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and Inland Fisheries

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RESEARCH PROJECT

Evaluating Age Determination for Opercula of European Perch (*Perca fluviatilis*)

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1 Introduction

1.1 Age determination

Age determination is a crucial aspect of fishery management, as it provides valuable information on growth rates and age structure. Age data can traditionally be obtained by observing age groups in length-frequency histograms over time, but this time- and cost-effective approach has its limitations. As fish age and their growth rates slow, the age groups start to overlap, requiring computational methods to accurately separate these groups and achieve a clearer analysis of size-frequency data *(Macdonald and Pitcher 1979)*.

This approach does not provide the age or growth rate of individual fish. To determine the age of a particular fish, examinations of specific biological structures are necessary. A widely used method is the examination of various hard structures, including scales, otoliths and skeletal bones like the opercular bone *(Le Cren 1947; Campana 2001; Maceina et al. 2007)*. These calcified structures exhibit a distinctive banded structure formed by alternating periods of rapid and slow growth. The distinct annuli in these structures result from seasonal variations in environmental factors such as temperature and food availability. During periods of favorable conditions, such as warmer temperatures and high prey density, fish exhibit accelerated growth, leading to the formation of wider bands on the structure. Conversely, narrower bands are attributed to slower growth during colder periods due to low metabolic rates or reproductive phases, where energy is allocated toward the development of gonads. Misleading false growth marks can also appear during physiologically demanding periods, such as food scarcity, changes in water quality or illness. During these times, fish growth rates can vary significantly, leading in unclear or altered band structures on the structure. Additionally, it can become difficult to discern the sequence of annuli when the structures of older fish exhibit considerable thickness or irregular growth patterns.

This uncertainty in examining hard structures underscores the inherently interpretative nature of age reading. Inaccuracies or systematic errors in this process can have substantial impacts on the estimated growth rates and age structure of fish populations, building a false basement for models leading to wrong management of fish resource *(Yule et al. 2008)*. For example, if the age of larger individuals is underestimated, it can result in high growth rates, which may mislead management strategies and increase the risk of overfishing or misdirected efforts to enhance recruitment rates. Therefore, potential errors in aging must be carefully considered when interpreting age data. Evaluating quality in age determination is crucial not only for drawing reliable conclusions but also for comparing research findings across studies *(Campana 2001)*.

For *Perca fluviatilis*, the operculum is commonly used to estimate age. The method described by *Le Cren (1947)* has been applied in several studies to assess age and growth patterns *(Le Cren 1958; Shafi and Maitland 1971; Gee 1978)*, even there are no formal validation studies that have been conducted for this method. While many studies have compared age estimates from various calcified structures in percids (*Percidae)*, relatively little research has been dedicated to evaluating the actual accuracy of these age estimates. Supporting the operculum as a suitable structure for age estimation, *Babaluk and Campbell (1987)* validated annual growth increments using tetracycline labelling on opercula of Walleye (*Stizostedion vitreum vitreum*), which have a similar structure and band sequence to those of European perch. For slower-growing yellow perch (*Perca flavescens*), the use of opercula for age determination has shown to be more precisely as using scales *(Baker and McComish 1998)*, attributed to greater clarity in identifying false annuli on opercula, as true and false annuli on scales can become crowded at the edges, complicating the distinction between them.

Regarding the formation of annual rings for percids, *Blackwell and Kaufman (2012)* documented that annulus formation in yellow perch (*Perca flavescens*) was typically completed by June for younger fish. While most age groups finished annulus formation by July, some older fish did not complete the process until August. By September and October, all fish had fully developed their annuli. Although this pattern may provide a hint for European perch, there are no studies specifically investigating the timing of annulus formation for this species. However, *Le Cren (1992)* noting that by mid-September *Perca fluviatilis* had completed their annual growth and annulus formation.

1.2 Aims of research

The primary aim of this project is to evaluate the reliability and quality of the age estimation method that involves measuring annuli on the opercula of *Perca fluviatilis*. Specifically, the study investigates potential systematic biases introduced by repeated measurements and explores whether the precision varies. It focuses on several key objectives:

1. **Consistency and quality of aging**: Investigating how reliable age estimates are when obtained through repeated measurements, with a focus on detecting potential systematic bias and the precision of annual ring length measurements across annuli.

2. **Comparison of pairs of opercula**: Comparing age estimates derived from the left and right opercula to identify any significant differences.

3. **Comparison with different reader**: Evaluating how these age estimates align with an independent set of age data from a previous study by Höhne et al. (2020), who also examined the same opercula for age determination.

2 Material and methods

2.1 Sampling and preparation

The gill covers of European perch (*Perca fluviatilis*) were collected from gravel pit lakes in Lower Saxony (Germany), during the period from late August to early October in the years 2016 and 2020. Fish were killed and measured to the nearest millimeter from the tip of the snout to the end of the caudal fin. Both gill covers were stored in protective envelopes labeled with the species, fish length, fish weight, lake, date, and type of fishing gear used. A unique identification number was assigned to every fish. Both gill covers from each specimen were placed in glass dishes and immersed in hot water for approximately five minutes. After soaking, the operculum was extracted from the gill cover using tweezers. Any remaining material was carefully wiped off with a paper tissue, ensuring the operculum was thoroughly cleaned. Whole opercula were viewed under a Leica MZ8 stereo microscope using transmitted light and a plan apochromat M objective ($f = 89$mm) and digitally recorded in high resolution (3648x2736 pixels) with a Leica MC190 HD camera via image acquisition software LeicaX. Larger structures (usually from perch >25cm) were recorded in separate images and merged using Inkscape software, adjusted only by rotating and shifting to align the images without distortions.

2.2 Sample selection and measurement

An initial training set ($N = 24$, single operculum) was selected to evaluate the age reading, allowing for the early identification of potential systematic biases before applying it to the larger dataset ($N = 66$, both opercula).

Both sets were selected along a broad length gradient, ensuring a representative distribution of age classes and capturing differences among different age groups. For comparison, both the left and right opercula were required to be present and undamaged. Most of the samples meeting this requirement were collected from Korlshorner See and Meitzer See, along with larger length classes of perch from other lakes. The samples were arranged in a randomized order for each new measurement series, and no information regarding their ID, origin, length, or catch date was available. Each annulus of every structure was measured as a radius in millimeters along a defined axis. A straight line was drawn along the outermost operculums edge, from which an orthogonal line was then constructed. This orthogonal was positioned to pass through the innermost point of the operculums origin, the point of initital growth as described in *Le Cren (1947)*.

Identifying the exact origin when the operculum is laid flat can be difficult, as it is obscured. Therefore, the structure was carefully examined beforehand to define the origin more accurately. In more challenging cases, or cases of thicker opercula, closely zooming in on the area of origin revealed subtle variations in shading, which helped estimate its location.

Another set of the measurement for the same structures were taken in *Höhne et al. (2020)* with a Mitutoyo digital caliper and image analysis software QSPAK. In this set, the information regarding which operculum was measured was not recorded.

