



# Attempted validation of the first annual growth zone in kingfish (*Seriola lalandi*) otoliths

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## EXECUTIVE SUMMARY

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The main objective of this study was to validate the location of the first annual growth zone in kingfish otoliths. Achieving this would allow us to determine which of two different ageing techniques is accurate for kingfish. The two ageing techniques result in a difference of one year in kingfish age estimates. We attempted to determine the ages of juvenile kingfish sampled from the wild using fish length and otolith increment counts. We also used fish and otolith length, and increment counts, from known-age, hatchery-reared kingfish for the same purpose. We posed two alternative hypotheses: first, that the cohort of wild fish belonged to the 0+ age class, and second, that the cohort belonged to the 1+ age class. We used five methods to test these two hypotheses, and the outcomes of each are summarised below:

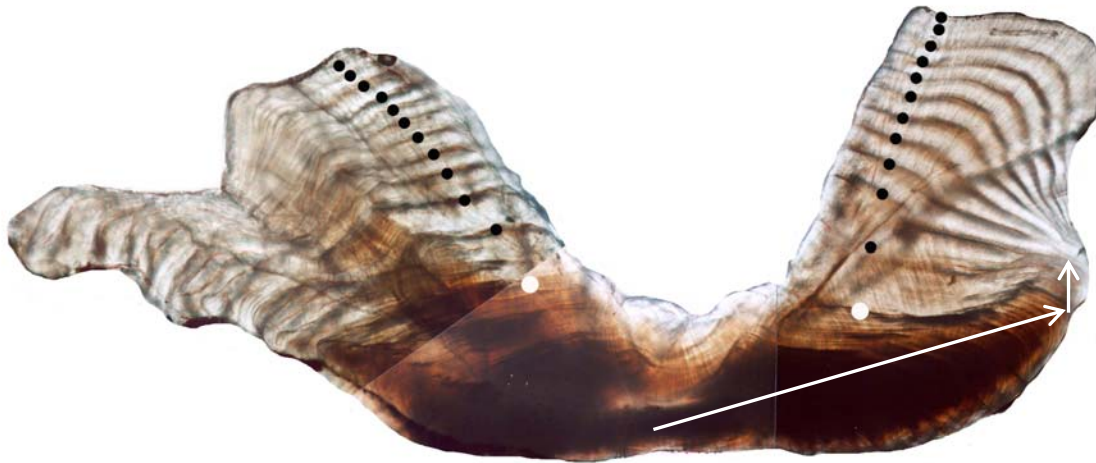
1. Growth curves were fitted to length-at-age data from a time series of samples of wild juveniles under the assumptions that the fish were either 0+ or 1+ fish. The results of this test were inconclusive, with neither hypothesis providing a more plausible growth curve.
2. Reared kingfish from Pah Farm hatchery grew very rapidly, consistent with the 0+ hypothesis. By contrast, reared kingfish from Bream Bay hatchery grew more slowly, consistent with the 1+ hypothesis. Whether the high rates observed at Pah Farm are possible in wild fish is unknown, but it is clear that growth of reared fish is highly variable, making this an unreliable method for discriminating between 0+ and 1+ hypotheses. However, the Pah Farm data demonstrate that the very high growth rate required by the 0+ hypothesis is not impossible.
3. Daily increments were counted in the otoliths of wild juveniles. Increment counts were too high for February and April fish to be consistent with the 0+ hypothesis, unless this cohort of juveniles was spawned 1–2 months before the theoretical birthday of 1 December. This is possible, because the spawning season of kingfish is not well known; spawning is likely to last several months and might easily occur 1–2 months before the theoretical birthday. Conversely, increment counts were too low for all fish to be consistent with the 1+ hypothesis unless increment numbers were grossly under-estimated. This is likely, as there were zones of uncountable increments in all otoliths examined. This test was therefore unable to distinguish between the two ageing hypotheses.
4. The dorsal growth axes of the reared Bream Bay 0.90 year old kingfish were smaller than those of the FAD juveniles sampled in February–July. While this may seem to suggest that the FAD juveniles were older than 0.9 years, and therefore support the 1+ hypothesis for FAD juveniles, there is generally a strong linear relationship between otolith size and fish size. Although that relationship is also affected by fish growth rate, with faster-growing fish having smaller otoliths than slow-growing fish of the same length, the larger otoliths of the FAD juveniles may simply reflect the fact that most of them had larger fork lengths than the Bream Bay fish.
5. Daily increments were counted in an otolith from a reared juvenile, and the count (about 120) was well below that expected from the age of the fish (327 days). This suggests that not all increments are visible in the otoliths of reared fish, and lends support to the suggestion (see point 3 above) that increment counts underestimate actual age in FAD juveniles. An important caveat however, is that other studies have reported that increments tend to be less distinct in reared fish than in wild fish. Consequently this test provides weak support for the 1+ hypothesis.

Four out of our five tests proved inconclusive. Test 5 provided weak evidence supporting the 1+ hypothesis rather than the 0+ hypothesis; i.e. the FAD juveniles were in their second year when caught in February–July. Overall, we conclude that there is still considerable doubt about whether the FAD juveniles are 0+ or 1+ fish, and therefore about the significance of the first growth zone. The annual increments counted when estimating age in older fish appear to be deposited in autumn–winter. Thus the first zone counted by Reader 1 is deposited at an age of about 6 or 18 months, depending on whether the 0+ or 1+ hypothesis is correct.

This study has failed to determine conclusively whether the first otolith zone is deposited during the 0+ or 1+ year. Until this is established, we suggest that kingfish ageing studies adopt the 1+ hypothesis, and assume that the first growth zone is formed during autumn–winter of the second year, at an age of approximately 18 months.

## 1. INTRODUCTION

In a previous study (McKenzie et al. 2014), we presented estimates of age and growth for New Zealand kingfish (*Seriola lalandi lalandi*) based on otolith zone counts. However, there was uncertainty about the location of the first annual growth zone, and as a result two otolith readers differed by one zone (on average) in their otolith counts. Reader 1 assumed that the core opaque region constituted the first zone, whereas Reader 2 started counting from the next zone (Figure 1).



**Figure 1: Thin transverse section of a sagittal otolith from a 92 cm FL kingfish (otolith K7-3) showing growth zones (image reproduced from (McKenzie et al. 2014)). Black dots indicate opaque zones counted by both readers, and white dots indicate an additional zone counted by Reader 1. Arrows indicate the growth axis measurements made in the present study.**

Apart from this systematic bias, the two readers achieved similar age estimates: if they had both used the same criterion for determining the first zone, they would have obtained exact agreement in zone counts for 76% of fish, and within one zone for 94% of fish. Thus kingfish otoliths can be consistently aged if the location of the first zone is accurately determined. Other researchers have experienced similar difficulty in determining the position of the first growth zone in both otoliths and scales from Australian kingfish (Gillanders et al. 1999, Stewart et al. 2001).

The aim of the present study was to validate the location of the first growth zone. A second implied aim, arising from the first, was to determine the age at which the first growth zone is deposited, as this is necessary for precise ageing. The time elapsed between the deposition of subsequent otolith zones was assumed to be one year by McKenzie et al. (2014), but this periodicity has not yet been validated and is not considered further in this report.

The overall project objective was “to characterise the kingfish (*Seriola lalandi lalandi*) fishery, and develop methods to monitor or assess the stocks”. The specific objective was “to validate the first annual ring in kingfish otoliths”.

## 2. METHODS

### 2.1 Growth of a juvenile cohort of wild kingfish

A presumed cohort of juvenile kingfish 23–42 cm fork length (FL) was sampled between February and July 2002. The first two samples (in February and April) came from a Fish Aggregation Device (FAD) off the Poor Knights Islands, east Northland, and two further samples (in June and July) were collected from the adjacent mainland coast at Ngunguru. These samples are hereafter referred to as FAD juveniles. Further collection details were provided by McKenzie et al. (2014).

