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Validation of age determination from otoliths of the King George whiting *Sillaginodes punctata* (Perciformes)

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Abstract The otoliths of the King George whiting *Sillaginodes punctata* were assessed for their usefulness in adult-ageing. Whole, transverse-sectioned and broken/burnt sagittae displayed alternating opaque and translucent zones. Some small whole otoliths contained accessory areas of pigment, whilst large otoliths were too thick to transmit light, resulting in ambiguous counts in each case. Both marginal-increment analysis and treatment of fish with tetracycline demonstrated that the first three opaque zones formed annually, the first during the spring/summer of the second year of life, and the second and third in each subsequent spring. Otolith thickness increased linearly with the completion of each new opaque/translucent zone. An ageing protocol based on whole otoliths, supplemented with the breaking/burning of ambiguous otoliths is recommended. An algorithm for calculating age in months from otolith counts based on a fixed birth-date of the mid-point of the spawning season is presented.

Introduction

Understanding population biology, assessing stock, and developing management protocols for fish populations are often best achieved through the interpretation of representative age structures based on the age of individual fish (Megrey 1989). Interpreting otolith structure is the best method for such age determination (e.g. Bagenal 1974; Pentilla and Dery 1988; Fowler 1995), but this technique must be validated to ensure that the estimates are accurate and to assess precision of the counts (Beamish and McFarlane 1983, 1995; Beamish 1992). Unvalidated otolith methodologies result in seriously biased age estimates, which ultimately undermine stock assessment and management processes (Beamish and McFarlane 1983, 1995; Lai and Gunderson 1987).

The process of validating the relationship between otolith growth and fish age initially involves three criteria (Fowler 1990): (1) otoliths must display an incremental structure which can be interpreted visually; (2) the optical pattern of alternating zones must correspond to a regular and determinable time scale; (3) the growth of otoliths along the axes examined must continue at a measurable rate throughout the life of the fish. When these criteria are fulfilled, zonal structure will be related to, but not necessarily equal, fish age, which depends on when the opaque and translucent zones are formed and the age at formation of the first opaque zone (Francis et al. 1992b).

This study assesses the usefulness of otoliths of the King George whiting *Sillaginodes punctata* of southern Australia, the largest and most valuable of Australian whitings (Kailola et al. 1993), by determining whether they fulfil the three criteria described above and by relating the otolith macrostructure to time of year and determining the age of formation of the first opaque zone. Assuming this to be the case, our final aim was to develop an efficient protocol for ageing large numbers of fish in a population study.

Materials and methods

Adult *Sillaginodes punctata* were sampled from the commercial catch at four sites, and juveniles were caught at two sites in South Australia (Fig. 1). The total length (TL) of each fish was measured and the fish was weighed. The sagittae were removed through a transverse slit across the back of the fish's head, and were cleaned, dried and stored in labelled plastic bags. The terms sagittae and otoliths are used synonymously throughout the paper.

Characteristics of sagittae

To assess Criterion 1, whole otoliths and transverse sections of otoliths from 380 fish collected from Sites A1, A2 and A3 (Fig. 1), were examined and the counts of opaque zones were compared.

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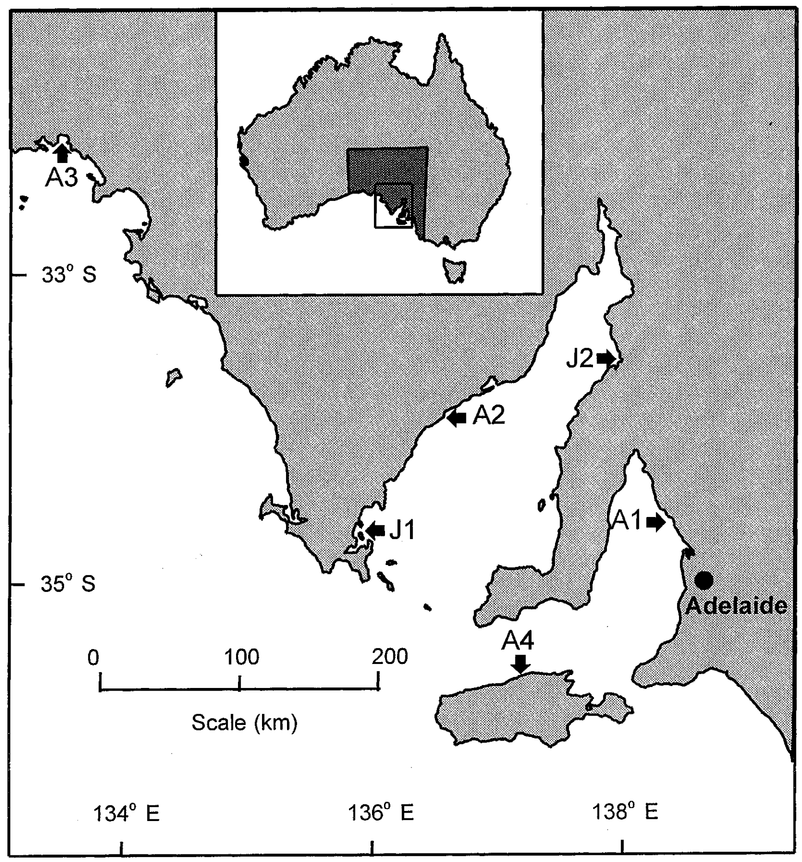

Fig. 1 Map of part of South Australia showing sampling sites of adult (*A1–A4*), and juvenile (*J1*, *J2*) *Sillaginodes punctata* (*Inset* area relative to Australian coastline)

Whole otoliths were immersed in aniseed oil and examined with a binocular microscope at ×6 magnification with transmitted light, and the opaque zones were counted. The second sagitta from the same fish was embedded in resin, and a transverse section was cut using an Isomet diamond saw. Sections were 400 μm thick and were cut so as to incorporate the otolith centre. The section was smeared with immersion oil to clear surface cracks, and was then examined and counted.

For some fish, the whole sagitta and transverse section were counted independently by two readers, who had no reference to each other's counts. The two methods occasionally produced different counts for the same fish; such counts were reconciled by examining the whole otolith and the transverse section together.

