560                                 | 518                                |
| <b>Pacific Bonito</b>                                                                                   |                       |                      |                                     |                                    |
| Los Angeles                                                                                             | 4                     | 4                    | 160                                 | 150                                |
| San Diego                                                                                               | 17                    | 17                   | 680                                 | 602                                |
| <b>Pacific Sardine</b>                                                                                  |                       |                      |                                     |                                    |
| Los Angeles                                                                                             | 11                    | 11                   | 440                                 | 402                                |
| NaN                                                                                                     | 9                     | 9                    | 360                                 | 326                                |
| <b>Vermilion Rockfish</b>                                                                               |                       |                      |                                     |                                    |
| Los Angeles                                                                                             | 18                    | 18                   | 720                                 | 665                                |
| Oceanside                                                                                               | 3                     | 3                    | 120                                 | 93                                 |
| San Diego                                                                                               | 17                    | 17                   | 680                                 | 621                                |
| Ventura/channel islands                                                                                 | 4                     | 4                    | 160                                 | 98                                 |

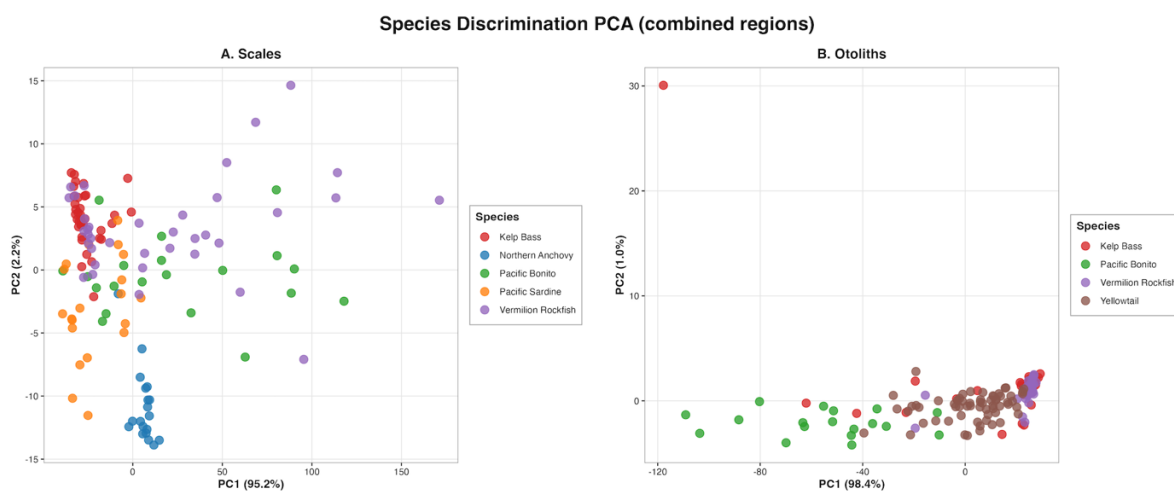
**Table 2.** Overview of otolith samples and FT-IR measurement points before and after quality control.

Fish sample counts and total FT-IR measurement points by species and geographic region before and after outlier removal. Measurement points from center and edge anatomical regions are combined.

| <b>Table 2. Overview of otolith samples and FT-IR measurement points before and after quality control</b> |                       |                      |                                     |                                    |
|-----------------------------------------------------------------------------------------------------------|-----------------------|----------------------|-------------------------------------|------------------------------------|
| <b>Geographic region</b>                                                                                  | <b>Fish before QC</b> | <b>Fish after QC</b> | <b>Measurement points before QC</b> | <b>Measurement points after QC</b> |
| <b>Kelp Bass</b>                                                                                          |                       |                      |                                     |                                    |
| Los Angeles                                                                                               | 1                     | 1                    | 20                                  | 18                                 |
| Oceanside                                                                                                 | 16                    | 16                   | 320                                 | 270                                |
| San Diego                                                                                                 | 8                     | 8                    | 160                                 | 112                                |
| Santa Catalina                                                                                            | 11                    | 11                   | 220                                 | 192                                |
| Ventura/channel islands                                                                                   | 2                     | 2                    | 40                                  | 29                                 |
| <b>Pacific Bonito</b>                                                                                     |                       |                      |                                     |                                    |
| Los Angeles                                                                                               | 4                     | 4                    | 80                                  | 62                                 |
| San Diego                                                                                                 | 17                    | 17                   | 340                                 | 289                                |
| <b>Vermilion Rockfish</b>                                                                                 |                       |                      |                                     |                                    |
| Los Angeles                                                                                               | 18                    | 18                   | 360                                 | 297                                |
| Oceanside                                                                                                 | 3                     | 3                    | 60                                  | 58                                 |
| San Diego                                                                                                 | 17                    | 17                   | 340                                 | 271                                |
| Ventura/channel islands                                                                                   | 4                     | 4                    | 80                                  | 75                                 |
| <b>Yellowtail</b>                                                                                         |                       |                      |                                     |                                    |
| Cabo Colonet                                                                                              | 24                    | 24                   | 480                                 | 393                                |
| Coronado Island                                                                                           | 12                    | 12                   | 240                                 | 231                                |
| Los Angeles                                                                                               | 15                    | 15                   | 300                                 | 252                                |
| San Diego                                                                                                 | 25                    | 25                   | 500                                 | 421                                |

*i. Species discrimination*

Principal component analysis (PCA) of scale spectra revealed multispecies clustering in PC1-PC2 space for the combined dataset (Figure 4). The Kelp Bass and Northern Anchovy formed distinct clusters, while Pacific Sardine, Pacific Bonito, and Vermilion Rockfish showed moderate overlap. In contrast, PCA of the combined dataset for otolith spectra showed less discrete separation among species (Figure 4). Pacific Bonito and Vermilion Rockfish formed separated clusters, while Kelp Bass and Yellowtail Jack exhibited greater overlap.



