

Investigating Seasonal Variability of Northern Anchovy Population Age Structure in Monterey Bay, California

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Abstract

The California Current System (CCS) is a highly productive marine ecosystem spanning from the Baja California peninsula northward to the Fraser River. The CCS is characterized by wind-driven upwelling which brings cold nutrient-rich water to the surface where it catalyzes primary productivity. Forage fish, like the Northern anchovy (*Engraulis mordax*) consume primary and secondary producers (phytoplankton and zooplankton) and serve as critical energy conduits to upper trophic level organisms (marine mammals, seabirds, other fish species). Despite playing an important role, Northern anchovy population structure is understudied. Monterey Bay is centrally located in the CCS and known for hosting a productive and diverse ecosystem. Northern anchovies have been collected weekly in the bay since August 2021. This effort is part of a larger project to generate a unique database with high temporal resolution to investigate seasonal variability of Northern anchovy in the central CCS. In this study, sagittal otoliths were used to age the anchovy, similar to tree rings. This study aims to answer the following questions: 1) Is there a seasonal pattern for anchovy ages in the bay?, 2) Can we determine relative growth rates based on otolith ring width? and 3) Is there a relationship between upwelling seasonality and anchovy age structure in the bay? This study will contribute to understanding how seasonal variability in productivity affects forage species' population structure as well as trophic relationships in marine ecosystems.

Introduction

Marine ecosystems are complex sources of biodiversity and contain organisms as small as bacteria and as large as blue whales. The California Current System (CCS) is an example of a highly productive marine ecosystem. The CCS is a narrow continental shelf that is responsible for coastal upwelling in regions like Monterey Bay (Thayer et al., 2013). Coastal upwelling pulls cold, nutrient-rich water to the surface to push forward primary productivity. Climatic events like global warming and upwelling can shift the availability of nutrients in a community. The annual upwelling event in Monterey Bay pushes cold, nutrient rich water up towards the surface, increasing the nutrient availability, productivity, and biodiversity of the area (Chavez et al., 2017). Although an understanding of how nutrients move through a marine ecosystem exists, it is difficult to examine the connections between this movement and predator-prey relationships within a single ecosystem.

The Northern anchovy (*engraulis mordax*) is a forage fish species throughout the CCS. This species is particularly important for studying marine ecosystems because they are major energy conduits between upwelling driven productivity and upper trophic levels. In other words, they exist at the center of their food chains (Thayer et al., 2013). Northern anchovies consume microorganisms as small as phytoplankton but also serve as prey for animals as large as blue whales. Anchovies are highly responsive and connected to their environment. Changes in their environment, such as an increase in nutrient availability during upwelling seasons, are reflected in their growth patterns as their food availability changes. Despite all of this, Northern anchovies remain largely understudied. This project aims to explore Northern anchovy population and age structure as part of a larger effort to develop a growing database. Though Northern anchovy ranges through the whole CCS, this project focuses on the population found in Monterey Bay,

which is centrally located in the CCS. Specifically, the sagittal otoliths of Northern anchovies will be used to age a population sample from Monterey Bay.

The sagittal otoliths are a pair of bones found in the head in all vertebrate fish. Composed mostly of calcium carbonate (CaCO_3) in the form of aragonite, otoliths are a natural record of the environment and food availability of anchovies (Mendoza, 2006). Not only can they be used to age organisms similar to tree rings, but temperature, pH, and food composition are known to affect the rate and type of otolith growth; thus, they are a reflection of environmental changes an anchovy encounters during its lifetime (Mille et al., 2016; Soares et al., 2021; Coll-Lladó et al., 2018; Karakiri et al., 1989). An annulus, one year of anchovy growth, corresponds to a combination of a complete opaque and a complete translucent band. The age of the anchovy is based on the number of complete annuli present in the otolith. The growth an anchovy experiences in one year can be divided into two periods corresponding to the opaque and translucent band. These periods are reflective of seasonal changes and food availability. The opaque band corresponds to an intense period of growth when there is higher food availability, such as during the upwelling cycle. The second period of growth is represented by the blue translucent band. The translucent band corresponds to a less intense period of growth when there is less food availability, such as in the winter months (Schwartzkopf et al., 2022)

Currently, there is no comprehensive database that profiles Northern anchovies based on their age across a high temporal range. Adult Northern anchovies have been caught and aged every spring and summer since 2015 in other studies; however, this project is unique because it achieves a full seasonal distribution of anchovy despite using a single location (Schwartzkopf et al., 2022). This project begins to generate a database centered around the age of anchovies in order to address both growth patterns and population structure with high resolution for

seasonality. This project aims to answer the following questions: 1) Is there a seasonal pattern for anchovy ages in the bay?, 2) Can we determine relative growth rates based on otolith ring width? and 3) Is there a relationship between upwelling seasonality and anchovy age structure in the bay?

