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Verification of annulus formation in the otoliths of captive yellowfin tuna in the WCPO

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Abstract

The age of yellowfin tuna in the western and central Pacific Ocean has been determined based on the number of complete opaque growth zones observed in thin sections of the transverse axis of otoliths. However, uncertainties remain regarding the periodicity and annual nature of these opaque zones. In this study, we conducted a captive experiment for yellowfin tuna to investigate the periodicity of opaque zone formation. Juvenile yellowfin tunas were reared in fish cages near Amami Oshima, Japan, for approximately 1–3 years. During the experiment, captive fish were recaptured from the fish cages 1–6 times to measure their fork length and inject oxytetracycline as otolith fluorescent markers, then released back into the fish cages. We collected otoliths from dead individuals and thin sections of the transverse axis of otoliths were prepared and imaged. On the images, we measured the distances from the primordium to each annulus, each oxytetracycline mark, and the outer edge. The dates of formation of clearly defined fluorescent marks and annulus measurements were used to estimate the periods during which annuli were formed and the approximate number of days between each annulus. Our results showed that the ages estimated using conventional methods were higher than the actual ages for all individuals. Furthermore, the estimated number of days between annuli was 349.0 ± 59.6 days between the first and second annuli but decreased with age after between the second and third annuli, reaching approximately 150 days between the fourth and fifth annuli. In addition, the estimated formation period of the annuli showed that many individuals were estimated to have formed during the fall and winter for the first and second annuli, but the proportion of individuals formed during the fall and winter decreased in the third annulus and thereafter. These findings suggest that ring formation does not always follow a strict annual cycle. The timing may differ between annulus, and some opaque zones may not reflect annual growth.

Introduction

The age of tuna is commonly estimated by counting opaque bands in their otoliths, which are small ear bones used in balance and hearing. In southern bluefin tuna (*Thunnus maccoyii*), individuals assessed to be more than 40 years old have been confirmed using this method (Gunn et al. 2008). In

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tropical tuna species such as yellowfin and bigeye tuna, age is also assessed using a similar method, and growth curves estimated from relationship between fork length and age are used for stock assessments (e.g. Vincent et al. 2020; Magnusson et al. 2023).

Manuals for reading otolith annulus have been published for bluefin tuna and southern bluefin tuna (Anonymous 2002; Shimose and Ishihara 2015). In contrast, although workshop reports exist for yellowfin and bigeye tuna, no manuals on annulus reading techniques have been published. Given the limited information currently available, validation studies of otolith annulus reading results become crucial.

So far, age validation studies for yellowfin and bigeye tuna have been conducted in the Atlantic, Indian, and Pacific Oceans. These studies used methods such as rearing experiments, tags and recapture of fish which are marked otoliths with $SrCl_2$ or oxytetracycline (hereafter as OTC), and then confirming the relationship between the growth section after marking and the annuli (hereafter as $SrCl_2$ marking and OTC injection, respectively) or using radiocarbon dating methods. Most of these studies have focused on evaluating the relationship between daily age increments and elapsed days, while verification of annuli has been conducted for yellowfin and bigeye tuna in the WCPO ($SrCl_2$ marking and radiocarbon dating: Farley et al. 2003; 2018; 2019, Andrews et al. 2024), and yellowfin and bigeye tuna in the Atlantic (OTC injection and radiocarbon dating: Andrews et al. 2020; Krusic-Golub and Ailloud 2022).

Studies on bigeye tuna in the WCPO and yellowfin and bigeye tuna in the Atlantic Ocean used sufficient sample sizes. In the WCPO bigeye tuna, the fish which days at liberty were 207-2420 days (Farley et al. 2018), in Atlantic yellowfin, the fish which days at liberty were 46-995 days, and in bigeye, the fish which days at liberty were 52-819 days, with the number of annuli corresponding to the number of years at sea for each (Krusic-Golub and Ailloud 2022), indicating that the band patterns counted are annuli. Radiocarbon dating was also used to validate annulus counts. The C14 profiles in otoliths of large yellowfin and bigeye tuna were compared with those from coral and young-of-year fish, showing consistent patterns. These comparisons showed no contradictions with the annulus counting results (Andrews et al. 2024).

In a study using $SrCl_2$ on yellowfin tuna in the WCPO, transverse sections were prepared and observed from otoliths of individuals recaptured 261 days after release. The results showed that one pair of opaque and transparent bands were formed after release, indicating that the age based on annulus reading was consistent with one year for yellowfin tuna, but only one example was included. Although validation studies on annuli have been conducted on yellowfin tuna in the WCPO, there are still limited number of studies. In addition, while previous studies have compared and verified the number of days between release and recapture and the number of annuli formed during that period, there has been no verification of the annularity or the timing of annulus formation.

