Comparative Precision and Bias of Five Different Ageing Methods for the Large Tropical Snapper *Lutjanus johnii*

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Abstract

Five common methods of age determination were compared to determine their usefulness in monitoring the catch structure of a small Australian sportfishery for *Lutjanus johnii*. Presumed annuli were visible on scales, whole otoliths, sectioned otoliths, whole vertebrae and sectioned vertebrae of *Lutjanus johnii* in a sample of 44 younger fish up to 793 mm FL and 12+ years of age. An inexperienced reader produced estimates of age with acceptable levels of precision after three replicate counts using all these methods with the exception of scale reading. The most precise methods used whole otoliths, sectioned vertebrae and sectioned otoliths. Linear comparisons showed promise for calibration of results from whole and sectioned otoliths, and sectioned otoliths and whole and sectioned vertebrae, for fish less than half the known longevity of the species. However, examination of the relative position of presumed annuli and tetracycline marks showed bias and underestimation of fish age in the interpretation of the margins of vertebral centra. Comparison of the scale and otolith readings amongst experienced and inexperienced readers revealed systematic bias in the interpretation of the position of presumed annuli for fish less than 3+. It was concluded that sectioned otoliths provide the best method to determine the age of *Lutjanus johnii*

Introduction

A wide variety of hard body parts have been used in studying the age and growth of lutjanids, including scales (Druzhinin 1970), vertebral

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centra (Ju et al. 1988), urohyal bones (Davis and West 1992) and whole otoliths (McPherson and Squire 1992). Recently, protocols have been developed to count annuli in thin sections of otoliths as precise and accurate estimators of lutjanid ages (Newman et al. 1996). Discrepancies have generally been reported amongst ageing methods for a variety of fish species (Beamish and McFarlane 1987), yet there have been few attempts at comparing methods for lutjanids (Newman et al., in press; Rocha-Olivares 1998).

With the exception of a minor commercial line fishery near Darwin, the spotted-scale sea perch *Lutjanus johnii* is exploited in northern Australia only by small recreational fisheries. Sportfishing interest in the species is expanding with both the development of charter operations in remote locations and growth in ownership of electronic fish-finding devices. The species is also cultured in Singapore, Malaysia and Australia (Williams and Russ 1994). Current knowledge requirements in Australia concern longevity, growth rates, age at first maturity and age composition of catches of this species.

Our objective was to compare five ageing methods to infer their relative precision and bias in monitoring the age structure of the catch of *L. johnii* in the inshore sportfishery of northeastern Australia. Precision was defined as the closeness of repeated age estimates and was measured by an inexperienced reader, unfamiliar with previous ageing of *L. johnii*, counting presumed annuli visible on otolith sections, whole otoliths, scales, whole vertebrae and sectioned vertebrae. Systematic bias can cause very precise estimates to be inaccurate and can occur because of differences in interpretation amongst readers and changes in the timing and true periodicity of presumed annuli on particular structures. Results for scales and otoliths were compared amongst readers to determine the effects of experience and bias in interpretation. Linear comparisons of best age estimates from each technique were made to determine the potential for calibration of techniques and the direction and magnitude of relative bias amongst techniques. The positions of presumed annuli relative to tetracycline (OTC) marks were measured for two young fish to help qualify this bias.

Materials and Methods

Filleted fish frames and skins from fillets were collected from sportfishing charter operators, anglers and spearfishers in mangrove estuaries of Cape York (10°48' 142°33' to 15°10' 145°12'; n = 13, 290 - 470 mm Fork length (FL)) and granite headlands of Cape Cleveland (19°10' 147° 05'; n = 31, 235 – 793 mm FL) in Queensland, northeastern Australia. Frames from two fish marked with OTC were collected near Cape Cleveland after completion of a validation study (Cappo et al., in press). The history of one fish (6021601/04 FL = 476 mm), at liberty between 324 and 712 days after marking, could not be accurately determined while the other (6021601/05 FL = 327 mm) was recaptured after 326 days.