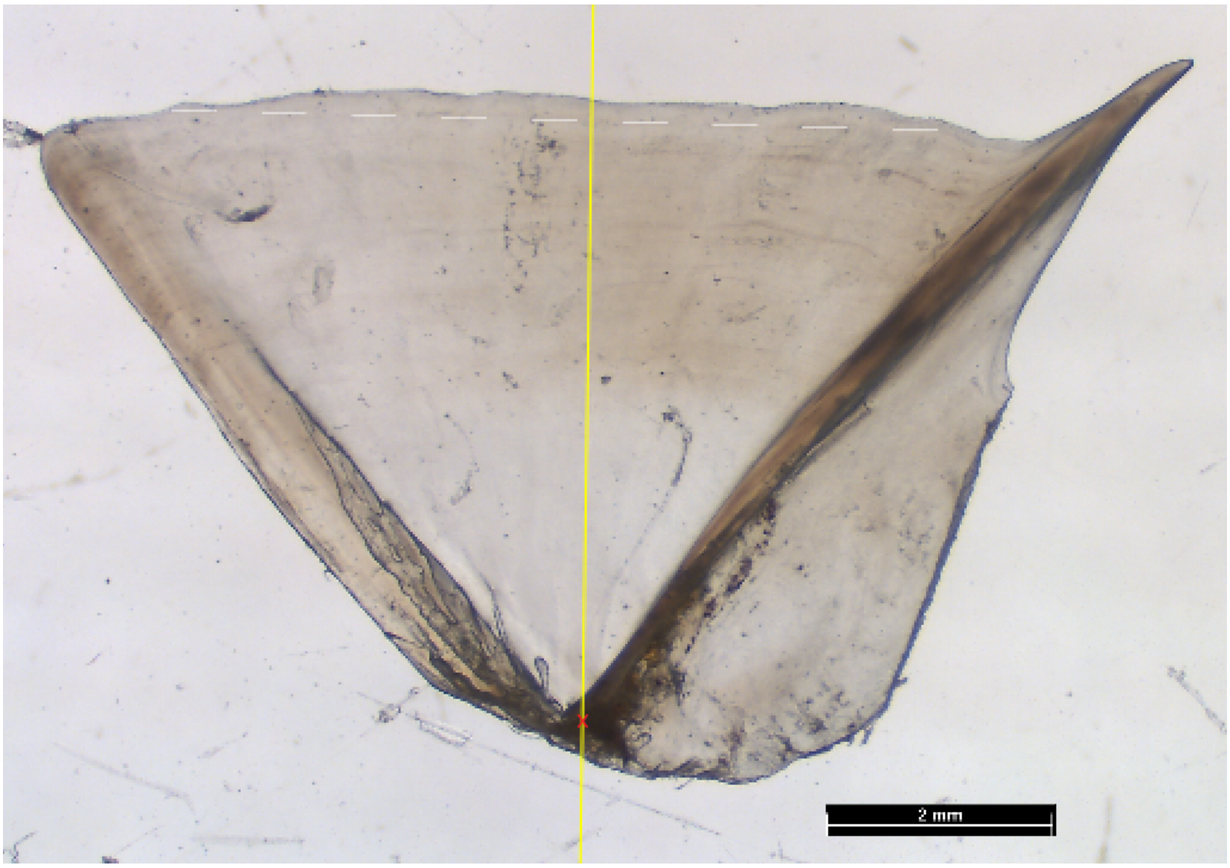


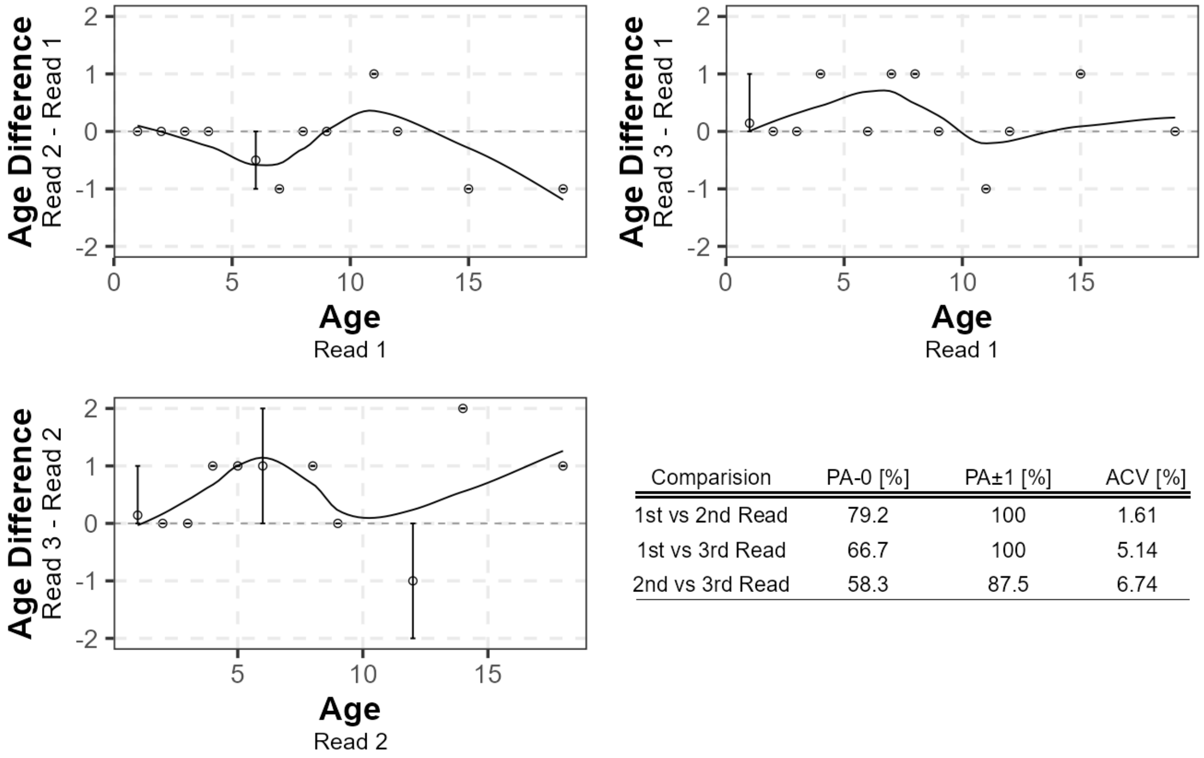
Figure 1: Positioning of the measurement axis. The white dashed line indicates the reference to the outermost edge of the operculum, while the yellow line represents the measurement axis, oriented orthogonally to the reference line. The red cross marks the starting point for measurements towards the respective annuli.