In this study, we accumulate knowledge about otolith annulus formation in the WCPO yellowfin tuna through long-term rearing experiment and repeated OTC injections, including whether the opaque band patterns are annuli, whether they are formed once a year, and if so, when they are formed.

Materials and methods

Rearing experiment of yellowfin tuna

In October 2020, May 2021, and May 2022, young yellowfin tuna were caught using pole-and-line fishing operations off the coast of Amami Oshima, Japan, and reared in the sea cages (18m and 20m diameters each) of Japan Fisheries Research and Education Agency, Fisheries Technology Institute, Amami Field Station (28°09'N, 129°15'E). Before release into the cage, the fork length of yellowfin tuna was measured, and oxytetracycline solution was injected as otolith fluorescent markers. Individual identification was performed using either dart tags or PIT tags. Following their release into the cage, we recaptured them every few months. Each time, we measured their fork length, injected them with OTC, and released them back into the cage. This process was repeated until the fish died. After the yellowfin tuna died, they were removed from the cage, their otoliths extracted, cleaned, dried, and stored in light-protected microtubes.

Otolith annulus and OTC mark reading and distance measurement (fluorescent marks and annuli)

The most complete otolith from each pair was selected for preparation, and the core was marked prior to it being embedded in Polyplex Clear Ortho Casting Resin (Allnex, Frankfurt, Germany). Otoliths were then set in resin blocks, oriented to allow a transverse section to be cut from the center of the otolith. Each row of otoliths was sectioned on the transverse axis. Using a modified high-speed gem-cutting saw equipped with a 250 μm-thick diamond-impregnated blade, we cut up to five serial sections, each approximately 300–320 μm thick, through the center of each otolith. Sections from each sample were cleaned, dried and mounted on clear glass microscope slides (50 x 76 mm) under glass coverslips using resin. The otolith sections were viewed with transmitted light at 20x magnification using a research grade Leica M125 stereo microscope. Prior to ageing, a single TIFF image was captured of the section cut closest to the primordium for each sample. The selected section was marked to ensure that the same section was chosen when conducting the age estimate procedure. The annual age of each section was estimated by counting opaque growth zones (which appear dark under transmitted light) along a transect that ran from the primordium to the outer edge adjacent to the inner edge of the ventral arm. The last fully completed opaque zone before the otolith edge was counted only if translucent otolith material was detected between the outer edge of the last opaque zone and the otolith edge. All age readings were made without knowledge of fish size or otolith weight. A customized image analysis system was used to count and measure the manually marked increments and to capture a single annotated jpg image. The otolith margin was classified as either opaque (O), narrow translucent (NT) or wide translucent (WT). The selected sections were then examined for the presence and position of the OTC marks by using a Leitz Diaplan compound microscope fitted with a 100-W incident ultraviolet light source and a Leitz D filter block (Leica Microsystems, with excitation filter of 450–520 nm) to suit the fluorescent properties of OTC. Images were taken at magnifications of 25× with a DFK 31AU03 digital camera (The Imaging Source, Charlotte, NC) attached to the microscope and the camera's corresponding image analysis software (IC Measure, vers. 2.0.0.245; The Imaging Source). Once imaged, the number of OTC marks present and the distance between the primordium to the first OTC mark, the distance between subsequent OTC marks and the distance between the last OTC mark and the edge was measured along the same transect used in the annual ageing procedure. We manually located the approximate positions of each annulus on the fluorescent images (Fig. 1). This was based on increment distances measured from transmitted-light images during routine ageing, along with anatomical landmarks on the inner and outer margins of the ventral arm in relation to completed opaque zones. These were marked with a temporary screen cursor on the ventral arm close to the inner margin (sulcal side). We measured the distances from the primordium to each OTC mark or annulus marker using a multiline measuring tool in IC Measure 2.0.0.286 (The Imaging Source©). The transect used for this measurement began at the primordium and passed through each temporary cursor to the otolith margin, following the order in which the markers were observed. This ensured that both OTC marks and annual increments were marked along the exact same transect and measurements were internally comparable. As the OTC measurements were conducted on the fluorescent images, the transect used was not exactly the same as that used for the annual ageing, however care was taken to ensure that the position of the two transects were as close as possible to each other. The fluorescent marking and annulus pattern analysis of these otoliths, as well as their position measurements, were performed by Fish Ageing Services.

Estimated daily ages based on the number of days reared and annulus reading protocol

The daily age at the 1st measuring was estimated for each individual using the fork length at the time of 1st measuring and the growth model which is constructed using the data from the output file (https://oceanfish.spc.int/en/other/cat_view/116-ofp-publications-a-documents/131-stock-assessment-and-modelling/181-stock-assessments/229-input-and-result-files/485-2020/487-yellowfin) downloaded on June 1, 2025. By adding the number of reared days until the time of finish rearing to daily age at start rearing, the daily age at finish rearing was estimated and converted to decimal age (age_cage). Additionally, the decimal age of each individual was estimated based on otolith annulus reading using the method described by Farley et al. (2020) (age_annulus).