Preparations for ageing

Fork length (FL mm) of the fish frames was measured. Scales, vertebrae and otoliths were removed. If possible, scales were selected from the skins in the vicinity of the pectoral fin base, washed and dried. Scales with interpretable ring patterns (Fig. 1f) and unbroken radial rays joined at the focus were chosen for estimating "scale age" using a microfiche reader at 10X magnification. A pilot study of the first 24 anterior vertebrae from five fish (442 - 697 mm FL), following the procedure and definitions of Ju et al. (1988), showed that vertebra number five had the largest mean diameter of the centrum and a moderate standard error (12.68 ± 0.46 mm). To obtain this vertebra for reading, a section of the vertebral column (numbers four to six) was boiled and the flesh was removed.

Whole and sectioned vertebrae were then prepared and read according to the procedure of Lai and Liu (1979) who presumed annuli to comprise continuous "translucent" zones, continuous or broken growth ridges, or growth ridges bordering continuous "translucent" zones on the centrum. Growth rings visible as ridges on the posterior centrum of vertebra five (Fig. 1c) were counted under reflected light at 50X to estimate "whole vertebra age". These vertebrae were sectioned in the longitudinal plane through the core. "Sectioned vertebra age" was estimated by counting only darker, denser increments on the section surface that were also visibly associated with the growth ridges recognised on the concave face of the centrum (Fig. 1d,e). These corresponded to the "translucent" zones shown in Lai and Liu (1979). Sagittae (hereafter referred to as the otoliths) were removed from under the gill covers, washed and dried. Whole, right-side otoliths were submersed in water and the concave proximal face from the nucleus to the posterior margin was examined to count opaque zones as an estimate of "whole otolith age" (Fig. 1a). Thin transverse sections of these otoliths were prepared according to the procedure of Newman et al. (1996) and read along the sulcal walls and ventral axis to count opaque zones as an estimate of "sectioned otolith age" (Figs 1b, 2a,b). Whole and sectioned otoliths were examined under a dissecting microscope at 50X on a black background with reflected light. The widths and positions of presumed annuli and OTC marks along axes from the centre to the edge of all five structures from two fish were measured using an eyepiece micrometer under both white and ultra-violet reflected light.

Data analyses

Samples were randomised among the three replicate readings or "counts" separated by several weeks by inexperienced Reader 1 (senior author) to compare the Coefficient of Variation (CV) and Average Percent Error (APE) indices of precision amongst methods (Campana et al. 1995). The non-parametric Median test (Steel and Torrie 1960; Choi 1978) was chosen to compare the CV index amongst methods, because the data were not normally distributed and contained values with an artificial limit of zero. Age

