Influence of sectioning otoliths on marginal increment trends and age and growth estimates for the flathead *Platycephalus speculator*

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Abstract. – Comparisons are made between estimates of ages and growth of the flathead *Platycephalus speculator* Klunzinger, from a temperate Western Australian estuary, using data obtained from whole and sectioned otoliths. The consistent annual trends shown by the width of the opaque zone on the periphery (marginal increment) of sectioned otoliths, irrespective of the number of translucent zones, demonstrate that the translucent zones in these otoliths correspond to annuli. While the marginal increments on whole otoliths also showed a similar marked and consistent annual trend when a single translucent zone was present, they were far less conspicuous when two or more translucent zones were observed. The large sample size and strong trends shown by marginal increments on otoliths exhibiting one translucent zone accounts for the fact that, when data for all whole otoliths are pooled, the marginal increment still shows a consistent annual trend. Sectioning of otoliths enhances the ability to differentiate between the outer opaque and translucent zones, and also often reveals one or more additional inner translucent zones in older fish. The use of whole otoliths frequently underestimated age by one year in 2+ to 4+ fish and two years in 5+ to 10+ fish, and by as much as five or six years in the oldest fish (11+ and 12+). The respective 95% confidence limits for the parameters L_∞, K, and t_0 in the von Bertalanffy growth equations for males, calculated using data from sectioned otoliths, overlapped those calculated from data for whole otoliths, and the same was true for K with females. This similarity in growth curves in particularly the first four years of life can be attributed to the fact that approximately 74 and 65% of the growth of males and females, respectively, occurred in the first three years, when underestimates of age were limited.

Manuscript accepted 18 February 1992.
Fishery Bulletin, U.S. 90:276–284 (1992).

Since assessments of fish stocks often rely on information on age composition, it is crucial that any age estimates used for such assessments are validated (Beamish and McFarlane 1983, Casselman 1987). Validation that growth zones on hard structures, such as otoliths, scales, and spines, are formed annually is often implied by establishing that the pattern of growth on the periphery of these structures follows a consistent annual trend (e.g., Johnson 1983, Maceina et al. 1987, Potter et al. 1988, Beckman et al. 1989). In otoliths of fish in temperate waters, an opaque zone generally starts to form in the spring immediately outside the translucent zone laid down during the preceding winter. The width of this opaque zone usually increases between spring and autumn. Although a subsequent retardation of growth during winter results in formation of the translucent zone at the edge of the otolith, this translucent zone frequently cannot be readily detected until the following spring, when it becomes delineated by the formation of a new opaque zone. The distance outside the outer translucent zone constitutes the marginal increment. Therefore, if the outer opaque and translucent zones are formed annually, the marginal increment should decline only once during the year. Verification that trends shown by the marginal increments follow a pattern consistent with annual growth is an important method for establishing that the alternating translucent and opaque zones each correspond to annuli and are thus appropriate for use in ageing (Brothers 1983).

Many workers have presented data which showed that an annual trend was followed either by the marginal increment, when data for all otoliths in each sample were pooled, or by the overall incidence of otoliths possessing either translucent or opaque zones on their outer edge. When these have shown a consistent annual trend, it has often been concluded that all translucent zones correspond to annuli (e.g., Nel et al. 1985, Reis 1986, Rincon and Lobon-Cervia 1989, Crozier 1990, Hayse 1990). However, trends shown in such pooled data will be strongly influenced by those of the dominant groups, *vis a vis* the number of translucent zones, and may not be representative of all groups. Furthermore, trends shown by the marginal increment in some long-lived fish become clear only after the otoliths have either been sectioned or broken and burnt (Campana 1984, Collins et al. 1988). This accounts for estimates of age sometimes being lower when whole otoliths have been used than when either sectioned or broken and burnt otoliths were employed (Beamish 1979a, Campana 1984, Collins et al. 1988).

Although the Platycephalidae occurs along the coasts and within estuaries throughout the Indo-west Pacific region, the majority of the 41 species of flathead found in Australia are restricted to its southern waters (Sri-ramachandra-Murty 1975, Paxton and Hanley 1989). Despite wide distribution and, in some cases, the commercial and recreational importance of the Platycephalidae, estimates of the age and growth of representatives of this family are limited to those obtained for *Platycephalus bassensis*, *P. castelnaui*, and *P. speculator* by Brown (1977) and for *P. richardsoni* by Colefax (1934), Fairbridge (1951), and Montgomery (1985), the populations of which were all located in southeastern Australia. The most abundant species of flathead on the temperate southern coast of Western Australia is *P. speculator*, a species which has been shown to breed within Wilson Inlet, the largest estuary of this region (Hyndes et al. In press).

Previous attempts to age platycephalids have used whole sagittal otoliths (Colefax 1934, Fairbridge 1951, Brown 1977, Montgomery 1985). However, a preliminary investigation of the translucent zones in the sagittal otoliths of *P. speculator* from southwestern Australia showed that the outer opaque and translucent zones on the otoliths of larger fish often became clear only when the otoliths had been sectioned.