3 Results

3.1 Consistency and aging quality

3.1.1 Training set

The comparison between first (Read 1) and second aging (Read 2) in the small set ($N = 24$, single operculum) which were conducted with a time interval of two days, showed no signs of a directional systematic bias. As shown in Figure 2, the ages of those readings are closely aligned, with 79.2% exact agreement rate (*PA-0*) and 20.8% agreement with 1 year difference (*PA-1*), suggesting strong consistency between the two measurements. The low average coefficient of variation (*ACV*) of 1.61% and the potential for recognizing some structures due to the short time interval between readings suggested the presence of a recognition effect. To test this, the same set was re-read after a few weeks, labelled as Read 3. When comparing this reading with the two before, the results showed a noticeable shift in precision. The agreement rate between Reading 1 and Reading 3 was 66.7% (*PA-0*), while Reading 2 and Reading 3 had an even lower exact agreement at 58.3% (*PA-0*). Additionally, the *ACV* increased to 5.14% for the 1st vs. 3rd Read and 6.74% for the 2nd vs. 3rd Read, supporting the idea that a recognition effect may have influenced the second reading. However, no directional systematic bias was found in the third reading, as the differences did not show a preference in one direction. Ageing was consistent, with no discrepancies exceeding 2 years.



Comparision	PA-0 [%]	PA±1 [%]	ACV [%]
1st vs 2nd Read	79.2	100	1.61
1st vs 3rd Read	66.7	100	5.14
2nd vs 3rd Read	58.3	87.5	6.74

Figure 2: Comparison of age estimates for the small training set ($N = 24$, single operculum). Age Bias Plots show the differences between repeated age readings on same structures. Precision metrics in the table define *PA-0*, representing the percentage of exact matches, and *PA±1*, indicating the percentage of readings within a 1-year difference. The Average Coefficient of Variation (*ACV*) reflects the relative variability of the readings, indicating how consistently the ages were estimated.

3.1.2 Project set

The comparison of the three readings performed on the larger set ($N = 66$, both opercula) showed no signs of a directional bias, as seen in the age bias plots on the left side of Figure 3. It is worth to mention that the third reading showed a slight negative deviation in the older individuals; however, this deviation remains within an acceptable range of -1 to -2 years. The maximum age discrepancy for the same structure was 3 years. Across all reading comparisons, a high proportion showed agreement within one year (i.e. -1, exact, or +1 year), with 97.7% agreement between Reading 1 and 2, 94.7% between Reading 1 and 3, and 96.2% between Reading 2 and 3. The *ACV* remained below 5% across all reading comparisons.

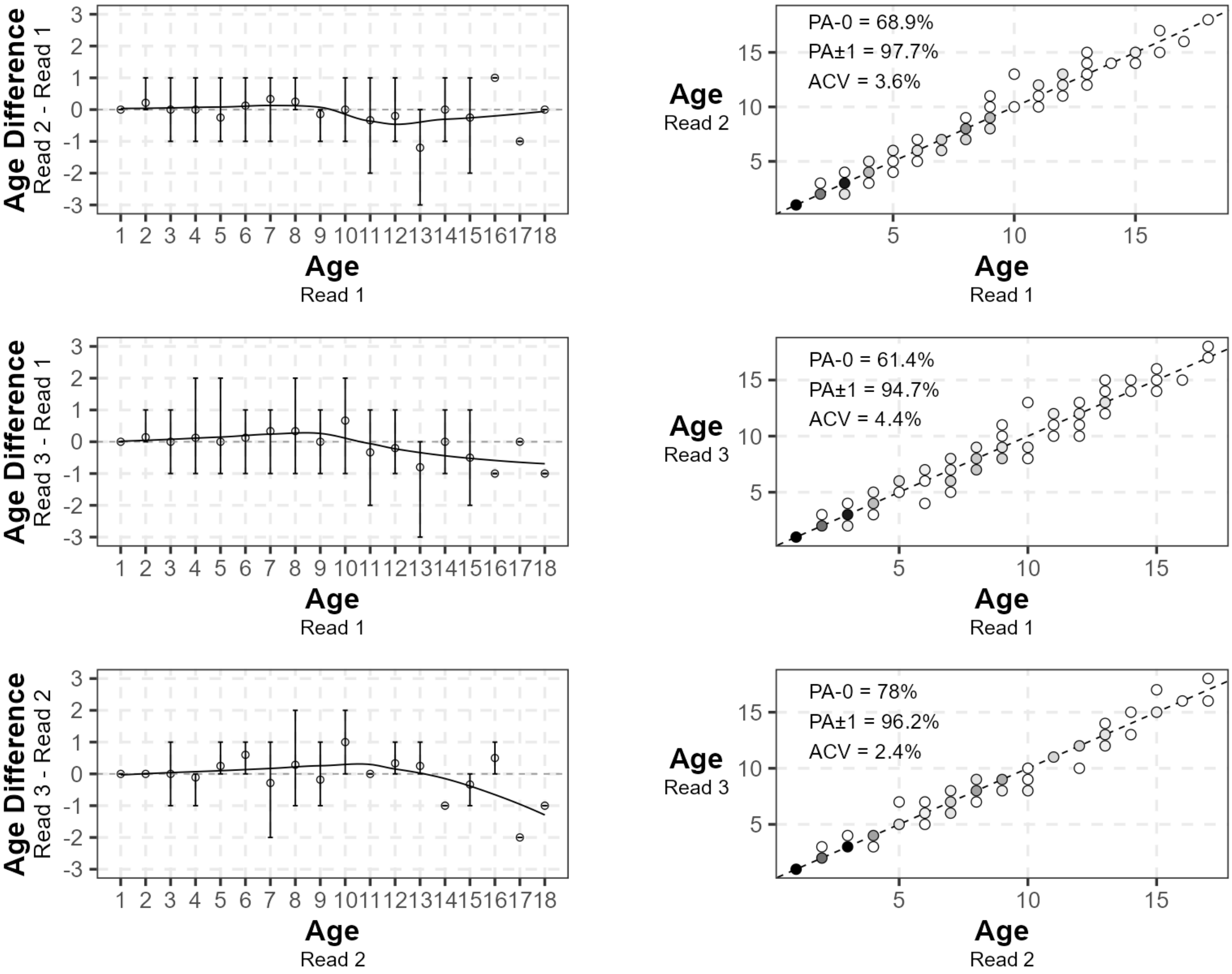


Figure 3: Analysis of age differences between repeated readings. Left side presents age bias plots highlighting the age differences between various readings. On the right, the relationship between the estimated ages from each reading is represented. The shading of the points in the scatter plots represents the sample size relative to the total dataset, giving an indication of the data distribution across different age ranges.

Understanding the degree of precision in identifying the same annulus across readings, cases where the age of the same structure matched in two readings were selected. The corresponding annulus lengths were plotted, with the higher value on the y-axis and the lower value on the x-axis (Figure 4). This setup visualizes the absolute deviation from the line of equality ($x = y$) and serves as an indicator of how precisely each annulus was identified. Only annuli up to the 12th were included, as data for older annuli became increasingly sparse, reducing the robustness of the relationship. The results reveal slightly lower precision for Annulus 1 (A1) ($R^2 = 0.854$) and for A2 ($R^2 = 0.873$) compared to following. The correlation improves for A3 ($R^2 = 0.938$) and A4 ($R^2 = 0.945$) and remains at high levels. For A9 ($R^2 = 0.899$) and A10 ($R^2 = 0.885$), the coefficient of determination then shows slightly lower values, comparable to those of first and second annulus.