Periodicity of opaque-zone formation

Marginal-increment analysis

The first validation procedure involved marginal-increment analysis. Fish were collected from Site A3 (Fig. 1) at approximately monthly intervals between February and December 1995. On each occasion, five fish from each of four size classes (≤309 mm, 310 to 349 mm, 350 to 389 mm, ≥390 mm TL) were randomly selected. One sagitta from each fish was sectioned transversely and examined at ×6 magnification. The distances between consecutive opaque zones and also from the last opaque zone to the outside edge of the otolith were measured along the short axis from the otolith centre to the proximal surface, using an image-analysis system. The marginal increment for each otolith was expressed as a proportion of the immediately preceding annulus, and plotted as a function of month of the year. Furthermore, the appearance of the otolith margin was recorded as opaque or translucent. Otoliths were processed without reference to fish size and in random order with respect to sampling occasion.

Tetracycline tagging

The second validation procedure involved treating live fish with tetracycline and then maintaining them in a large tank for up to 2 yr. Fish were netted in an estuary near Adelaide (Fig. 1) on 6 March 1995 and transported in a large oxygenated tank to the laboratory facility. Tetracycline was administered in the form of Terramycin/MA injectable solution (oxytetracycline hydrochloride at 100 mg ml^{-1}). Appropriate doses were determined on the basis of the length/weight relationship $\log_{10} W = (3.0482 \log_{10} L - 2.3091)$ from Robertson (1977). The tetracycline was administered by injection into the dorsal musculature and coelomic cavity. It was used in its original concentration and the volume appropriate to provide a dose of 50 mg kg^{-1} body weight.

On 29 September 1995, the 29 surviving fish were treated with tetracycline for a second time to double-mark the otoliths. On this occasion, tetracycline was used at a lower concentration of 50 mg ml^{-1}, with the volume determined as above. On 20 March, 8 November 1996 and 5 March 1997, 10, 8 and 6 fish were sampled from the tank, respectively. Their otoliths were sectioned in the transverse plane, examined, and photographed under both UV and transmitted white-illumination, and the positions of the fluorescent bands were identified in relation to the location of the opaque zones.

Fish were maintained in a 40 000-litre tank with flow-through seawater pumped from the nearby gulf. The water temperature was not artificially modified, and thus reflected the normal seasonal temperature-cycle. Although a low light regime was used to minimise algal growth, several windows in the tank allowed a natural day/night length cycle. The fish were generally fed once a day, initially on cockles (*Donax deltoides*, Bivalvia: Donacidae) at a rate of 5% of the total weight of live fish, and later on dry fish pellets at 2% of total weight per day.

Formation of first increment

Juvenile fish from the 1+ year-class were sampled at either of two sites (J1, J2: Fig. 1), on eight occasions between March 1995 and December 1996: this provided samples from two different year classes at different times of the year. Their sagittae were examined whole, using transmitted light to identify the presence or absence of an opaque zone and the nature of the otolith margin.

Growth of otoliths

The fish collected from Site A4 displayed the greatest range in size and age; therefore, their otoliths were used in the analysis of otolith growth. These were weighed (to the nearest milligramme) and then sectioned. For each transverse section the otolith thickness, i.e. the minimum distance from the otolith centre to the proximal surface, was measured. Relationships of best fit between otolith weight and thickness, and the number of opaque zones were determined.

Results

Characteristics of sagittae

Whole sagittae of *Sillaginodes punctata* are typical of those of teleost fishes in overall shape and orientation (Pannella 1980). When examined whole in aniseed oil or water, they displayed alternating opaque and translucent zones which were clearest along the short axis square of the otolith centre towards the ventral margin (Figs. 2A). The transverse sections of sagittae under transmitted light were essentially translucent, but contained opaque regions (Fig. 2C, D; 3B, D). The otolith centre was distinctly opaque, as were the thin zones out towards the proximal surface from the centre, which became less distinct near the dorsal and ventral margins.

Comparison of counts between whole otoliths and transverse sections

Although a large number of otolith pairs from Sites A1 to A3 were compared, they all displayed only 1 to 3 opaque zones. Reader 1 recorded the same count for whole and sectioned otoliths for 77.9% of 281 fish (Table 1), suggesting that features were generally consistent between the pair. Nevertheless, the 22.1% for which a difference was recorded is surprisingly high given the low numbers of opaque zones counted. For most of these, the count for the whole otolith was higher than that for the transverse section (Table 1). Whole otoliths were then compared between readers for 287 fish, for which the interpretation was the same for 86% (Table 2). That 14% were interpreted differently indicates that the structure of otoliths is occasionally ambiguous.

To interpret the differences in counts evident in Tables 1 and 2, some whole otoliths and transverse sections were directly compared. This revealed that some of the former displayed intermediate opaque regions running from the anterior to posterior tip, parallel to opaque zones that were being included in the count (Fig. 3C). In the transverse sections these were evident as accessory areas of pigment along the dorsal/ventral axis, unrelated to real opaque zones (Fig. 3D). Since transverse sections were not interpreted along this axis, these features did not compromise the interpretation, as was the case for whole otoliths. We learned to identify these misleading features based on their colour, appearance and location with respect to real zones.

Later in the study, a sample of large fish became available from Site A4. The otoliths of these large individuals were so thick that they transmitted little light, and could not be interpreted directly (Fig. 4A). Counts were made along the ventral margin whilst turning the otoliths through 90° (Ferreira and Russ 1994), but this method underestimated counts from sectioned otoliths (Fig. 5). Little confidence could therefore be placed in such counts.

Periodicity of opaque-zone formation

Marginal-increment analysis

The marginal-increment analysis considered fish from four size classes, collected on nine occasions over 11 mo, whose otoliths displayed between 1 and 3 opaque zones. All otoliths collected between February and June had translucent margins, with the marginal increment increasing through this period (Fig. 6). Some collected in August and most from September and October had opaque margins, indicating that the opaque zone formed from late winter to at least mid-spring. By December, otoliths had translucent margins, and the formation of the new opaque zone had been completed (Fig. 6).

Tetracycline tagging

The fish treated with tetracycline in March 1995 were from two non-overlapping size classes (Fig. 7). All those from the small size class and 12 fish from the larger size class died within several days of capture and treatment. The otoliths from the former were all completely translucent, whilst those from the latter displayed one clear opaque zone.