For this cohort of juveniles, we wished to test two alternative hypotheses:

- 0+ hypothesis – that these fish were of the 0+ age class, being spawned on the theoretical birthday 1 December 2001.
- 1+ hypothesis – that these fish were of the 1+ age class, being spawned on the theoretical birthday 1 December 2000.

The theoretical birthday of 1 December was chosen as the midpoint of the peak spawning season (McKenzie et al. 2014).

For the 0+ hypothesis, ages were assigned to the FAD juveniles using the time elapsed between 1 December 2001 and the date of capture. A von Bertalanffy growth curve was fitted to the data, forcing it through the biological origin (zero length at age zero) (Simpfendorfer et al. 2000):

$$L_t = L_0 + (L_\infty - L_0)(1 - e^{-Kt})$$

where  $L_t$  is the expected length at age  $t$  years,  $L_0$  is the length at age zero,  $L_\infty$  is the asymptotic maximum length,  $K$  is the Brody growth coefficient, and  $t$  is the fish age in years.

For the 1+ hypothesis, the above procedure was repeated after adding one year to all the ages. Growth curves for the 0+ and 1+ hypotheses were inspected for plausibility, and goodness of fit based on patterns in the residuals, in an attempt to determine whether the data supported either hypothesis better.

### 2.2 Growth of hatchery-reared juvenile kingfish

Length-at-age data from reared kingfish were obtained from two sources. Walsh et al. (2003) provided growth data for kingfish reared at Pah Farm, Kawau Island, up to 190 days old. The raw data were not available, so we estimated the lengths and ages from Walsh et al.'s figure 21.

We also obtained four kingfish reared in the NIWA Bream Bay hatchery; these fish were spawned on 20 February 2004, and were aged 0.90 years (327 days) at the time of death on 12 January 2005. Attempts to obtain further growth data for kingfish reared in hatcheries in Australia and South Africa were unsuccessful.

### 2.3 Otolith increments in a juvenile cohort of wild kingfish

Otoliths were selected from FAD juveniles for examination of microincrements (hereafter called 'increments'). Six juveniles were sampled, two each from the February, April and July samples. One otolith from each pair was sectioned transversely after mounting in epoxy resin. The section was then mounted on a glass slide and progressively ground with fine carborundum paper. A series of photomicrographs was taken between the otolith core and the dorsal margin following each grinding stage. For each otolith, a composite image was created from the best grind (judged to be that which showed the clearest sequence of increments between the core and margin) by merging them in Adobe Photoshop image processing software. Sometimes images from different grinds were used to create the composite image, in order to produce the clearest possible sequence of increments. The core region is generally more opaque than the margin, so a grind that is optimal for marginal increments is



not necessarily optimal for core increments. Increments were counted between the core and dorsal margin from the merged images, and from the final grind of each otolith viewed with transmitted light under a compound microscope.

For each of the six FAD juveniles, the second otolith of each pair was sectioned, ground to expose the core, and then etched with 0.1 M HCl. The etched section was mounted on a scanning electron microscope (SEM) stub with a carbon tab and silver paint (to improve conduction of charge and reduce flaring), sputter coated with carbon and gold/palladium, and digitally photographed under a Leo 440 SEM. Three etching times of 40, 80 and 120 s were tested. Increments were counted from the digital images.

## **2.4 Otolith increments in reared kingfish**

Otoliths from four young kingfish reared at the NIWA hatchery in Bream Bay were prepared for increment examination by light microscopy as described above. The kingfish were aged 0.90 years at death.

## **2.5 Otolith measurements**

In young kingfish, the otolith initially grows dorso-ventrally (to the left and right in Figure 1), and the dorsal growth axis is approximately linear. Some time before the deposition of the first zone counted by Reader 1, the direction of otolith growth curves medially (upwards in Figure 1), thus creating a deep sulcus in older fish. This makes measurement of the dorsal growth axis difficult except in fish younger than the age at deposition of the first zone.

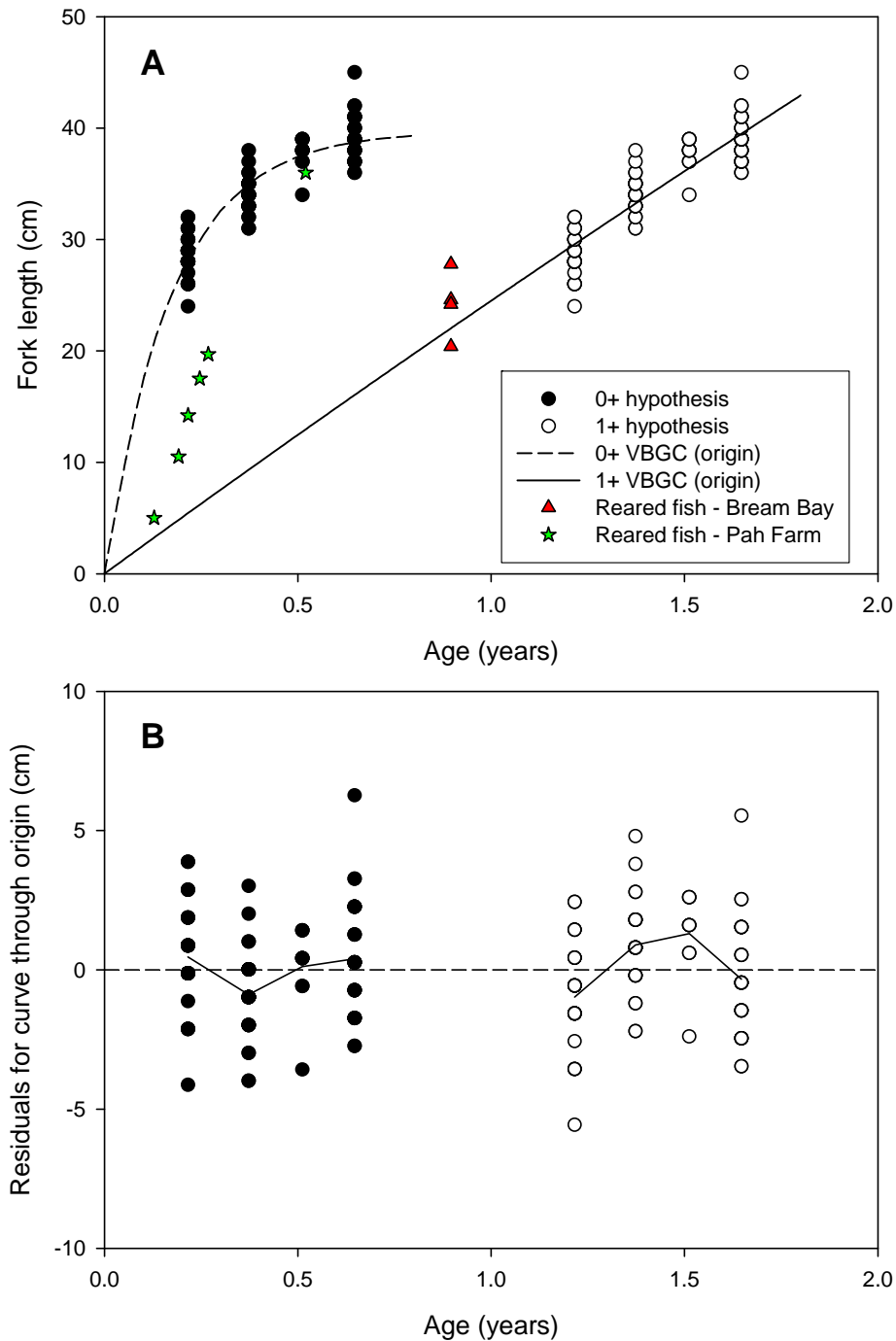
We made two sets of otolith measurements in order to compare the relative sizes of the otoliths from FAD juveniles, and the first and second growth zones of otoliths from older kingfish. For the six FAD juveniles examined for increments, the dorsal growth axes were approximately linear, or had a small amount of curvature near the dorsal edge. The dorsal growth axis of each otolith section was measured in a straight line between the core and the dorsal margin under a compound microscope.