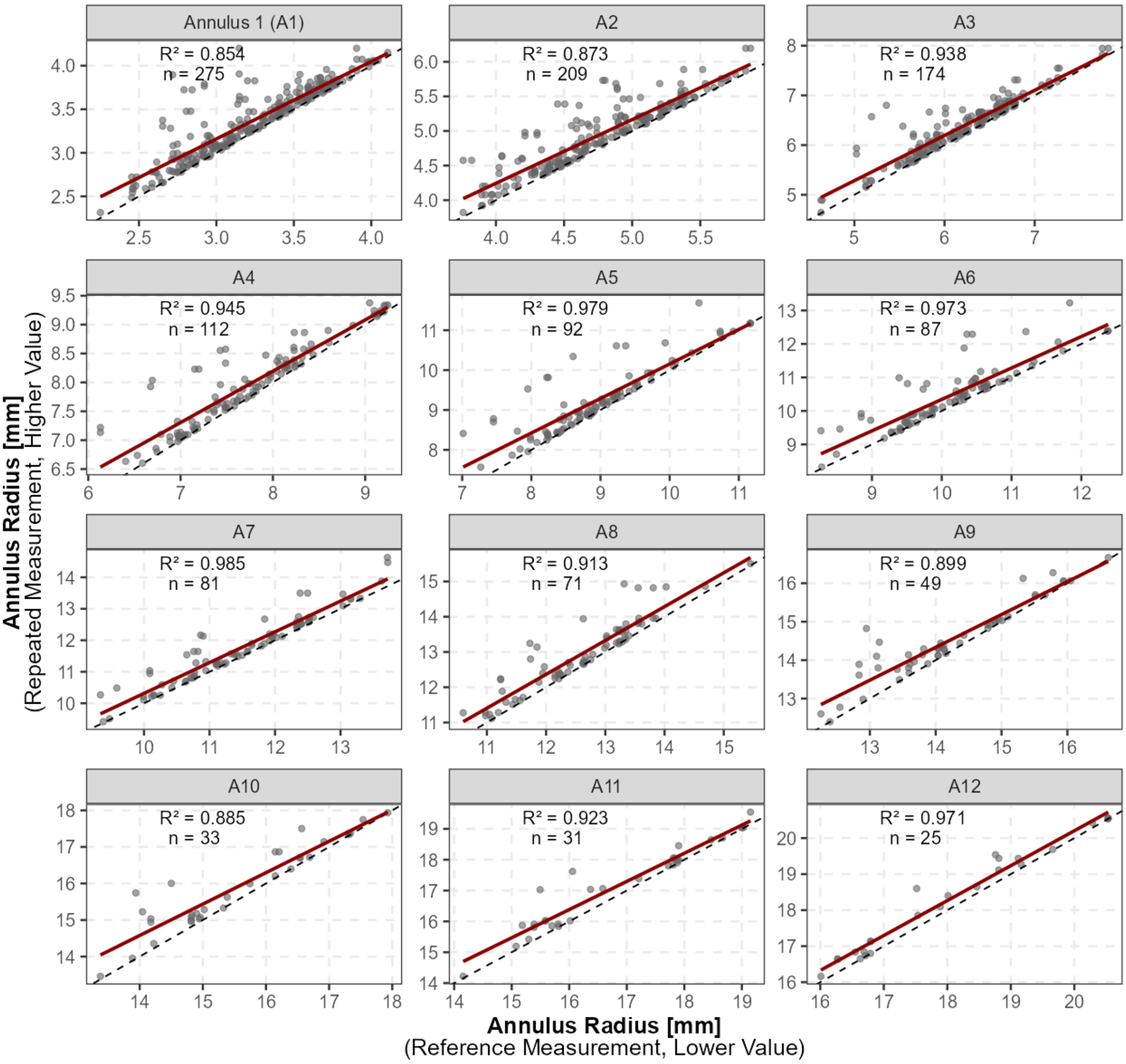


Figure 4: Analysis of determining the same annulus. Comparing annulus measurements across readings with same aging result, higher values are plotted on the y-axis against lower values on the x-axis. Deviation from the line of equality ($x = y$) indicates the precision in identifying the same annulus in different readings.

3.2 Comparison of pairs of opercula

The comparison of age estimates between both opercula of the same individual revealed no directional systematic bias across readings (Figure 5). In approximately half of the cases, the age was determined identically for both opercula (Read 1: *PA-0*$=50\%$, Read 2: *PA-0*$=48.5\%$, Read 3 *PA-0*$=53\%$). A large proportion of the age estimations fell within a 1-year interval across the three reads (Read 1: *PA±1*$=87.9\%$, Read 2: *PA±1*$=89.4\%$, Read 3: *PA±1*$=89.4\%$).