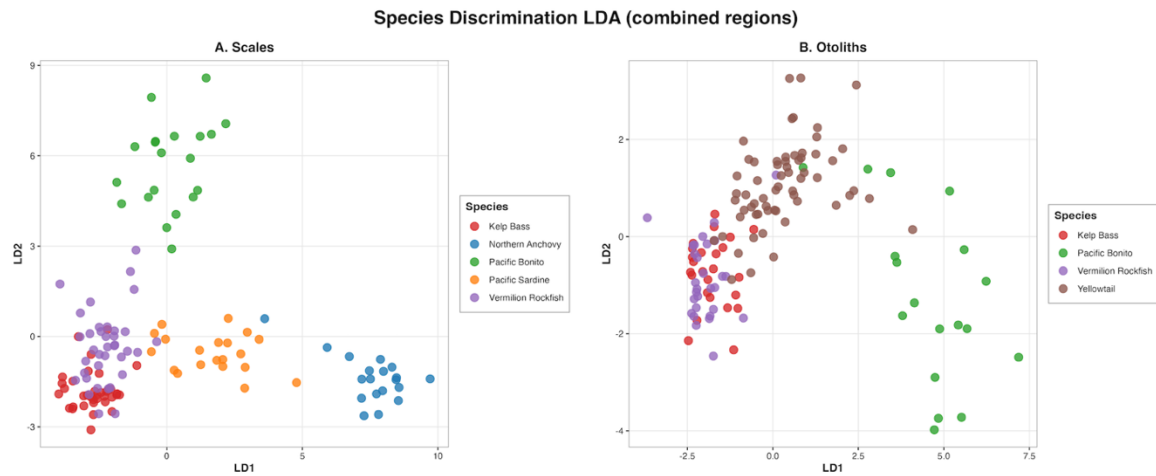
**Figure 4.** Unsupervised Principal Component Analysis (PCA) on scales and otoliths.

(A) PCA score plot (PC1 vs. PC2) of combined, fish-averaged scale spectra following quality control. (B) PCA score plot (PC1 vs. PC2) of combined, fish-averaged otolith spectra following quality control.

The LOO-CV accuracies from the LDA model varied among species and tissue types. In the scale dataset, Northern Anchovy and Pacific Sardine were both classified with 100% accuracy, while Kelp Bass and Pacific Bonito achieved lower but still consistently high accuracies (Table 3; Figure 5). Vermilion Rockfish showed the lowest classification accuracy among scale samples at 80% (Table 3). In the otolith dataset, Yellowtail Jack exhibited the highest classification accuracy at 91%, followed by Pacific Bonito and Vermilion Rockfish (Table 3; Figure 5). Kelp Bass exhibited the lowest accuracy among otolith samples at 52% (Table 3).

**Table 3.** Per-species classification accuracy for scale and otolith spectra by region input. Accuracy values are based on leave-one-out cross-validation (LOO-CV).

| Species            | Scale Combined | Scale Center-only | Scale Edge-only | Otolith Combined | Otolith Center-only | Otolith Edge-only |
|--------------------|----------------|-------------------|-----------------|------------------|---------------------|-------------------|
| Northern Anchovy   | 100%           | 100%              | 95%             | -                | -                   | -                 |
| Pacific Sardine    | 100%           | 85%               | 75%             | -                | -                   | -                 |
| Kelp Bass          | 97%            | 92%               | 87%             | 52%              | 63%                 | 45%               |
| Pacific Bonito     | 85%            | 71%               | 85%             | 83%              | 76%                 | 39%               |
| Vermilion Rockfish | 80%            | 68%               | 79%             | 68%              | 76%                 | 59%               |
| Yellowtail         | -              | -                 | -               | 91%              | 89%                 | 73%               |



**Figure 5.** Linear Discriminant Analysis (LDA) canonical score plot (LD1 vs. LD2) for scales and otoliths.

(A) LDA plot (LD1 vs. LD2) of combined, fish-averaged scale spectra following quality control.  
 (B) LDA plot (LD1 vs. LD2) of combined, fish-averaged otolith spectra following quality control.

Based on the LDA classification for anatomical region of analysis within each scale or otolith sample, scale spectra achieved high species classification accuracy across all anatomical region inputs, with the combined dataset performing best at 89.6% (Table 4). Center-only and edge-only scale datasets yielded slightly lower but comparable accuracies. The otolith spectra showed lower overall species classification accuracy, with the combined dataset performing the best at 76.7% (Table 4). The center-only dataset for otolith spectra produced results similar to the combined dataset and the edge-only dataset for otolith spectra performed the lowest (Table 4). This indicates that the inclusion of edge-only measurements did not significantly improve the classification performance for species discrimination.

**Table 4.** Species classification accuracy by tissue type and region input.

Leave-one-out cross-validation (LOO-CV) accuracies are reported with 95% Wilson confidence intervals for scale and otolith datasets using combined, center-only, and edge-only spectral inputs. Chance classification accuracy was 20% for scales (five species) and 25% for otoliths (four species).

| <b>Tissue Type</b> | <b>Region Input</b> | <b>N Fish</b> | <b>N Species</b> | <b>LOO-CV Accuracy.<br/>[95% CI]</b> | <b>Chance (%)</b> |
|--------------------|---------------------|---------------|------------------|--------------------------------------|-------------------|
| <b>Scales</b>      | Combined            | 115           | 5                | 89.6%<br>[83–94%]                    | 20%               |
|                    | Center-only         | 139           | 5                | 82.0%<br>[75–88%]                    | 20%               |
|                    | Edge- only          | 139           | 5                | 83.5%<br>[76–89%]                    | 20%               |
| <b>Otoliths</b>    | Combined            | 150           | 4                | 76.7%<br>[69–83%]                    | 25%               |
|                    | Center-only         | 177           | 4                | 77.4%<br>[71–83%]                    | 25%               |
|                    | Edge-only           | 150           | 4                | 60.0%<br>[52–67%]                    | 25%               |