Methods

The methods that were used in this study can be divided into three components: retrieval, dissection, and aging. Beginning in August 2021, Northern anchovies were captured through weekly surveys across Monterey Bay. These surveys were conducted on the R/V Paragon via MBARI. During each survey, ten anchovies were caught using hook and line within 10 miles of the shore. This method was used in order to prevent by-catch products and increase selectivity. Current databases profiling anchovies based on age conduct annual surveys during the summer months, preventing them from exploring the impact of seasonal variability on anchovy growth (Schwartzkopf et al., 2022).

The anchovies were dissected to harvest the sagittal otoliths from the head. Before dissecting, the standard length of the anchovy (cm) and its weight were measured. Standard surgical materials were used during the dissection: scalpel, tweezers, gloves, etc. The otoliths were dry stored in microcentrifuge tubes until analysis. To age the otoliths, they were submerged in water in a petri dish that had a black background to increase the contrast of the otolith. Then, the otoliths were lit from above and photographed by the Olympus DP74 microscopic camera. It was dependent on the reader to photograph the side of the otolith that presented the clearest depiction of its rings. Upon being photographed, the CellSens software was used to measure the width of each opaque and translucent portion of an annulus. Each measurement began at the core

of the otolith and extended to the edge of the nearest band at the point farthest away from the core (generally near the bottom of the otolith). **Figure 1** depicts how the measurement was taken for the first opaque (A) band for an otolith sample. The width of each band, the age of the anchovy (total number of complete annuli), the edge type (opaque or translucent), and the reader's confidence level (1-least confident, 3-most confident) were recorded.

Results

The results of this project fall into three different categories: seasonal patterns of northern anchovy populations, otolith types and complexity in presentation, and predictive growth models. Different variables were compared ranging from anchovy weight, length, age, the date each anchovy was caught, and the otolith size defined as otolith width. These results are drawn from a developing database of northern anchovies that used a total of 178 samples.

The population and age structure of Northern anchovy are explained in graphs that examine relationships within anchovy populations and across temporal ranges. Figure 2 displays the temporal distribution of Northern anchovy otolith size and age from August 2021-July 2022. There is high variability in the population structure; there is not an isolated age group of anchovies present at a specific time during the year.

The age structure within the Northern anchovy population of Monterey Bay is explained through Figure 3: a plot comparing the age of the anchovy with otolith size. There is high variability within the age structure.

Figures 4-A and 4-B compare a more literal measurement, such as fish length, against anchovy age and otolith size, respectively. Figure 4-A compares anchovy length to age. Here, there is no significant correlation between the two variables. On the other hand, Figure 4-B

compares otolith size to fish length, and there is much more positive correlation present. As the length of the fish increases, the size of its otolith increases as well.

Otolith presentation was one of the challenges of this project because of its inconsistency from anchovy to anchovy. Figure 5 displays four different otolith pairs taken from the sample that exhibit different presentation types (from left to right): standard, opaque edge, split checks, cracked or “waterlogged.” The first pair is a standard pair of otoliths that has a clear distinction between the opaque and translucent bands. The second pair is a pair of otoliths exhibiting an opaque edge. An anchovy with otoliths presenting an opaque edge was caught during a period of intense growth (Schwartzkopf et al., 2022). The third pair is a pair of otoliths exhibiting a split check. A check is any irregularity in the otolith presentation that could be mistaken for a band or an annuli when it is unrelated to the age of the anchovy. A split check is a type of check where an anchovy undergoing weak growth, as marked its translucent band, suddenly experiences a burst of food consumption high in energy that causes its translucent band to be interrupted by a short opaque sliver before resuming back to the translucent band (Schwartzkopf et al., 2022). The final pair on the far right is a pair of otoliths that has no precise distinction between the opaque and translucent bands. This lack of distinction is due to two factors: cracking and “waterlogging.” Cracking is when cracks appear in the otolith surface as a result of being damaged during dissection or storage. “Waterlogging” takes place when, upon being submerged in water, the otolith band presentation becomes warped, distorted, or blurry in a way that complicates the distinction between opaque and translucent bands.