The present study was undertaken to determine the age structure and growth of *P. speculator* in Wilson Inlet, where this species is abundant and contributes to the local commercial and recreational estuarine fisheries (Lenanton and Potter 1987). Emphasis has been placed on elucidating the degree to which sectioning the otoliths influences marginal increment trends, age estimates, and growth equations. In addition, marginal increment data were pooled for both whole and sectioned otoliths to examine whether the resultant overall annual marginal increment trends were strongly influenced by that of a group(s) of otoliths with a particular number(s) of translucent zones.

Materials and methods

Sampling locality and regime

Wilson Inlet (117°25′E and 34°50′S) has a narrow entrance channel which opens into a wide basin (48 km²) supplied by two main tributary rivers. Water depth in the basin is generally less than 2 m. *Platycephalus speculator* was collected monthly from within the basin of Wilson Inlet between September 1987 and April 1989 using beach seines (mesh size in pocket 9.5 mm) during the day and gillnets (six stretched-mesh sizes, 38–102 mm), otter trawls (mesh size in pocket 25 mm) and plankton trawls at night (mesh size 1 mm).

Bottom water temperatures near the entrance channel of Wilson Inlet and 12 km further up the estuary near the top end of the basin were recorded at the time of sampling.

Age determination

Each fish was measured (total length) and weighed to the nearest 1 mm and 0.1 g, respectively. Sex was recorded when the gonad could be identified as either ovary or testis, which was usually possible in fish >100 mm in length. Both of the sagittal otoliths of 1305 juvenile and adult fish were cleaned, dried, and stored in gelatin capsules.

Whole otoliths were placed in methyl salicylate solution and examined microscopically under reflected light against a black background. The marginal increment, i.e., the distance between the outer edge of the outermost translucent zone and the periphery, was measured on one of the otoliths of each fish and expressed either as (1) a proportion of the distance between the focus and the outer edge of the translucent zone when only one translucent zone was present, or (2) as a proportion of the distance between the outer edges of the two outermost translucent zones when two or more translucent zones were present. Measurements were always made along the same axis, to the nearest 0.05 mm (Fig. 1). The number of translucent zones on each otolith was recorded.

These otoliths were later mounted and embedded in black epoxy resin (Bedford 1983, Augustine and Kenchington 1987) and cut into 1.5–2 mm transverse sections using the diamond saw described by Augustine and Kenchington (1987). Sections were mounted on glass slides and their surfaces ground on sequentially finer grades (400–1200) of carborundum paper. Sections were then coated with clear nail polish and examined microscopically under reflected light. Measurements of the marginal increment and counts of the number of translucent zones in these sectioned otoliths were carried out in precisely the same manner as described above for whole otoliths.

Mean marginal increment values were plotted separately for both whole and sectioned otoliths with 1–4 and ≥5 translucent zones to ascertain if they follow a consistent annual trend and thus permit the translucent zones to be considered as annuli. Width and

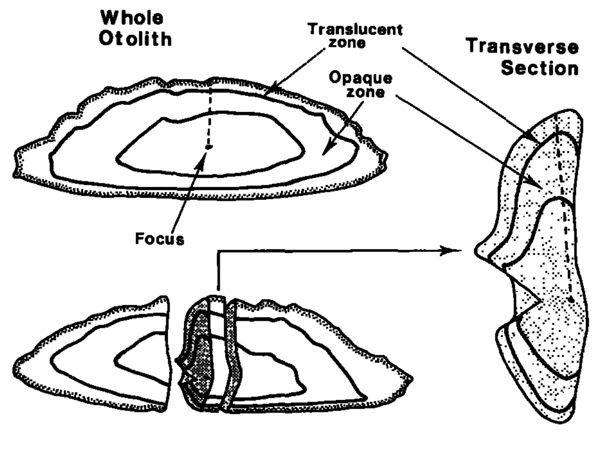


Figure 1
Schematic diagram showing the translucent and opaque zones in a whole and sectioned sagittal otolith of *Platycephalus speculator*. Broken line represents the axis along which measurements were taken.

thickness of the whole otoliths of 123 fish, covering the full range of sizes, were measured to the nearest 0.01 mm to examine the relationship between otolith width and thickness. The number of translucent zones in 140 otoliths, of which up to 20 otoliths came from each age-class (estimated from sectioned otoliths), were counted in whole and sectioned otoliths by a second 'reader' who had no previous experience in examining otoliths of this species. The reproducibility of age estimates for each method was determined by using the coefficient of variation (Sokal and Rohlf 1981, Chang 1982).