For 10 randomly selected adult kingfish, we measured the dorsal growth axis between the core and the first zone counted by Reader 1 (see long arrow in Figure 1). This measurement corresponds with the dorsal growth axis measurement in FAD juveniles. In the same 10 adult kingfish, we also measured the distance between the first and second zones along the medial axis (short arrow in Figure 1). The distance from the core to the second zone was calculated as the sum of these two measurements.

# **3. RESULTS**

## **3.1 Growth of a juvenile cohort of wild kingfish**

Length-at-age data for the FAD juveniles are shown in Figure 2A for both the 0+ and 1+ cohort hypotheses. The 0+ hypothesis requires very fast growth of juveniles from hatching to reach an average length of 28 cm after 2.5 months (assuming that this cohort was spawned on the theoretical birthday). By contrast, the 1+ hypothesis implies almost linear growth from hatching to 28 cm in 14.5 months. The residuals from both models are reasonably random, with a slightly better fit for the 0+ hypothesis (Figure 2B). Neither growth model incorporated parameters allowing seasonally variable growth rates, so this analysis is somewhat oversimplified. The true trajectory of the growth curve for the 1+ hypothesis would probably have faster growth in summer and autumn, and reduced growth in winter and spring, resulting in a kinked curve with a better spread of residuals; however the overall interpretation would not be substantively affected.



**Figure 2: A. Length-at-age relationship for a cohort of juvenile kingfish sampled in east Northland in 2002 (FAD juveniles). Data were plotted for hypotheses that the fish were from either the 0+ or the 1+ age class. Von Bertalanffy growth curves (VBGC) forced through the origin are fitted for both hypotheses. Also shown are the lengths of kingfish aged 0.90 years reared at Bream Bay, and kingfish reared at Pah Farm. B. Residuals from the VBGCs in A. The solid lines indicate the mean residuals.**

### 3.2 Growth of hatchery-reared juvenile kingfish

Published growth data from juvenile kingfish reared at Pah Farm hatchery, Kawau Island, showed a very high growth rate, with fish reaching 36 cm FL in only 190 days (0.52 years) (Walsh et al. 2003). This rapid growth is consistent with the 0+ hypothesis for FAD juveniles (Figure 2A).

Four reared Bream Bay kingfish that were sacrificed at an age of 0.90 years measured 20.4–27.8 cm. The lengths of these fish straddled the growth curve fitted to the FAD juvenile data under the 1+ growth hypothesis (Figure 2A).

### 3.3 Otolith increments in a juvenile cohort of wild kingfish

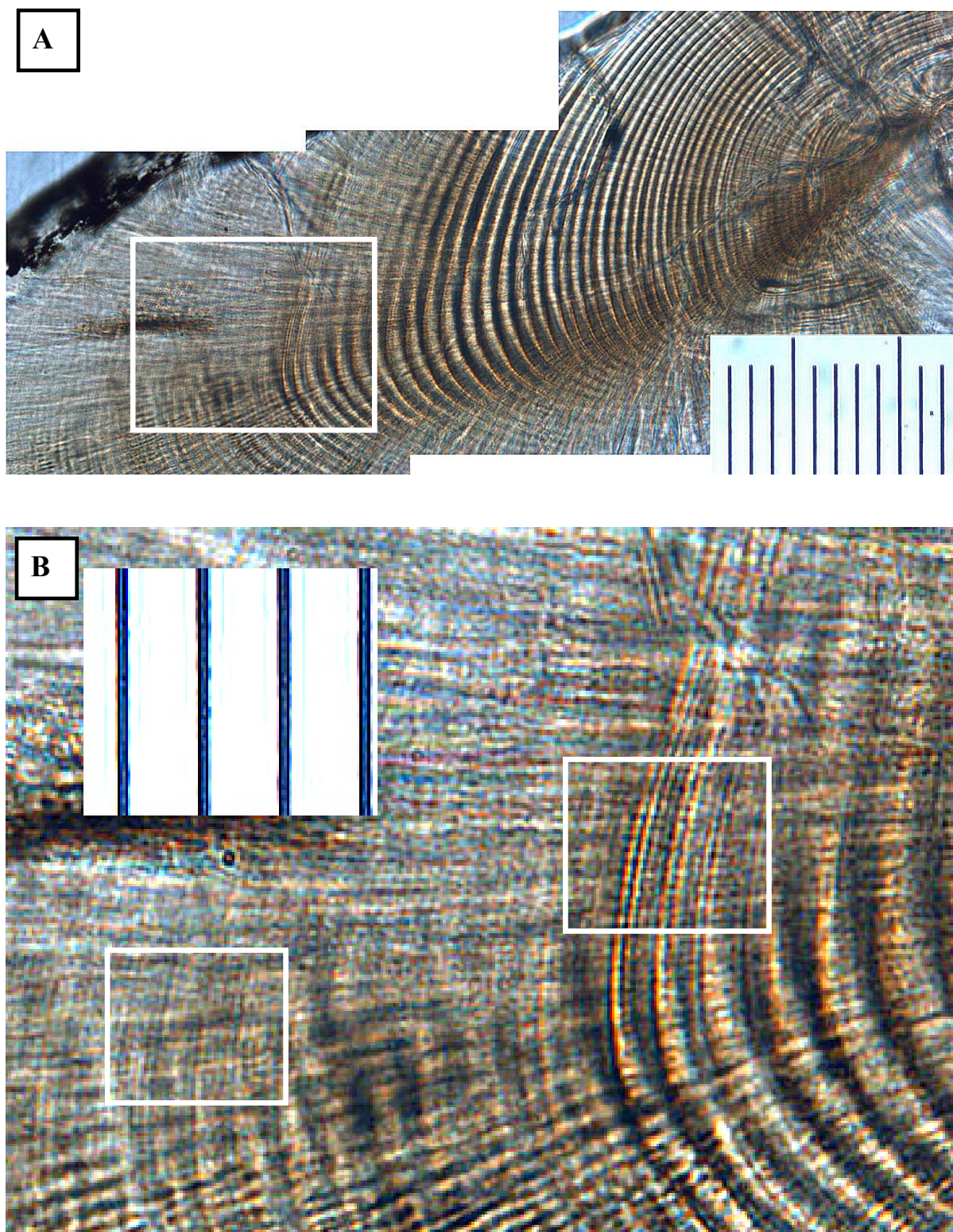
Increments in juvenile kingfish otoliths were highly variable in width (spacing) and appearance, both along the otolith growth axis, and among different fish. Increments near the core were usually very distinct, wide and easily counted (although some sections did not include the core, resulting in the loss of some increments) (Figures 3–7). Increment width increased from less than 2  $\mu\text{m}$  near the core to a maximum of 10  $\mu\text{m}$  at a distance about 35–40 increments from the core (Figure 3A). Increment width then declined towards the margin, with increments smaller than 1  $\mu\text{m}$  being visible in some sections (e.g. Figure 5).