Timing of annulus formation and annual periodicity

To estimate the formation date of each annulus, we used the dates of two oxytetracycline (OTC) marks that were located immediately before and after the annulus, along with the measured distances from the primordium. Assuming that each OTC mark corresponds to the injection date, the date of annulus was estimated by linear interpolation:

$$D_{ann} = D_{t-1} + \left(\frac{D_t - D_{t-1}}{X_t - X_{t-1}}\right) \times (X_{ann} - X_{t-1})$$

where D_t and D_{t-1} are the dates of two consecutive OTC marks, and X_t and X_{t-1} are their respective distances from the primordium. X_{ann} represents the distance from the primordium to the annulus.

Of course, annuli are not formed on a specific "day" but rather in bands that are formed over several months, but for the sake of convenience, we treated them as specific date included in the band of annuli.

To verify the annual periodicity of annulus formation, we compared the number of days between successive annuli—specifically, from the first to the second, second to third, third to fourth, and fourth to fifth—for each individual. In addition, the year was divided into four periods: February to March, April to May, June to August, and September to November, and the timing of ring formation from the first to fifth annuli was verified.

Results

A total of 39 young yellowfin tunas were fished and released into a sea cage. Information on each individual (such as the start date of rearing, fork length at the start of rearing, and OTC injection date) is shown in Table 1. The rearing period ranged from 295 to 1160 days, during which OTC was injected 1 to 7 times. The interval between OTC injections was 135.3 ± 72.6 days (57–573 days) and varied by individual and number of injections. The estimated age at the first measurement based on the fork length-daily age relationship was 266.8 ± 62.6 days (184–407 days, N=39), and almost all were estimated to be young-of-year fish (Table 2). When comparing the decimal age based on the age at the first measurement and rearing days with the decimal age based on annulus reading results, the decimal age based on anulus reading showed higher values than the decimal age based on rearing days for all individuals (Fig. 2).

The formation periods of each annulus were estimated for each individual based on the relationship between the OTC mark formation date, for which the exact date was clear, and the position of the annuli. The number of days elapsed between the first and second annuli, the second and third annuli, the third and fourth annuli, and the fourth and fifth annuli was calculated (Fig. 3). The interval between the first and second annuli was 349.0 ± 59.6 days (range: 233–449 days, N=22), suggesting that one annulus is formed during the same period each year. However, from the second to the third annulus onward, the interval between annuli gradually shortened:

− Second to third: 278.9 ± 82.5 days (134–481 days, N=28)

– Third to fourth: 205.1 ± 36.2 days (160–292 days, N=14)

– Fourth to fifth: 148.3 ± 22.4 days (109–171 days, N=6).
Regarding the estimated annulus formation period, for the first-year annuli, 82.6% were estimated to have formed between December and February, indicating that many individuals formed annuli during this period of low water temperatures. From the second year onwards, this proportion decreased to 43.2%, 14.3% (third year), and 14.3% (fourth year), and was 0% in the fifth year, suggesting that the formation period of annuli from the second year onwards does not correspond to the low-temperature period (Fig. 4). Furthermore, no specific quarter consistently accounted for a large proportion annulus formation, including the low-temperature period, the seasonal timing of formation showed no clear pattern.