Another goal of this project was to develop some sort of predictive growth model that could return the hypothetical ages of anchovy upon inputting their length. This project used the Gompertz model, which is typically used by fisheries to model growth rates of species using age

and length (Tjørve et al., 2017). Figure 6-A shows anchovy length data plotted against age with a generic Gompertz curve plotted on top of it to compare the data's fit. Figure 6-B is a plot that compares fish length with otolith size and shows a Gompertz curve that was calculated and fitted precisely to the data.

Discussion

Generally, the results of this project across all three categories: seasonal population patterns, otolith presentation, and predictive growth models, showed a high amount of variability and lacked an adequate sample size to accurately reflect the population and age structure of the Northern anchovy in Monterey Bay. There was no significant trend in the temporal distribution of anchovy population structure, which implied that anchovy population structure does not depend on seasonality. However, this is not necessarily the case. Though it is clear that there is high variability, this could be explained by existing understandings of anchovy spawning behavior. Research suggests that Northern anchovy spawn throughout the year, and that their spawning behavior is not isolated to a specific season (Thayer, et al., 2013). Anchovy spawning patterns could serve as an explanation for the variability in population and age structure. Additionally, existing databases that profile Northern anchovy based on a monthly, semi-annual, or annual seasonality have sample sizes of up to 12,000 (Schwartzkopf et al., 2022). The high amount of variability present in Figure 2 does not necessarily imply a lack of trend behavior among Northern anchovy populations in Monterey Bay. Instead, more anchovy samples may be needed to accurately reflect the population composition.

The variability reflected in the temporal distribution extends to age structure as well. When comparing otolith size or anchovy age, no significant trend was observed. Where a strong

correlation between increasing otolith size and anchovy age was expected, high variability was shown to suggest that otolith size does not correspond to anchovy age. Despite all of this, few anchovies >2 years old were collected, suggesting that there is a lack of data to accurately describe the age structure of the Monterey Bay population.

Age structure was also analyzed through its relationship with fish length. A positive correlation was observed between otolith size and fish length but not between fish length and age. The former observation is consistent with what is expected of an anatomical structure responsible for representing growth patterns. However, why is there a strong correlation between fish length and otolith size, but not age? This question is particularly intriguing when age should be a direct reflection of otolith presentation a.k.a size. One explanation for this discrepancy is that otolith size is a relatively objective measurement. The reader is responsible for measuring the otolith width from the core to the farthest edge, and there is very little left to interpret. Unlike otolith size, the age of the anchovy is dependent on the reader's interpretation of the bands presented in the otolith and therefore subject to more inaccuracy.

As shown previously, there was high variability in otolith presentation that directly contributed to the variability in anchovy age structure. Examining the standard pair of otoliths, the reader would be able to confidently assume that this anchovy is one year old because there is a clear distinction between the bands, enabling an accurate read of the annuli. Similarly, the pair of otoliths with an opaque edge clearly depicts this edge type. However, many other otoliths with opaque edges could easily have been mistaken for a refraction of the microscope light or a crack in the otolith, ruining the reader's ability to accurately record the age. The pair of otoliths demonstrating the split check present a split check small enough for the reader to confidently assume that the opaque sliver does not correspond to an actual band (and therefore, an annuli)

but is instead a check. However, many otoliths presented split checks where the opaque sliver was large enough to be mistaken as a complete band, causing the reader to inaccurately age the anchovy. Finally, the pair of otoliths with the cracked and “waterlogged” presentation displays a common event where several otolith samples were photographed with several cracks along the surface resulting from damage during the dissection process or from the impact of hitting the sides of the microcentrifuge tubes used to store them. This pair would prevent the reader from accurately aging the fish with high confidence because there is no distinction between the band types significant enough to observe an annulus. These four pairs of otolith depict the variability in otolith presentation that decreased the accuracy of anchovy aging. Aging subjectivity may be a plausible explanation for why trends were seen with otolith size but not age.