In some parts of the otolith sections, close inspection revealed smaller increments nested within the larger increments (Figure 3B). The small increments were about 1.1–1.3  $\mu\text{m}$  wide. We interpret the wide increments as presumed daily increments, and the smaller nested increments as presumed subdaily increments, based on the following considerations:

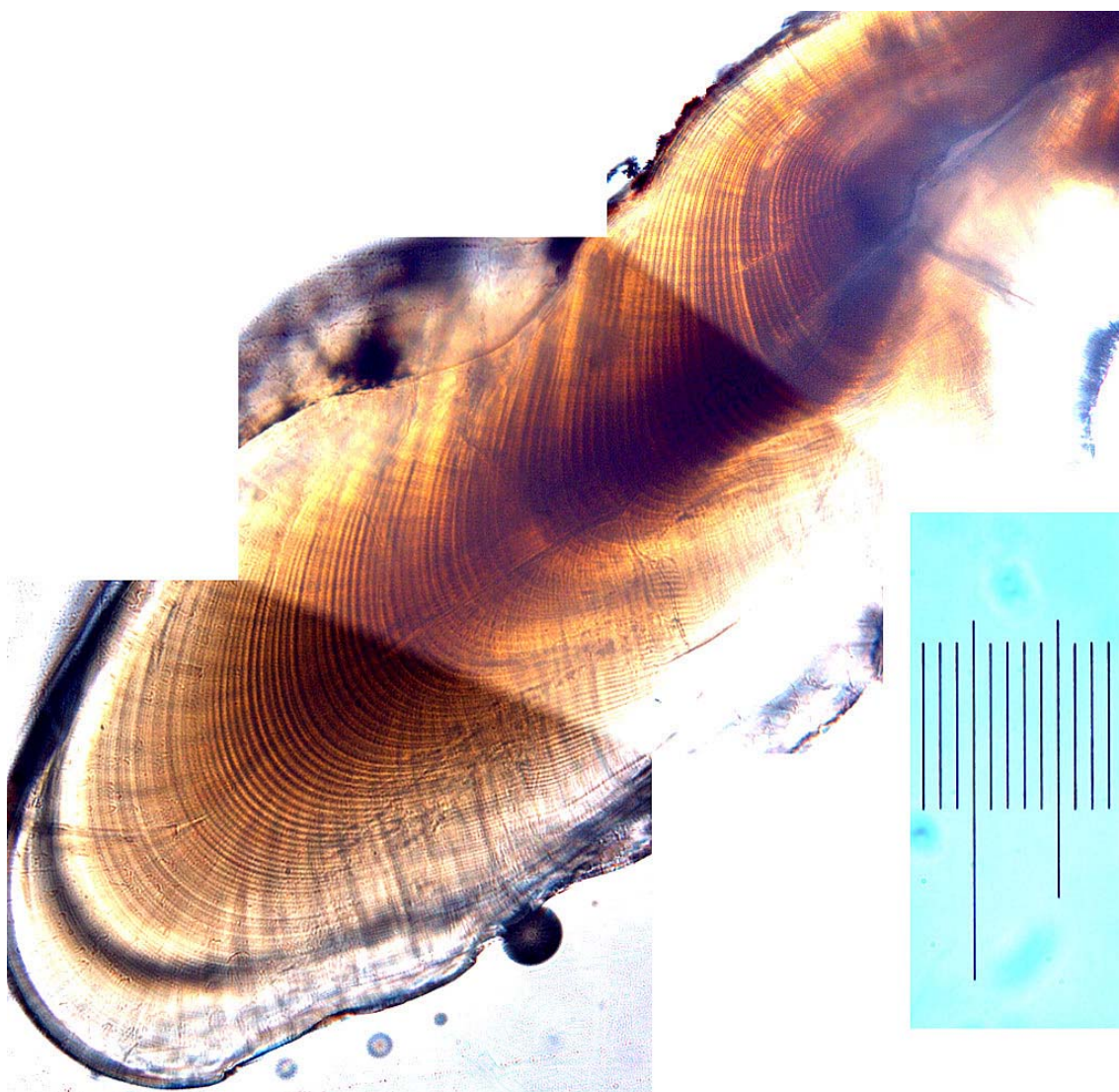
- Small increments were present in only a small proportion of the otolith section, and were not seen in SEMs of etched otoliths.
- The small increments ‘disappear’ when the microscope is not sharply focused. This ‘off-focus’ technique has been used intentionally to avoid counting sub-daily increments (Tsukamoto & Kajihara 1987, Sakakura & Tsukamoto 1997).
- Similar sub-daily increments have been found in many other fish species in which the larger increments have been validated as daily (S. E. Campana, Department of Fisheries and Oceans, Canada, pers. comm.). Faint sub-daily increments are present in the otoliths of Japanese yellowtail (*Seriola quinqueradiata*), a species closely-related to New Zealand kingfish. The larger yellowtail increments have been validated as daily in reared fish up to 60 days old (Sakakura & Tsukamoto 1997).

In this study, we have assumed that the larger increments are daily increments, but validation is required to confirm this. The cause of the sub-daily increments is not known (S. E. Campana, pers. comm.), but because they are not apparent in SEMs of the surface of an otolith, they may be an optical artifact of viewing a thick section under a light microscope. Subdaily increments are not considered further in this report.

Daily increments were not present across the entire growth axis of any of the six FAD kingfish examined (though they were always visible over a much higher proportion of the section than were subdaily increments). Areas with unclear or uncountable daily increments included the core (usually because the core was missing from the section plane; Fig 4), the central region (Figure 6), and the margin (Figures 4, 6A). Juveniles with the clearest marginal daily increments were caught in February, with fish from April and July having uncountable or unclear marginal increments (Table 1). The loss of daily increments from the core would have produced a small underestimate of the number of daily increments (bias probably less than 10 increments), whereas missing increments from the central and marginal portions of the section would have produced larger errors.