Discussions

In this study, we clarified the formation cycle and timing of annuli in the WCPO yellowfin tuna by analyzing fork length at the start of rearing, the number of subsequent rearing days, and the positional relationship between otolith annuli and OTC marks. Since the interval between the first and second rings was approximately one year, it is highly likely that these annuli represent annual growth. However, the interval between the formation of the second and third annuli, as well as the subsequent annuli, was shorter than one year. This suggests that, in many individuals, annulus formation occurs mainly during the fall and winter seasons up to age two, but timing varies among individuals thereafter. A study conducted on the Atlantic yellowfin tuna reported that release and recapture of OTC-injected individuals resulted in a concordance between the number of years at liberty and the number of annuli (Krusic-Golub and Ailloud 2022). In that study, 27 of the 32 individuals whose OTCs were detected were 1 or 2-year-old fish, and the results of the present study, in which the interval between the first and second rings was about 365 days, were consistent with that study. However, for the older individuals, Krusic-Golub and Ailloud (2022) found a general agreement between the number of years at liberty and the number of annuli, while the present study tended to identify more than the expected number of annuli. Several hypotheses can explain this discrepancy. First, this study was conducted in a temperate-region fish cage, while the release and recapture location in Krusic-Golub and Ailloud (2022) are unknown. Given that yellowfin tuna are primarily distributed in tropical areas, it is likely that the environmental conditions differed between the two studies. Tropical and temperate regions, as well as the Atlantic Ocean and WCPO, experience vastly different water temperature environments, as well as prey species, salinity, and other environments (Yu et al. 2021). In fish, water temperature (Jobling 1983; Carmona-Osalde et al. 2004) and photoperiod (Geffen 1983; Thrush et al. 1994; Duncan et al. 1999) are generally noted to affect growth rates. It has been reported that annuli formation in the Atlantic bluefin tuna during periods of warm water temperature (Rodriguez-Marin et al. 2022), and it is undeniable that water temperature may have affected the formation of annuli in yellowfin tuna, or that the timing of annulus formation may have differed between the Atlantic Ocean and WCPO. For the same reason, environmental differences between the previous study conducted on wild individuals and this study conducted in a closed offshore fish cage may have contributed to these differences. The fish cage used in this study was located in a bay with a maximum depth of about 15 meters, limiting the fish's diving depth to the bottom of the cage net. The water temperature fluctuates, averaging around 30°C at its highest during August and September, and slightly below 20°C at its lowest during December and February. Spring and fall are intermediate between these values, with distinct seasonal variations. However, if these environmental factors affect the otolith annulus formation schedule, then individual differences will occur even among wild individuals, since not all individuals migrate in the same manner. Some species of fish have been reported to have a more indistinct otolith ring structure in captivity than in the wild (Compana 1984; Laroche et al. 1984), but the yellowfin tuna in this study, as in the surrounding natural environment, has annual fluctuations in water temperature. In addition, it has been confirmed in previous studies that fork length growth differs between high and low water temperature seasons (Okamoto et al. 2022; Hasegawa et al. 2023), and if fork length and otolith growth are correlated, otolith growth may also be correlated with water temperature and contribute to annulus formation. However, this study found no consistent of annulus formation among individuals, indicating that the underlying factors influencing annulus development in this species remain unclear.

Other possible factors include rapid environmental changes associated with catch for measurement and OTC injection. It is known that in the formation of daily rings, rapid changes in the environment, such as water temperature, can result in the formation of many daily rings (Hagen et al. 1995). It is possible that annulus patterns were formed in the yellowfin tuna in this study due to stresses such as shock at the time of catch for measurement and OTC injection. In this case, annulus patterns would have formed at the time of the OTC mark (when the fish were fished out of the fish cage), but no annuli were formed in such a position in otoliths. In addition, many individuals were observed to have formed annuli in the period between the last OTC injection and the end of rearing. From these facts, it was considered unlikely that false annuli were formed due to shock at the time of catch for measurement and OTC injection.

From another perspective, this may suggest that existing methods for age annulus reading of otoliths cannot be applied to the WCPO yellowfin tuna, and that a special annulus pattern reading method may be necessary. In fact, the ages assessed by annuli reading were all higher than the estimated age at the end of rearing, which is a highly credible figure in this study. The timing of the formation of the first and second annuli was different from that of subsequent annuli, but the first and second annuli had a formation cycle of about one year, so the reading method seemed to be acceptable. Since the first and second annuli formed between fall and winter, it can be assumed that the third and later annuli also formed between fall and winter, and since the approximate location of the second and third annuli in winter can be indicated, the age estimation method can be adjusted using these data. Therefore, when assessing age using transverse sections of otoliths from individuals considered to be two years old or older, it is advisable to consider other methods in addition to counting the number of band patterns consisting of opaque and transparent zones.

For example, an equation could be constructed to relate age and fork length based on the distance from the primordium to the edge and the area of the transverse section using otoliths from individuals of a known age. Although this study was conducted under limited conditions in captivity, it provided fairly accurate otolith annuli pattern analysis results, suggesting the possibility of the need to pay attention to age assessment based on the transverse section of otoliths in the WCPO yellowfin tuna. If the current annulus reading method cannot be applied to wild WCPO yellowfin tuna, further study on a new age assessment method will be needed in future.

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Table 1. Date of start rearing for each individual, fork length at start of rearing, date of each OTC injection, date of end of rearing, number of OTC injections.
Blank spaces for OTC injection dates 1 to 7 indicate that no OTC injections were given. The date range shown in the “Date of start rearing” and “Date of 1st OTC injection” columns (e.g. 2020/10/12–14) indicates that the specific dates could not be identified because the individual identification tags were lost.

