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An assessment of otoliths, dorsal spines and scales to age the long-finned gurnard, *Lepidotrigla argus*, Ogilby, 1910 (Family: Triglidae)

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Summary

Sagittal otoliths, dorsal spines and scales were critically assessed as structures to potentially determine the age of the long-finned gurnard, *Lepidotrigla argus*. Counts were made of opaque growth increments and a readability score was assigned to each structure. Comparisons of growth increment counts were made between structures and between readings. All three structures showed some degree of readability and quantifiable growth increments, but this varied within fishes and between structures. Initial results showed that whole otoliths were more suitable to determine age estimates than dorsal spines and scales. Scales were considered unsuitable due to between reading ageing bias, variation in age estimates between structures, low precision and poor readability for this species. Dorsal spines showed evidence of loss of growth increments due to hollowing of the vascular core, which resulted in underestimation of older individuals in comparison to whole otoliths. Further analysis showed that growth increment counts from whole otoliths were lower for older individuals in comparison to sectioned otoliths. It is suggested that this is because of decreased clarity of growth increments towards the outer margin of whole otoliths in older individuals; this problem was not present with sectioned otoliths. It was concluded that sectioned otoliths were a more suitable structure from which to estimate age of *L. argus* than were whole otoliths, dorsal spines and/or scales.

Introduction

Age-based information plays an important role in determining the population dynamics and life history characteristics of an organism (Winemiller and Rose, 1992; Worthington et al., 1995; Campana, 2001). In particular, age determination assists in quantifying the rates of key processes such as growth, recruitment and mortality, which are essential for assessing the status of exploited populations, such as commercially and recreationally caught fish (Lai et al., 1996; Myers et al., 1997; Campana, 2001; Berkeley et al., 2004; Lewin et al., 2006). Age-based information can therefore support the sustainable management of such organisms (Mace, 1994; Jennings et al., 1999; Reeves, 2003; Berkeley et al., 2004).

An important step in any ageing study is to firstly establish a suitable method for age determination. The use of calcified structures to determine the age of fish is a well-accepted method (Lai et al., 1987; Campana, 2001). This involves counting periodic growth increments (alternating opaque and translucent zones) that are formed by accretion as the fish grows (Morales-Nin, 1992). Sagittal otoliths are the most widely used structure for ageing fish as they have been found to provide the most accurate and precise estimates of age over the largest age and size ranges (Secor et al., 1995; Campana, 2001). Other bony structures such as vertebrae, dorsal spines and scales have also successfully been used to age fish (Lai et al., 1987; Metcalf and Swearer, 2005; Rifflart et al., 2006). However, all methods of ageing are problematic. For example, small otolith size and/or the clarity of growth increment structures can preclude their use (Ihde and Chittenden, 2002; Isermann et al., 2003; Phelps et al., 2007). Otolith growth and clarity has been shown to be variable within species, between locations and at different life history stages (Fowler and Short, 1996; Caldow and Wellington, 2003; Fischer et al., 2004; Jessop et al., 2004). When assessing the potential of a structure for age determination, it is important to take into account the numbers aged, as well as the efficiency and cost effectiveness of the steps involved (Worthington et al., 1995).

In this study we examine the utility of otoliths, dorsal spines and scales to potentially age the long finned gurnard, *Lepidotrigla argus* (Family: Triglidae). This species forms an important by-catch component of the Ocean Prawn Trawl Fisheries in eastern Australia (NSW D.P.I., 2004). Currently, there exists little life history information for this species, thus hindering the assessment of the potential impacts of fishing. Several studies have assessed age in triglids, but mostly for the larger growing genus Chelidonichthys (Booth, 1997). Like other members of the genus Lepidotrigla, *L. argus* are of small size and possesses relatively small otoliths which can make age determination difficult. The specific aims of this study were to: (i) examine the presence and utility of growth increments in otoliths, dorsal spine and scales, and (ii) compare the amount of variation in the counts and readability of growth increments between structures. This information is used to identify the most suitable structure for ageing *L. argus* for future demographic studies.

Materials and methods

Collection of samples

Samples of *Lepidotrigla argus* were collected monthly between April 2006 and April 2007. Fish were collected aboard a chartered commercial prawn trawl vessel at locations ranging from 20 to 100 m depths offshore from the Clarence River, NSW (Lat. 29.424°S; Long. 150.373°E).