*ii. Location discrimination*

Scale spectra showed promise of within-species geographic discrimination for some species (Figure 6). Based on the LOO-CV accuracies, the Pacific Bonito scale dataset exhibited the strongest location signal, with the highest classification accuracy of 75.0% from the combined dataset, exceeding the species' 50% chance level (Table 5). Vermilion Rockfish showed moderate location discrimination, with its highest accuracy of 46.3% from the scale center-only dataset, which exceeds this species 25% chance level (Table 5). Overall, both Pacific Bonito and Vermilion Rockfish spectra showed stronger geographic discrimination signal within the scale dataset than Kelp Bass. Kelp Bass did not consistently exhibit a reliable location signal,

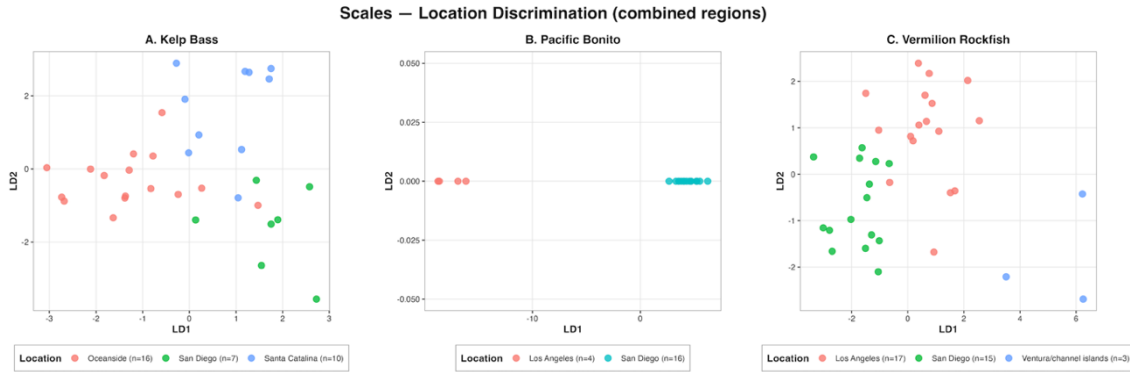
with classification accuracy all near this species' chance level of 33% (Table 5). It is important to note however, the wide 95% confidence intervals that were evident in every dataset.

Differences among anatomical region inputs were generally small, although the center-only scale dataset produced slightly higher accuracies for Vermilion Rockfish, the combined scale dataset produced slightly higher accuracies for Pacific Bonito, and the edge-only scale dataset produced slightly higher accuracies for Kelp Bass.

**Table 5.** Per-species geographic classification accuracy for scale and otolith spectra by region input.

Chance-level classification accuracy is shown in the second column. Accuracy values are based on leave-one-out cross-validation (LOO-CV).

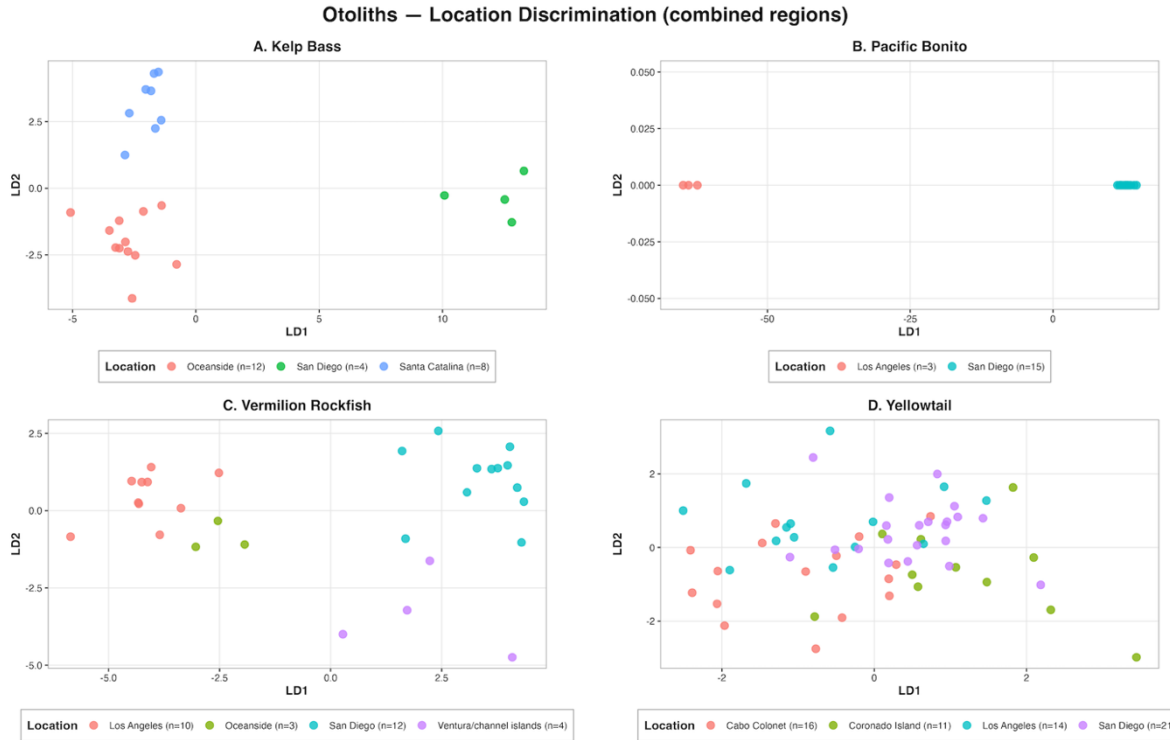
| Species            | Chance (%) | Scale Combined [95% CI] | Scale Center-Only [95% CI] | Scale Edge-Only [95% CI] | Otolith Combined [95% CI] | Otolith Center-Only [95% CI] | Otolith Edge-Only [95% CI] |
|--------------------|------------|-------------------------|----------------------------|--------------------------|---------------------------|------------------------------|----------------------------|
| Kelp Bass          | 33%        | 34.3%<br>[21-51%]       | 31.4%<br>[19-48%]          | 40.0%<br>[26-56%]        | 41.4%<br>[26-59%]         | 45.7%<br>[30-62%]            | 41.4%<br>[26-59%]          |
| Pacific Bonito     | 50%        | 75.0%<br>[53-89]        | 66.7%<br>[45-83%]          | 70.0%<br>[48-85%]        | 66.7%<br>[44-83%]         | 71.4%<br>[50-86%]            | 72.2%<br>[49-88%]          |
| Vermilion Rockfish | 25%        | 39.0%<br>[26-54%]       | 46.3%<br>[32-61%]          | 40.5%<br>[27-55%]        | 52.9%<br>[37-69%]         | 42.9%<br>[29-58%]            | 38.2%<br>[24-55%]          |
| Yellowtail         | 25%        | -                       | -                          | -                        | 31.3%<br>[22-43%]         | 39.5%<br>[29-51%]            | 22.4%<br>[14-34%]          |