In comparison, all fish sampled from the tank between March 1996 and March 1997 displayed between 2 and 4 opaque zones, as well as 2 fluorescent bands (Fig. 8). The inner band generally corresponded to the second translucent zone; the outer band, resulting from treatment with tetracycline on 29 September, was always closely associated with the second opaque zone, suggesting that the latter was forming about this time. The otolith material outside the second tetracycline band differed, according to when the fish were sampled: for those collected in March 1996 this part of the otolith was completely translucent, whereas otoliths from fish sampled in November 1996 and March 1997 displayed one

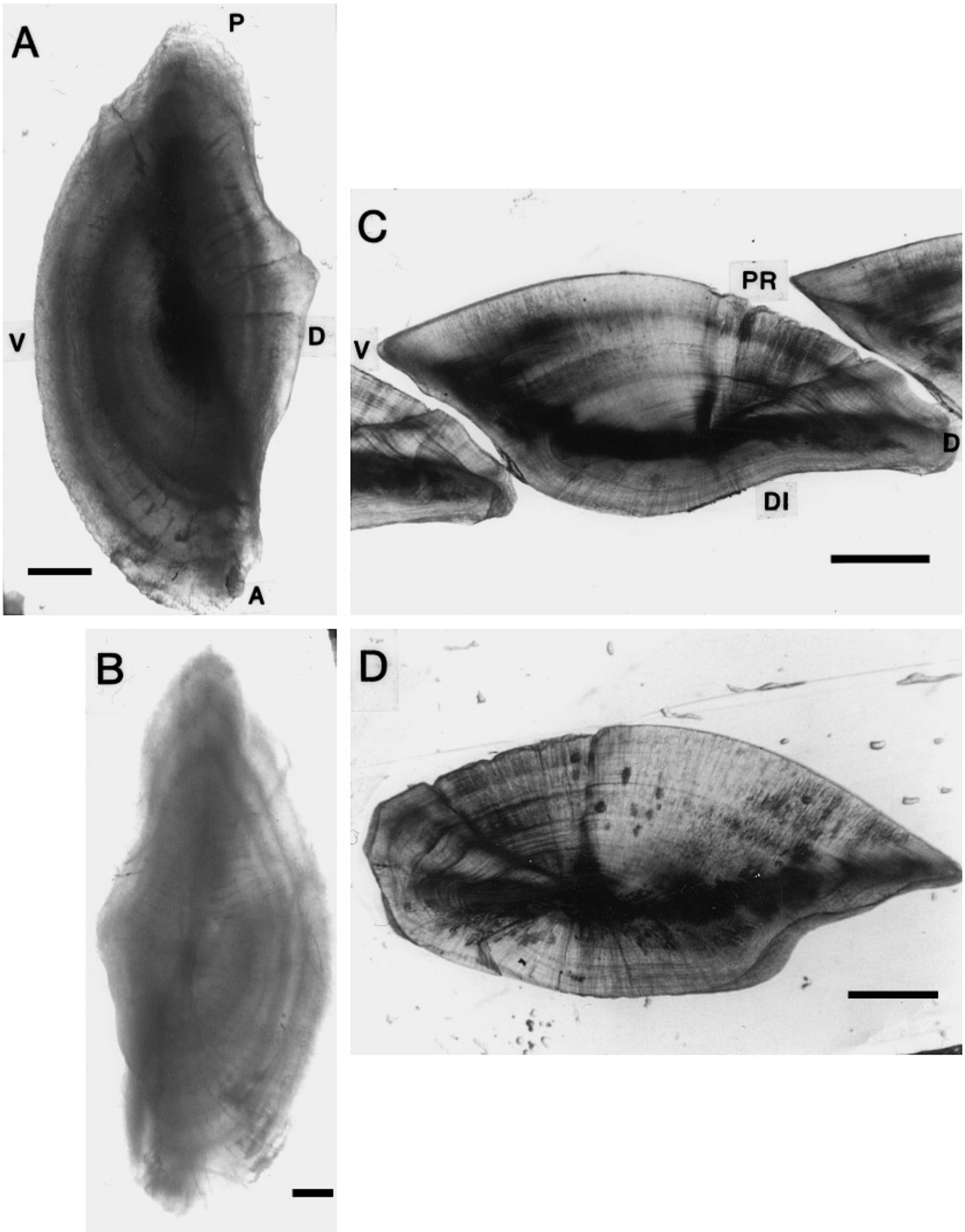

Fig. 2 *Sillaginodes punctata*. **A** Whole sagitta displaying two opaque zones; **B** whole sagitta with four opaque zones; **C** transverse section of sagitta with two opaque zones towards proximal surface; **D** transverse section of otolith with four opaque zones. Labelling indicates orientation of otolith with respect to its position in fish's head, using terminology of Secor et al. (1995) (*A* anterior; *P* posterior; *D* dorsal; *V* ventral; *PR* proximal surface; *DI* distal surface; *scale bars* = 1 mm)

further opaque zone that had probably formed during the spring of 1996. All results are consistent with the annual deposition of an opaque zone, deposited during spring and completed by early summer.

Formation of first increment

Juveniles were collected on different occasions between March 1995 and December 1996. All were a minimum of 12 mo old and originated from spawning between March and June in the year prior to their capture. Those collected in March and August 1995 had completely translucent otoliths (Fig. 9); those from November 1995 were either translucent or had a distinct opaque edge; the 47 fish collected in January 1996 each had one opaque zone distinguishable in its own right. In the otoliths of fish sampled the following year also, the