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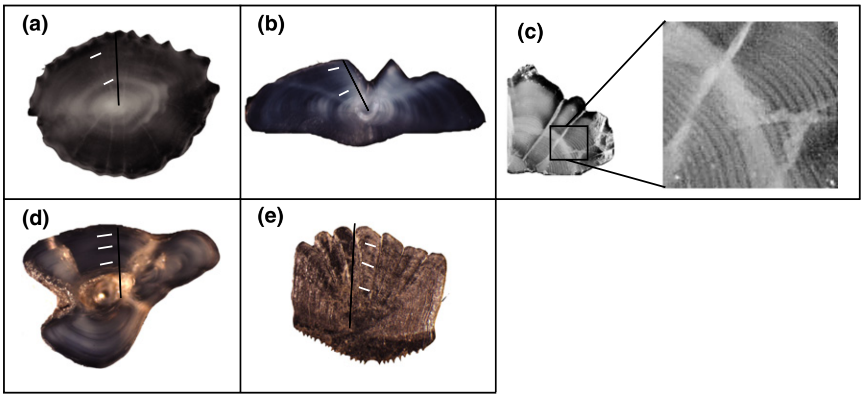

Fig. 1. Structures used for age estimates. Black line = axis along which counts and measurements were made. White marks = growth increments. (a) Whole oil immersed otolith displaying two growth increments. (b) Cross-sectioned otolith displaying two growth increments. (c) Polished otolith showing presence of possible daily growth increments. (d) Sectioned dorsal spine displaying three growth increments. (e) Ctenoid scale displaying three growth increments.

Two experiments were conducted to assess the utility of different structures for ageing. Firstly, samples of whole sagittal otoliths, dorsal spines and scales were examined from fish over a short time period (3 months) to determine a suitable structure from which to estimate age. A second experiment was conducted to examine variations between ages estimated from whole and sectioned otoliths collected across the entire sampling period (1 year). This second experiment was conducted after the first experiment due to the advances in otolith processing, which resulted in the ability to obtain visibly clearer sections from much smaller otoliths than was previously possible. Therefore, sectioned otoliths were not included in the initial experiment.

Experiment 1: Assessment of whole otoliths, dorsal spines and scales

Sagittal otoliths, dorsal spines and scales were collected from 139 fish, between September and November 2006. Each otolith was weighed (0.0001 g) and the left otolith from each fish immersed whole in lavender oil and viewed under a dissecting microscope with reflected light against a black background. The second and larger dorsal spine was removed, cleaned and dried. Each spine was set in clear casting polyester resin blocks in groups of five, and multiple cross sections were cut from the base of the spine using a diamond edge single bladed saw. Sections were set onto slides using clear casting polyester resin and cover slips. Spine sections were viewed using a compound microscope and reflected light against a black background. Three to five scales were removed from each fish posterior to the pectoral fin to reduce the likelihood of selecting regenerated or damaged scales. Scales were cleaned, dried, pressed and secured between two microscope slides, and viewed under a dissecting microscope using transmitted light.

Otoliths, spines and scales were assessed for their utility as a means of ageing *L. argus*, based on counts of opaque growth increments. Samples of each structure were viewed in random order with no information on the length of the fish available. Counts were made of opaque growth increments and a readability score of 1–5 was assigned to each sample (1 = unambiguous, excellent ring clarity; 2 = unambiguous, minor faults in ring clarity; 3 = ambiguous, counts may vary by ±1; 4 = ambiguous, counts may vary by greater than ±1; 5 = unreadable). Each structure was aged twice to determine a co-efficient of variation and to determine potential ageing bias. A third read was conducted for any fish that had different estimates between age readings 1 and 2. A final consensus age was determined for each structure based on the final read. Measurements were taken from the centre or core of the structure to the first assumed opaque ring, between opaque rings and from the final opaque ring to the edge of each structure. Additional measurements of the vascular core radius were taken for dorsal spines. To determine if hollowing of the vascular core for dorsal spines could lose growth increments, comparisons were made between measurements of the vascular core and growth increments of dorsal spines and growth increments of otoliths. Opaque ring counts were made along the same axis corresponding to each structure (Fig. 1).