ID	Date of start rearing	FL (cm) of start rearing	Date of 1st OTC injection	Date of 2nd OTC injection	Date of 3rd OTC injection	Date of 4th OTC injection	Date of 5th OTC injection	Date of 6th OTC injection	Date of 7th OTC injection	Date of finish rearing	No. of OTC injections
1	2020/10/12	39.5	2020/10/12	2020/12/10	2021/3/15	2021/10/11				2021/12/6	4
2	2020/10/12	35.2	2020/10/12	2020/12/10	2021/3/15	2021/10/11				2022/1/7	4
3	2020/10/12	35.5	2020/10/12	2020/12/10	2021/3/15	2021/10/11	2022/2/22	2022/4/25	2022/10/11	2023/8/11	7
4	2020/10/12	36.1	2020/10/12	2020/12/10	2021/3/15	2021/10/11	2022/2/22			2022/3/8	5
5	2020/10/12	35	2020/10/12	2020/12/11	2021/3/17	2021/10/11	2022/2/22	2022/4/25		2023/7/11	6
6	2020/10/13	39.2	2020/10/13	2020/12/11	2021/4/12	2021/10/11	2022/2/22	2022/4/25	2022/10/13	2023/8/11	7
7	2020/10/13	44.5	2020/10/13	2020/12/11	2021/3/15	2021/10/11	2022/2/22			2022/3/8	5
8	2020/10/13	39.8	2020/10/13	2020/12/10	2021/4/12	2021/10/11	2022/2/21			2022/3/8	5
9	2020/10/13	36.5	2020/10/13	2020/12/10	2021/3/15	2021/10/11	2022/2/22			2022/3/8	5
10	2020/10/13	39.8	2020/10/13	2020/12/10	2021/3/15	2021/10/14	2022/2/22	2022/10/11		2023/8/11	6
11	2020/10/13	41.3	2020/10/13	2020/12/10	2021/3/15	2021/10/11	2022/2/24	2022/4/25	2022/10/11	2023/8/27	7
12	2020/10/13	39.2	2020/10/13	2020/12/10	2021/3/15	2021/10/11	2022/2/22			2023/9/12	5
13	2020/10/14	38	2020/10/14	2020/12/10	2021/3/15	2021/10/11	2022/2/22	2022/4/25	2022/10/11	2023/12/18	7
14	2020/10/12-14	—	2020/10/12-14	2021/3/15	2021/10/11	2022/2/22	2022/4/25	2022/10/12		2023/8/11	6
15	2020/10/12-14	—	2020/10/12-14	2020/12/10	2021/3/15	2021/10/11	2022/2/22	2022/4/28	2022/10/11	2023/9/25	7
16	2020/10/12-14	—	2020/10/12-14	2021/3/15	2021/10/11	2022/2/22	2022/4/25	2022/10/11		2022/10/31	6
17	2020/10/12-14	—	2020/10/12-14	2020/12/10-11	2021/4/12	2021/10/11	2022/2/22	2022/10/11		2023/8/11	6
18	2020/10/12-14	—	2020/10/12-14	2020/12/10-11	2021/3/15	2021/10/11	2022/4/25	2022/10/11		2023/7/17	6
19	2020/10/12-14	—	2020/10/12-14	2020/12/10-11	2021/3/17	2022/10/11				2023/9/12	2
20	2020/10/12-14	—	2020/10/12-14	2020/12/11	2021/3/16	2021/10/11	2022/4/25	2022/10/11		2023/9/11	6
21	2020/10/12-14	—	2020/10/12-14	2021/3/15	2021/10/11	2022/2/25	2022/4/25	2022/10/11		2023/10/13	6
22	2020/10/12-14	—	2020/10/12-14	2020/12/10	2021/3/19	2021/10/13	2022/2/21	2022/4/25	2022/10/18	2023/10/13	7
23	2021/5/13	53.7	2021/5/13	2021/10/11	2022/2/21					2022/3/11	3
24	2021/5/13	44.5	2021/5/13	2021/10/11	2022/2/22	2022/4/25	2022/10/13			2023/8/11	5
25	2021/5/13	47.9	2021/5/13	2022/10/11						2023/12/26	2
26	2021/5/13	47.8	2021/5/13	2021/10/11	2022/2/22	2022/4/25	2022/10/11			2023/7/14	5
27	2021/5/13	50	2021/5/13	2021/10/11	2022/2/22	2022/4/25	2022/10/11			2023/7/18	5
28	2021/5/13	47.4	2021/5/13	2021/10/11	2022/2/21	2022/4/25	2022/10/11			2023/7/18	5
29	2021/5/13	47.6	2021/5/13	2021/10/11	2022/2/22	2022/4/25	2022/10/11			2023/9/6	5
30	2021/5/17	46.8	2021/5/17	2021/10/11	2022/2/21	2022/4/25	2022/10/11			2023/8/27	5
31	2021/5/17	44.8	2021/5/17	2021/10/11	2022/2/21					2022/3/8	3
32	2021/5/17	46.4	2021/5/17	2021/10/11	2022/2/22	2022/4/25	2022/10/11			2023/10/11	5
33	2022/5/11	—	2022/10/11							2023/7/26	1
34	2022/5/11	—	2022/10/11							2023/7/24	1
35	2022/5/11	—	2022/10/11							2023/7/18	1
36	2022/5/11	—	2022/10/11							2023/7/24	1
37	2022/5/11	—	2022/10/11							2023/7/30	1
38	2022/5/11	—	2022/10/11							2023/12/12	1
39	2022/5/11	—	2022/10/11							2023/7/14	1