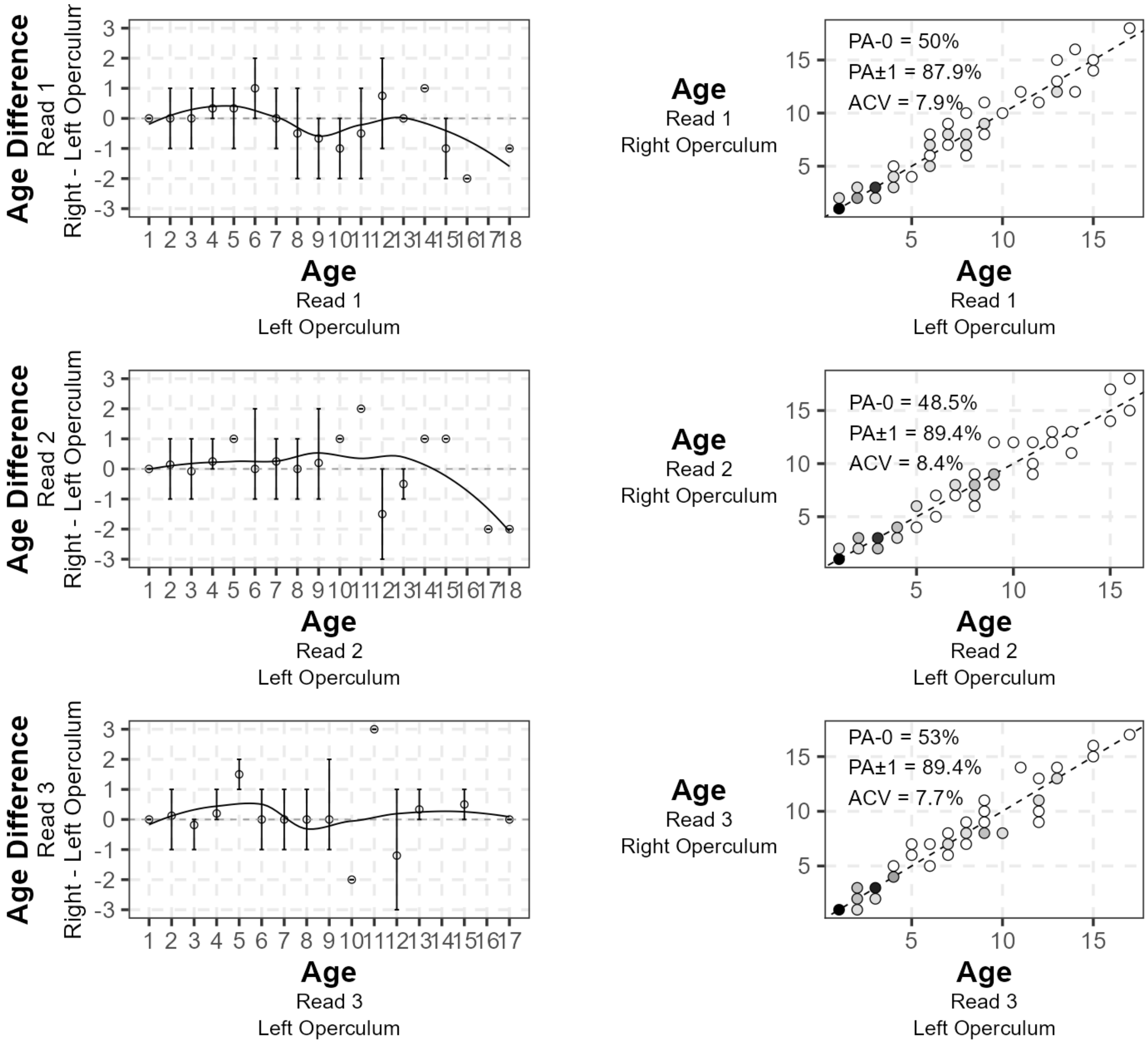


Figure 5: Comparison of repeated age estimates by operculum type (left/right). Age bias plots on left show differences in age estimates between left and right operculum. Plots on right provide a more detailed comparison of the estimated ages for both opercula. Point shading reflects the sample size relative to the total dataset.

The maximum deviation observed between the two structures was three years. Discrepancies in agreement were more pronounced for older age classes. The *ACV* remained across all reading comparisons above 5% (Read 1: *ACV*$= 7.9\%$, Read 2: *ACV*$= 8.4\%$, Read 3: *ACV*$= 7.7\%$), suggesting that there is a notable amount of variation in the age estimates between the two structures. While the differences may not be extreme, they indicate a degree of variability and uncertainty in the age estimation of both opercula derived from the same individual.

3.3 Comparison with different reader

Allowing comparison of age determination results with another reading, the rounded mean of three measurements from the left and right operculum was calculated. This average age was then compared with the age set of the same fish provided by another reader (Figure 6). The left and right operculum were analysed separately because the ages differed and dataset of Höhne et al. (2020) did not specify which operculum was used for age determination. However, both analyses present a similar perspective. There are significant discrepancies in age determinations, particularly for individuals with age 5 and above. The largest observed difference is 9 years. In the higher age classes, the ageing differs largely, although there are some cases where the age differs by 1 or 2 years (detailed in Figure 7).For younger age classes, there is not such a difference; the ages are either estimated exact or slightly below the age. The agreement rates for exact matches are 30.3% and 27.3%, and for a tolerance of ±1 year within, they are 60.6% and 56.1%. These agreement values are quite low, indicating a notable level of discrepancy between the two sets of readings. Additionally, high average coefficient of variations of 19.6% and 21.3% suggesting a significant level of variability comparing both measurements.

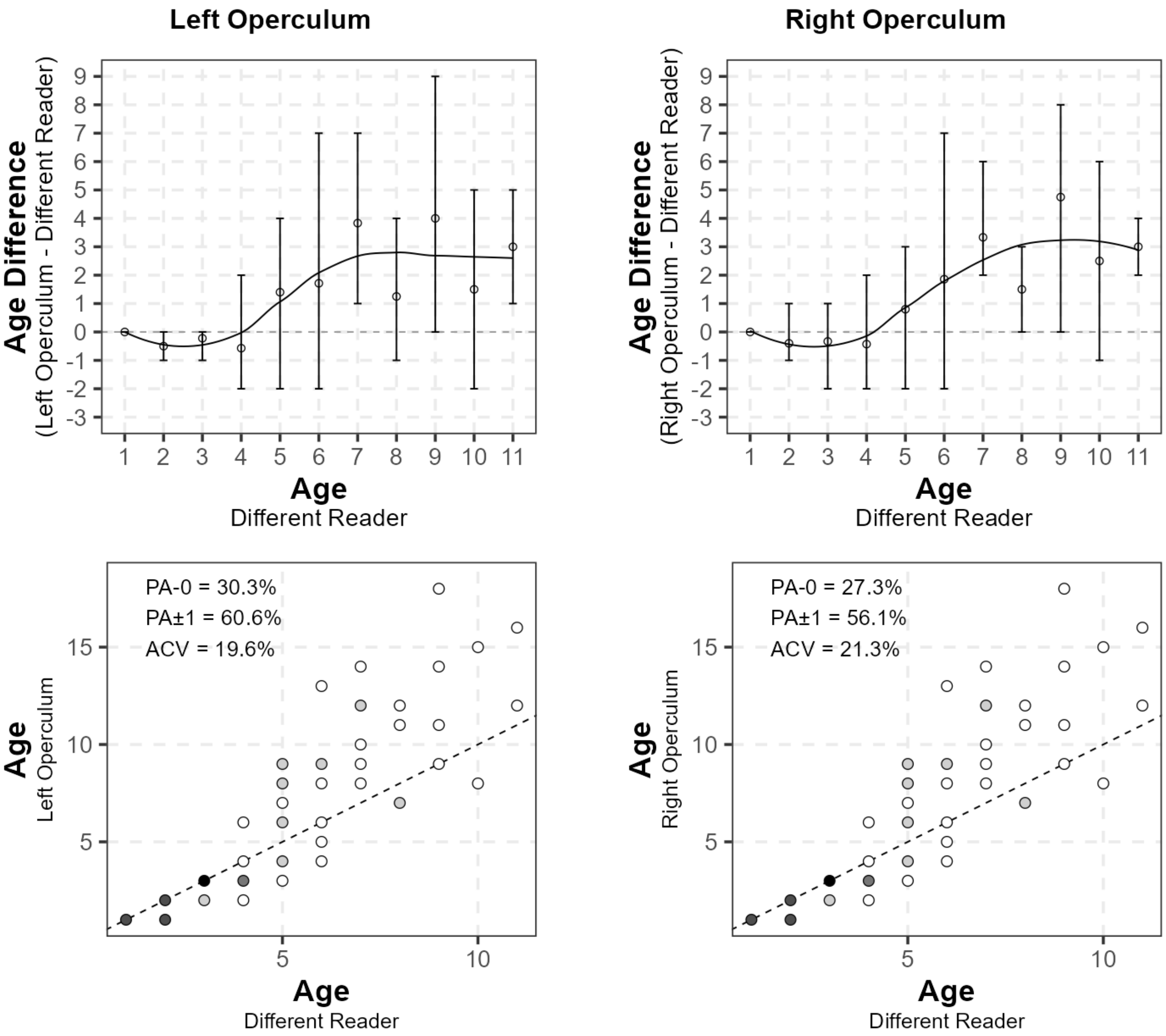


Figure 6: Comparison of age estimations on the same structures by different readers. Figure is divided in age bias plots on top and more detailed plots on the bottom. The different age estimations reveal large discrepancies for older age classes, with low overall agreement and high variation.