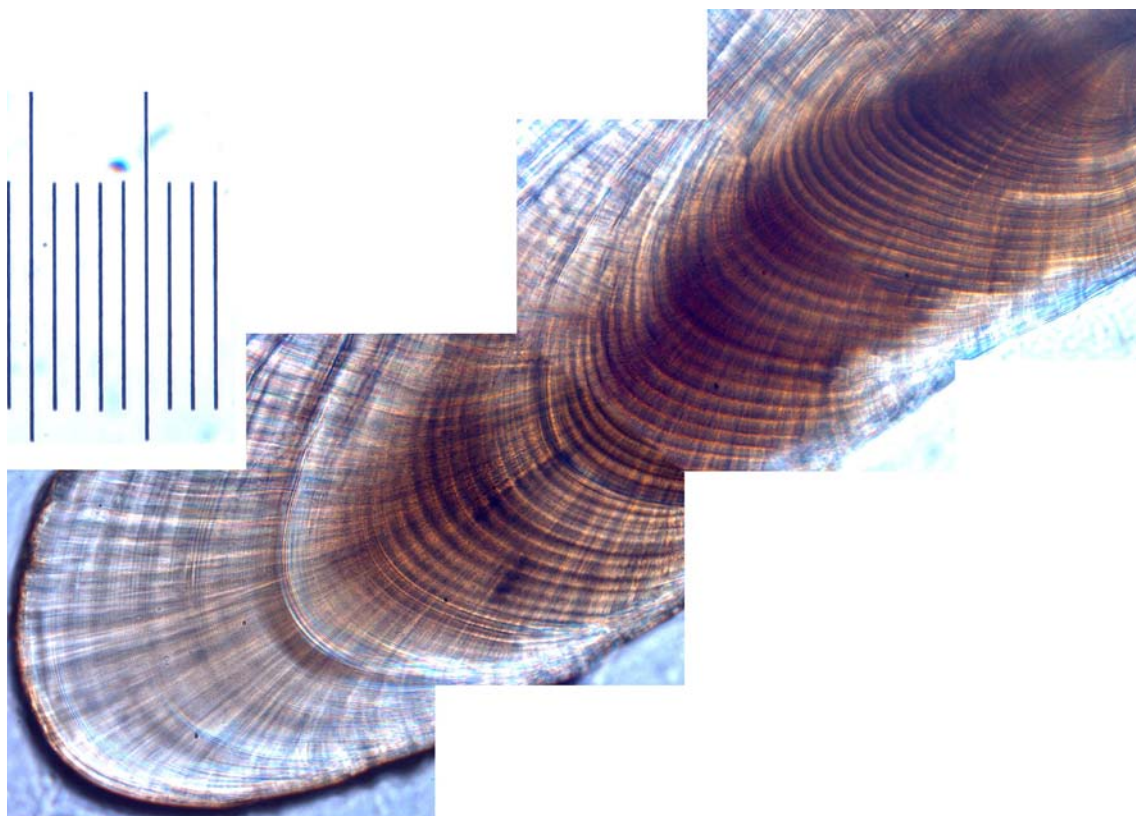


**Figure 3: Composite light micrograph of an otolith section from a kingfish FAD juvenile (KIN5, 301 mm male, February 2002) showing increments. A. Dorsal growth axis from core (right) towards margin (left). Area inside white box is enlarged in B. B. Enlargement of boxed part of image in A. The two white boxes indicate regions of prominent, narrow (1.1–1.3 µm wide) presumed sub-daily increments within the wider 6–7 µm wide presumed daily increments. The smallest scale tick marks in both images are 10 µm apart.**

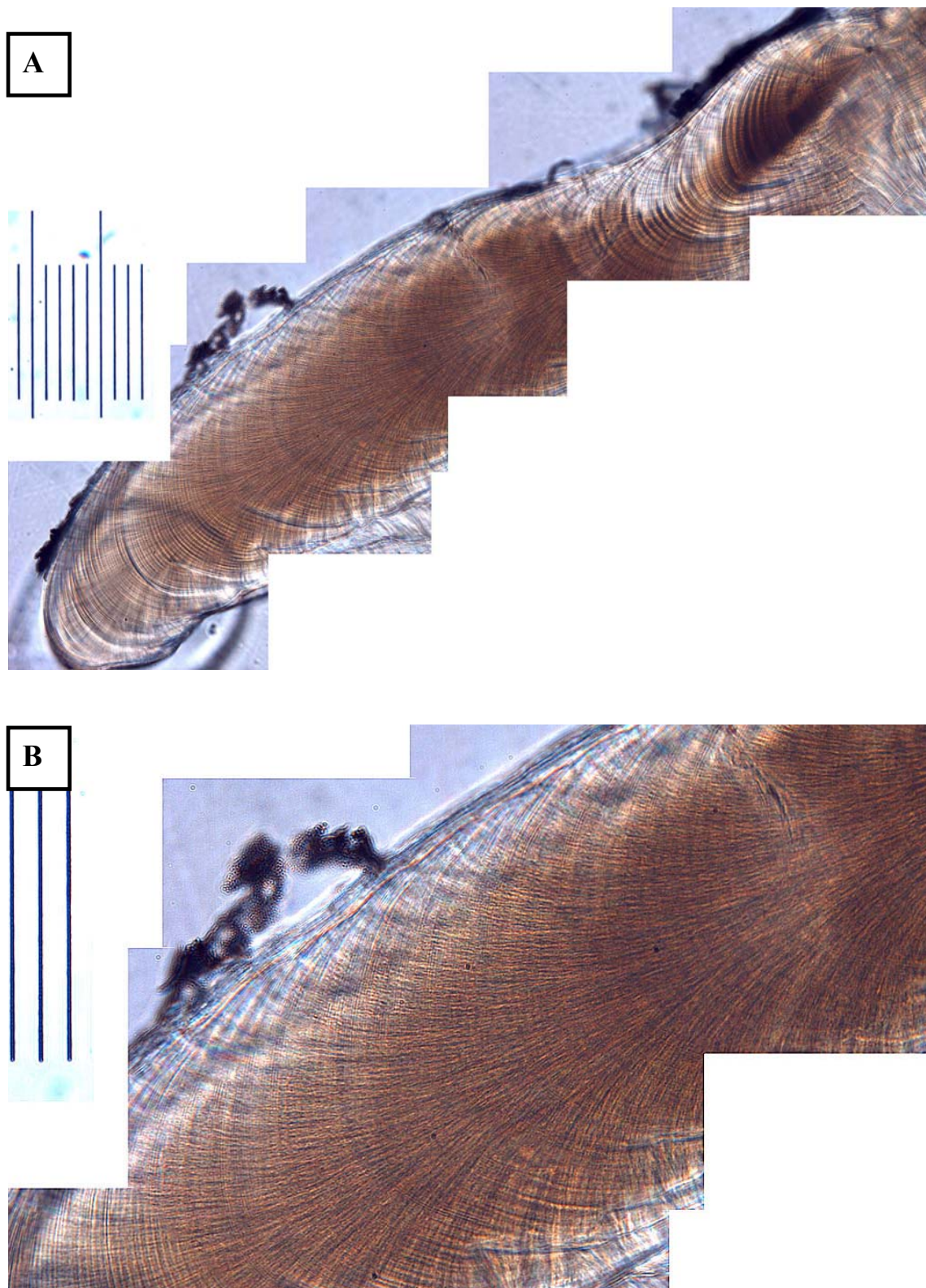


**Figure 4: Composite light micrograph of an otolith section from a kingfish FAD juvenile (KIN1, 347 mm male, April 2002) showing increments. The smallest scale tick marks are 10  $\mu$ m apart.**