Developing a predictive growth model for this project involved a novel method that was not typical of Gompertz models. As shown previously, there was no visible correlation between length and anchovy age and this data did not fit the generic Gompertz curve at all. The data’s inability to fit the Gompertz curve was caused by the way otoliths were used to measure age. Because age was measured based on the number of complete annuli (and not partial), multiple fish could be at different stages of the third year of growth, but all would be recorded as two years old because that is how many complete annuli were present. The aging method inherently prevents the anchovy age from being recorded precisely enough to fit the Gompertz curve. To evade this problem, otolith size was used as a proxy for age since otolith size is another representation of anchovy growth. As seen previously in Figure 4-B, there was a stronger positive correlation between fish length and otolith size than there was with age. Therefore, it was inferred that the data in Figure 4-B would fit the Gompertz model much better. In fact, Figure 6-B shows the fit of the Gompertz curve to the data after being calculated precisely, and it

presents a good model of the data besides the lack of an asymptote at the top. Instead of assuming a lack of asymptote means the Gompertz model cannot be used in this case, it can be attributed to a lack of data points from older and longer fish, suggesting that more data is needed to truly discredit the model. Additionally, it might be beneficial to look towards lab-grown Northern anchovy so that the age can be recorded more precisely in order to better fit the data. Research has shown that Gompertz models can be used to model growth rates in lab-grown fish when comparing fish length and weight (Sakagawa et al., 1976). Thus, looking at lab-grown anchovies or a different comparison between weight and age might be promising.

Conclusion

Based on the results of this project, there is no seasonal pattern for population structure among Northern anchovy in Monterey Bay, nor is there a clear relationship between upwelling seasonality and anchovy age structure. It is important to note that this conclusion may not be reflective of the true composition of Northern anchovy population and age structure in Monterey Bay, especially considering the small sample size compared to other studies. Instead, this project can be considered the results of a preliminary stage of a larger project aiming to profile the anchovy composition in Monterey Bay. Though a predictive growth model was developed using otolith size as a proxy for age, this is not a reliable method for modeling anchovy growth on the basis of age in the future. Instead, relationships between age and weight should be studied, or a more precise method to age the anchovy must be developed (possibly through lab-grown fish).

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Appendix: Tables and Figures

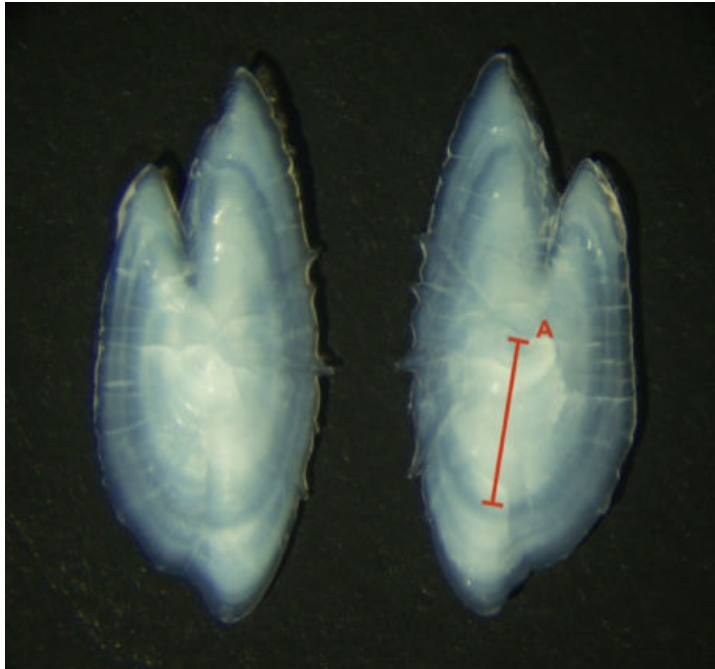


Figure 1: A pair of otoliths harvested from an anchovy part of this study. Measurement A corresponds to the first measurement for otolith width starting at the center (core) of the otolith and extending to the edge of the first opaque band.