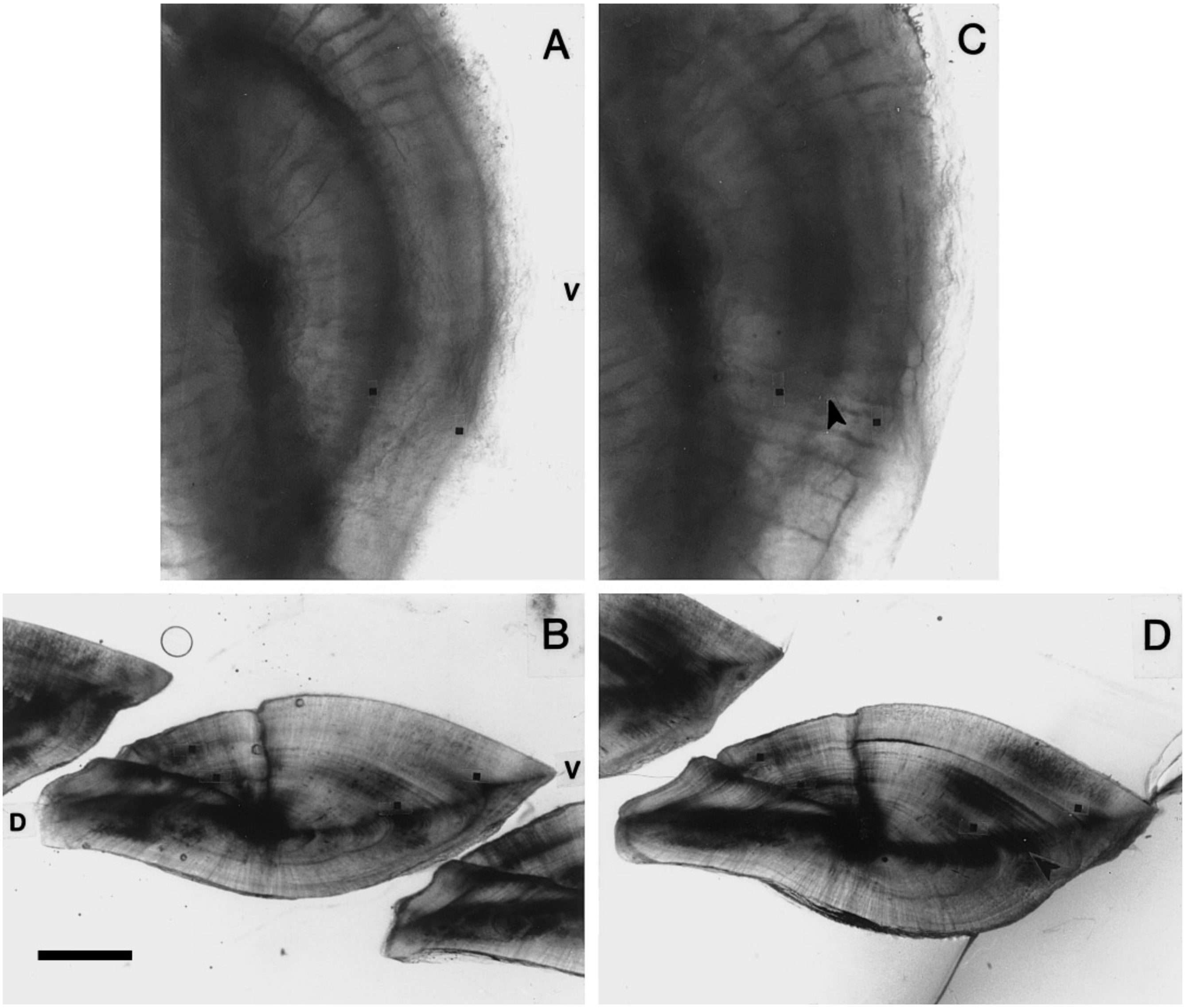

Fig. 3 *Sillaginodes punctata*. Comparison between whole and transverse sections of otoliths. **A** Whole otolith with two opaque zones; **B** transverse section of second sagitta from same fish as in **A** with two opaque zones towards proximal surface; **C** whole otolith from different fish with ambiguous annulus (*arrowhead*) and real opaque zones; **D** transverse section of otolith from same fish as in **C** with opaque zones and accessory area of pigment (*arrowhead*) (■ opaque zones; *lettering* as in Fig. 2)

opaque zone had formed through November and December (Fig. 9). All these results suggest that the first opaque zone is initiated late in spring in the fish's second year and is completed by mid-summer.

Growth of otoliths

Two measurements of otolith size were considered here: otolith weight and thickness of the transverse sections. Both were related by highly significant linear relationships to the number of opaque zones counted on the transverse section (Fig. 10). These relationships were: otolith weight $= (0.0323)$ no. of zones $+\ 0.1282$ ($r^2 = 0.8918, n = 55, p < 0.001$); otolith thickness $= (0.1429)$ no. of zones $+\ 1.1369$ ($r^2 = 0.8457, n = 55, p < 0.001$).

Development of ageing protocol

This study revealed that counts from whole otoliths could be misleading, and that some would need to be sectioned to obtain an accurate count of opaque zones. However, preparing transverse sections with a diamond saw is labour-intensive and limits the total sample that can be aged. An alternative method for such preparations is to break and burn the otoliths (Christensen 1964). Both sagittae were removed from 56 large fish from Site A4, and one was prepared by breaking and burning while the other was sectioned; the counts of opaque zones were then compared. For the two readers, $>80\%$ of the transverse sections and broken/burnt otoliths gave the same count, whilst the remainder differed by a count of only one (Table 3). There was $>80\%$ agreement between the two readers for both

Table 1 *Sillaginodes punctata.* Comparison of counts between whole and sectioned otoliths by Reader 1 (*Difference* difference between whole and sectioned counts; *Variation* percentage of fish for which counts varied by amount indicated; *Total* total number of fish in each difference category)

Opaque zones in TS	Difference					Variation between methods (%)		
	−2	−1	0	1	2	0	± 1	± 2
1	1	16	51	0	0	75	23.5	1.5
2	8	28	164	7	0	79.2	16.9	3.9
3	0	0	4	2	0	66.7	33.3	0
Total	9	44	219	9	0			

Table 2 *Sillaginodes punctata.* Comparison between Readers 1 and 2 for whole otoliths for fish collected from three sites (*Difference* difference between Reader 1 and Reader 2; other details as in legend to Table 1)

Site	Difference					Variation between methods (%)		
	−2	−1	0	1	2	0	± 1	± 2
A1	1	1	78	9	1	86.7	11.1	2.2
A2	0	1	83	9	6	83.8	10.1	6.1
A3	3	7	86	2	0	87.8	9.2	3.1
Total	4	9	247	20	7			

techniques (Table 4), indicating that both methods involved some inherent error and ambiguity. Nevertheless, the counts did not suggest that the transverse sections were more accurate or precise than breaking and burning. Therefore, the latter method constitutes a quick, accurate and precise way of preparing otoliths for more accurate examination.