**Figure 6.** Geographic location discrimination from scale FT-IR spectra.

Linear discriminant analysis (LDA) score plots for geographic location discrimination using combined-region scale FT-IR spectra averaged per fish. Panels show (A) Kelp Bass, (B) Pacific Bonito, and (C) Vermilion Rockfish. Points represent individual fish colored by collection location. Axes correspond to the first two linear discriminants (LD1 and LD2).

Otolith spectra showed varying levels of within-species geographic discrimination based on results from the LOO-CV (Figure 7). The Pacific Bonito datasets exhibited classification that all exceeded the 50% chance level, and the edge-only dataset displayed the highest classification accuracy of 72.2%, out of the otolith datasets for this species (Table 5). The strongest evidence of a geographic signal for Vermilion Rockfish was seen in the otolith combined dataset, which achieved 52.9% accuracy, relative to a 25% chance level (Table 5). The strongest location signal for the Yellowtail Jack datasets was shown in the center-only otolith dataset which achieved 39.5% accuracy, while the Yellowtail Jack combined and edge-only otolith datasets performed near or below the 25% chance level (Table 5). In contrast, Kelp Bass otoliths did not show a consistent location signal, with accuracies all near the 33% chance level for the species (Table 5).

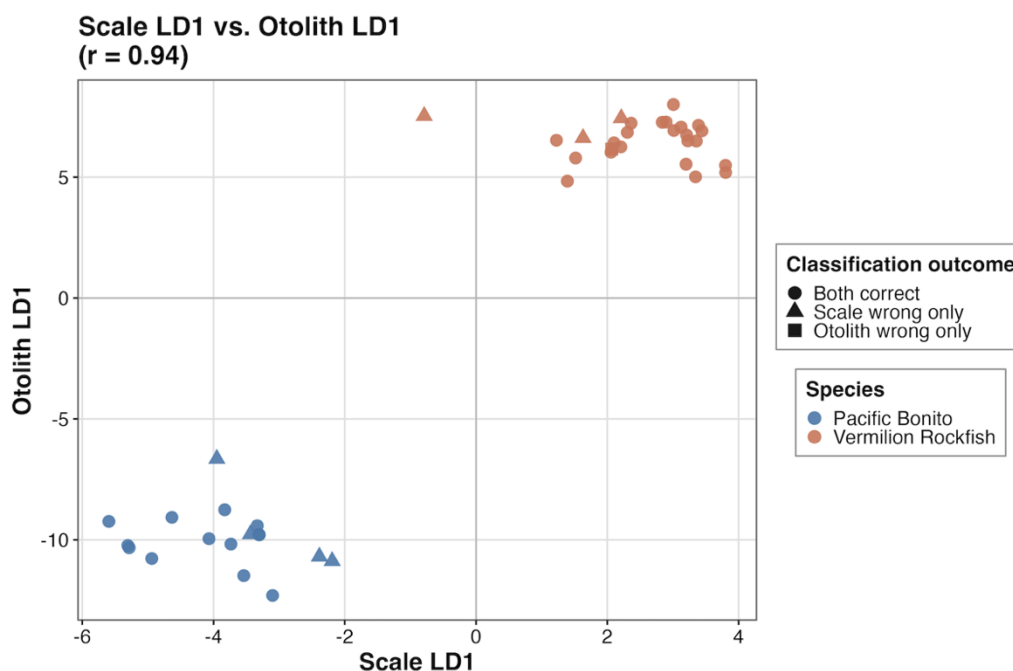


**Figure 7.** Geographic location discrimination from otolith FT-IR spectra. Linear discriminant analysis (LDA) score plots for geographic location discrimination using combined-region otolith FT-IR spectra averaged per fish. Panels show (A) Kelp Bass, (B) Pacific Bonito, (C) Vermilion Rockfish, and (D) Yellowtail. Points represent individual fish colored by collection location. Axes correspond to the first two linear discriminants (LD1 and LD2).

### *iii. Comparing scales and otoliths from species with paired samples*

I conducted paired analysis on 50 fish for which both scale and otolith data were available (33 Vermilion Rockfish, 17 Pacific Bonito). Scale-based species classification accuracy for these matched samples was 88%, with otolith-based accuracy reaching 96%. Across these samples, eight total classification errors were observed, and no individual fish was misclassified by both tissue types simultaneously (Figure 8). Instead, classification errors were tissue-specific, with one tissue type correctly identifying samples misclassified by the other. Discriminant scores

were highly correlated between tissues for species classification, with a Pearson correlation of  $r=0.94$ . This indicates that both scales and otoliths separated species similarly in discriminant space and managed to differ in which individual fish produced the classification errors.



**Figure 8.** Scale and otolith LD1 scores for paired fish samples. Relationship between LD1 scores from FT-IR spectra of scales and otoliths from the same individuals of Pacific Bonito and Vermilion Rockfish. Colors indicate species while shapes indicate classification outcome. Both correct means the species was correctly classified in both tissues, scale wrong only means the scale misclassified and the otolith correctly classified, otolith wrong only means the otolith misclassified and the scale correctly classified.