Von Bertalanffy growth curves were fitted to individual lengths of males and females at the estimated age-at-capture by a nonlinear technique (Gallucci and Quinn 1979) using a nonlinear subroutine in SPSS (SPSS 1988) and assuming a 'birth date' of 1 January. This date corresponds approximately to the midpoint of the period when, on the basis of gonadosomatic indices and trends shown by oocyte development, *P. speculator* exhibited peak spawning activity in Wilson Inlet (Hyndes et al. In press). The von Bertalanffy equation is $L_t = L_\infty\ [1 - e^{-K(t-t_0)}]$, where L_t is the length at age t (yr), L_∞ is the mean asymptotic length predicted by the equation, K is the growth coefficient, and t_0 is the hypothetical age at which fish would have zero length if growth followed that predicted by the equation. Comparisons have been made between the age estimates and von Bertalanffy growth curves, calculated from data obtained using whole and sectioned otoliths and assuming that, in both cases, the translucent zones correspond to annuli.

Results

Marginal increments

Annual trends in the mean marginal increments for whole and sectioned otoliths with one translucent zone were similar (Fig. 2). However, the sharp decline which occurred in the marginal increment after the winter (June–August) of 1988 was detected earlier in sectioned otoliths (October) than in whole otoliths (December). Although the data for 1987 were not as extensive, they still exhibited a similar marked decrease at the same time of year. In both years, the marginal increment on both whole and sectioned otoliths subsequently rose consistently through the summer, before leveling off in the late autumn and winter (Fig. 2).

Annual trends in mean marginal increments of sectioned otoliths with two, three, and four translucent zones parallel those in sectioned otoliths with one translucent zone, with marginal increments falling sharply in the spring (October) of both 1987 and 1988 (Fig. 2). Although the marginal increment on whole otoliths with two, three, and four translucent zones also declined in spring, the decrease was far less pronounced and the trends less consistent.

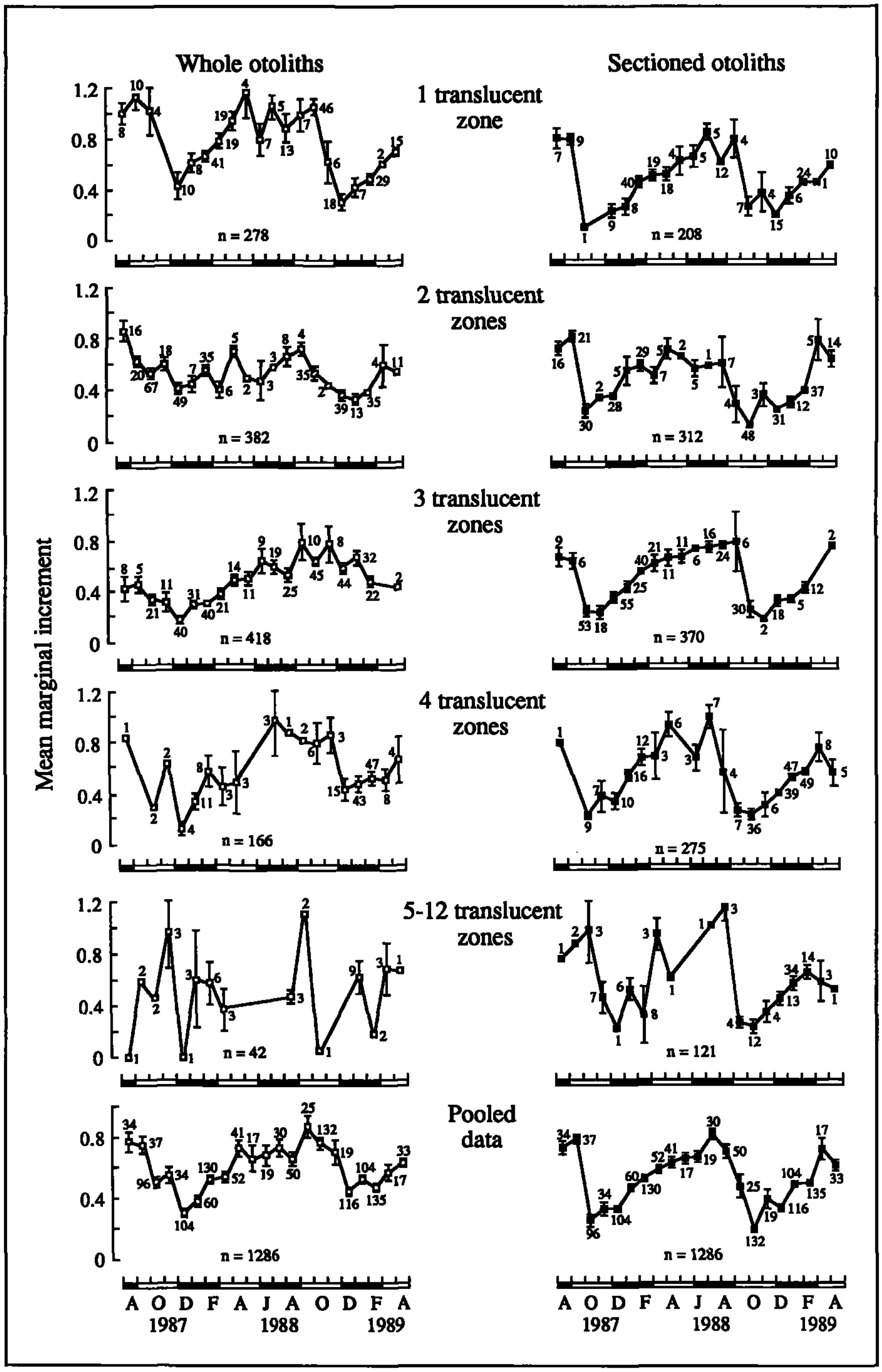
Since the number of otoliths with five or more translucent zones was small, values for the marginal increments on all such otoliths were pooled. Although seasonal trends shown by the marginal increment in sectioned otoliths with $\geqslant$5 translucent zones were slightly less consistent than in those with 1–4 such zones, they still followed a similar annual trend (Fig. 2). Furthermore, the translucent zones were still clearly visible and had the same appearance as those in otoliths with 1–4 translucent zones. No clear annual trend could be seen in the marginal increments of whole otoliths displaying $\geqslant$5 translucent zones (Fig. 2).