Experiment 2: Comparison of whole and sectioned otoliths

Whole and sectioned otoliths were compared from 227 fish randomly selected from samples collected between April 2006 and April 2007. The right otolith from each fish was examined whole and the paired left otolith was sectioned. Sectioned otoliths were obtained by setting the otolith in clear casting resin blocks in groups of six. Between two and five transverse sections were made through the core of the otoliths. The side of the section which was closest to the core was marked and set with the core side facing down onto the microscope slide using clear casting polyester resin. The sections were then polished using a labopol gem-polishing wheel, until thin readable sections which incorporated the core of the otoliths were obtained. Cover slips were then applied over the sections using polyester resin. Counts of opaque zones, readability scores and measurements of sectioned and whole otoliths were obtained as per methods described in experiment 1.

Analysis

The mean difference in age estimation between paired structures was tested using a paired t-test. Age bias plots were used to determine if the estimated age from each structure was overestimated at one end of the age range and underestimated at the other (Campana et al., 1995). The age bias plots graphed the age estimate from one sample against the mean age estimate for the other showing the 95% confidence interval at about the mean. Comparisons were made between

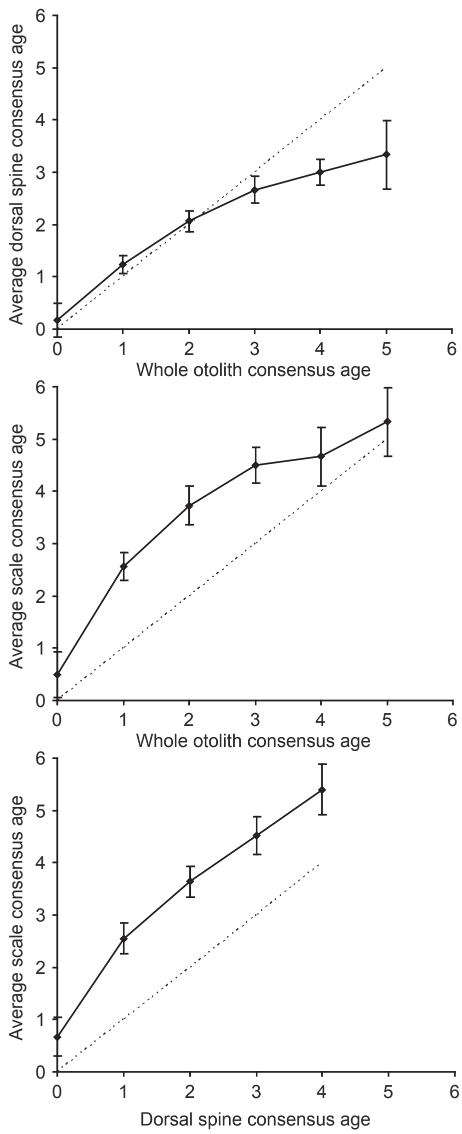

Fig. 2. Age bias plots for whole otoliths, dorsal spines and scales comparing differences between consensus ages for structures. X axis = age estimates, Y axis = average age estimates, error bars = 95% confidence interval about the average age estimate for the Y axis. Dashed line = expected 1:1 aging ratio about which deviation of 95% confidence interval shows aging bias.

structures and between age readings. Pairwise comparisons showing counts of the difference between age readings were also determined between age readings and structures. Coefficient of variation (Chang, 1982) was determined between age readings for each structure to ascertain precision of age readings.

Constrained and non-constrained von Bertalanffy growth curves were fitted to the final age estimation determined for each structure. Constrained growth curves were forced to pass through the size at settlement as this can give a more accurate representation of growth in rapid growing species (Berumen, 2005). An approximate size at settlement of 10 mm was derived from similar-growing *Lepidotrigla* species (Neira et al., 1998). Comparisons of growth curves were conducted between whole otoliths, dorsal spines and scales; whole otoliths and sectioned otoliths; and between sexes for sectioned otoliths using the likelihood ratio test (Kimura, 1980).

Results

Characteristics of bony structures

Whole sagittal otoliths displayed characteristics typical for those of teleost fish in overall shape and orientation. Whole and sectioned otoliths displayed an opaque core with alternating translucent and opaque growth increments. In general the first and second growth increments consisted of two wide opaque zones and two narrow translucent zones. Increment spacing then became more regular and narrow, with opaque and translucent zones displaying similar widths. Estimates of age were based on counts of completed opaque zones. Counts were made along the long axis, square of the otolith centre, towards the posterior margin for whole otoliths (Fig. 1a), and from the core to the posterior edge of the sulcul grove for sectioned otoliths (Fig. 1b). Finely polished small otoliths displayed what appeared to be daily growth increments (Fig. 1c).