### *Acknowledgements*

Chapter 2, in full, is part of a paper currently being prepared for submission coauthored with Swalethorp, R., Ben-Aderet, N., Sleugh, T., Semmens, B.X. The author of this thesis was the primary investigator and author of this material.

### Chapter 3. DISCUSSION

FT-IR spectroscopy successfully differentiated between multiple fish species using both scale and otolith samples. This study supports past findings that FT-IR can be a viable tool for fisheries identification. Overall, the scales displayed higher species classification accuracy than the otoliths, which may suggest that scales exhibited more consistent or stronger biochemical signals necessary for species discrimination. Within the otolith dataset, measurements taken at the center of the otolith generally outperformed the measurements that were taken at the outer edge of the otolith. Additionally, slight geographic differentiation was detected within several species; however, the performance of classification varied among the different tissue types and species. Lastly, the scales and otoliths seemed to provide complementary information for classification which suggests that each structure may reflect distinct biochemical or physiological signals. The implications of these findings and the potential mechanisms underlying these patterns are discussed in the following sections.

#### *i. Species Discrimination*

The PCA and LDA results showed that FT-IR successfully differentiated between species using the scale-derived data as well as the otolith-derived data. This demonstrates that the FT-IR spectral data contained measurable biochemical differences among fish species. Several species, such as Northern Anchovy and Pacific Sardine in the scale dataset and Yellowtail Jack in the otolith dataset, formed relatively distinct clusters within PC1-PC2 space. The species that showed more distinct clustering in PCA generally also exhibited higher LDA classification accuracy. For example, Yellowtail Jack exhibited classification success from both the PCA and LDA whereas Kelp Bass showed overlap in the PCA as well as lower classification performance in LDA. This suggests some species may be classified by spectra more consistently than others

and also shows consistency between the PCA and the LDA analyses. It is important to note that PCA is an unsupervised method that summarizes the major sources of variation in the dataset regardless of species identity, whereas LDA is a supervised method that explicitly maximizes separation among predefined groups, so LDA may detect species different that are less apparent in the PCA space.

These findings are consistent with previous studies that investigated spectroscopy-based approaches for identification of fish species. Previous work has demonstrated FT-NIR can accurately classify fish species and populations based on spectral signatures from otoliths (Benson et al., 2020). Although fewer studies have analyzed fish scales for classification purposes, spectroscopy-based studies have shown that scales contain detectable biochemical variation which can be spectroscopically identified and characterized. (Ravago, 2022; Santana et al., 2015). Additionally, previous studies that have investigated spectroscopy-based approaches in species identification often showed consistently high classification accuracies, usually exceeding 90% (Goldstein et al., 2021; Wedding et al., 2014). The results of this study are consistent with these findings, as several species achieved high classification performance. The varying levels of species discrimination performances among taxa may suggest that FT-IR-based classification is be influenced by biochemical differentiation among the different fish species.

#### *ii. Location discrimination*

The geographic discrimination performances within species varied across taxa and tissue types. This suggests that the strength of location-specific biochemical signals differed among species. Pacific Bonito displayed the strongest overall geographic discrimination signal as separation was observed in both scale and otolith datasets. Additionally, Vermilion Rockfish displayed some evidence of geographic discrimination, particularly in the scale center-only

dataset and the otolith combined dataset. A similar pattern was observed for Pacific Bonito, which also exhibited some geographic discrimination in both scale and otolith tissues. In contrast, Kelp Bass did not exhibit reliable geographic discrimination in either tissue type as the classification accuracies all remained near the chance level across all anatomical region inputs. Yellowtail Jack displayed geographic discrimination only within the otolith center-only dataset. Overall, these varying findings suggest that the geographical discrimination ability of FT-IR spectroscopy may vary according to species, tissue type, and anatomical region. However, it is important to consider the wide confidence intervals in the results and low, as well as imbalanced, sample sizes in this study. Further steps are needed to truly investigate FT-IR application to geographic discrimination performance using this method.

Previous studies have demonstrated that fish hard structures incorporate chemical information from the environment which then may reflect specific locations (Gillanders, 2005; Tzadik et al., 2017). Both scales and otoliths have been found to function as chronological biochemical archives that reflect habitat use, environmental exposure, and the resulting physiological processes across the lifespan of the fish (Tray et al., 2022; Campana, 1999). Because of this, I hypothesized that individuals of the same species collected from different geographic locations may develop distinguishable biochemical signatures which could then be detected spectroscopically. In species exhibiting prolonged environmental exposure or greater site fidelity, such as Vermilion Rockfish, I expected the accumulation of location-associated biochemical influences to increase the likelihood of detecting geographic differences among populations.

Detecting differences in the geographic discrimination among species could help infer conclusions about variations in ecology, habitat use, and migration patterns between populations.

Environmental conditions, habitat use, and diet can influence the biochemical and chemical composition of fish tissues over time, resulting in location-associated signatures that may be preserved within scales and otoliths (Gillanders, 2005; Tzadik et al., 2017). FT-IR spectroscopy may be capable of detecting some of this variation through differences in the molecular and mineral composition of these structures. Overall, these findings suggest that FT-IR spectroscopy may capture biochemical variation associated with environmental history and could have potential for geographic discrimination in some species. However, the results from this study show that geographic classification was more variable than species-level discrimination, indicating that performance could vary based on species, tissue type, or sampling design. Because geographic sampling and sample sizes were limited and uneven, additional studies across broader spatial scales are needed before drawing more general conclusions about this method's utility for geographic discrimination.