Table 2. Fork length at initial measurement, estimated daily age at that time, estimated daily and decimal age at the end of rearing, and total annual increment count.

The IDs correspond to those shown in Table 1, and the same ID indicates the same individual.

ID	FL at initial measurement (cm)	FL at finish rearing (cm)	Estimated daily age at initial measurement	No. of reared days since initial measurement	Estimated daily age at finish rearing	Estimated decimal age at finish rearing	Total annual increment count
1	39.5	68.3	216	420	636	1.7	1
2	35.2	—	185	452	637	1.7	2
3	35.5	106.5*	188	1033	1221	3.3	4
4	36.1	80	192	512	704	1.9	2
5	35	114.2	184	1002	1186	3.2	3
6	39.2	118.1**	214	1032	1246	3.4	4
7	44.5	75	252	511	763	2.1	2
8	39.8	81.6	218	511	729	2	2
9	36.5	82.4	195	511	706	1.9	2
10	39.8	108.9	218	1032	1250	3.4	3
11	41.3	120.2	229	1048	1277	3.5	5
12	39.2	116.4	214	1064	1278	3.5	4
13	38	121.9*	205	1160	1365	3.7	5
14	47.7	111.0**	275	879	1154	3.2	3
15	40.7	115.9	225	1019	1244	3.4	3
16	47.3	92	272	595	867	2.4	2
17	49.6	109.2	288	851	1139	3.1	3
18	46	108.4	263	854	1117	3.1	3
19	43.4	111.2	244	909	1153	3.2	5
20	42	117.8	234	1004	1238	3.4	4
21	45	122.5	255	942	1197	3.3	5
22	42.9	120.5	240	1036	1276	3.5	5
23	53.7	71.9	318	302	620	1.7	2
24	44.5	109.4**	252	820	1072	2.9	3
25	47.9	123.1	276	957	1233	3.4	4
26	47.8	118.4	275	792	1067	2.9	4
27	50	117.7	291	796	1087	3	4
28	47.4	—	273	796	1069	2.9	4
29	47.6	113.4	274	846	1120	3.1	5
30	46.8	117	268	832	1100	3	3
31	44.8	71.7	254	295	549	1.5	1
32	46.4	117.6	265	877	1142	3.1	3
33	57.6	92.4	345	288	633	1.7	2
34	66.3	101.2	407	286	693	1.9	3
35	63.7	94.2	389	280	669	1.8	2
36	63.5	95.2	387	286	673	1.8	3
37	62.9	98.9	383	292	675	1.8	3
38	58.2	100.3	350	427	777	2.1	3
39	64.1	100.4	392	276	668	1.8	3