The above trends in marginal increments of sectioned otoliths (with a sharp decline only occurring at one time of the year, i.e., in the spring) show that the first four translucent zones on otoliths of *P. speculator* are laid down annually. Since the same trends were exhibited in pooled data for the fifth and subsequent translucent zones, these zones were presumably also, at least in most of these cases, laid down annually. We thus consider the translucent zone on sectioned otoliths as an annulus which can be used for ageing *P. speculator* from Wilson Inlet. The data also show that the outer opaque zone starts to form when water temperatures are rising from their winter (July) minima of about 11°C towards their summer (December–February) maxima of ~22°C (c.f. Figs. 2,3).

The annual trend shown by the mean marginal increment based on all sectioned otoliths, irrespective of the number of translucent zones, was essentially

Figure 2

Mean marginal increments ± SE for whole and sectioned sagittal otoliths of *Platycephalus speculator*. Note that the marginal increment is given as a relative value, i.e., as a percentage of the distance between the focus and the outer translucent zone when only one zone was present, or as a percentage of the distance between the outer edges of the two outermost translucent zones when two or more such zones were present. In this Figure and in Figs. 3 and 6, the black bars on the x-axis refer to winter (June–Aug.) and summer (Dec.–Feb.) and the open bars to spring (Sept.–Nov.) and autumn (March–May).



the same as that of otoliths with 1–4 translucent zones (Fig. 2). Mean marginal increments based on all whole otoliths followed similar annual trends, but they were not as pronounced or consistent, and the variation about the means was greater (Fig. 2).

Length-frequency data

Few fish < 180 mm in length were caught (Fig. 4), reflecting the fact that smaller individuals of this benthic species did not tend to be collected by seine and gillnets.

The three fish caught in the middle of spring (October) of 1987, which had otoliths with a single translucent zone bounded by a very narrow opaque zone, measured 91–106 mm in length (Fig. 4). This group is assumed to represent the 0+ age-class, i.e., the result of spawning which peaked in early January 1987 and can therefore be referred to as the 1987 year-class. The larger fish, which produced modal length-classes at 325–349 mm and 400–424 mm in October 1987 (Fig. 4), had otoliths with a narrow opaque zone bounding two and three translucent zones, respectively. The groups with two and three translucent zones therefore represent the 1+ and 2+ age-classes, or the 1986 and 1985 year-classes, respectively. Fish with otoliths exhibiting one, two, and three translucent zones in December 1987 and February 1988 are designated as representing the 1987, 1986, and 1985 year-classes, which is consistent with their length distributions (Fig. 4). The marked difference between the lengths of the 1987 year-class in October and December 1987 suggests that this year-class underwent remarkable growth between these months. However, the three fish caught in October were taken by beach seine in the shallows and are thus presumed to represent the lower end of the length range of this year-class, whereas those fish of the

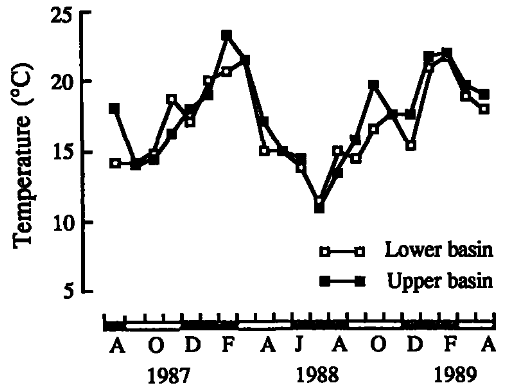


Figure 3
Bottom-water temperatures recorded at sites at lower and upper ends of the basin of Wilson Inlet, Western Australia, August 1987–April 1989.