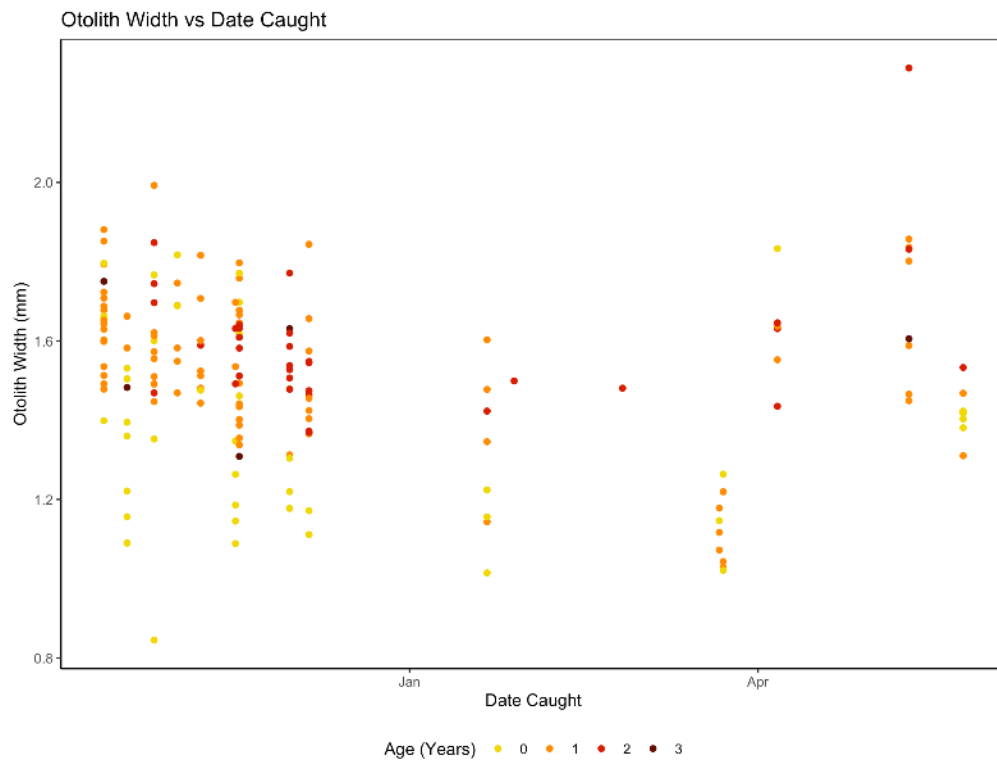


Figure 2: A plot depicting the temporal distribution of otolith size (mm) from August 2021-July 2022, color coded by the interpreted age of anchovy (years).

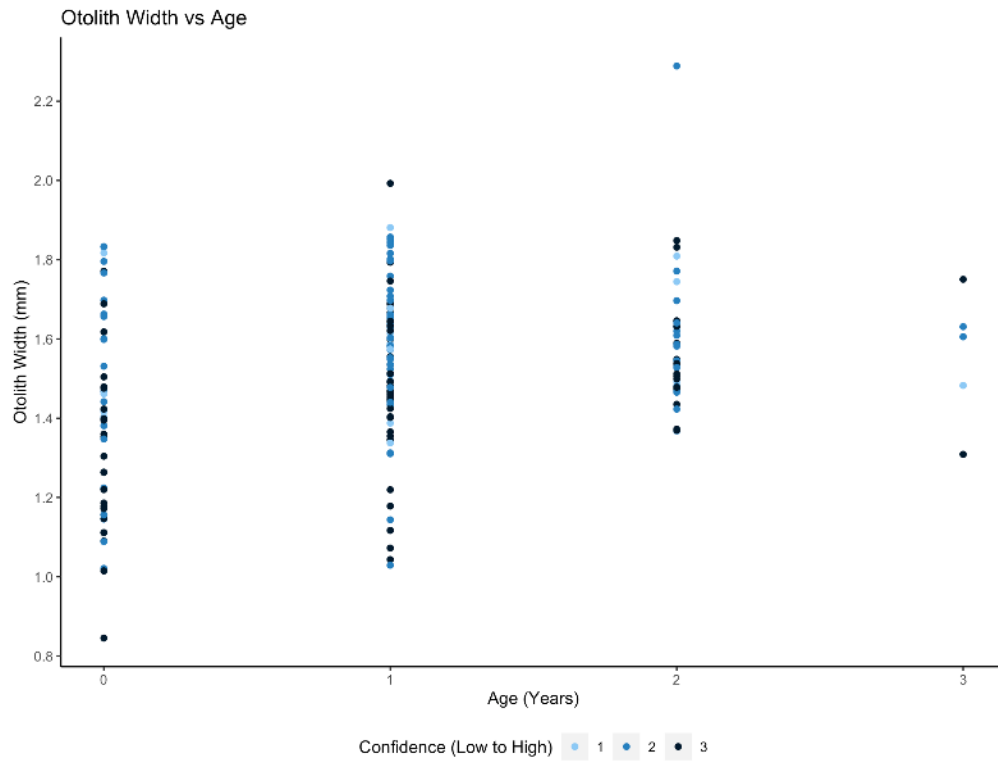


Figure 3: A plot comparing the age of anchovy (years) and otolith size, color coded by the reader's confidence in their otolith reading.