bias plots were constructed to identify trends and sources of bias in discrepancies between age estimates among successive readings (Campana et al. 1995). To make direct comparisons of the five methods for each fish, a set of "best" age estimates was obtained by Reader 1. If count 2 and count 3 were equivalent, then count 3 was accepted as a best age estimate. If they were
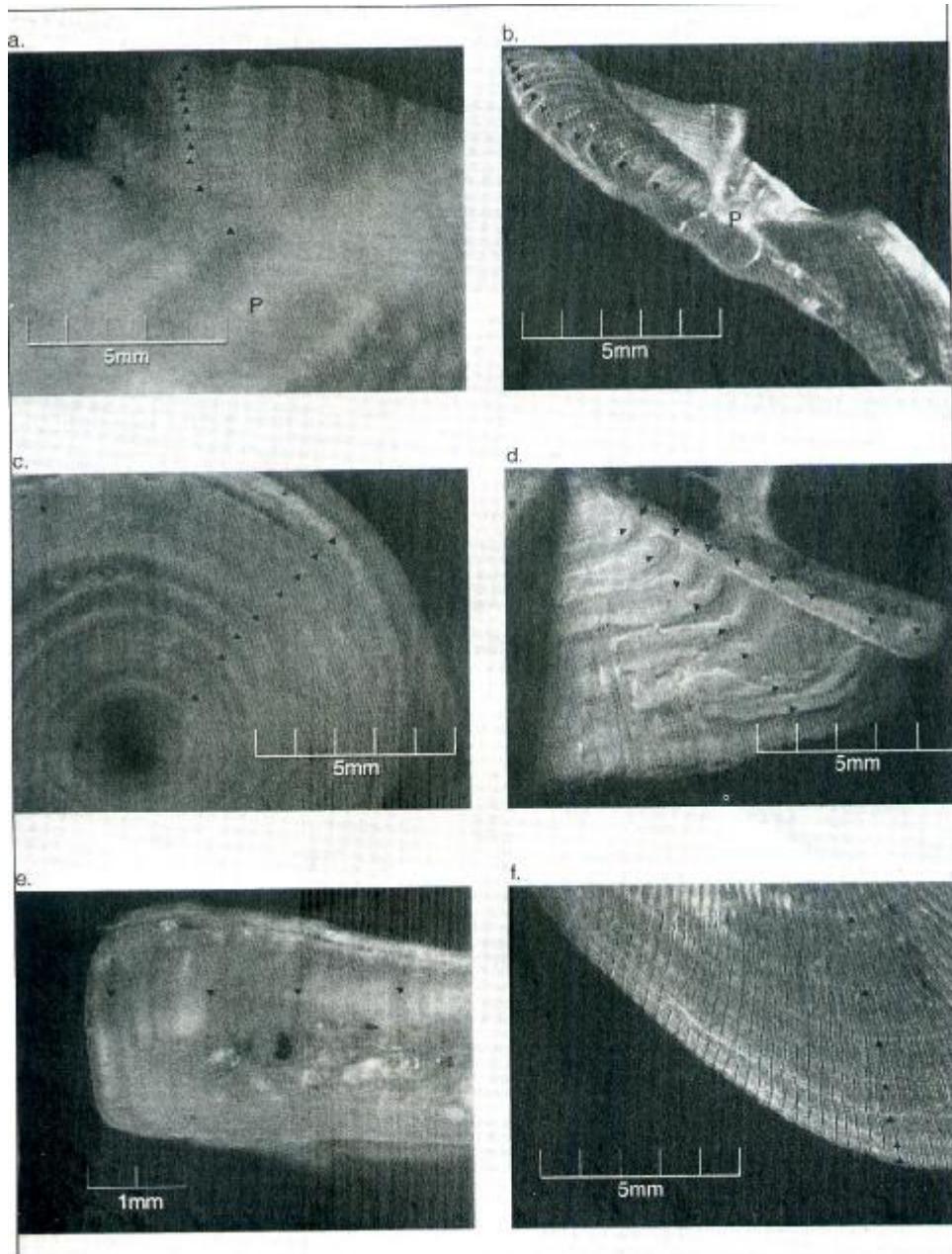

Fig. 1. Photomicrographs showing presumed annuli on the a) whole otolith, b) sectioned otolith, c) whole vertebra, d),e) sectioned vertebra and f) scale of a *Lutjanus johnii* of 793 mm FL estimated to be 12+ years old. P marks the otolith primordium.

not equivalent, further independent counts were made until successive counts produced the same age estimate. If agreement was not achieved after more than six successive counts the sample was excluded from the analysis. Best age estimates from each method were compared using linear regression models. The least squares estimates for b and *a* from these models were tested for significant departure from a "no difference" or "Y = X" model at 5% significance level using Bhattacharyya and Johnson (1977). Experienced Reader 2 (second author) provided two readings of scales, whole otoliths, and otolith sections. To make inferences about discrepancies amongst readers, count 2 age estimates of Reader 2 were compared with count 3 age estimates of Reader 1 using age bias plots.