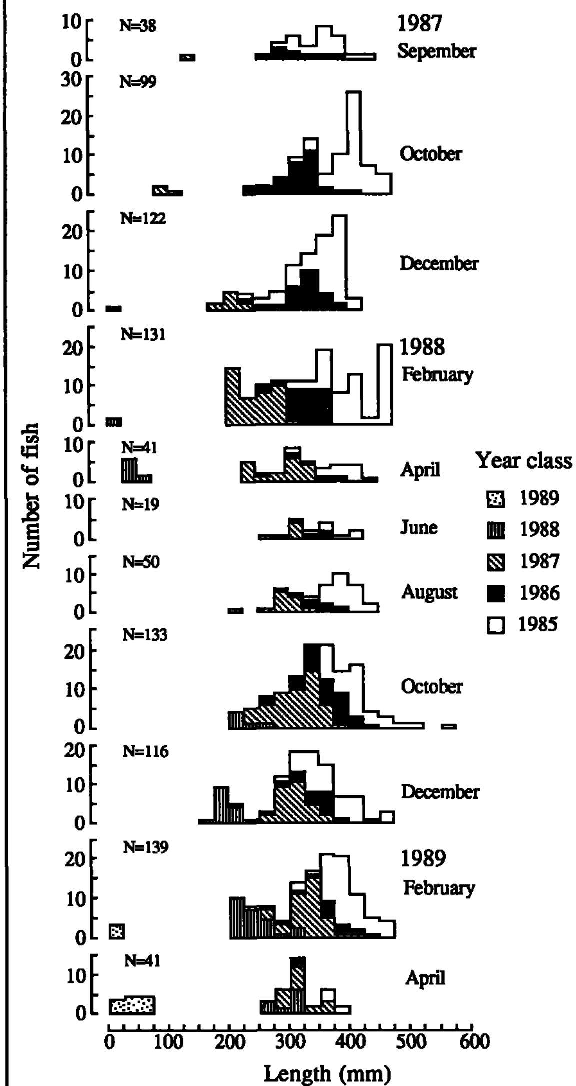


Figure 4
Length-frequency histograms for the 1985–89 year-classes of *Platycephalus speculator*.

corresponding cohort caught in December were taken in gillnets which, because of the mesh sizes of these nets, would have taken only the larger members of that year-class.

Larval *P. speculator*, ranging up to a length of 13 mm, were collected in plankton trawls in December 1987, February 1988, and February and April 1989 (Fig. 4). Otoliths of juveniles caught in April 1988 and 1989, and which from their lengths (23–89 mm) clearly corresponded to the 0+ age-class, did not have a translucent zone. The group of fish representing the 1988 year-class had reached 198–231 mm by December 1988, and 258–323 mm by April 1989. These length ranges are similar to those attained by the previous (1987) year-class in December 1987 and April 1988 (Fig. 4). The modal length of the 1+ age-class in October 1988 (= 1987 year-class) was identical to that of the 1+ age-class in October 1987 (= 1986 year-class). Six year-classes were usually found in samples from each month, and as many as eleven year-classes were present in February 1988 (older year-classes are not shown in Fig. 4). Maximum lengths for each sex were 696 mm for a 10+ female and 545 mm for a 12+ male.

Estimated ages and growth curves using whole and sectioned otoliths

All of the 197 otoliths which showed one translucent zone (= annulus) in sectioned otoliths also displayed a single zone prior to sectioning (Fig. 5). However, 44% of otoliths with two or more translucent zones after sectioning produced underestimates using whole otoliths. Between 23% and 57% of the otoliths with 2–4 annuli each showed one less translucent zone prior to sectioning and thus underestimated ages by 1 year. Discrepancies between the number of translucent zones in sectioned and whole otoliths were even more marked in otoliths with 5 or more translucent zones. Indeed, the numbers of annuli were as many as 5 or 6 less in whole otoliths than the 11 or 12 annuli observed in sectioned otoliths (Fig. 5). The proportion of underestimates using whole otoliths was greatest in spring for those otoliths which showed 2 or 3 translucent zones after sectioning (Fig. 6).