### *iii. Scales outperformed otoliths*

Comparing the two calcified structures analyzed in this study, the scale spectra consistently showed higher species classification in comparison to the otolith spectra. This aligns with expectations based on the biochemical composition of the two tissues, as scales are primarily composed of collagen along with other organic components including proteins, carbohydrates, lipids, and tissues associated with the epidermal and mucus layers that cover the scale surface (Qin et al., 2022), whereas otoliths are composed mostly of calcium carbonate minerals (Yasmeen et al., 2025). Thus, the FT-IR scale spectra were likely dominated by the relatively consistent amide I (approximately  $1700\text{-}1600\text{ cm}^{-1}$ ) and amide II (approximately  $1565\text{-}1520\text{ cm}^{-1}$ ) functional groups, rather than the varying carbonate polymorphs as observed in otoliths (Aloglu & Harrington, 2018). The collagenic composition of scales likely produced

stronger and more easily distinguishable absorbance features compared to the spectra in otoliths because FT-IR spectroscopy is particularly sensitive to organic molecules.

Additionally, the reduced performance of otolith spectra may be due in part to the greater structural and compositional heterogeneity present in otolith material. As a fish ages, otoliths grow through the accretion of calcium carbonate and an organic matrix in discrete increments (Campana, 1999). This results in layered growth structures which reflect environmental and physiological changes over time (Gillanders, 2005). As the otolith grows, more carbonate polymorphs such as aragonite or vaterite are displayed within the otolith, and changes in the environment can cause variation in polymorphs and crystal structures within a single otolith (Gillanders, 2005; de Vries et al., 2005). These variations in carbonate structures may be directly linked to the differences displayed in the spectral readings between otoliths. Carbonate minerals exhibit absorption features associated with  $\text{CO}_3^{2-}$  vibrational modes and changes in crystal structure or composition can cause shifts in spectral peaks (Gunasekaran et al., 2006). Thus, the possibility of heterogeneity in mineral composition in each single otolith may have caused the otoliths to have increased variability in FT-IR spectral signatures compared to scales.

Differences in surface characteristics during spectral acquisition may have also contributed to the differing discrimination performance between tissues. Because each scale was intact, dried, and laid flat, their surfaces allowed for more consistent contact, whereas for otoliths, there is a possibility that their surfaces were more irregular due to the sanding to remove mounting resin. This nonuniform surface could have led to increasing variability in spectral measurements because ATR measurements are sensitive to the contact quality between the crystal and the sample thus, increased surface variability could have added additional noise to the otolith spectral measurements. Overall, the combined effects of tissue composition, mineral

heterogeneity, and surface variability likely contributed to the improved performance of scales for species discrimination as opposed to otoliths.

It can be inferred that classification errors in this study were tissue-specific, as demonstrated by the absence of overlapping misclassifications between scales and otoliths. This shows that analyzing both tissues had complementary strengths in species discrimination, as each tissue successfully identified samples that were misidentified by the other. This interpretation is further supported by the high correlation observed between tissues for species-level classification. Together, these results suggest that scales and otoliths may display different information and combining data from both tissues, or possibly more, could improve overall classification performance.

#### *iv. Regional differences within structures*

The anatomical regions of the two tissue types displayed differing levels of discrimination. When comparing the anatomical regions (center or edge) within otoliths, the center-only spectra consistently outperformed the edge-only spectra for species discrimination. The edge-only otoliths measurements displayed increased variability and failed quality control more frequently which may suggest the otolith edge provides a less stable spectral signal than the center does for spectroscopy-based classification. This finding is somewhat surprising as the edge region of the otoliths are often considered very informative since it reflects the more recent environmental conditions surrounding the fish (Gillanders, 2005). However, this higher compositional heterogeneity within the newly deposited material on the outer ring of the otolith and the variability in crystal structure could have contributed to the high levels of spectral inconsistency observed in the edge-only measurements. Additionally, the sanding to prepare the otolith surface for spectral acquisition may have inadvertently added more irregularities to the

edges of the otolith relative to the center. It is interesting to note that Pacific Bonito, a relatively mobile species with lower site fidelity, exhibited its highest geographic classification accuracy in the edge-only otolith dataset. In contrast, Vermilion Rockfish, a longer-lived species with greater site fidelity, showed stronger location discrimination in the center-only and combined otolith datasets. This could indicate that different otolith regions may capture distinct temporal aspects of an individual's environmental history and the center could have utility for identifying geographic origin of individuals in some species. However, these patterns should be interpreted cautiously given the variable sample sizes among datasets.

In contrast to the otolith results, the scale spectra show that center-only and edge-only measurements exhibited relatively similar classification performance. This suggests that the biochemical composition of scales may be more uniform across the surface than otoliths. Scales are primarily composed of collagen-rich organic material (Qin et al., 2022), which does not show the same degree of mineral heterogeneity over time as otolith growth increments do. Thus, the spectral measurements collected from the center and the edges of the scales may display more consistent biochemical signatures with individuals of the same species, as there is less variability introduced. These findings suggest selecting specific regions of a sample to analyze may be more critical for otolith-based FT-IR studies than for scale-based studies, as the center and edge measurements seem to be similarly informative.

#### *v. Methodological Limitations and Uncertainties*

The limitations of this study should be considered when interpreting the results and evaluating the broader applicability of the findings. Overall, the sample sizes of fish were uneven across locations and species. These uneven groups may have reduced statistical power as well as added variability in classification performance. In addition, differences in sample collection

methods and sample origin across contributing projects may have introduced additional variability. Along with sampling limitations, several technical limitations of the ATR mode for FT-IR analysis should be considered. Because the ATR crystal probe only analyzes a shallow surface layer of the sample (roughly 1.66  $\mu\text{m}$  deep) the resulting spectra primarily reflect the composition of the material immediately beneath the crystal rather than the entire structure. Consequently, multiple replicate measurements were necessary to account for within-sample heterogeneity. This limitation is particularly relevant for otoliths, which required additional preparation to expose internal regions such as the center prior to spectral acquisition. Variability in surface preparation and contact quality between the ATR crystal and the sample may have contributed additional variation to the spectral measurements.