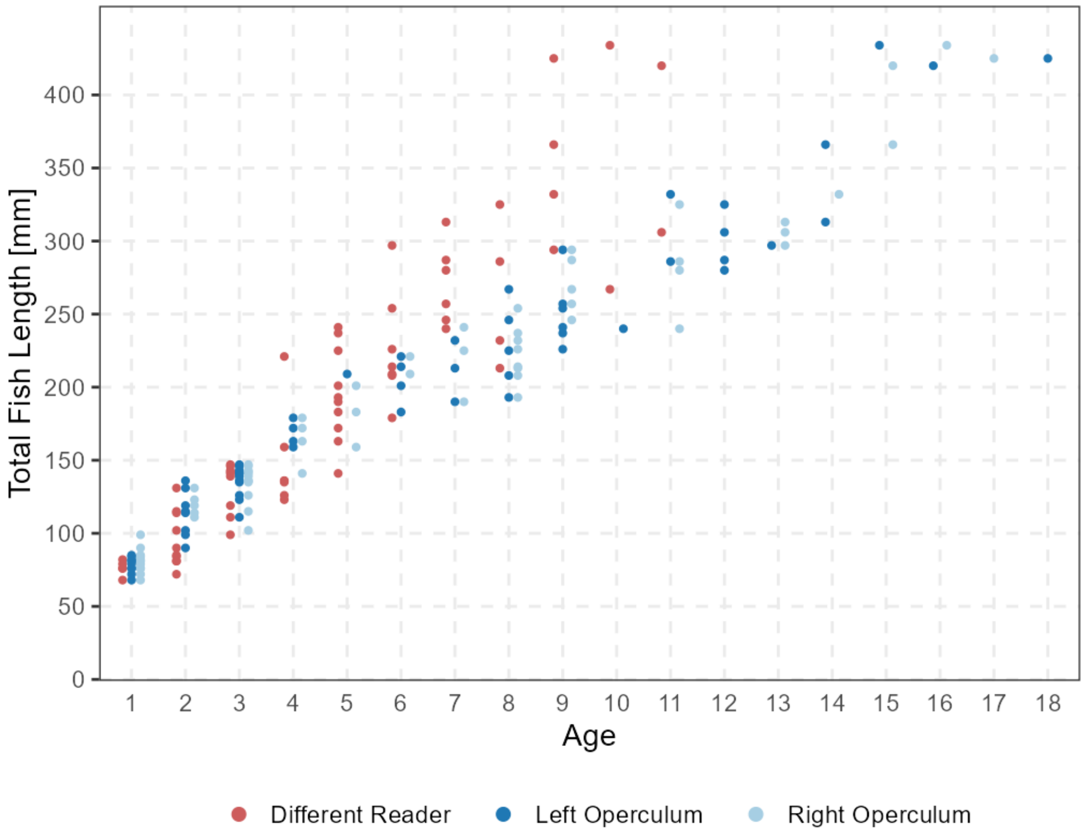


Figure 7: Detailed representation of age estimates corresponding to total fish length. For each age, data points are categorized and color-coded by measurement series.

Linear regression analysis of the maximum operculum Radius (*MOR*) in relation to total fish length (*TL*) demonstrated a strong linear relationship (Figure 8). The relationship is described by the following equation

$$\mathit{TL}\,[\mathrm{mm}] = 15.46 \cdot \mathit{MOR}\,[\mathrm{mm}] + 31.34$$

with $R^2 = 0.985$ and sample size $n = 132$. Based on this, the lengths of the determined radii of the annual rings were converted to fish length (total Length in mm) and plotted against the corresponding annual rings in Figure 9. This analysis was conducted on the same individuals, though the comparison is slightly distorted due to variations in length measurements caused by different optical devices used during the analysis. The age boundaries determined by another reader were notably broader than the reference.

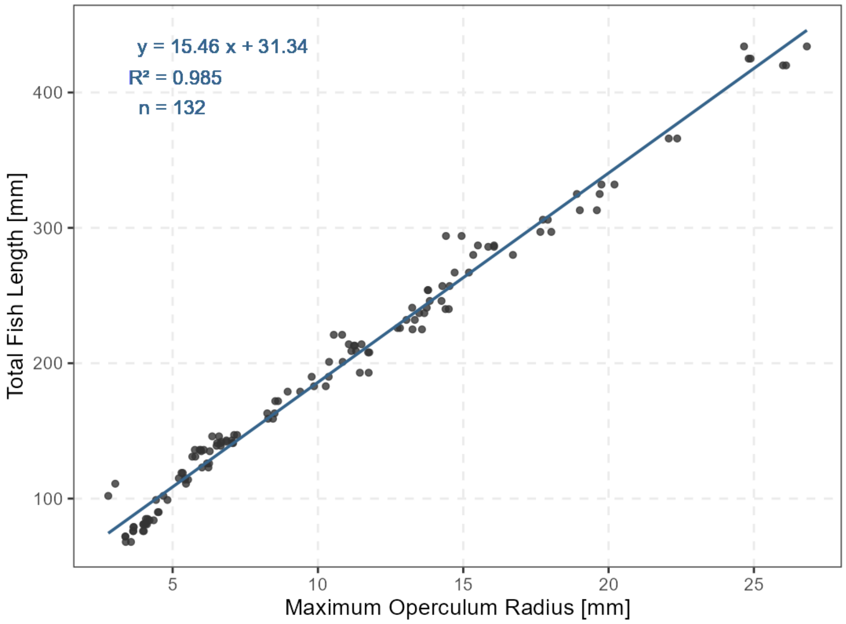


Figure 8: Linear regression analysis of the maximum operculum radius and total fish length. Maximum opercular radii from the first measurement were plotted against the corresponding total lengths of the fish.

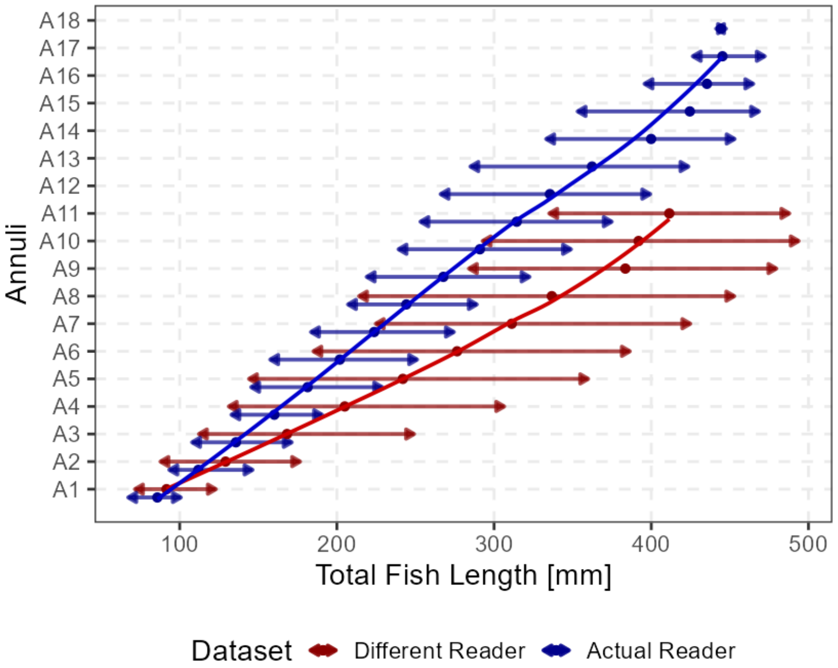


Figure 9: Fish growth progression based on annuli formation and fish Length. Each segment with arrows at both ends marks the minimum and maximum fish size for the respective annulus, while the point in the middle represents the mean length, illustrating the fish length range at each age and growth progression to the next year. Total Fish Length was converted using linear correlation equation with measured maximum operculum lengths ($R^2 = 0.985,\ n = 132$). Data sets are color-coded by different readers. A trendline is set to aid in comparing line of growth in both datasets.