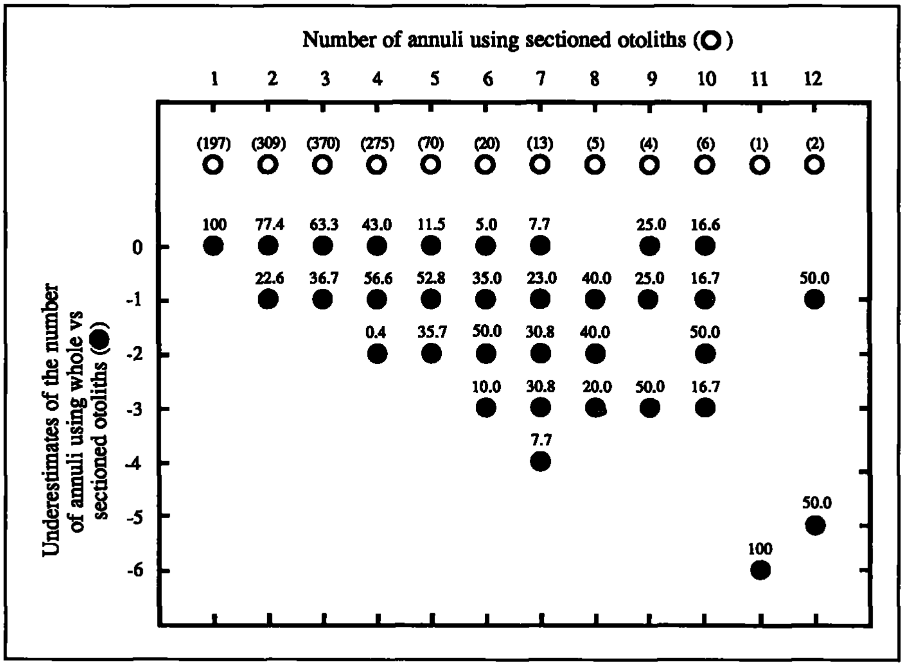


Figure 5
Number of otoliths with 1–12 annuli based on sectioned sagittal otoliths of *Platycephalus speculator*, and underestimates of the number of annuli observed on the same whole sagittal otoliths. Numbers in parentheses indicate the number of fish of different ages based on sectioned otoliths, while numbers above the closed circles indicate the percentage of underestimates using whole otoliths.

The coefficient of variation for replicate age estimates between readers was far less for sectioned otoliths (1.2%) than for the same otoliths prior to sectioning (8.7%). While the estimated age varied by only 1 year for each of the six sectioned otoliths for which there was disagreement, the estimated ages varied by as much as 3 years for the 53 whole otoliths for which there were discrepancies.

The relationship between width and thickness of sagittal otoliths of *P. speculator* is curvilinear, demonstrating that width does not increase proportionately with thickness (Fig. 7). The relationship between otolith width (W) and otolith thickness (T) is described by the following polynomial equation:

$$W = -0.283 + 4.635T - 1.175T^2 \quad (r^2\ 0.82,\ n\ 123).$$

The von Bertalanffy growth parameters for both male and female flathead were initially determined using individual lengths at estimated age (Table 1). Examination of the length-at-age plots showed that the curve for both sexes fell below the majority of the points for fish >5 years old, i.e., the asymptote was too pronounced to accommodate lengths of the older fish. Individual lengths of the fish were grouped into intervals of 0.1 years and the curves determined again by weighting the data by the inverse of the sample size for each age interval (Beckman et al. 1990). This procedure resulted in a better fit of the curve (Fig. 8). Although the values for t_0 for males and females were shifted slightly away from zero (namely from −0.134 to −0.332 and from −0.056 to −0.423, respectively), differences in the lengths of males and females at

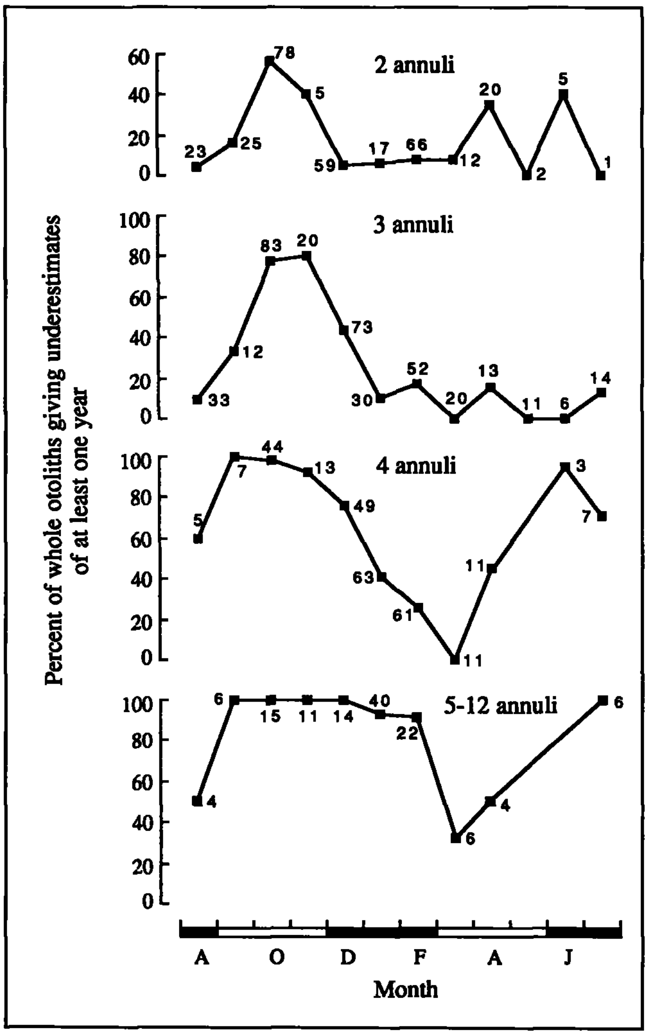


Figure 6
Seasonal incidence of underestimates by one or more annuli when using whole vs. sectioned sagittal otoliths of *Platycephalus speculator*.