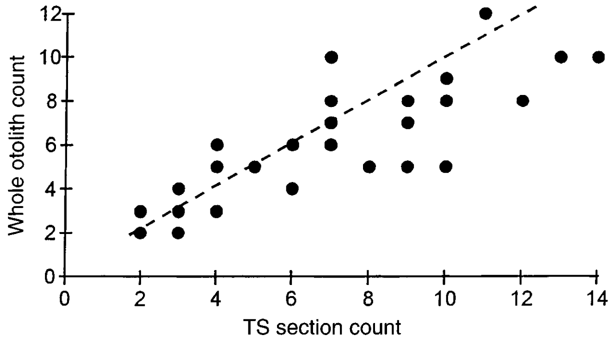

Fig. 5 *Sillaginodes punctata.* Comparison of counts of whole and transverse section (*TS*) of sagittae from large fish sampled from Site A4 (*dashed line* line of equal counts)

Discussion

Characteristics of sagittae

The sagittae of *Sillaginodes punctata* were examined in different ways – whole sagittae and transverse sections were illuminated by transmitted light, and broken/burnt sagittae by reflected light. Both techniques revealed that the internal structure of the otoliths consists of alternating opaque and translucent zones. The zonal structure revealed by both methods was comparable, but the transverse preparations were clearest (a result typical for such studies; Beamish 1992). Some small whole sagittae contained accessory areas of pigment which were similar in appearance to opaque zones, and which complicated interpretation. These were apparent in transverse sections between the otolith centre and the ventral margin, but they did not influence the count of opaque zones.

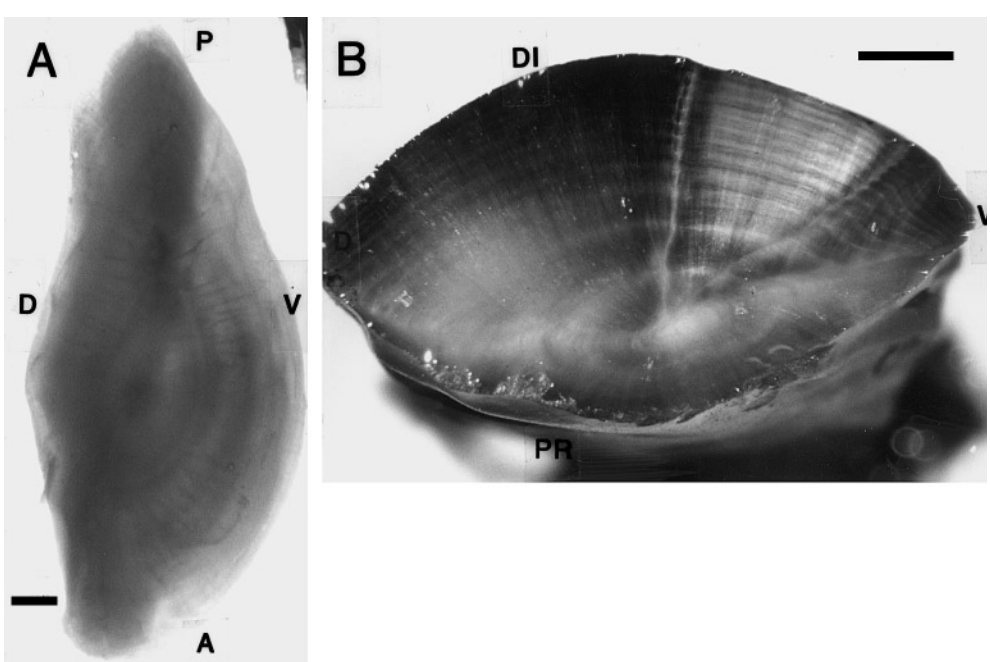

Fig. 4 *Sillaginodes punctata.* Comparison between whole and broken/burnt otolith from same individual. **A** Whole otolith from large fish; **B** broken/burnt otolith containing 12 opaque (*white*) zones (*Lettering* as in Fig. 2)

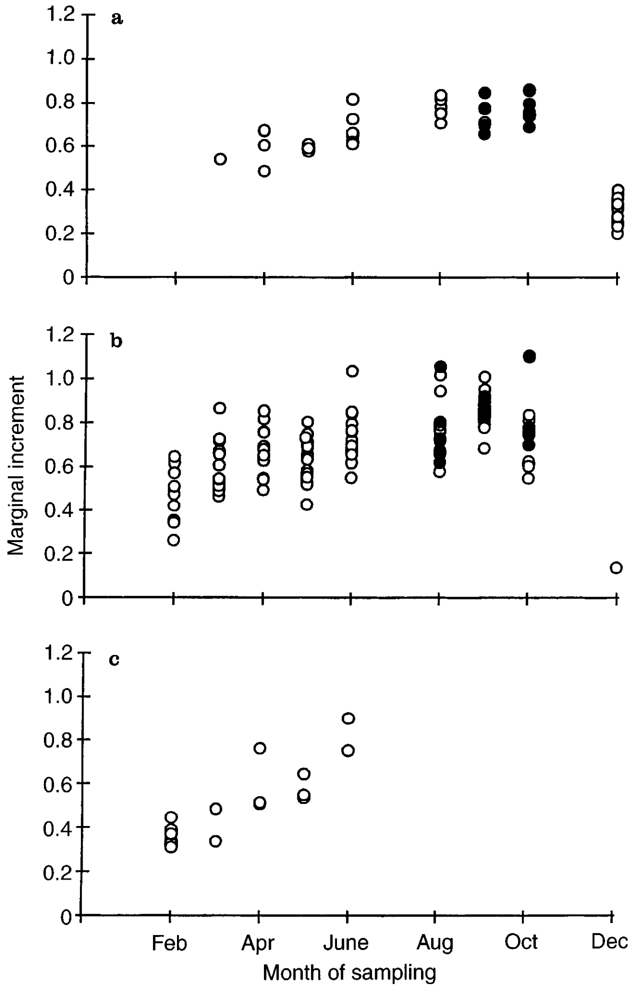

Fig. 6 *Sillaginodes punctata.* Marginal-increment analysis showing relative widths of marginal increment for fish sampled on nine occasions over 11 mo. **a** Formation of second opaque zone; **b** formation of third opaque zone; **c** otoliths with > 3 opaque zones (● otoliths with opaque margins; ○ otoliths with translucent margins)

Whole otoliths from all large individuals were very thick. This hindered the transmittance of light and restricted the view of the internal zonal structure. In ageing studies performed primarily before the 1980s, workers consistently used counts from such ambiguous, whole otoliths, failing to recognise that such counts were serious underestimates of the true age (Beamish 1992; Beamish and McFarlane 1995). In contrast to whole otoliths, the transverse sections and broken/burnt faces of large otoliths from King George whiting displayed clear zones which were relatively easy to count.