*Approximate value of standard length

**Approximate value

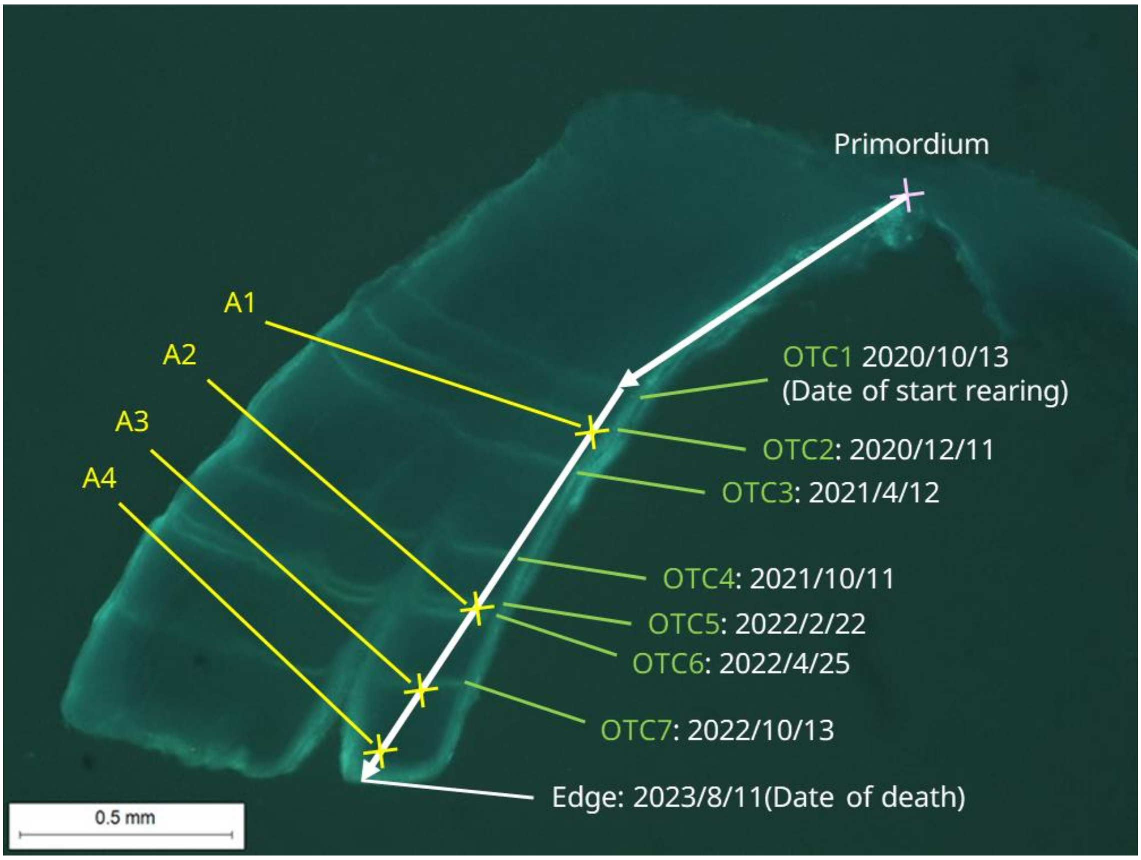

Fig. 1. Representative fluorescence image of an otolith transverse section.

The image shows the location of the primordium, OTC marks (OTC1-7), and annuli (A1-4) in the fluorescence image of a transverse section of otolith from a single individual (ID: 6), as well as the formation date of each OTC mark and the axis using measurement for the distance from the primordium to each OTC mark, each annulus, and the edge.

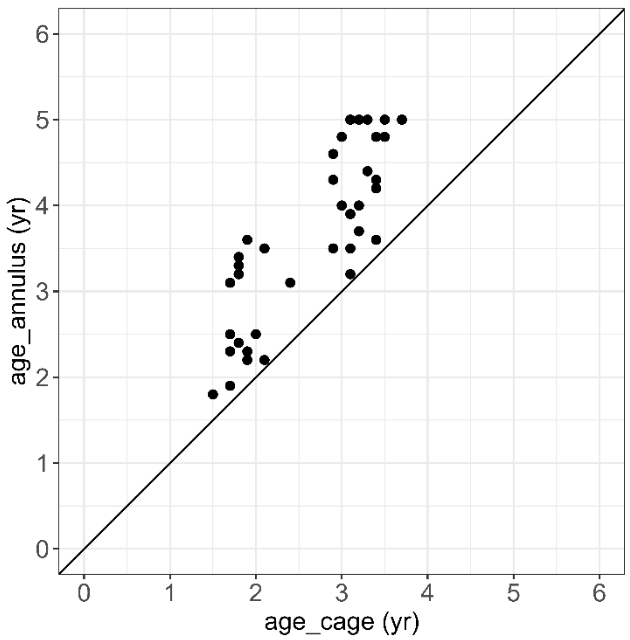

Fig. 2. Comparison of estimated age based on age determined from annuli reading method and age determined from number of days reared.

A black solid line represents a 1:1 relationship. Points above this line indicate higher values for the age estimated from annuli reading (age_annulus), while those below indicate higher values for the age estimated from the number of rearing days (age_cage).

For all individuals, the age determined from annuli reading method was higher.

Noting that individuals plotted with age_annulus = 5.0 are individuals aged 5 years or older, as their decimal age could not be calculated using the method described by Farley et al. (2020).