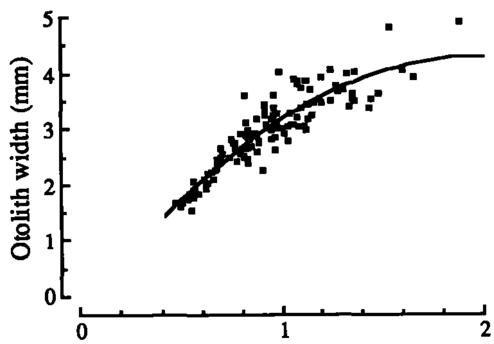


Figure 7
Relationship between width and thickness of the sagittal otoliths of *Platycephalus speculator*.

Table 1
Parameter estimates (95% confidence limits) for the von Bertalanffy growth model fitted to 630 male and 711 female *Platycephalus speculator*, determined from whole and sectioned otoliths using individual lengths-at-age and weighted lengths of each age-group in each month.

Sex	Parameter	Individual lengths Est.	Individual lengths Lower	Individual lengths Upper	Weighted lengths Est.	Weighted lengths Lower	Weighted lengths Upper
Sectioned otoliths							
Male	L_∞	429.2	419.9	438.5	477.4	468.6	486.2
	K	0.573	0.525	0.621	0.408	0.380	0.437
	t_0	-0.134	-0.201	-0.067	-0.332	-0.411	-0.253
Female	L_∞	481.8	469.4	494.2	601.0	588.2	619.8
	K	0.593	0.547	0.639	0.309	0.283	0.335
	t_0	-0.056	-0.109	-0.003	-0.423	-0.515	-0.331
Whole otoliths							
Male	L_∞	426.9	417.3	436.4	484.9	473.5	496.3
	K	0.700	0.641	0.760	0.466	0.429	0.502
	t_0	-0.068	-0.125	-0.012	-0.244	-0.292	-0.156
Female	L_∞	457.8	445.6	470.0	659.6	626.1	693.2
	K	0.767	0.694	0.840	0.264	0.231	0.296
	t_0	-0.088	-0.148	-0.028	-0.698	-0.837	-0.560

ages 1, 2, 3, and 4 were never altered by more than 22 mm and the change was generally less than 12 mm. The coefficient of determination (r^2) for the von Bertalanffy curve was 0.85 for both sexes using individual lengths-at-age, and 0.93 for males and 0.89 for females using the weighted procedure.

The respective parameters L_∞, K, and t_0 in the von Bertalanffy growth equations for males determined using weighted data obtained from sectioned otoliths overlapped those when the same approach was employed for whole otoliths, and the same was true for K with females (Table 1, Fig. 8). The von Bertalanffy growth-equation parameters using weighted data from sectioned otoliths show that female *P. speculator* grow towards a larger asymptotic size (L_∞) than males (Table 1, Fig. 8). Individual lengths of *P. speculator* in December–February at the end of their first and second years of life were 190–310 mm and 210–370 mm, respectively, for males, and 210–300 mm and 250–400 mm, respectively, for females (Fig. 8).

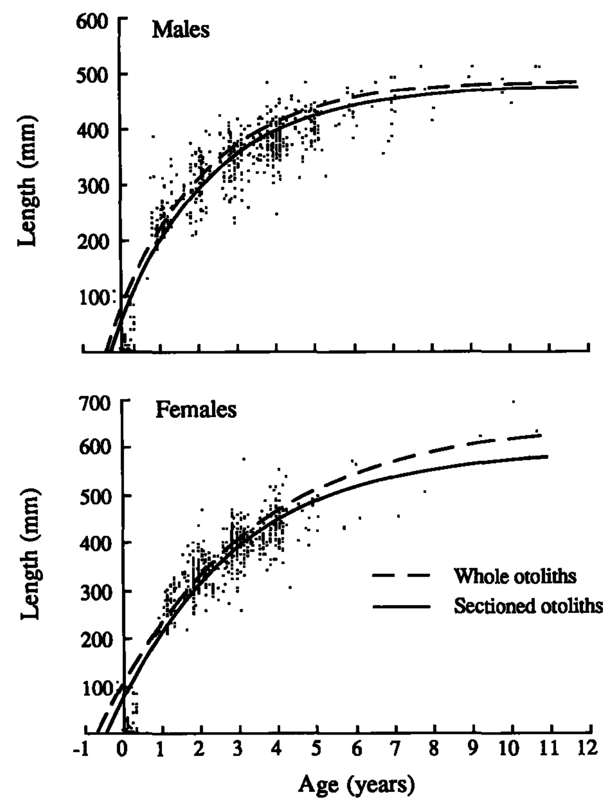


Figure 8
Von Bertalanffy growth curves fitted to length-at-age data, weighted by the inverse of the sample size of each 0.1 year age-group, using whole and sectioned otoliths of male and female *Platycephalus speculator*.