Results

Concentric marks that were interpreted as annuli were visible using all five methods. Presumed annuli on scales were usually identified as narrow, more or less continuous bands of different optical density (Fig. 1f) that comprised circuli which cut over each other in the lateral field of the scale. Whole otoliths showed an opaque nucleus with one or two broad, diffuse bands of translucent and opaque material, followed by narrow, equidistant translucent and opaque zones in younger fish and then a series of sharp, densely opaque lines or ridges at the ventral edge (Fig. 1a). The sulcal and ventral axes of otolith sections showed a densely opaque nucleus with an alternating pattern of translucent and opaque zones (Fig. 1b). These zones were approximately equal in width along the sulcal axis (Fig. 2a), but along the ventral axis translucent zones were wider and opaque zones were more distinct (Fig. 2b). Presumed annuli on vertebral centra appeared as ridges which changed from a regular, continuous series associated with continuous darker, denser zones in the inner centrum to a more diffuse, broken pattern on the margins (Fig. 1c,d). Sectioning of vertebrae revealed darker zones at the margins of the centra and allowed additional annuli to be distinguished within the discontinuous series of outer growth ridges on the centrum face. However, only those darker growth checks along the plane of the section
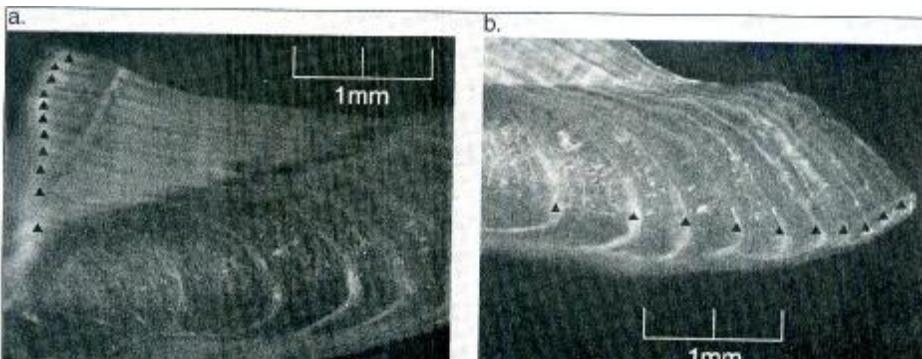

Fig. 2. Photomicrographs showing presumed annuli on the a) sulcal axis and b) ventral axis of the sectioned otolith of a *Lutjanus johnii* of 793 mm FL estimated to be 12+ years old.

that were visibly associated with semi-continuous ridges on the centrum face could be counted under our definition of presumed annuli (Fig. 1d, e).

The most precise age estimates were obtained by Reader 1 from whole otoliths, sectioned vertebrae and sectioned otoliths, with no significant differences detected in precision amongst these methods. Least precision was attained using whole vertebrae and scales to estimate age (Table 1). The age bias plots comparing successive counts showed learning by Reader 1 in increasing estimates of age for older fish using sectioned otoliths (Fig. 3e, f) and an overall bias for higher estimates and less precision at all ages using scales (Fig. 3a, b). There were no clear trends in bias or precision for whole otoliths (Fig. 3c, d) and sectioned vertebrae (Fig 3i, j), but precision improved from count 2 to count 3 using whole vertebrae (Fig. 3g, h).