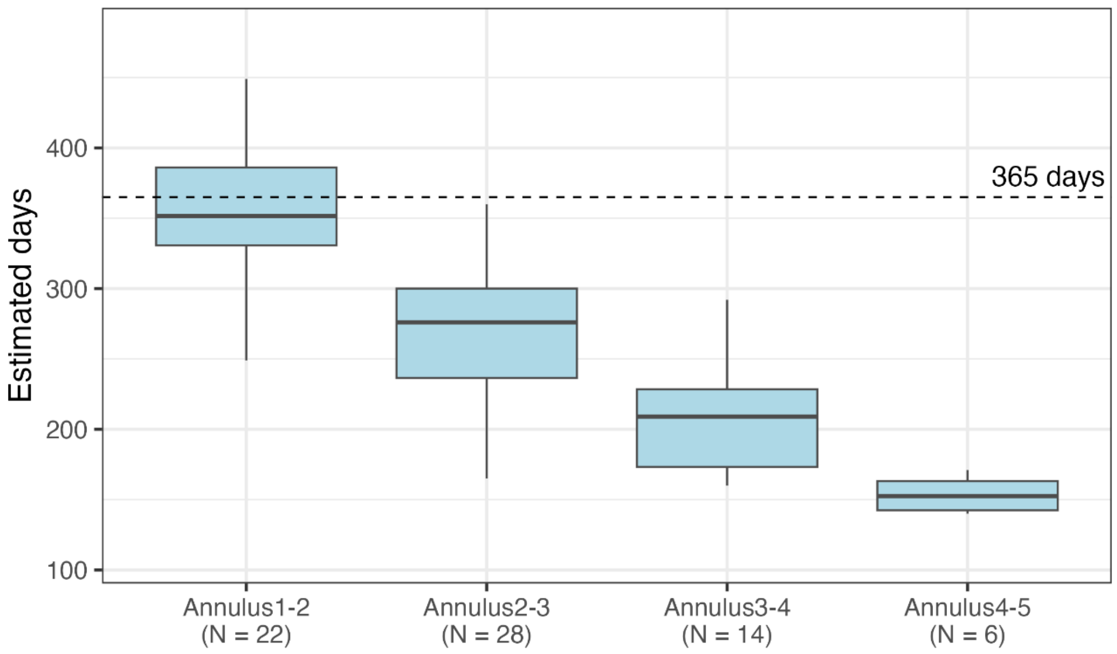

Fig. 3. Estimated number of days between annuli.

Annulus 1-2 means the estimated number of days between the first and second annuli. Similarly, 2-3, 3-4, and 4-5 mean the second and third annuli, the third and fourth annuli, and the fourth and fifth annuli, respectively. The dashed line indicates 365 days, which represents the expected interval if annuli were formed once per year. If the annuli had annual periodicity, the number of days between any two annuli would scatter around this line. However, the observed intervals, particularly for older annuli, are often shorter than 365 days, suggesting that annuli were not always formed annually.

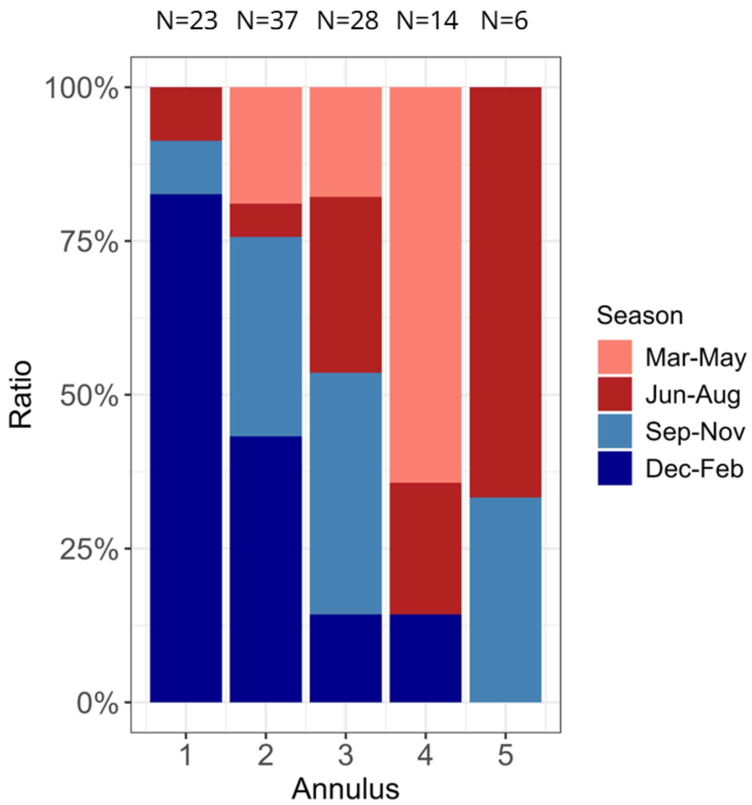

Fig. 4. Percentage of estimated timing of formation for each annulus.

Each bar shows the percentage of individuals that formed a given annulus during each season represented by color (Dec–Feb: dark blue; Sep–Nov: light blue; Mar–May: light red; Jun–Aug: dark red). N above the graph indicates the number of samples analyzed for each annulus. Many individuals formed their first and second annuli in fall and winter, but the number of individuals for which the timing of formation was estimated to be in fall and winter decreased significantly with age thereafter.