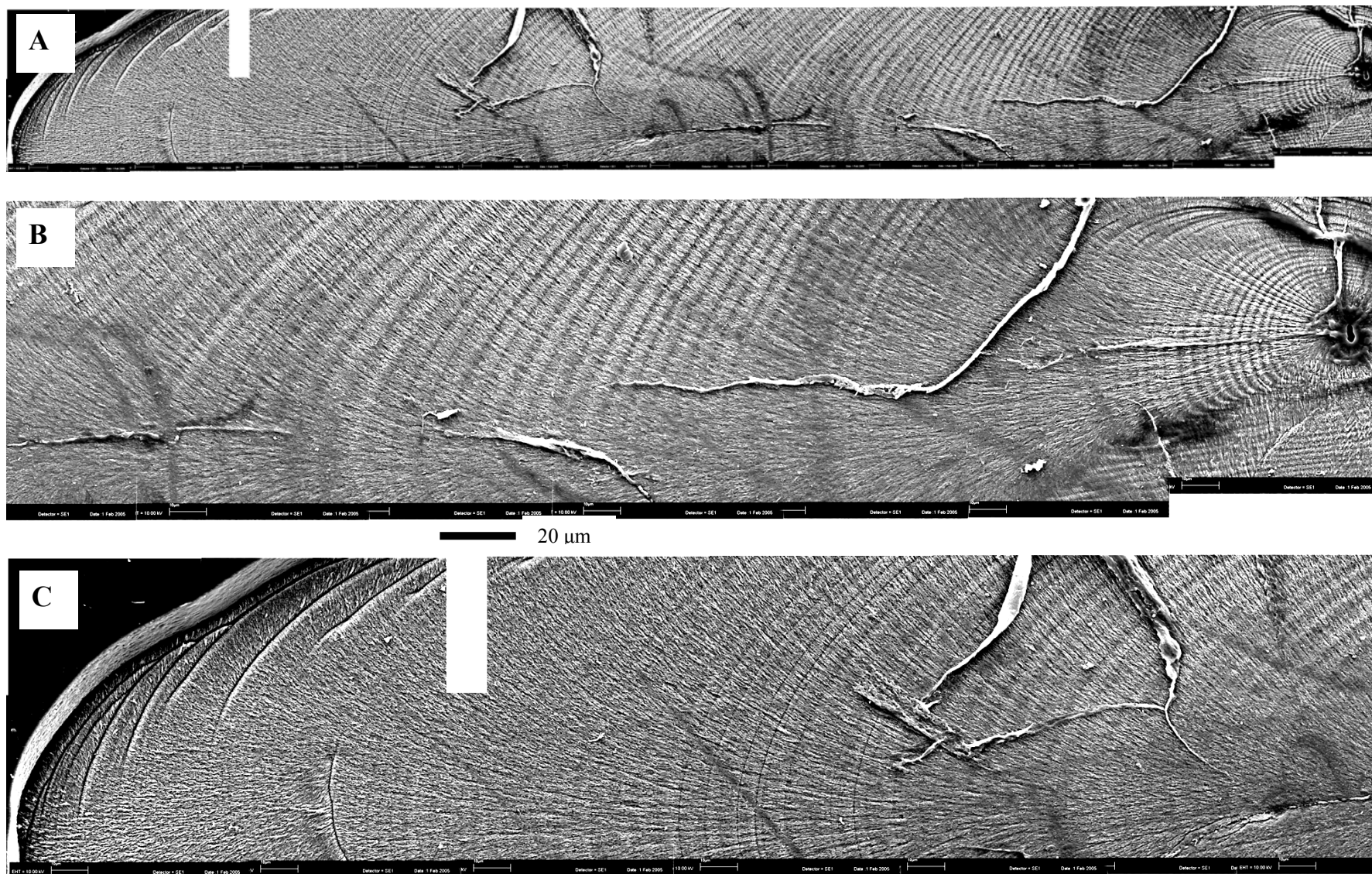


**Figure 5: Composite light micrograph of an otolith section from a kingfish FAD juvenile (KIN6, 292 mm female, February 2002) showing increments. The smallest scale tick marks are 10  $\mu\text{m}$  apart.**



**Figure 6 Composite light micrograph of an otolith section from a kingfish FAD juvenile (KIN4, 369 mm male, July 2002) showing increments. A. Dorsal growth axis. B. Enlargement of the central portion of A. The smallest scale tick marks are 10  $\mu$ m apart in both images.**





**Figure 7: Scanning electron micrograph of an otolith section from a kingfish FAD juvenile (KIN1, 347 mm male, April 2002) showing increments.**  
**A.** Dorsal growth axis from core (right) to margin (left) (length = 0.735 mm). Image is a composite of 26 separate images. **B.** Enlargement of right half of A. **C.** Enlargement of left half of A. Scale bar for B and C is 20 μm. The image in A is approximately twice the scale of the images in B and C.



**Table 1: Increment counts from thin sections of otoliths from FAD juvenile kingfish. Counts were made along the dorsal axis from a photomontage, and along dorsal and ventral axes under a compound microscope. A count was also made from an SEM of one otolith. Ranges indicate separate counts; all counts were approximate only and often underestimated the full number because parts of the section were uncountable. The last two columns provide the expected number of daily increments under the 0+ and 1+ hypotheses (see text for details). > indicates a minimum count (some increments could not be counted); –, not countable.**

					Light microscope				Expected number of increments	
FAD juvenile	Fork length (mm)	Sex	Capture date in 2002	Increment readability	Count from photo montage	Count under microscope Dorsal	Count under microscope Ventral	SEM count from digital image	0+ hypothesis	1+ hypothesis
6	292	F	18 February	Core faint, central increments difficult	142	120	–	–	79	444
5	301	M	18 February	Marginal increments faint, better in ventral than dorsal axis	124–141	116	149	–	79	444
1	347	M	16 April	Core uncountable, margin very thin and difficult to count	> 149	> 103	> 188	156	136	501
2	351	M	16 April	Core and margin uncountable, central increments faint	169	> 121	–	–	136	501
4	369	M	25 July	Central and marginal increments difficult	175–199	> 169	–	–	236	601
3	393	M	25 July	Central increments difficult, margin uncountable	–	>> 115	164	–	236	601

The number of increments counted under the light microscope varied depending on whether the count was made from a photomicrograph, or directly under the microscope from dorsal or ventral growth axes (Table 1). Counts were often incomplete because part of the otolith could not be counted. They ranged from more than 103 to 199. Maximum counts obtained were 149 in February, more than 188 in April, and 199 in July. A count of 156 increments was obtained from an SEM of an otolith from a kingfish caught in April (Figure 7), and this was of the same order as the light microscope counts from the same fish (Table 1). Counts could not be made on the SEMs for the other five kingfish because of poor etching of the increments.

The increment counts for FAD juveniles caught in February and April were higher than expected under the 0+ hypothesis (Table 1). The differences between the maximum increment counts and the expected ages were in the range 33–70 days. However, the counts for the juveniles caught in July were lower than expected under the 0+ hypothesis. The maximum increment counts for all juveniles were well below the number expected under the 1+ hypothesis (average 33% of the expected amount, range 27–38%) (Table 1).

### **3.4 Otolith increments in reared kingfish**

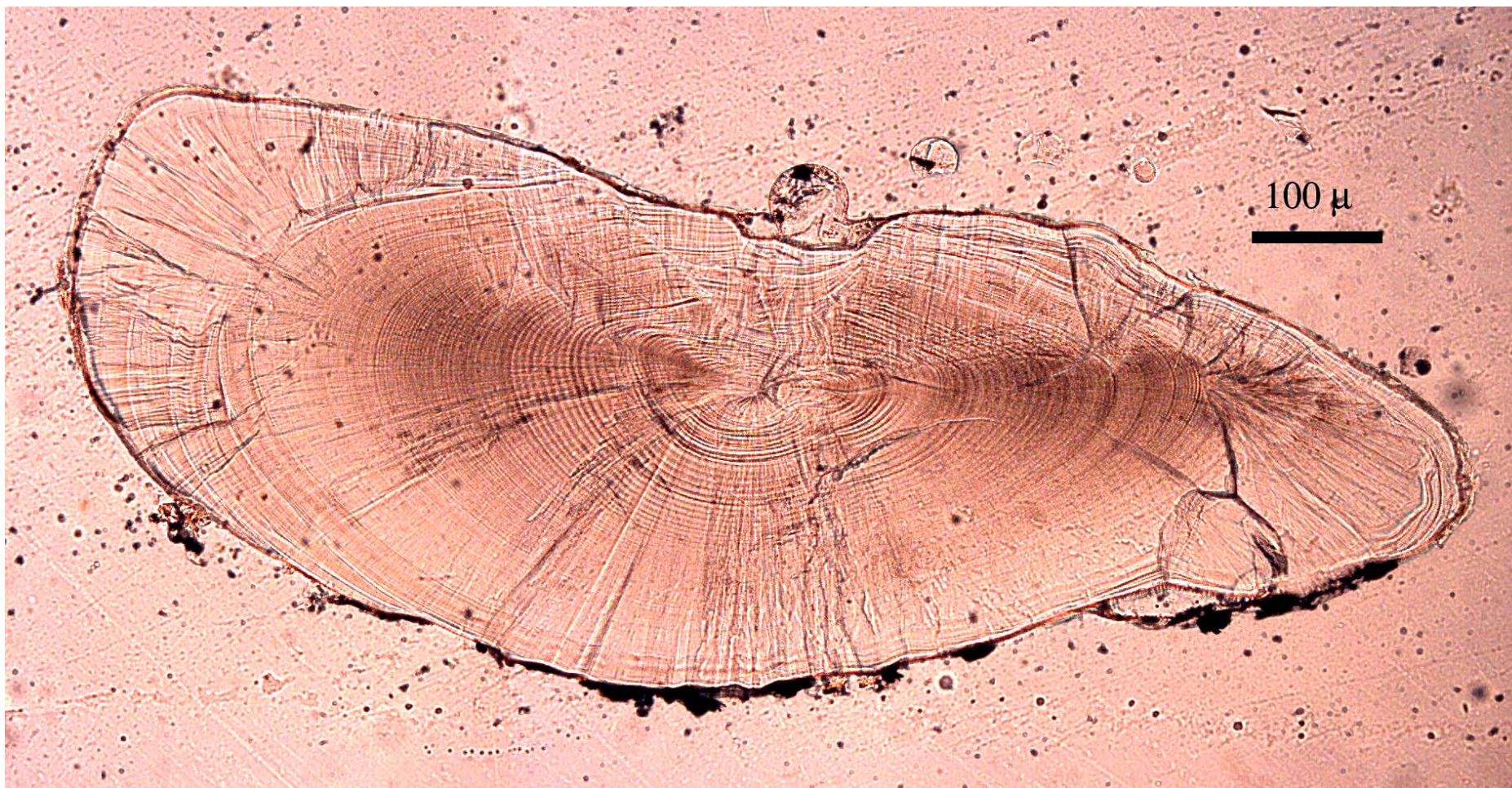
Otoliths from four reared kingfish that were sacrificed at an age of 0.90 years were examined. The small size and fragility of the otoliths meant that removal and preparation were difficult and not always successful. The dorsal growth axes of three of these fish were 0.52–0.54 mm (mean 0.53 mm) long. Increments were generally poorly defined (low contrast), and could not be counted in three of the four fish. About 120 increments were counted on the inner 75% of the section from the fourth fish; the outer 25% of the section did not have any visible increments, though there were some major check marks (Figures 8 and 9).