Comparisons of the best age estimates from the five methods are shown in the linear regressions in Table 2. There were weak relationships between scale reading and the other four methods, with less than 67% of the variation explained by the linear comparisons. We therefore chose a limit of $r^2 \geq 0.70$ for tests for significant departures of the intercept from zero and the slope from one. The most informative comparisons showed that sections of otoliths produced higher estimates of age for older fish than both whole otoliths and whole vertebrae (Fig. 4a, b), and that sectioning vertebrae significantly increased age estimates by about one year for all age classes (Fig. 4c).
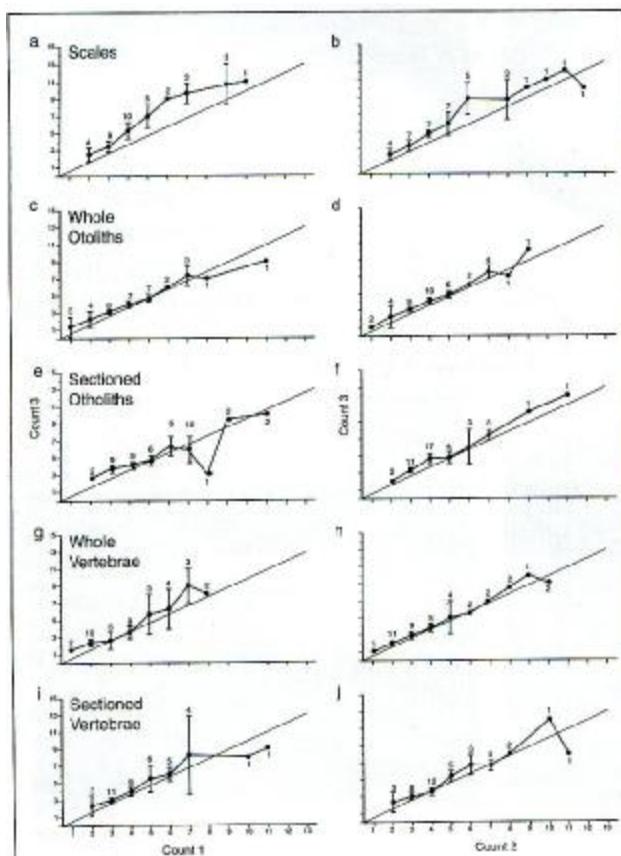

Fig. 3. Age bias plots comparing age estimates from first and third and second and third counts by Reader 1 for scales (SC), whole otoliths (WO), sectioned otoliths (SO), whole vertebrae (WV) and sectioned vertebrae (SV).

Table 1. Mean indices of precision : Coefficient of variation (CV) and average percentage error (APE), and results of median test for significant difference of distribution of CV amongst ageing methods using whole otoliths (WO), sectioned vertebrae (SV), sectioned otoliths (SO), whole vertebrae (WV) and scales (SC). _____ no significant difference at p = 0.05.

	Most Precise				Least Precise
	WO	SV	SO	WV	SC
Mean CV	8.0 ± 1.55	9.5 ± 1.50	12.7 ± 1.97	16.0 ± 1.97	16.5 ± 1.54
Mean APE	6.2 ± 1.19	6.9 ± 1.07	9.5 ± 1.48	12.0 ± 1.34	12.1 ± 1.11
Median Test					

Table 2. Intercept a, slope β, and coefficients of determination r^2 of the linear regression between ageing methods and Reader 1 (R1) and Reader 2 (R2) for n fish. Where $r^2 \geq 0.70$ tests for H_0: a = 0 and H_0: β = 1 were made and significant results (*** p<0.05) are shown.

Y	X	a	β	n	r^2
Scales	Whole Otoliths	0.56	1.09	26	0.66
Scales	Sectioned Otoliths	-0.06	1.06	28	0.60
Whole Vertebrae	Scales	1.18	0.45	22	0.48
Sectioned Vertebrae	Scales	1.37	0.61	25	0.67
Whole Otoliths	Sectioned Otoliths	0.44	0.76***	36	0.79
Whole Vertebrae	Whole Otoliths	0.46	0.79	26	0.70
Sectioned Vertebrae	Whole Otoliths	0.73	0.94	30	0.74
Whole Vertebrae	Sectioned Otoliths	-0.29	0.83***	31	0.87
Sectioned Vertebrae	Sectioned Otoliths	0.08	0.95	35	0.87
Sectioned Vertebrae	Whole Vertebrae	0.56***	1.09	31	0.90
Sectioned Otoliths (R1)	Sectioned Otoliths (R2)	2.25***	0.80***	30	0.88
Scales (R1)	Scales (R2)	1.385	1.23	28	0.64
Whole Otoliths (R1)	Whole Otoliths (R2)	1.96	0.81	26	0.56

Inexperienced Reader 1 obtained consistently higher age estimates than Reader 2 using otolith sections, whole otoliths and scales (Fig. 5a, b, c). These differences were consistent amongst age classes for scale reading, but also showed evidence of some severe bias for fish estimated by Reader 2 to be 2+ or less in age. This may have inflated the average indices of precision (mean CV) amongst readers for the otolith section, whole otolith and scale reading methods, which were two to three times as high (Fig. 5a, b, c) as those achieved amongst replicate counts by Reader 1 (Table 1).