Although the otoliths from adults of many teleost species from different ecosystems display a zoned macrostructure, the nature of this optical pattern is not yet understood (Beckman and Wilson 1995; Fowler 1995). It must in some way be related to the process of daily microincrement formation, which varies seasonally and results in differences in: the widths of daily microincrements; the relative deposition rates of protein and $CaCO_3$; the deposition rates of trace elements; and the

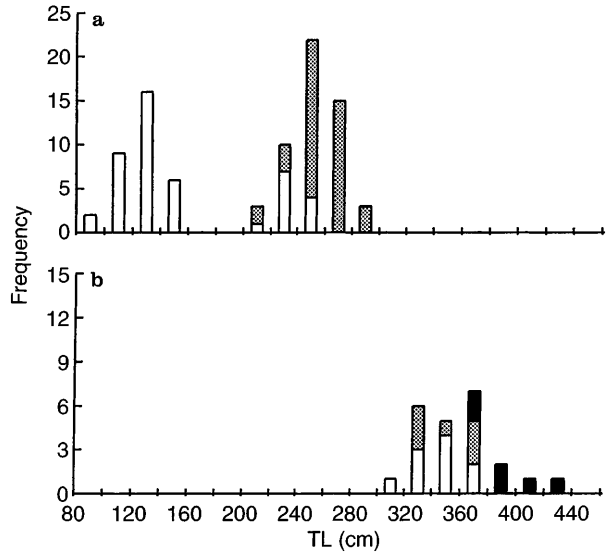

Fig. 7 *Sillaginodes punctata.* Size-frequency distributions of fish in tetracycline validation study. **a** size of fish at initial capture (*open bars* fish that died soon after capture; *shaded bars* survivors); **b** fish size at time of sampling (*open bars* fish sampled 20 March 1996; *shaded bars* fish sampled 8 November 1996; *black bars* remainder sampled 5 March 1997) (*TL* total length)

characteristics of the aragonite crystals that are part of the daily microincrements (reviewed by Fowler 1995). One, or a combination of these factors, must result in the integrated effect that is manifested as the zonal macrostructure.

Periodicity of opaque-zone formation

Two validation methods were used to assess this criterion, i.e. marginal increment analysis and tetracycline tagging.

The first method indicated that one sequence of opaque/translucent zones was formed per year. Each opaque zone was formed over a relatively short period through late winter/spring and completed by early summer, with the translucent material being deposited throughout the remainder of the year. An exception was the first opaque zone, which formed during late spring through into summer.

Tetracycline is usually used as a time-marker in otoliths of adult fish, in association with field-based tagging programmes (e.g. Beamish and Chilton 1982; Fowler 1990; Francis et al. 1992b; McFarlane and Beamish 1995). This was not possible in the present study, and a tank-based validation procedure was used as a compromise (cf. Ferrell et al. 1992; Ferreira and Russ 1994). Although this procedure confined fish in unnatural circumstances, they nevertheless still experienced natural water temperature and day-length regimes. Our analysis indicated that one opaque zone was formed during the spring, and thereby corroborated the results of the marginal increment analysis.

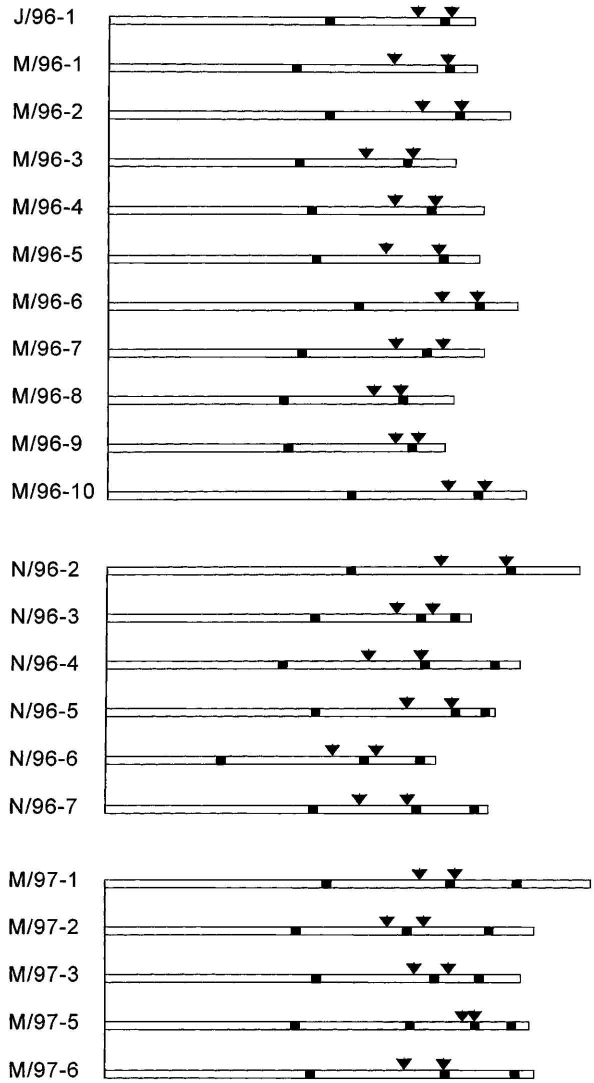

Fig. 8 *Sillaginodes punctata*. Photomicroscopy analysis of otoliths from 23 fish treated with tetracycline and maintained for different periods between 1 and 2 yr after treatment. Each horizontal bar represents relative radius of one otolith, measured between otolith centre (*left-hand axis*) and proximal surface (*dark segments* locations of opaque annuli; *arrows* tetracycline bands; *fish codes* month and year of death: *J/96* January 1996; *M/96* March 1996; *N/96* November 1996; *M/97* March 1997)