### **3.5 Otolith measurements**

The length of the dorsal growth axis in FAD juveniles increased from about 0.6 mm in February to about 0.75 mm in April, and 0.72–0.92 mm in July (Figure 10, dots and curve). However, for the July fish having a 0.72 mm long axis (arrowed in Figure 10), the dorsal half of the otolith appeared to be deformed, being much shorter than the ventral half (the dorsal axis is usually similar in length to the ventral axis). The growth of the dorsal axis in the remaining FAD juveniles is well described by a simple exponential curve (Figure 10).

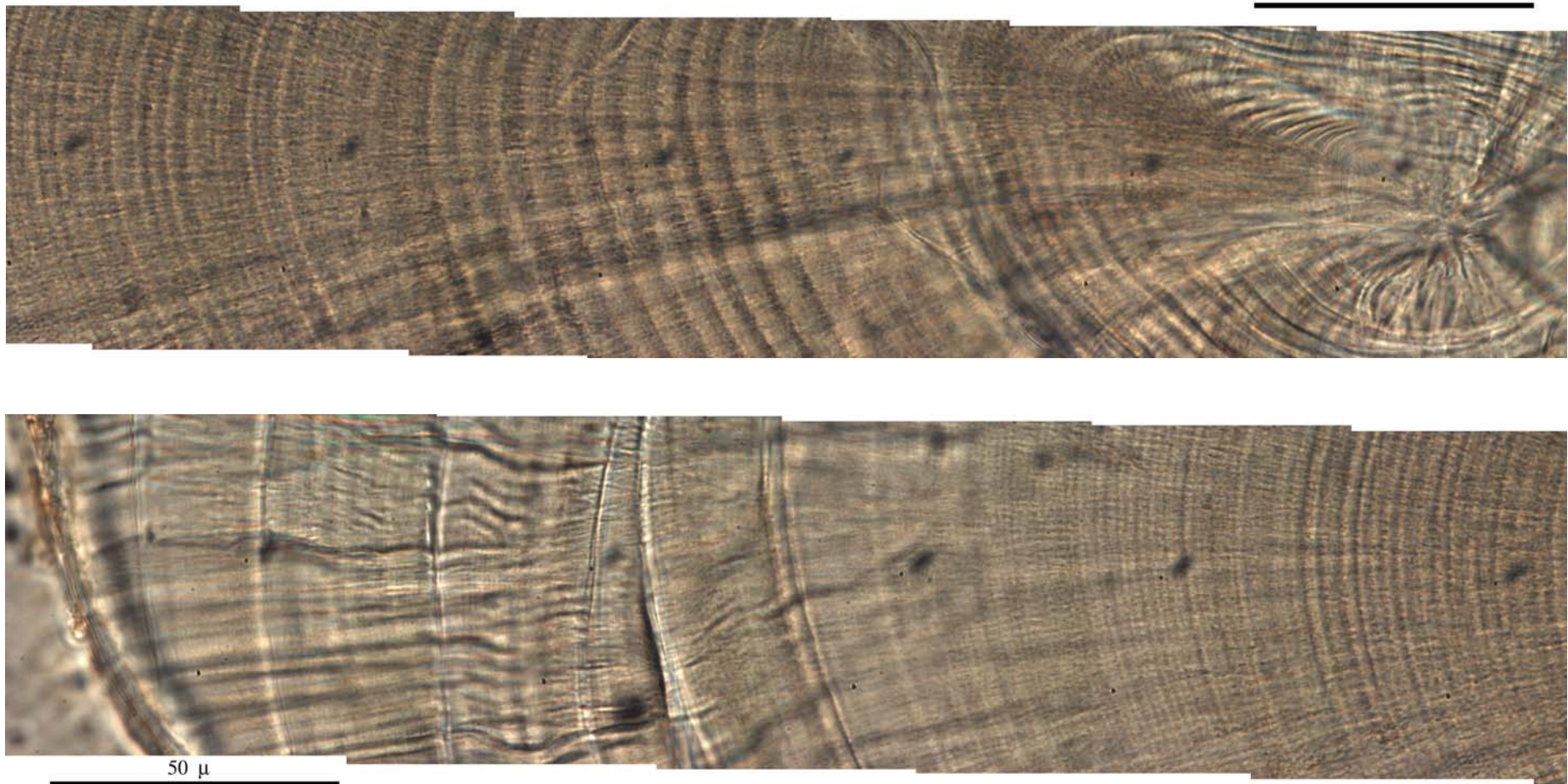
The length of the dorsal growth axis measured to the first otolith zone counted by Reader 1 in older kingfish averaged 0.74 mm (Figure 10, black histogram bars), which is almost the same as the FAD juvenile axis length in April. This suggests that the first growth zone counted by Reader 1 in older kingfish is deposited in or after autumn. However, the distance between the first and second otolith zones was small (mean = 0.11 mm, range 0.09–0.14 mm), so the axis length from the core to the second zone (white histogram bars) was only slightly larger than the axis length to the first zone. The distances between the core and the first and second zones in adult fish overlap considerably.

Although the distance to the second zone in adult fish is not directly comparable with the dorsal axis length in FAD juveniles (because the former is the sum of two distances measured along different axes), the April FAD juveniles appear to have otolith lengths consistent with both the first and second zones. Taking into account the very small sample sizes, this indicates that otolith size does not effectively discriminate between the 0+ and 1+ hypotheses.