The margins of calcified body parts marked with OTC showed important differences in number, location and width of increments recognised using the five different ageing methods (Fig. 6). Most importantly, there was one less increment visible than expected outside the OTC mark on whole and sectioned vertebrae of both fish. In the case of the fish known to have passed one winter/spring period at liberty (6021601/05) there were no increments visible outside the OTC mark on vertebrae, but the other structures showed increments as expected for an annual periodicity of deposition. The other fish without an identifiable tag (6021601/04) had two increments outside the OTC mark on otolith preparations and scales that were consistent with the upper limit of possible time at liberty.

Discussion

Comparisons of age estimates from sectioned otoliths, whole otoliths and scales have generally shown discrepancies that increase with age and length of fish – often with a marked divergence at a threshold age, where annuli counted on otolith sections were not visible on the other structures. This divergence was as high as a factor of two or three for a range of species (Ferreira and Russ 1994; Lowerre-Barbieri et al. 1994; Secor et al. 1995; Rocha-Olivares 1998). Our results for *L. johnii* showed a similar trend without the marked divergence in age estimates for very old fish, which were lacking from the study. We believe the largest *L. johnii* in our sample (793 mm FL and age <=12+) was just below the threshold length at which
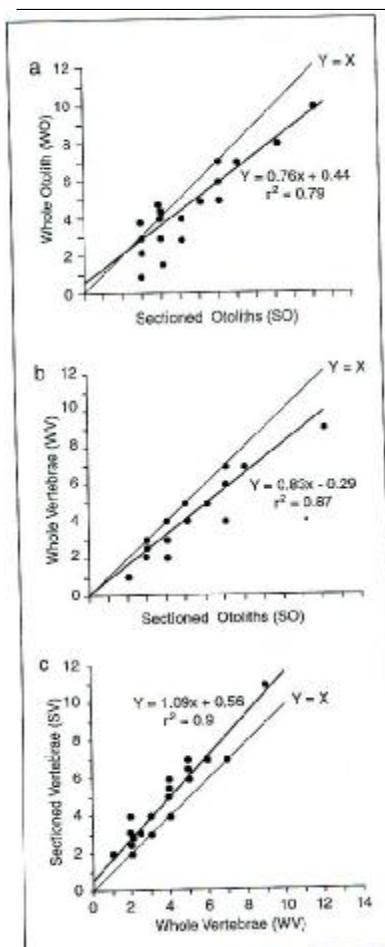

Fig. 4. Linear comparisons of best estimates of age by Reader 1 from Table 2 where significant differences were found in slope or intercept from a Y=X relationship.
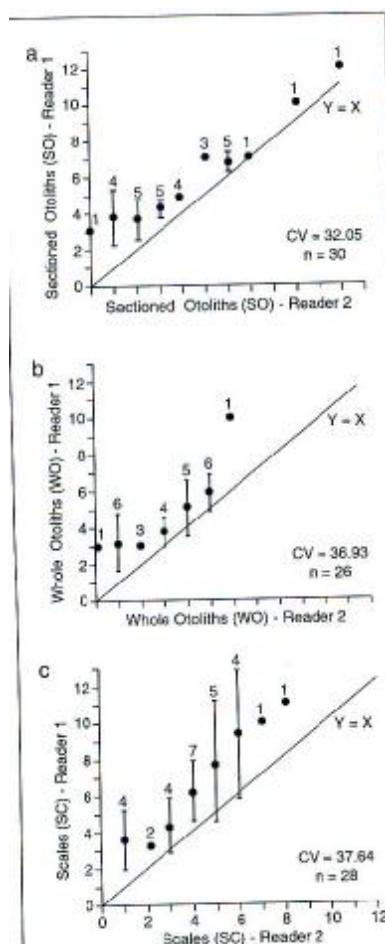

Fig 5. Age bias plots comparing estimates by Reader 2 and Reader 1 for sectioned otoliths, whole otoliths and scales, showing an index of precision (mean CV) and sample size *n*. Error bars are 95% Confidence Intervals for the mean, shown only when $n > 2$. Lines through the origin represent Y=X.

marked divergence amongst methods could be expected. Age estimates of other, larger *L. johnii* from sectioned otoliths were 24+ for an 800 mm fish, 25+ for an 810 mm fish and 28+ for an 850 mm fish from Cape Cleveland (Cappo, unpubl. data).