Dorsal spines possessed a smooth anterior edge and a serrated posterior edge. Cross-sections of dorsal spines displayed a hollow vascular core with alternating opaque and translucent increments. The increments did not form a complete uniform loop around the centre, instead possessing 3–5 lobed areas, in which growth increments spread radially out from the centre. Estimated age was based on counts of opaque zones, which were clearest along the largest lobed section, across an axis extending perpendicular to spine orientation in the fish (Fig. 1d). The posterior and anterior

Table 1
Pairwise comparison showing counts of difference between age readings for whole otoliths, dorsal spines and scales

	Difference between age read for structures									
	−3	−2	−1	0	1	2	3	4	5	Total
	Dorsal spine/Whole otolith difference									
Whole otolith age										
0				5	1					6
1			7	36	17	2				62
2			3	21	5					29
3			12	12	3					27
4		1	10	1						12
5		2	1							3
Total		3	33	75	26	2				139
	Whole otolith/Scale difference									
Whole otolith age										
0				3	3					6
1			3	6	18	23	12			62
2				1	12	12	3		1	29
3	1			4	8	11	3			27
4				7	3	1	1			12
5				2	1					3
Total		1	3	23	45	47	19		1	139
	Dorsal spine/Scale difference									
Dorsal spine age										
0				5	6	1				12
1			1	3	16	13	7			40
2		1		1	23	21	4		1	51
3			1	3	11	12	3	1		31
4					3	2				5
5										0
Total		1	2	12	59	49	14	1	1	139

Differences grouped by age estimates derived from whole otoliths and dorsal spines.