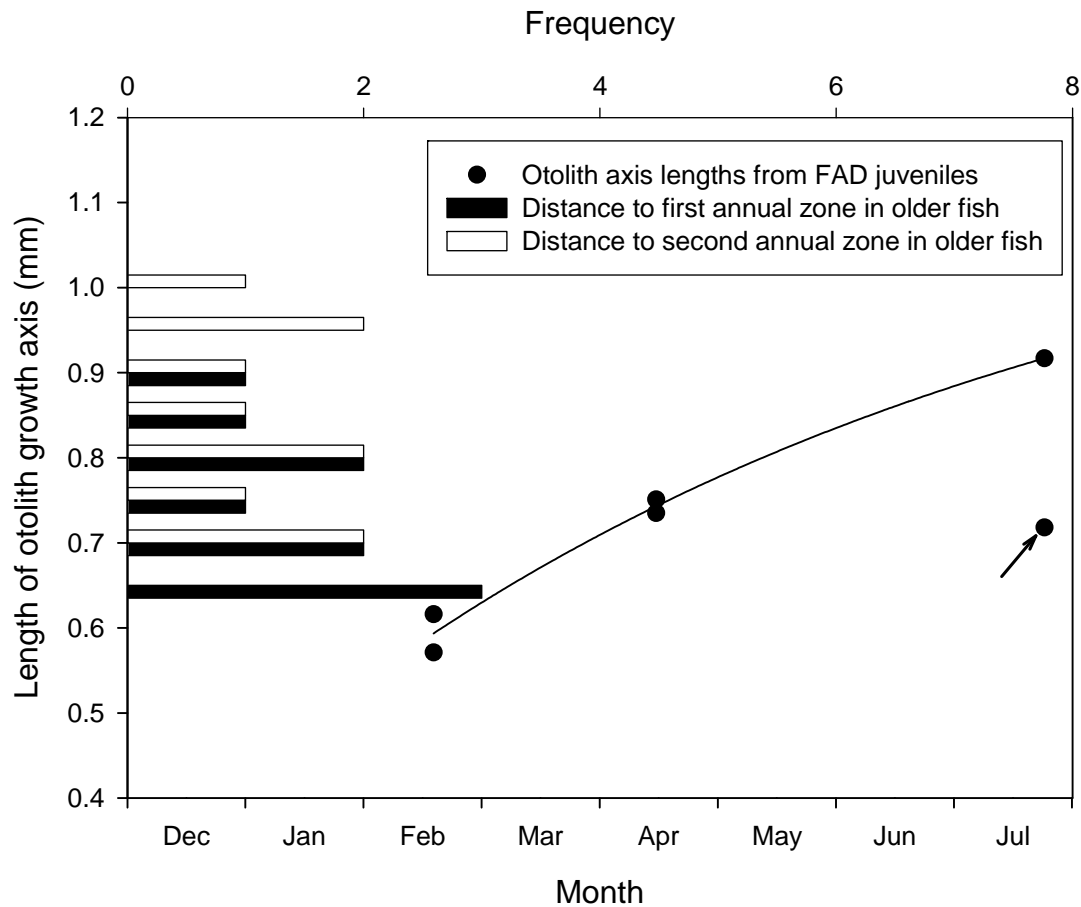


**Figure 8:** Light micrograph of an otolith section from a reared 0.90 year old kingfish (KIN1BB, 204 mm, January 2005) showing increments. Scale bar = 100 μm.





**Figure 9: Composite light micrograph enlargement of the dorsal growth axis of the otolith section from Figure 8 showing increments. Scale bars = 50  $\mu$ m.**



**Figure 10: Comparison of the length of the otolith dorsal axis in FAD juveniles (dots with fitted curve) with the distance between the core and the first and second otolith growth zones of Reader 1 measured in 10 older kingfish (horizontal histograms).**

#### 4. DISCUSSION

The main objective of this study was to validate the location of the first annual growth zone in kingfish otoliths. Achieving this would allow us to determine which of two different ageing techniques is accurate for kingfish. The two ageing techniques result in a difference of one year in kingfish age estimates (McKenzie et al. 2014).

We attempted to determine the ages of juvenile kingfish sampled from the wild using fish length and otolith increment counts. We also used fish and otolith length, and increment counts, from known-age, hatchery-reared kingfish for the same purpose. We posed two alternative hypotheses: first, that the cohort of wild fish belonged to the 0+ age class, and second, that the cohort belonged to the 1+ age class. We used five methods to test these two hypotheses, and the outcomes of each are summarised below:

1. Growth curves were fitted to length-at-age data from a time series of samples of wild juveniles under the assumptions that the fish were either 0+ or 1+ fish. The results of this test were inconclusive, with neither hypothesis providing a more plausible growth curve.
2. Reared kingfish from Pah Farm hatchery grew very rapidly, consistent with the 0+ hypothesis. By contrast, reared kingfish from Bream Bay hatchery grew more slowly, consistent with the 1+ hypothesis. Whether the high rates observed at Pah Farm are possible in wild fish is unknown, but it is clear that growth of reared fish is highly variable, making this an unreliable method for

discriminating between 0+ and 1+ hypotheses. However, the Pah Farm data demonstrate that the very high growth rate required by the 0+ hypothesis is not impossible.

3. Daily increments were counted in the otoliths of wild juveniles. Increment counts were too high for February and April fish to be consistent with the 0+ hypothesis, unless this cohort of juveniles was spawned 1–2 months before the theoretical birthday of 1 December. This is possible, because the spawning season of kingfish is not well known; spawning is likely to last several months and might easily occur 1–2 months before the theoretical birthday. Conversely, increment counts were too low for all fish to be consistent with the 1+ hypothesis unless increment numbers were grossly under-estimated. This is likely, as there were zones of uncountable increments in all otoliths examined. This test was therefore unable to distinguish between the two ageing hypotheses.
4. The dorsal growth axes of the reared Bream Bay 0.90 year old kingfish were smaller (average 0.53 mm) than those of the FAD juveniles sampled in February–July (0.57–0.92 mm). While this may seem to suggest that the FAD juveniles were older than 0.9 years, and therefore support the 1+ hypothesis for FAD juveniles, there is generally a strong linear relationship between otolith size and fish size. Although that relationship is also affected by fish growth rate, with faster-growing fish having smaller otoliths than slow-growing fish of the same length, the larger otoliths of the FAD juveniles may simply reflect the fact that most of them had larger fork lengths than the Bream Bay fish (see Figure 2).
5. Daily increments were counted in an otolith from one reared juvenile, and the count (about 120) was well below that expected from the age of the fish (327 days). This suggests that not all increments are visible in the otoliths of reared fish, and lends support to the suggestion (see point 3 above) that increment counts underestimate actual age in FAD juveniles. An important caveat however, is that other studies have reported that increments tend to be less distinct in reared fish than in wild fish. Consequently this test provides weak support for the 1+ hypothesis.

Four out of our five tests proved inconclusive. Test 5 provided weak evidence supporting the 1+ hypothesis rather than the 0+ hypothesis; i.e. the FAD juveniles were in their second year when caught in February–July. Overall, we conclude that there is still considerable doubt about whether the FAD juveniles are 0+ or 1+ fish, and therefore about the significance of the first growth zone. The annual increments counted when estimating age in older fish appear to be deposited in autumn–winter. (Although otolith size suggests zone formation in autumn, otoliths growth probably declines substantially in autumn–winter, and the growth zone may not be completed and become apparent in otoliths until the following spring.) Thus the first zone counted by Reader 1 (white dots in Figure 1) is deposited at an age of about 6 or 18 months, depending on whether the 0+ or 1+ hypothesis is correct.

This study has failed to determine conclusively whether the first otolith zone is deposited during the 0+ or 1+ year. Until this is established, we suggest that kingfish ageing studies adopt the 1+ hypothesis, and assume that the first growth zone is formed during autumn–winter of the second year, at an age of approximately 18 months.

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