Compared to other methods, FT-IR offers a rapid and non-destructive approach to biochemical analysis. Although genetic approaches may provide high taxonomic resolution and are useful for population structure analysis, FT-IR may be better suited for large-scale sample processing, due to its scalability and application to existing sample archives. Similarly, elemental and isotopic fingerprinting methods, although providing more detailed environmental and migratory information, often require much more specialized preparation and instrumentation, and can be costly (Campana et al., 2005). FT-IR is emerging as a viable addition to traditional methods such as genetic or elemental and isotopic fingerprinting methods in the field of fishery stock assessment.

### ***Acknowledgments***

Chapter 3, in full, is part of a paper currently being prepared for submission coauthored with Swalethorp, R., Ben-Aderet, N., Sleugh, T., Semmens, B.X. The author of this thesis was the primary investigator and author of this material.

## Chapter 4. CONCLUSION

Rapid, non-destructive identification methods are highly valuable for fisheries management and ecological studies. The non-destructive nature of FT-IR spectroscopy increases its utility for fisheries research because it can be applied to many archived samples without damaging existing collections. This capability may facilitate retrospective analyses using previously collected material, although samples that have undergone chemical preservation may be less suitable for FT-IR analysis due to potential alterations in their spectral signatures. In addition, these methods could support stock assessment and monitoring efforts by enabling faster, more scalable data collection across large sample sets.

The results of this study further demonstrate the utility of FT-IR spectroscopy for distinguishing among ecologically and commercially important fish species using both scales and otoliths. This method may be valuable for fisheries studies involving isolated structures, such as predator diet analysis, sediment core analysis, or fossil records, where there are only minimal amounts of remaining hard tissues available for analysis. Future work should increase sample sizes and balance more effectively across species and geographic locations to help strengthen classification performance. An expansion of this analysis to additional species and environments may help determine the broader applicability of FT-IR spectroscopy within fisheries research and management.

### *Acknowledgements*

Chapter 4, in full, is part of a paper currently being prepared for submission coauthored with Swalethorp, R., Ben-Aderet, N., Sleugh, T., Semmens, B.X. The author of this thesis was the primary investigator and author of this material.

## REFERENCES

- Adamack, A. T., Antaya, K., Dhaliwal, S., Dwyer, K., Bourne, C. M., & Forbes, G. (2025, May). *Exploring the use of Fourier transform near infrared spectroscopy for aging Newfoundland and Labrador Atlantic cod (Gadus morhua)*—*ScienceDirect*. <https://www.sciencedirect.com/science/article/pii/S0165783625000682>
- Aloglu, A. K., & Harrington, P. de B. (2018). Differentiation of Bovine, Porcine, and Fish Gelatins by Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIRS) Coupled with Pattern Recognition. *Journal of AOAC INTERNATIONAL*, 101(1), 221–226. <https://doi.org/10.5740/jaoacint.17-0244>
- Attenuated Total Reflectance (ATR) | Bruker*. (n.d.). Retrieved April 27, 2026, from <https://www.bruker.com/en/products-and-solutions/infrared-and-raman/ft-ir-routine-spectrometer/what-is-ft-ir-spectroscopy/atr-attenuated-total-reflectance.html>
- Ben-Aderet, N., Johnston, E. M., Cravey, R., & Sandin, S. A. (2020). Revisiting the life history of yellowtail jack (*Seriola dorsalis*) in the Southern California Bight: New evidence for ontogenetic habitat shifts and regional differences in a changing environment. *Fishery Bulletin*, 118(2), 158–170. <https://doi.org/10.7755/FB.118.2.5>
- Benson, I. M., Barnett, B. K., & Helser, T. E. (2020, July 5). *Classification of fish species from different ecosystems using the near infrared diffuse reflectance spectra of otoliths*—*Irina M Benson, Beverly K Barnett, Thomas E Helser, 2020*. <https://journals.sagepub.com/doi/full/10.1177/0967033520935999>
- Berthomieu, C., & Hienerwadel, R. (2009). Fourier transform infrared (FTIR) spectroscopy. *Photosynthesis Research*, 101(2), 157–170. <https://doi.org/10.1007/s11120-009-9439-x>
- Campana, S. E. (1999). Chemistry and composition of fish otoliths: pathways, mechanisms and applications. *Marine Ecology Progress Series*, 188, 263–297. <https://doi.org/10.3354/meps188263>
- Campana, S. E. (2004). *Photographic Atlas of Fish Otoliths of the Northwest Atlantic Ocean*. Canadian Science Publishing. <https://doi.org/10.1139/9780660191089>
- Campana, S. E., Cadrin, S. X., Friedland, K. D., & Waldman, J. R. (2005). Otolith Elemental Composition as a Natural Marker of Fish Stocks. In *Stock Identification Methods Applications in Fishery Science* (pp. 227–245). Elsevier. <https://doi.org/10.1016/B978-012154351-8/50013-7>
- Dahl, K., O'Malley, J., Barnett, B., Kline, B., & Widdrington, J. (2024, April). *Otolith morphometry and Fourier transform near-infrared (FT-NIR) spectroscopy as tools to discriminate archived otoliths of newly detected cryptic species, Etelis carbunculus and Etelis boweni*—*ScienceDirect*. <https://www.sciencedirect.com/science/article/pii/S016578362300320X>