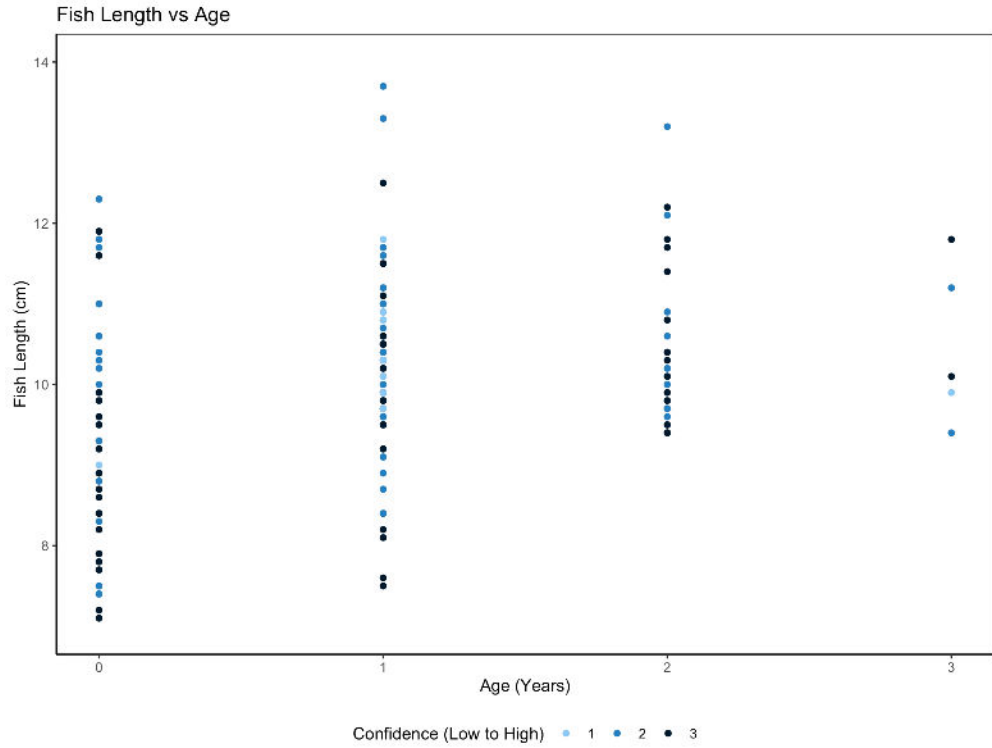


Figure 4-A: A plot comparing the age of the anchovy (years) against the standard length of the fish (cm), color coded by the reader's confidence in their measurement.