4 Discussion

4.1 Learning set

The minimal variance observed in Reading 1 and Reading 2, along with a high level of exact agreement, suggests a probable recognition effect. This is supported by the greater variation observed in the third reading compared to the first two. To avoid this potential bias in future analyses, it is recommended that small sets be left undisturbed at least for a week before subsequent readings. This practice could help prevent minimizing recognition bias from affecting the results.

4.2 Quality of aging and comparison of reading both structures

The consistency of age readings was generally high, with strong agreement across the same structures. Age estimates remained stable over time, with no signs of drift in one direction. Precision of these estimates held steady, with no noticeable decrease in agreement as time progressed. Precision varied slightly among the individual annuli, with overall high agreement rate. In larger structures, identifying of the initial growth ring can be challenging, as thickness can cause the earliest rings to appear darker and less distinct. For instance, during the first reading, the first annulus may be identified correctly, but in subsequent readings, it may be unrecognized, resulting in the second annulus being misinterpreted as the first. Observed higher variation between left and right opercula could be attributed to differences in their appearance, as some structures showed unique colors, varying shades, inclusions, or more or less pronounced banding patterns. In the analysis comparing readings from both opercula of the same individual, factors like the degree of cleaning and the quality of the digital images may have also played a role. This aligns with the repeatability tests, which were conducted on the same photo and structure, showing some higher consistency. Nevertheless, the within-year agreement remained within acceptable limits, indicating that the method used is still reliable. This suggests that, in future studies, it may be sufficient to examine only one structure, using the other only to cross-check the banding pattern when necessary. This could simplify the process while maintaining reading precision.

4.3 Aging comparision

In this study, significant discrepancies in age estimation were found in older age classes when compared to *Höhne et al. (2020)*. A potential source of this discrepancy lies in the use of different optical devices. The digital images used in this study provided high-resolution, well-lighted images of the structures, allowing for detailed observation of subtle differences in shade and structure. The ability to zoom in on the entire structure made it easier to identify and track annual rings, as well as spot false checks. In contrast, the use of a digital caliper with projector brought challenges, such as lower lighting quality and inability to view the full structure at once. This may have contributed to the divergence in age estimations, particularly for older individuals. This assumption is supported by the fact that there is still very high agreement for smaller, thinner opercula from fish up to 150mm (Figure 8).

Further, comparisons with other studies on aging *Perca fluviatilis* using opercular bones indicate that, although there are some variations in age estimates, the results are generally consistent within documented range for length-at-age perch. For example, data is consistent with *Shafi and Maitland (1971)*, which reports for Scottish lakes a 7-year-old female perch at approximately 20 cm and males slightly smaller. The results for 7-year-old perch in this research are in line with those, where sizes ranges from 17.3 to 25.7cm (mean 21.3cm) for fish forming 7th annuli. For Loch Lomond, reported slightly larger female perch, 32cm at 10 years and 34cm at 11 years, whereas my study records 22.5cm to 32.7cm (mean 27.2cm) for age 10 and the range 23.8cm to 35.1cm (mean 29.6cm) for age 11. In *Shafi and Maitland (1971)*, the growth of the observed perch populations can be categorized as moderate, when compared to the growth curves of faster-growing populations in lakes like Bodensee *(Haakh 1929)* or Lake Krugloe *(Svetovidov 1929)* and slower-growing populations, such as those in Swedish lakes *(Alm 1946)*. Other studies documented substantial variability in age-length estimates for Perca fluviatilis in Windermere, even within the same lake across different years *(Le Cren 1958)*. Here, female perch with a total length of 25cm were determined to be either 7 or 15 years old in different years. This variability was attributed to factors such as population density and annual temperature fluctuations, as well as an experimental population reduction *(Worthington 1950)*. In case for gravel pit lakes, rapid growth was documented in two of the three gravel pit lakes in south-east England, where perch reached 19cm by age 4 *(Gee 1978)*. The results of the aging in this present study indicate a range of 12.6cm to 18.0cm (mean 15.2cm) for 4th annulus formation. However, it is important to note that comparing regional growth rates among different perch populations can be complicated, as they inhabit distinct aquatic ecosystems with their own unique ecological dynamics and environmental factors.

Providing context for maximum age, the oldest perch in this study was estimated at 18 years based on the left operculum and 17 years on the right for a 425mm individual. Other large perch were aged at 16 years (left operculum) and 15 years (right operculum) with lengths of 420mm and 434mm. These findings are consistent with maximum age published in the study *Le Cren (1958)* for perch reaching up to 17 years. Here, it is suggested that mortality for large, old perch is likely low due to reduced predation, a hypothesis supported by *Frost (1954)*, who found no perch above 270mm in pike stomachs. This suggests that the survival of older, larger individuals is promoted by their size, reducing their vulnerability to predators. In final consideration, these studies provide support for the reliability of age assignments. The accuracy of the estimated ages remains uncertain, as no validation study has been performed.

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6 Appendix

The following figure provide a more detailed representation of Figure 3, with the results further separated by left and right operculum.