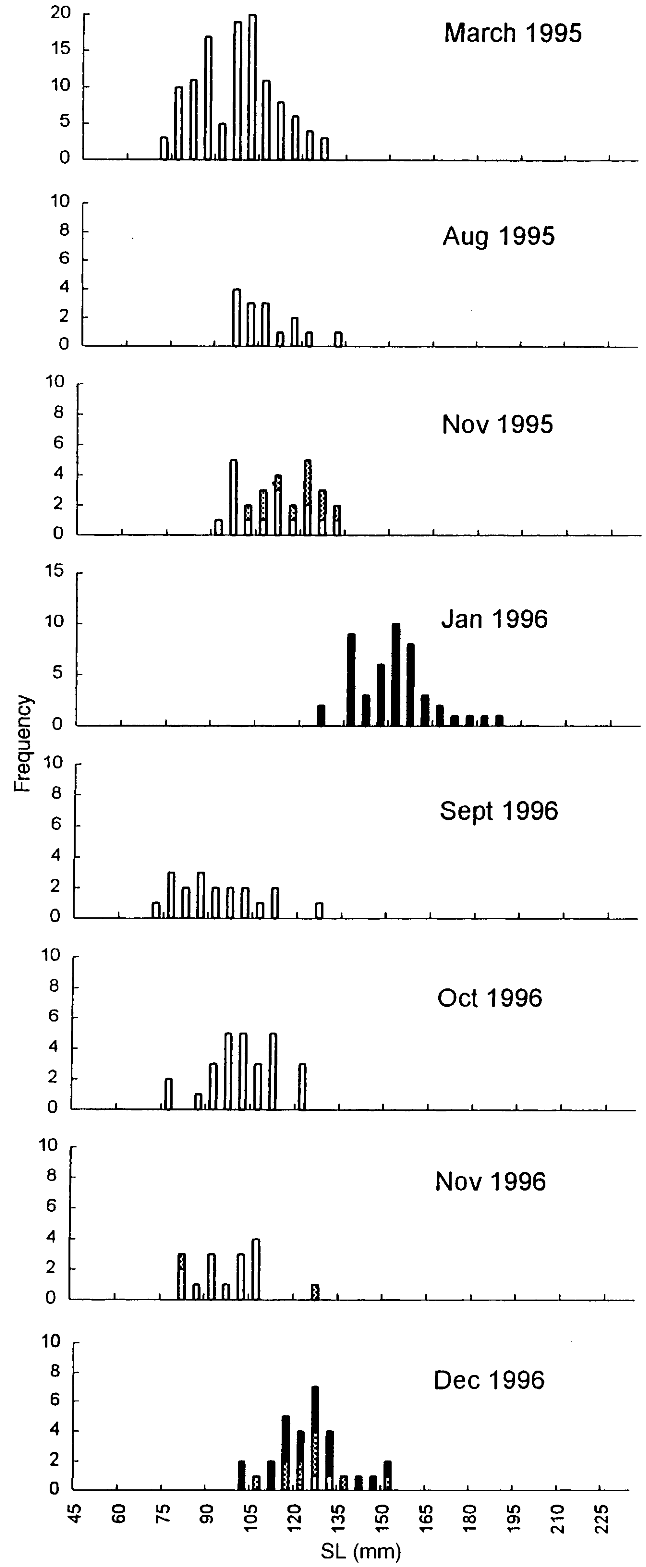

Fig. 9 *Sillaginodes punctata*. Formation of first increment in otoliths from fish ≥12 mo old (*Stippled bars* otoliths with an opaque edge; *open bars* otoliths with a translucent edge; *black bars* fish with complete opaque zone and translucent edge; *SL* standard length)

Despite the similar conclusion from the two independent techniques, validation of the periodicity of opaque-zone formation was only achieved for the first three opaque zones, although counts of up to 12 were obtained. This arose from our being initially unaware of the large-scale differences in distribution patterns of this species as a function of age, and that in order to capture old individuals we would have to target particular areas. Although, for the time being, those zones subsequent to the third must remain unvalidated, there is indirect evidence to suggest that they also form annually. Firstly, such opaque zones are identical in appearance to those formed earlier, suggesting that their underlying nature

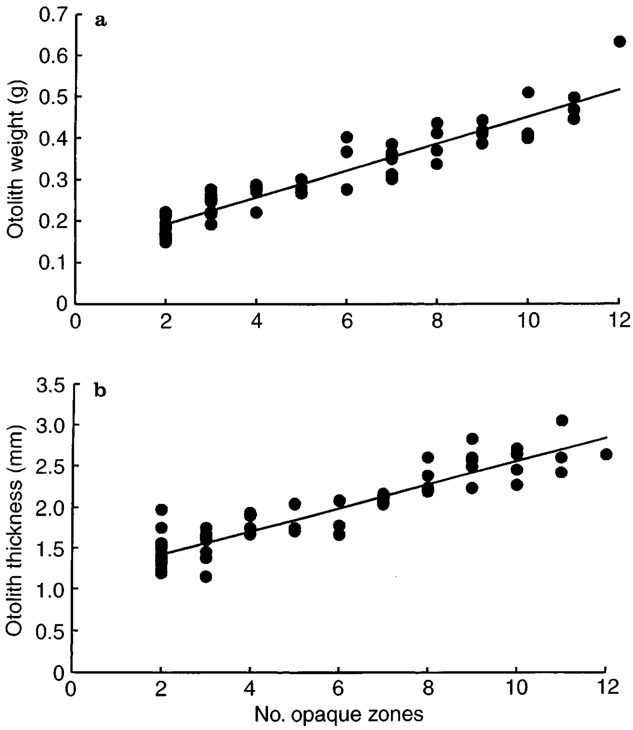

Fig. 10 *Sillaginodes punctata.* Relationships between otolith weight and thickness and number of opaque zones

Table 3 *Sillaginodes punctata.* Comparison of counts between transverse sections and broken/burnt otoliths for Readers 1 and 2 (*Difference* difference between counts from transverse sections and broken/burnt otoliths; *Variation* percentage of fish for which counts varied by amount indicated)

Reader	Difference					Variation between methods (%)		
	−2	−1	0	1	2	0	± 1	± 2
1	0	2	47	7	0	83.9	16.1	0
2	0	3	45	8	0	80.4	19.7	0

Table 4 *Sillaginodes punctata.* Comparison of counts between Readers 1 and 2 for transverse sections (*TS*) and broken/burnt (*B/B*) otoliths (*Difference* difference in counts between Reader 1 and Reader 2 for either preparation method; *Variation* percentage of fish for which counts varied by amounts indicated)

Method	Difference					Variation between methods (%)		
	−2	−1	0	1	2	0	± 1	± 2
TS	1	7	46	2	0	82.1	16.1	1.8
B/B	0	8	47	1	0	83.9	16.1	0

and causation are similar. Secondly, the rate of addition of new material to the otolith surface as a function of the number of opaque zones (subsequent to the second zone), did not deviate from linearity. Some deviation would be expected were the rate of formation to change over time.