Sectioning of *L. johnii* otoliths and vertebrae enhanced the ability to differentiate opaque zones in otoliths and interpret growth checks in vertebrae, and produced estimates of age higher than those obtained from whole vertebrae and otoliths. This occurred for otoliths because deposition of material occurs mainly on the interior proximal surface, along the plane of the sulcus, after a certain age - and growth increments there are best discerned on sections (Hyndes et al. 1992). Growth checks crowded at the margins of vertebrae are revealed by sectioning. Sectioning also increased precision (CV) by nearly a factor of two for vertebrae, but not for otoliths. The lack of improvement in precision using sectioned otoliths probably resulted from a learning process in the interpretation of sections by the unfamiliar reader. This learning was detected as a trend toward higher estimates of age from scales and lower estimates from sectioned otoliths between successive counts. Scale reading results were the least precise and could not be consistently related to any other method, preventing calibration of our results with a maximum age of only 9 years (913 mm FL) proposed for *L. johnii* by Druzhinin (1970) from scale reading.

Our results were similar to the comparisons made for *Lutjanus peru* by Rocha-Olivares (1998), for which sectioned otoliths were the most precise
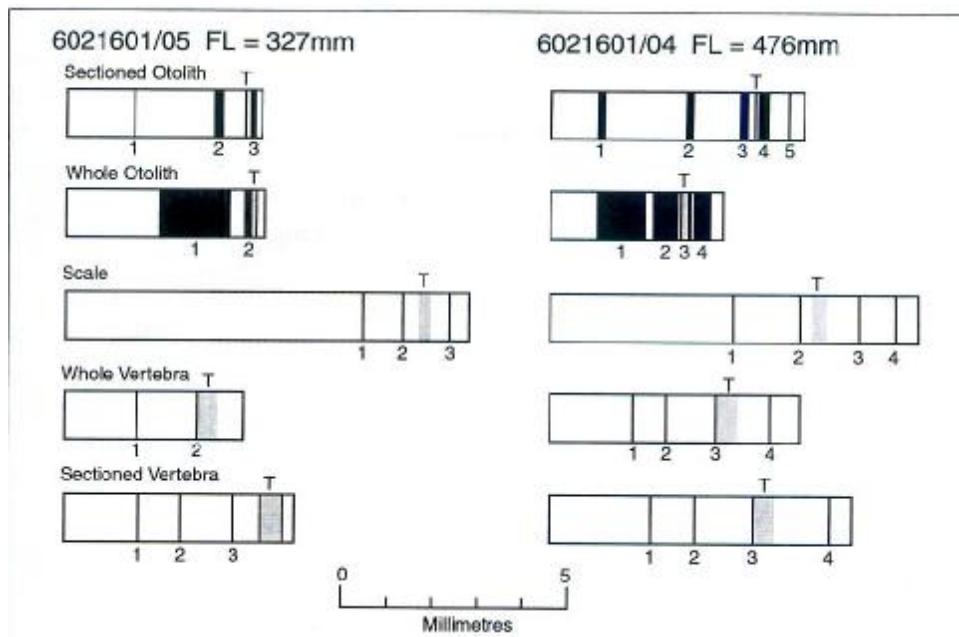

Fig. 6. Relative positions of tetracycline marks and presumed annuli on scales, otoliths and vertebrae of two fish marked and recaptured after 326 days (6021601/05) and 324 - 712 days at liberty (6021601/04). Where possible, widths of OTC marks (light shading) and presumed annuli (dark shading) are shown, otherwise the measurements are to the outermost edge of the OTC marks or presumed annuli.

and scales the least precise structures for age determination. The average percentage error for the most and least precise method differed by a factor of two for both *L. peru* and *L. johnii*, but the magnitude of the errors was much different. The errors for *L. johnii* were about 1.5 times (whole otoliths), three times (scales) and four times (sectioned otoliths) greater than those reported by Rocha-Olivares (1998) for the same methods, probably due to the small sample sizes in our study and learning by the unfamiliar reader.