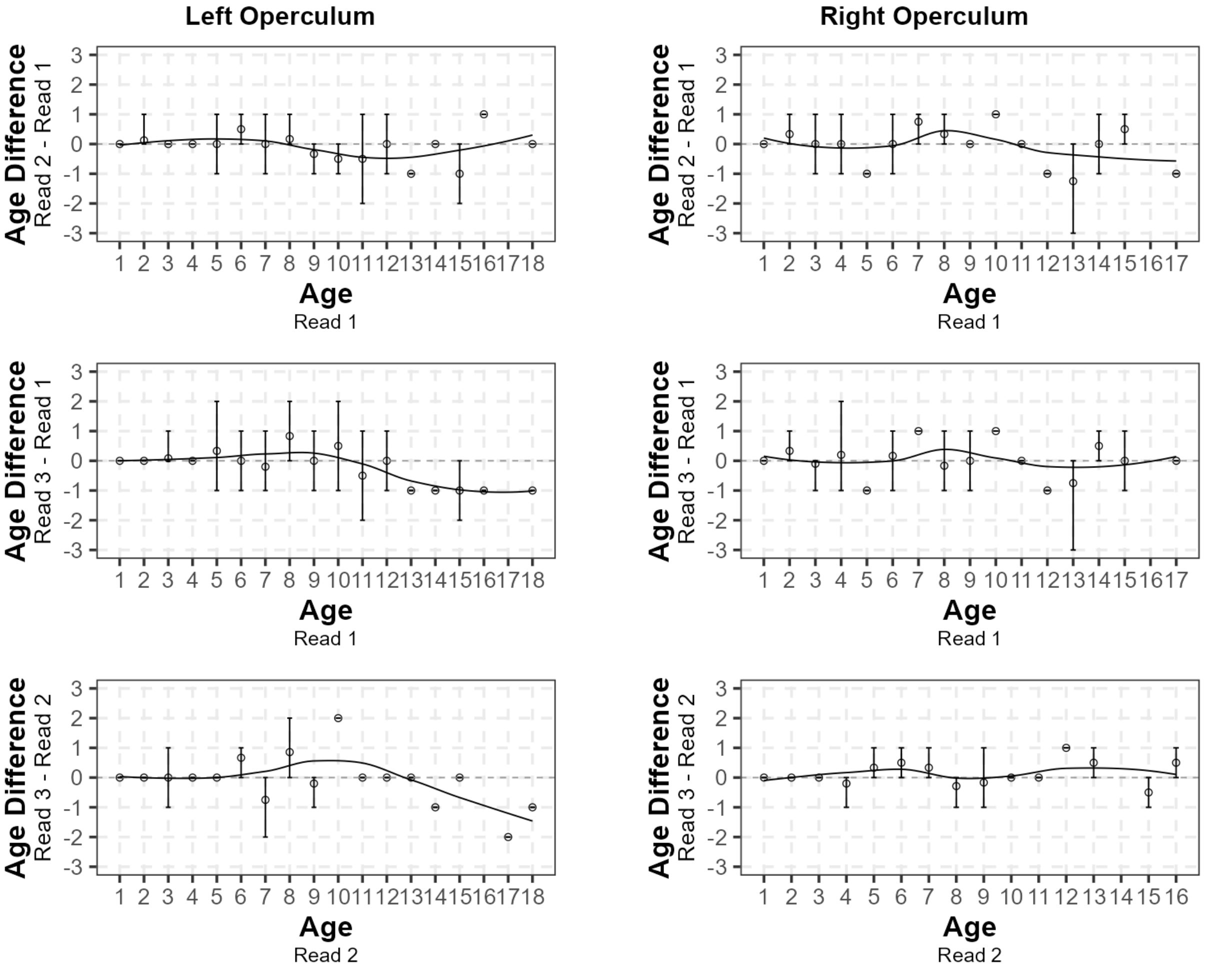

Figure 10: Detailed comparision of age estimates by operculum type (left/right) for the large project set ($N = 66$, both opercula). Age bias plots present the differences between individual age readings. The plots on the left correspond to the left operculum, while those on the right to the right operculum. While there is no theoretical basis to suggest a systematic bias towards either operculum type, it is worth noting that left opercula tend to show a consistent deviation in the same direction at higher ages for Read 3. However, since this trend is not observed in the right opercula within the same series of measurements, it seems to be a random occurrence rather than a systematic bias.

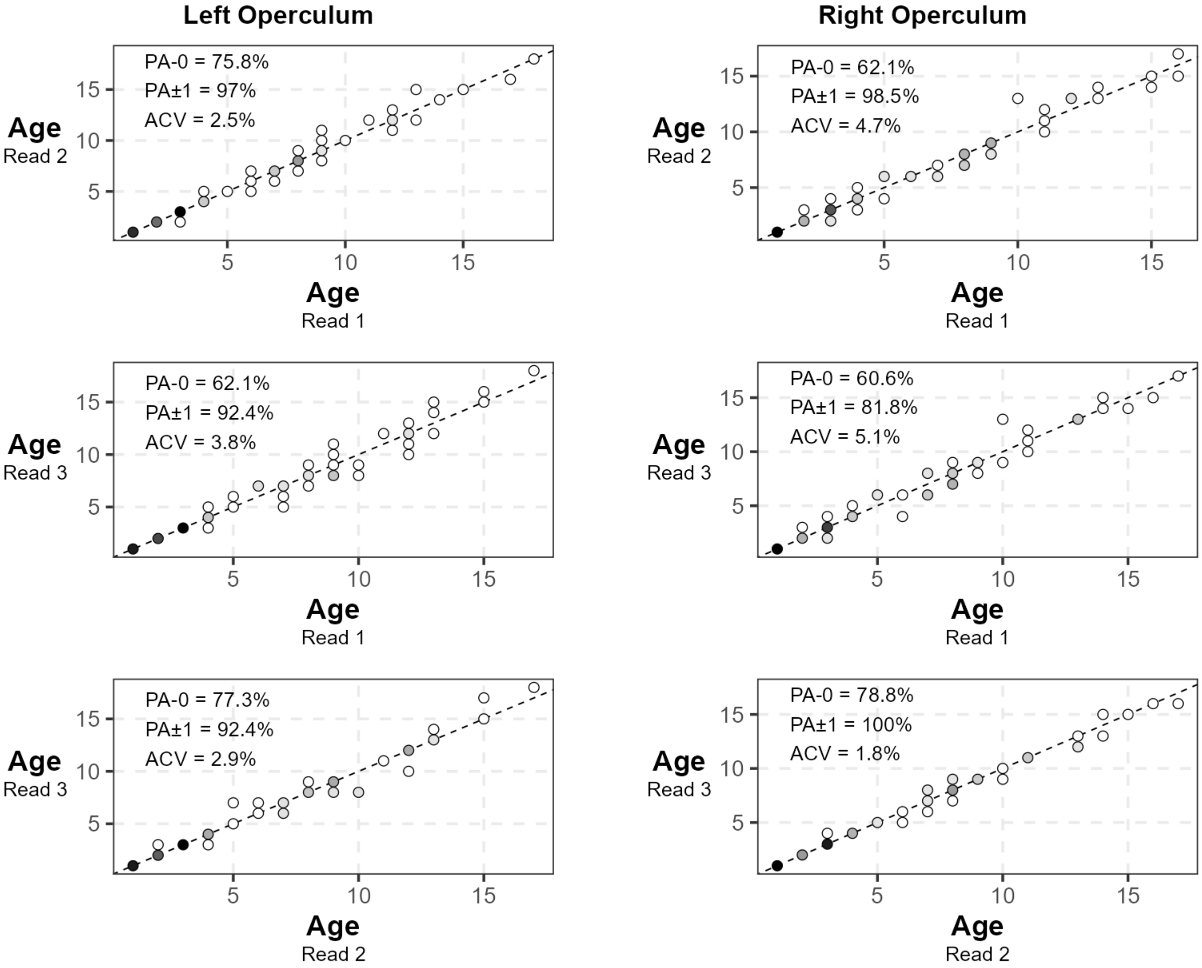

Figure 11: Detailed precision of age estimates by operculum type (left/right) for the large project set ($N = 66$, both opercula). Agreement of age estimates for the left and right operculum across different reading sessions. The results for each operculum are shown separately, for the left operculum on the left side and for the right operculum on the right side.