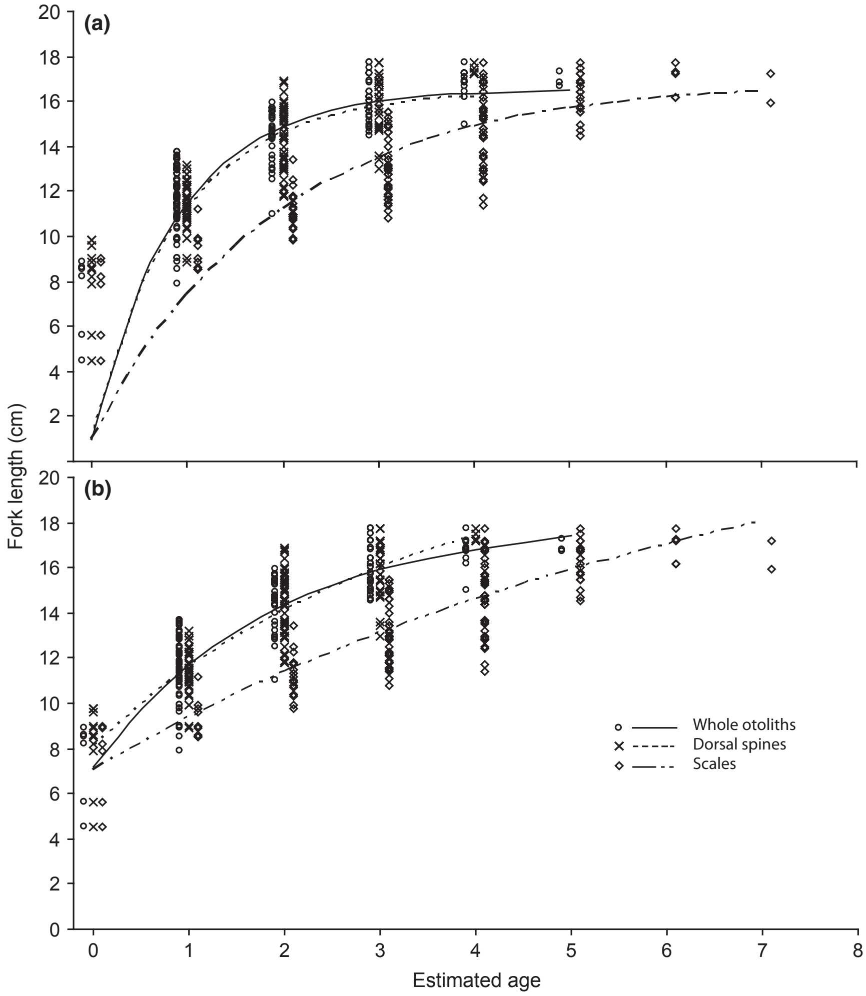

Fig. 3. Length at age estimates and von Bertalanffy growth curves derived for whole otolith, dorsal spines and scales. (a) Growth curve estimates determined with age 0 constrained to size at settlement (FL = 1 cm). (b) Growth curve estimates determined with age 0 not constrained. Otolith age estimates offset by −0.1 and scale age estimates offset by +0.1 to clearly display age estimates. Growth curves have not been offset.

lobes of the dorsal spine did not show complete growth increments. The centre of the hollow vascular core was considered the centre of the spine. Both otoliths and dorsal spines displayed the presence of sub-annular growth increments, which made age estimation difficult.

L. argus possessed ctenoid scales, each having 3–9 radial groves. Regenerated scales were distinguished by having an unstructured centre, which lacked circuli. Regular patterns of growth increments did not appear evident in a number of scales. Estimates of age were made on the anterior area of the scale, across an axis extending from the centre of the scale to the middle of the anterior edge (Fig. 1e).

Experiment 1

Comparison of growth increment counts for bony structures. Comparisons between whole otoliths and scales and between dorsal spines and scales identified that scales showed significantly higher average age estimates. There was no significant difference in age readings between whole otoliths and dorsal spines.

Age bias plots showed that there was no significant bias between readings 1 and 2 for whole otoliths and dorsal spines (Fig. 2). Ageing bias was evident between consecutive reads for scales, with age reading 2 overestimating ages which corresponded to age <3 for age reading 1 and conversely underestimating ages >5 (Fig. 2).

Comparisons between readings for the different structures showed that otolith readings had the highest percent agreement (84.2%), followed by dorsal spines (65.5%) and scales (38.1%). Variations in counts of greater than ±1 were low for whole otoliths (1.4%) and dorsal spines (1.4%), with more variation occurring for scales (25.9%). The precision between readings was best for otoliths (c.v. = 5.5), followed by dorsal spines (c.v. = 19.4) and scales (c.v. = 24.1).

Comparison of counts between bony structures. All comparisons of counts between bony structures were made using the final consensus ages and paired comparisons were made between structures from the same fish. Comparison of estimated age between whole otoliths and dorsal spines from the same fish, indicated that counts were the same for 54% of samples, with 42% showing a ± 1 variation in counts and 4% of samples showing a ± 2 variation in counts (Table 1). No significant difference was found for the average age counts between otoliths and dorsal spines, however comparison of age bias plots and paired age differences showed an ageing bias between the two structures (Table 1, Fig. 2). Spines were found to have higher age estimations when compared to otoliths with an age estimation of zero to two and consistently

Table 2
von Bertalanffy growth curve parameters derived from each structure and between sexes

	Constrained					
	Whole otoliths	Dorsal spines	Scales	Otoliths/Spines[a]	Otoliths/Scales[a]	Spines/Scales[a]
L_{inf}	16.53	16.41	16.87	16.44	15.16	15.19
K	1.13	1.08	0.52	1.11	1.21	1.14
t_0	−0.06	−0.06	−0.12	−0.06	−0.06	−0.06
R^2	0.71	0.68	0.68	0.68	0.51	0.52
RSS	456.26	857.58	521.46	1317.88	1358.79	1711.63
n				276	276	276
df				3	3	3
χ^2				0.848	90.838	59.631
P				0.838	0.000	0.000

	Non-Constrained					
	Whole otoliths	Dorsal spines	Scales	Otoliths/Spines[a]	Otoliths/Scales[a]	Spines/Scales[a]
L_{inf}	18.18	21.44	24.12	19.15	16.95	16.85
K	0.53	0.30	0.15	0.42	0.43	0.44
t_0	−0.96	−1.60	−2.35	−1.24	−1.47	−1.46
R^2	0.80	0.79	0.76	0.79	0.61	0.60
RSS	202.20	209.91	236.65	425.30	780.69	789.29
n				276	276	276
df				3	3	3
χ^2				8.693	158.984	157.200
P				0.034	0.000	0.000