- de Vries, M. C., Gillanders, B. M., & Elsdon, T. S. (2005). Facilitation of barium uptake into fish otoliths: Influence of strontium concentration and salinity. *Geochimica et Cosmochimica Acta*, 69(16), 4061–4072. <https://doi.org/10.1016/j.gca.2005.03.052>
- Gillanders, B. M. (2005). Using elemental chemistry of fish otoliths to determine connectivity between estuarine and coastal habitats. *Estuarine, Coastal and Shelf Science, Connectivity in the Life Cycles of Fishes and Invertebrates That Use Estuaries*, 64(1), 47–57. <https://doi.org/10.1016/j.ecss.2005.02.005>
- Goldstein, E. D., Helser, T. E., Vollenweider, J. J., Sreenivasan, A., & Sewall, F. F. (2021). Rapid and Reliable Assessment of Fish Physiological Condition for Fisheries Research and Management Using Fourier Transform Near-Infrared Spectroscopy. *Frontiers in Marine Science*, 8. <https://doi.org/10.3389/fmars.2021.690934>
- Gunasekaran, S., Anbalagan, G., & Pandi, S. (2006). Raman and infrared spectra of carbonates of calcite structure. *Journal of Raman Spectroscopy*, 37(9), 892–899. <https://doi.org/10.1002/jrs.1518>
- Lewis, L. S., Huang, J. L., Willmes, M., Fichman, R. A., Hung, T.-C., Ellison, L. T., Stevenson, T. A., Teh, S. J., Hammock, B. G., Schultz, A. A., Grimsich, J. L., Huyskens, M. H., Yin, Q.-Z., Cavole, L. M., Botto, N. W., & Hobbs, J. A. (2022, December 1). *Visual, spectral, and microchemical quantification of crystalline anomalies in otoliths of wild and cultured delta smelt* | *Scientific Reports*. <https://www.nature.com/articles/s41598-022-22813-w>
- Mohamed, M. A., Jaafar, J., Ismail, A. F., Othman, M. H. D., & Rahman, M. A. (2017). Fourier Transform Infrared (FTIR) Spectroscopy. In *Membrane Characterization* (pp. 3–29). Elsevier. <https://doi.org/10.1016/B978-0-444-63776-5.00001-2>
- Movasaghi, Z., Rehman, S., & ur Rehman, Dr. I. (2008). Fourier Transform Infrared (FTIR) Spectroscopy of Biological Tissues. *Applied Spectroscopy Reviews*, 43(2), 134–179. <https://doi.org/10.1080/05704920701829043>
- Neat, F. C., Wright, P. J., & Fryer, R. J. (2008). *Temperature effects on otolith pattern formation in Atlantic cod Gadus morhua*. <https://onlinelibrary.wiley.com/doi/10.1111/j.1095-8649.2008.02107.x>
- Qin, D., Bi, S., You, X., Wang, M., Cong, X., Yuan, C., Yu, M., Cheng, X., & Chen, X.-G. (2022, January 15). *Development and application of fish scale wastes as versatile natural biomaterials—ScienceDirect*. <https://www.sciencedirect.com/science/article/abs/pii/S138589472102684X>
- Ravago, R. (2022). Exploring FT-NIR Spectroscopy to Determine the Age of Black Sea Bass and White Grunt with Otoliths and Scales [M.S., College of Charleston]. In *ProQuest*

- Rumengan, I. F. M., Suptijah, P., Wullur, S., & Talumepa, A. (2017). Characterization of chitin extracted from fish scales of marine fish species purchased from local markets in North Sulawesi, Indonesia. *IOP Conference Series: Earth and Environmental Science*, 89(1), 012028. <https://doi.org/10.1088/1755-1315/89/1/012028>
- Saas, E., Dorval, E., Porzio, D. L., & Schwartzkopf, B. D. (2024). *Development of methods for collecting spectral data from Fourier transform near-infrared spectroscopy to age three small coastal pelagic species (Pacific Sardine, Pacific Mackerel, Northern Anchovy) in the northeast Pacific Ocean*. <https://doi.org/10.25923/GP34-Z216>
- Santana, C. A., Andrade, L. H. C., Suárez, Y. R., Yukimitu, K., Moraes, J. C. S., & Lima, S. M. (2015). Fourier transform-infrared photoacoustic spectroscopy applied in fish scales to access environmental integrity: A case study of *Astyanax altiparanae* species. *Infrared Physics & Technology*, 72, 84–89. <https://doi.org/10.1016/j.infrared.2015.07.005>
- Thompson, A. R., Chen, D. C., Guo, L. W., Hyde, J. R., & Watson, W. (n.d.). *Larval abundances of rockfishes that were historically targeted by fishing increased over 16 years in association with a large marine protected area | Royal Society Open Science | The Royal Society*. <https://royalsocietypublishing.org/rsos/article/4/9/170639/93633/Larval-abundances-of-rockfishes-that-were>
- Tray, E., Brophy, D., Maoiléidigh, N. Ó., Finlay, R., de Eyto, E., Poole, R., & Crowley, Q. G. (2022). Investigating post-depositional alteration of trace elements in fish scales using tagged and recaptured wild salmon. *Fisheries Research*, 248, 106207. <https://doi.org/10.1016/j.fishres.2021.106207>
- Tzadik, O. E., Curtis, J. S., Granneman, J. E., Kurth, B. N., Pusack, T. J., Wallace, A. A., Hollander, D. J., Peebles, E. B., & Stallings, C. D. (2017, February 11). *Chemical archives in fishes beyond otoliths: A review on the use of other body parts as chronological recorders of microchemical constituents for expanding interpretations of environmental, ecological, and life-history changes*. <https://aslopubs.onlinelibrary.wiley.com/doi/10.1002/lom3.10153>
- Vasconcelos, J., Jurado-Ruzafa, A., Otero-Ferrer, J. L., Lombarte, A., Riera, R., & Tuset, V. M. (2021). Thinking of Fish Population Discrimination: Population Average Phenotype vs. Population Phenotypes. *Frontiers in Marine Science*, 8. <https://doi.org/10.3389/fmars.2021.740296>
- Wedding, B. B., Forrest, A. J., Wright, C., Grauf, S., Exley, P., & Poole, S. E. (2014). A novel method for the age estimation of Saddletail snapper (*Lutjanus malabaricus*) using Fourier Transform-near infrared (FT-NIR) spectroscopy. *Marine and Freshwater Research*, 65(10), 894–900. <https://doi.org/10.1071/MF13244>

Yasmeen, L., Sadiq, M., Hamza, A., Nargis, B. R., & Afzal Khan, M. (2025, November).  
*Evidences of formation of predominant calcite polymorphs in otoliths of the fishes of  
riverine environment—ScienceDirect.*  
<https://www.sciencedirect.com/science/article/abs/pii/S0165783625002899>