The variation between the unfamiliar and experienced readers was much greater than that measured amongst replicate counts by the unfamiliar reader, indicating the presence of bias in determining the age of *L. johnii*. This was probably due partly to differences between readers in the interpretation of the position of the indistinct first annulus on otoliths and scales, but there may have been an unexpected effect in the area of collection. Half of the sample of fish aged 2+ by the experienced reader were collected from locations 4º - 8º north of the others, and regional differences in the ease of interpretation of otolith macrostructure have been reported in tropical species separated by only four degrees of latitude (Fowler 1995).

The lack of an expected increment between an OTC mark and the edge of whole and sectioned vertebrae showed inherent inaccuracy in the use of vertebrae to age *L. johnii*, despite the precise nature of estimates obtained using sectioned vertebrae. This inaccuracy was probably due to both a lag time in the appearance of an identifiable growth check and to errors in interpretation of the rings at the margins of the centra. For example, Francis et al. (1992) showed that the annulus on sections of *Pagrus auratus* otoliths was formed in spring, but was only first visible among older fish in late summer. There may be similar or greater time lags in the interpretation of presumed annuli on the outer margins of the vertebral centra. This produced a bias for age under-estimation of at least one year among the younger *L. johnii*. This may be greater among the older fish.

Direct validation of an annual periodicity of opaque zone formation on sections of *L. johnii* otoliths has been demonstrated using OTC mark-recapture (Cappo et al., in press). We conclude that sectioned otoliths can provide the most accurate and precise ageing method for *L. johnii* to produce growth curves and estimates of natural mortality, provided the position and timing of formation of the first annulus and periodic calibration amongst readers is well-defined. However, it is also desirable to develop reliable, cost-effective ageing methods for the routine monitoring of age structures of heavily fished populations, which are often truncated to a few younger year classes. This may require the use of quicker techniques, such as whole otolith and scale readings, especially in island states lacking laboratory infrastructure, or in catch-and-release sportfisheries where the removal of scales and measurement before release offers the only chance of estimating age. Landings of *L. johnii* in the Australian sportfishery produced very limited opportunity for sampling in our study because they are widespread, often very far from population centres and fish of various sizes are retained or released depending on the prevailing ethics of anglers.

Accurate application of a combination of ageing techniques might be possible for long lived tropical species given a knowledge of the inherent errors, the threshold age and length at which they become unreliable and a robust relationship for calibration throughout all age classes. For example, Rocha-Olivares (1998) demonstrated that scale reading was reliable until *L. peru* reached 500 mm LCF or an age of 5+, and whole otoliths could be used for fish up to 16+ and 800 mm LCF, but for fish beyond this age to 32+ the otoliths must be sectioned to observe annuli. Secor et al. (1995) also found that scale ages were, on the average, 9 years less than the ages estimated from sectioned otoliths among fish older than 20 years, but concluded that scales could be used to estimate the age *of Morone saxatilis* adequately up to an age of 12 years.

We found poor relationships between scale readings and any other method, with all coefficients of determination less than 67%, but if this relationship could be improved a calibration curve between estimates from scale reading and sectioned otoliths could be constructed for younger *L. johnii*. Scales collected by anglers from such fish may then provide age estimates in catch-and-release sportfisheries. The development of a scale-based method would require an extension of our study to all older *L. johnii* age classes and the determination of the best sites from which to remove scales, as well as direct validation of the timing and periodicity of presumed annuli.

Acknowledgments

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