	Constrained			Non-Constrained		
	Whole otoliths	Sectioned otoliths	Whole/Sectioned[a]	Whole otoliths	Sectioned otoliths	Whole/Sectioned[a]
L_{inf}	17.51	18.01	17.67	21.30	22.34	21.65
K	0.69	0.73	0.72	0.34	0.33	0.34
t_0	−0.09	−0.08	−0.08	−0.77	−0.81	−0.80
R^2	0.72	0.79	0.75	0.76	0.84	0.79
RSS	1099.47	1102.92	2242.79	728.37	489.38	1258.17
n			454			454
df			3			3
χ^2			8.252			14.824
P			0.041			0.002

	Constrained sectioned otoliths		
	Males	Females	Male/Female[a]
L_{inf}	18.96	18.92	18.92
K	0.64	0.63	0.63
t_0	−0.08	−0.09	−0.09
R^2	0.76	0.78	0.77
RSS	1141.08	1074.31	2216.77
n			410
df			3
χ^2			0.255
P			0.968

Constrained estimates derived with age 0 constrained to size at settlement of species (0 years = 10 mm FL).
[a]Combined growth curve estimates and likelihood ratio test results comparing structures and sexes.

gave lower age estimations in comparison to otoliths with age three to five (Table 1, Fig. 2).

Comparison of age estimates between whole otoliths and scales showed that counts were the same for only 17% of the samples, with 34% showing a ± 1 variation in counts, and 49% showing greater than ±2 variation in counts (Table 1). Scales consistently showed higher counts throughout the age range when compared to whole otoliths (Table 1, Fig. 2). A similar trend was found for the comparison between dorsal spines and scales, with scales consistently showing higher growth increment counts compared to dorsal spines (Table 1, Fig. 2). Age estimates were the same for only 8% of samples, with 43% varying by ±1 and 49% varying by greater than ±2 (Table 1).

Constrained and non-constrained von Bertalanffy growth curves showed similar trends for whole otoliths and dorsal spines (Fig. 3). Higher age estimates for scales resulted in a lower length at age compared to whole otoliths and dorsal spines. This reduced the slope of the curves (K) and resulted in a different shaped growth curve derived for scales when compared to whole otoliths and dorsal spines (Fig. 3). Constrained growth curves derived from otoliths and dorsal