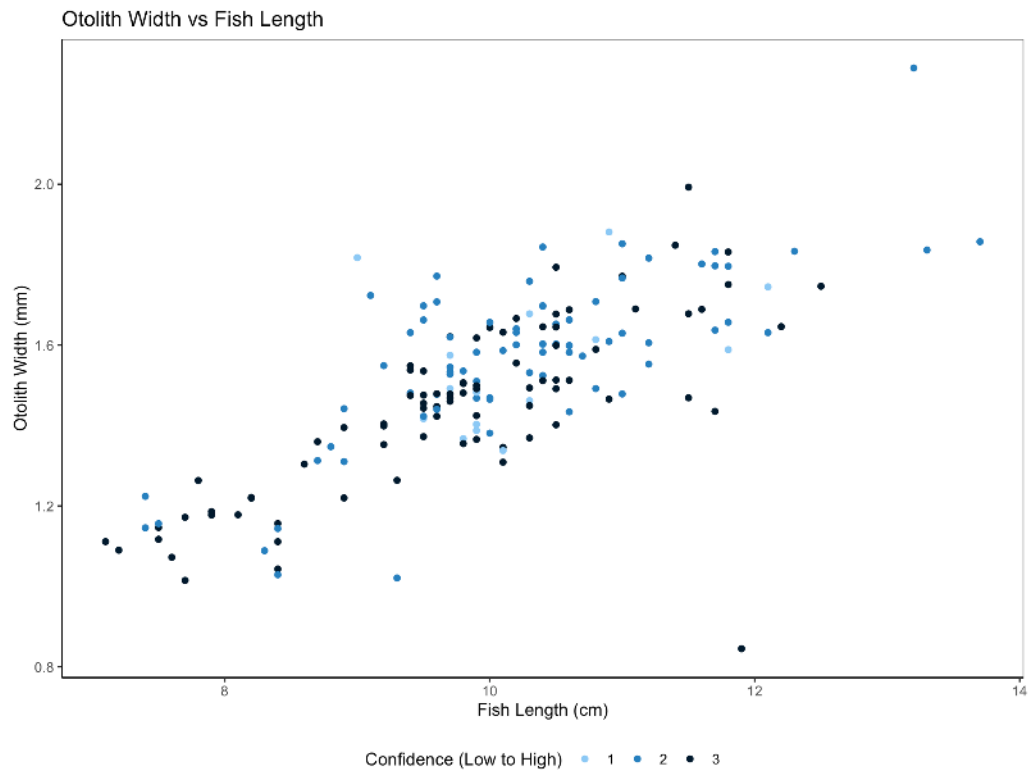


Figure 4-B: A plot comparing the standard length of the anchovy (cm) against the otolith size (mm), color coded by the reader's confidence in their measurement.

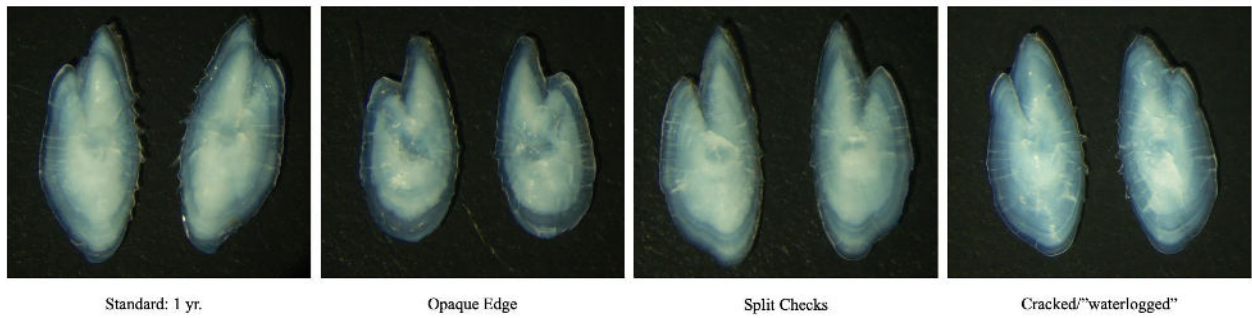


Figure 5: Four different otolith pairs from this project depicting variability in otolith presentation (left to right): clear, confident, and standard pair; pair with an opaque edge type; pair depicting split checks in the first translucent band; and a pair depicting cracked, “waterlogged”, and unclear band patterns.