Discussion

Marginal increments

Marginal increments of the otoliths of *P. speculator* with two or more translucent zones exhibited conspicuous trends only after the otoliths were sectioned. This is largely due to the fact that sectioning of otoliths results in more accurate measurement of their peripheral and/or penultimate opaque zones, because one or both of the two outermost translucent zones have become more clearly delineated. This is similar to the situation with starry flounder *Platychthys stellatus*, in which annual trends in the marginal increments of otoliths with four or more annuli were observed only after otoliths had been broken and burnt (Campana 1984). Likewise, in the case of king mackerel *Scomberomorus cavalla*, the percentage of otoliths with an opaque zone on their edge exhibited an annual trend only after the otoliths were sectioned (Collins et al. 1988).

The consistency in annual trends of marginal increments among sectioned otoliths of *P. speculator*, despite differing numbers of translucent zones, accounts for the clear annual trend in marginal increments when data for all otoliths in each of the monthly samples were pooled. The contrast between the conspicuous trend shown in pooled data for whole otoliths, and the relatively poor trend exhibited in whole otoliths with two or more translucent zones, shows how trends can be unduly influenced by those of a relatively large sample size of otoliths exhibiting a particularly strong annual trend, such as was present with those otoliths having one translucent zone. For validation of the use of translucent zones as annuli, it is thus important to establish that the trends shown by the marginal increments on otoliths with differing numbers of translucent zones each follow a consistent annual trend (Johnson 1983, Maceina et al. 1987, Potter et al. 1988, Beckman et al. 1989).

Age and growth estimates

Our results demonstrate that, while age estimates between sectioned and whole otoliths corresponded when one translucent zone was present, ages of older fish were underestimated by 2–4 years or as much as 5 or 6 years using whole otoliths. Increased resolution of the growth zones after sectioning is reflected in the far lower variability between age estimates made by two independent readers using sectioned otoliths.

Although otoliths with two or three translucent zones frequently yielded counts of one less zone prior to sectioning, many underestimates occurred with the otoliths taken from fish between mid-spring and early summer (October–December). In other words, they were collected during the period when sectioning enabled the new opaque zone to be detected approximately 2 months earlier than was possible with whole otoliths.

Our inability to detect all of the translucent zones in whole otoliths can in part be attributed to the growth pattern of the otolith. Whereas the first translucent zone can be easily detected in whole otoliths, the disproportionate increase in otolith thickness relative to its width results in the translucent zones becoming increasingly more closely apposed and therefore difficult to distinguish from one another. This parallels the situation recorded by Beamish (1979a,b) for Pacific hake *Merluccius productus*, and for several species of rockfish (*Sebastes*), and also by Campana (1984) for starry flounder *Platychthys stellatus*.

Despite the fact that a large proportion of ages were underestimated using whole otoliths, the von Bertalanffy growth curves derived from data using whole otoliths, particularly of males, did not differ markedly from those obtained using sectioned otoliths. This can be attributed to the fact that approximately 74 and 65% of growth for males and females, respectively, occurred in the first 3 years of life when underestimates of age were limited.

Implications for management

The vast majority of male *P. speculator* reach sexual maturity at the end of their first year of life (Hyndes et al. In press).Since males have attained only 190–310 mm by this time (Fig. 8), they will only occasionally have reached 300mm, the minimum legal length for capture of this species. However, the majority of females do not first attain sexual maturity until they are 2 years old (Hyndes et al. In press), by which time they have reached 250–400mm. Thus, the females of *P. speculator* can be exploited before they have had the opportunity to spawn.

In summary, this study has demonstrated that, in the case of the flathead *P. speculator*, it is crucial to section its otoliths in order to obtain an accurate estimate of age. Sectioning reduces the problems of distinguishing between peripheral translucent zones which, due to the growth pattern of the otolith, become increasingly more closely apposed with increasing size. While the results presented in this paper refer only to *P. speculator*, they parallel in some respects those obtained for *Platychthys stellatus* and *Scomberomorus cavalla* (Campana 1984, Collins et al. 1988). Such age underestimates have obvious implications in estimating mortalities for use in fisheries management. Our results also demonstrate the importance of plotting marginal increments for otoliths with different numbers of translucent zones, to establish that such zones are laid down annually on the otoliths of fish representing each presumed age-group. Since the females of *P. speculator* are being caught before they have spawned for the first time, there is a case for increasing the minimum legal size for capture.

Acknowledgments

We thank F. Baronie, D. Gaughan, P. Geijsel, P. Humphries, L. Laurenson, and F. Neira for their assistance with sampling. The sectioning saw was kindly provided by S. Blaber of the CSIRO Marine Laboratories at Cleveland, Queensland. Helpful discussions were provided by N. Hall, L. Laurenson and L. Pen. L. Pen also estimated the number of translucent zones in otoliths for the precision estimates. Gratitude is expressed to J. Casselman, R. Fletcher, M. Moran, N. Hall, two anonymous referees, and the scientific editor for constructive criticism of our work and this paper. Financial support was provided by the Western Australian Department of Fisheries.

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