The characteristics of the duration and timing of zone formation documented here for King George whiting are common to many taxa from various regions (Beckman and Wilson 1995; Fowler 1995). Since the period of opaque-zone deposition was relatively short, the otoliths had the glassy appearance common to many temperate and tropical fish (Beckman and Wilson 1995; Fowler 1995); this contrasts with the densely opaque otoliths from high latitudes, where the opaque zone is deposited over much of the year (Bagenal 1974; Beckman and Wilson 1995).

Some studies have indicated that the opaque zone is formed during the winter (Ferrell et al. 1992; Francis et al. 1992b; Ferreira and Russ 1994) and reflects a reduction in the width of daily increments (Francis et al. 1992a). However this model is not generally applicable, since the opaque zone is usually formed in spring, a period of accelerating somatic growth (reviews: Beckman and Wilson 1995; Fowler 1995). Furthermore, daily increments in opaque zones are generally wider and more consistent than increments from translucent zones (Victor and Brothers 1982; Brothers and Mathews 1987; Morales-Nin and Ralston 1990). Two alternative, but closely related physiological hypotheses relate otolith growth and zone deposition to seasonal environmental variation (Fowler 1995):

The first hypothesis suggests that otolith formation is linked with the overall physiology of the fish, and that the opaque zone is directly related either to the reproductive season or to a period of accelerating somatic growth. The second hypothesis suggests that otolith zone formation is an independent physiological process, directly responsive to environmental variation. In recent years, considerable evidence has accumulated in support of the latter hypothesis, demonstrating that otoliths grow in an incremental manner independent of somatic growth (Casselman 1990). This includes the non-isometric relationship between fish and otolith growth (Casselman 1990), their decoupling through experimental procedures (Mosegaard et al. 1988; Mugiya and Tanaka 1992), and a relationship between otolith growth and metabolic rate that is not necessarily manifested as somatic growth (Wright 1991).

Growth of otoliths

For the otoliths of the King George whiting to be useful for ageing, they must continue to grow through the lives of the fish at a rate that allows us to distinguish the zonal structure. To quantify this, it would be preferable to estimate the annual rate of otolith growth across a broad range of ages as has been done in similar studies (Fowler 1990; Fowler and Doherty 1992). However, since here validation was limited to the first three zones, we were restricted to relating otolith growth to the number of opaque zones. The two linear relationships between otolith size and number of zones suggest that there was no substantial diminution in the amount of otolith material added to the proximal surface of the otolith during the formation of each new complete opaque/translucent zone sequence. This is apparent from Fig. 4, which shows that all such sequences on the proximal surface of the older otoliths were of similar thickness. On average, each new opaque/translucent sequence added 32.3 mg weight and 0.143 mm thickness to the otolith surface.

Development of ageing protocol

Direct age-determination of fish from otoliths is a two-stage process: analysis of the otolith structure, and subsequent interpretation of this count.

For King George whiting, since many individuals were relatively young, age was accurately reflected by their whole otoliths. However, for some young fish and all the older ones it was necessary to expose the internal structure of the otoliths. Breaking and burning proved an accurate and efficient means of achieving this. Consequently, for this species, whole otoliths should be used as much as possible, and ambiguous counts quickly validated by breaking/burning.

The otolith count must be interpreted in reference to the time of otolith-zone formation and the life-history of the fish (Fig. 11). The first opaque zone did not form during the first year, but became apparent on the otolith margin in November/December of the second year, 16 to 20 mo after the time of spawning. New opaque zones were then formed between August and November of the following years. Based on this relationship between otolith growth and life-history (Fig. 11), we developed an algorithm to estimate the age of fish in months. A universal birth date of 1 May, i.e. the middle of the spawning season, was assigned. This algorithm is: $age_m = (N \times 12) + m_b + m_c$, where age_m = age in months, N = number of opaque zones, m_b = number of months from assigned birth date to end of year (i.e. May to December), and m_c = number of months from start of year to month of capture of the fish. The algorithm functions when the opaque zone that is completed around November or Decemeber is included in the count of opaque zones from the following January onwards. For example, for a fish caught in November with three opaque zones, the last of which has only recently formed, $N = 2$, $m_b = 8$, and $m_c = 11$, giving an estimate of age_m of 43 mo. Alternatively, for a fish caught in February with three opaque zones, $N = 3$, $m_b = 8$ and $m_c = 2$, giving an age_m of 46 mo. Such age estimates indicate the appropriate age-group and year-class for each fish considered (Williams and Bedford 1974).

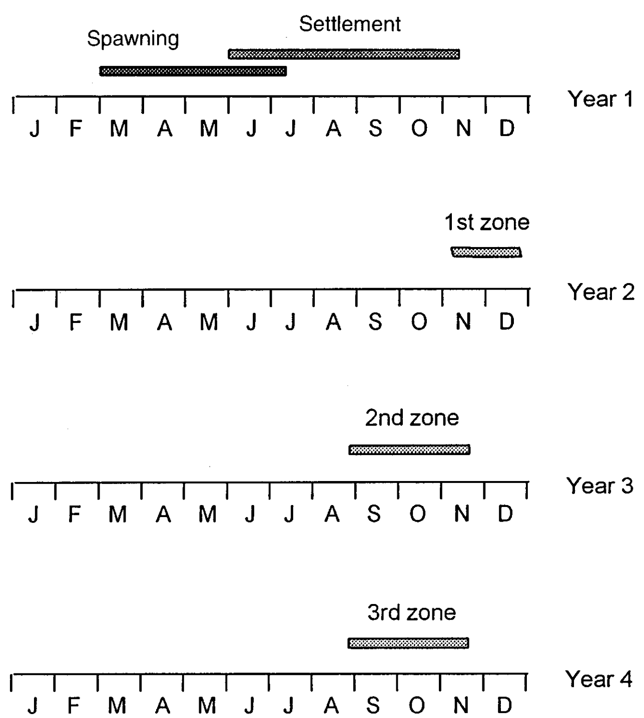

Fig. 11 *Sillaginodes punctata*. Model relating otolith zone-formation with life history and time of year

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