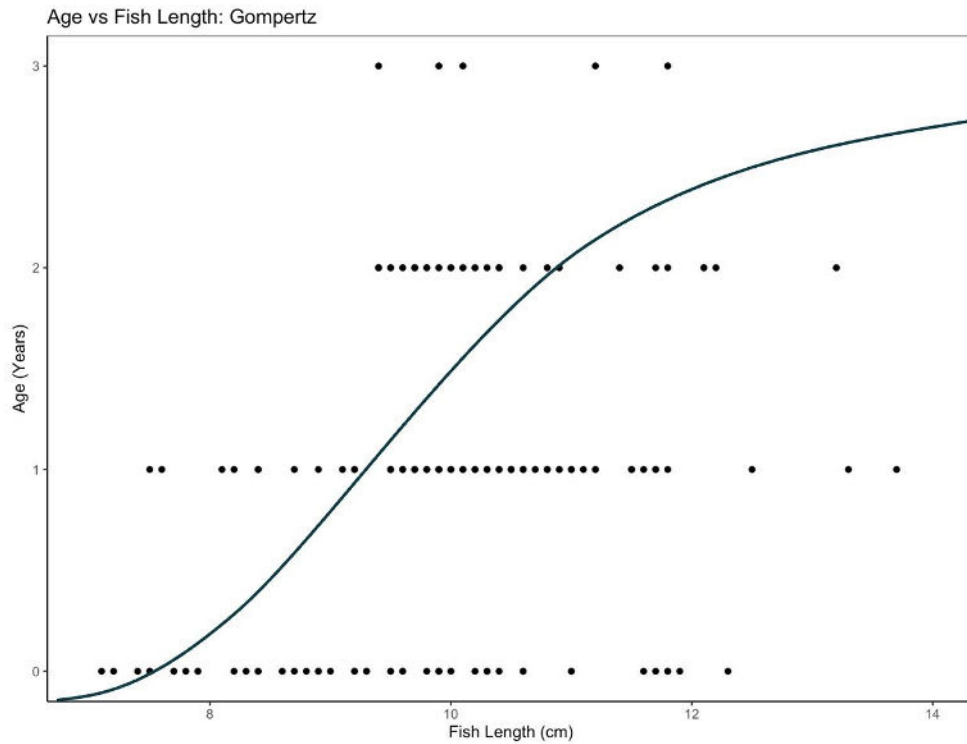


Figure 6-A: A plot comparing the standard length of the anchovy (cm) against the interpreted age of the fish (years). On top of this plot, the generic shape of a Gompertz Model curve is shown to compare the fit of the data to the model type.

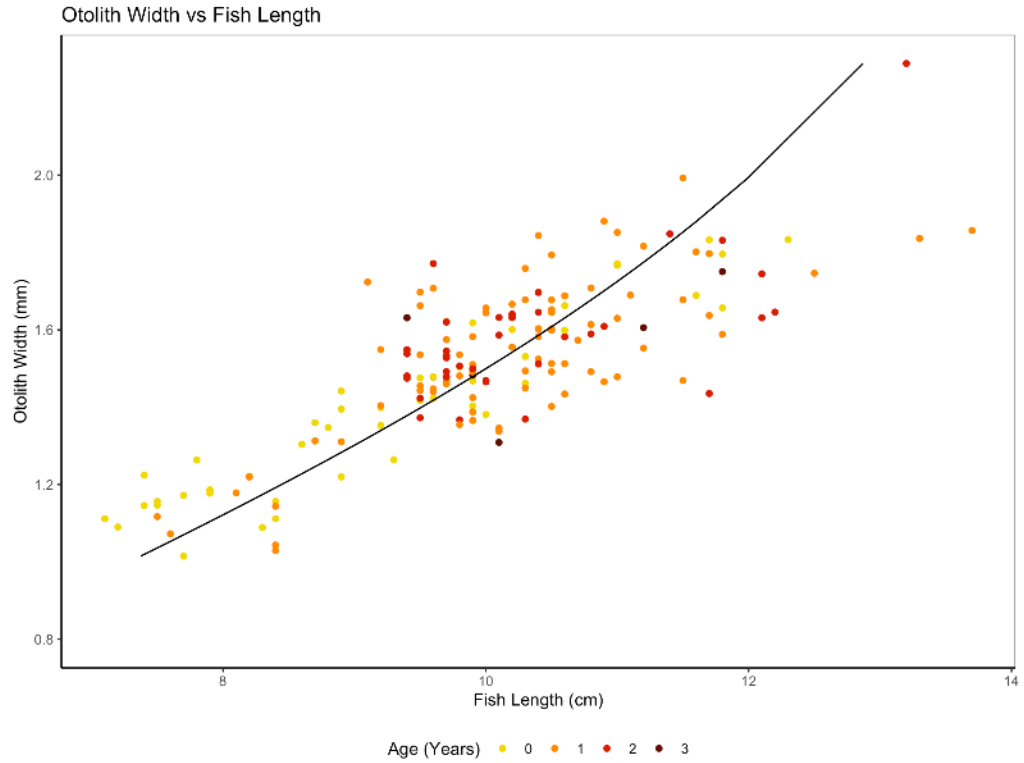


Figure 6-B: A plot comparing the standard length of the anchovy (cm) against the otolith size (mm). A Gompertz Model curve was calculated and fitted to the data precisely.