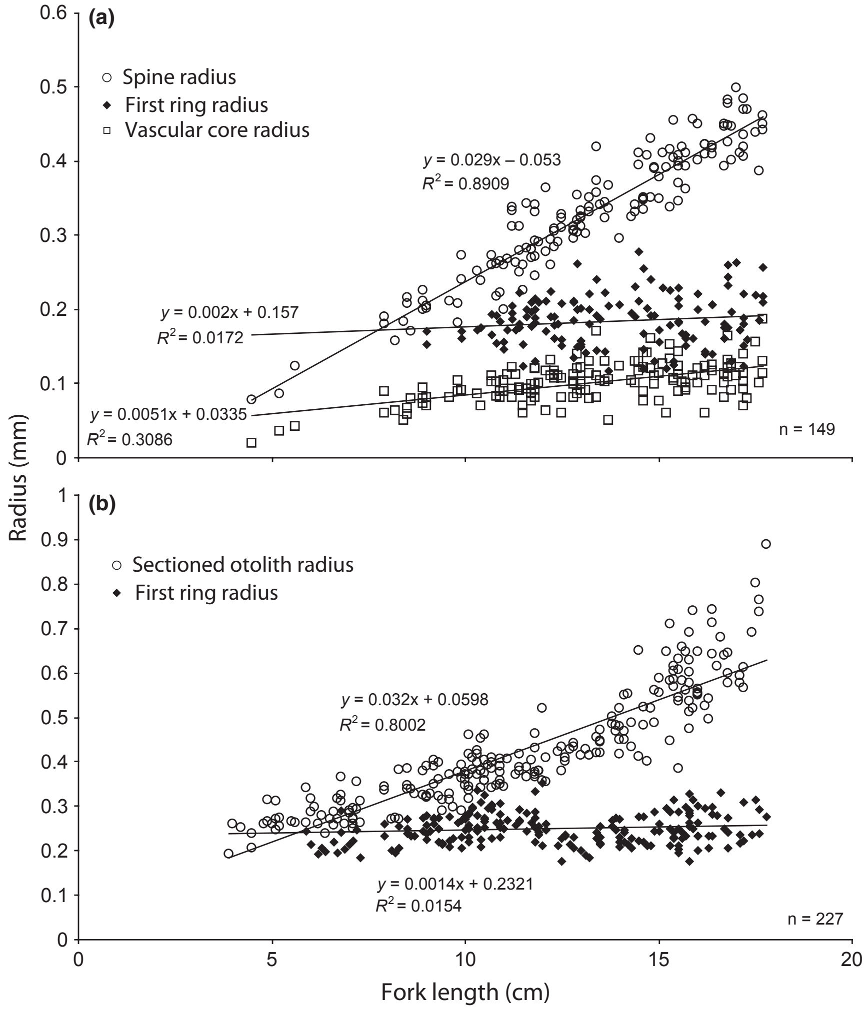

Fig. 4. (a) Relationship between fork length (cm) and radius (mm) of vascular core, first growth increment, and whole structure for dorsal spines and (b) relationship between fork length (cm) and radius (mm) of first growth increment and whole structure for sectioned otoliths.

spines did not differ significantly, whereas those from otoliths and scales and from dorsal spines and scales did differ significantly (Table 2). When growth curves were not constrained, then all structures gave significantly different growth curves (Table 2).

Comparisons of measurements of the vascular core and growth increments between dorsal spines and whole otoliths showed different results. The radius of the dorsal spine vascular core increased with fork length and there was also an increasing trend between the radius of the first ring and fork length (Fig. 4). There was an overlap between the maximum radius of the vascular core and the minimum radius of the first growth increment (Fig. 4). Measurements of the radius of the first growth increment in relation to fork length for otoliths showed no increasing trend (Fig. 4).

Experiment 2

Comparison of whole and sectioned otoliths. No significant ageing bias was detected between age readings 1 and 2 in both sectioned and whole otoliths. Overall, 82% of sectioned otoliths and 80% of whole otoliths returned the same age readings, with all discrepancies differing by ±1 count (Table 3). The coefficient of variance suggest that whole otolith age readings (c.v. = 0.095) were more precise than sectioned otolith age readings (c.v. = 0.122). Comparisons of paired final age readings indicated that estimates from whole otoliths over-aged the fish <3 and under-aged the fish >3 compared to sectioned otoliths (Table 3, Fig. 5).

The constrained and non-constrained growth curves differed significantly for whole and sectioned otoliths (Table 2, Fig. 6). Parameter estimates for L_{inf}, K and t_0 were found to be greater for sectioned otoliths compared to whole ototiths for constrained growth curves (Table 2). Similarly, for non-constrained growth curve estimates, sectioned otoliths gave a higher L_{inf} value than whole otoliths (Table 2). In contrast, both K and t_0 values were lower for sectioned otoliths than whole otoliths for non-constrained growth curves (Table 2). The r^2 values for sectioned otoliths were higher compared to whole otoliths for both constrained and non-constrained growth curves (Table 2).

Discussion

This study examined differences in growth increment counts within and between three bony structures commonly used to estimate the age of fish as a means to determine the most suitable structure for estimating the age of *L. argus*. Validation of estimated ages was not conducted in this study as it assessed the counts of visible growth increments prior to more comprehensive ageing studies. All structures showed some degree of readable and quantifiable growth increments,

Table 3
Pairwise comparison showing counts of difference between age readings 1 and 2 (25% reread) for whole and sectioned otoliths, differences between final ages for sectioned and whole otoliths

	Difference between age reads							
	3	−2	−1	0	1	2	3	Total
Sectioned otoliths	Age reading 2/Age reading 1							
Age reading 1								
0				4	1			5
1			2	24	3			29
2			1	7	1			9
3			1	8				9
4				2	1			3
Total			4	46	6			56
Whole otoliths	Age reading 2/Age reading 1							
Age reading 1								
0				8				8
1			1	18	2			21
2			1	10	1			12
3			3	6	2			11
4			1	2				3
5				1				1
Total			6	45	5			56
	Whole otoliths/Sectioned otoliths							
Sectioned otolith age								
0				17	13			30
1			4	79	20			103
2			2	38	13	1	1	55
3			7	21	5	1		34
4			1	2				3
5		1	1					2
Total	0	1	15	157	51	2	1	227

Differences grouped by age estimates for age reading 1 and by sectioned otoltihs.

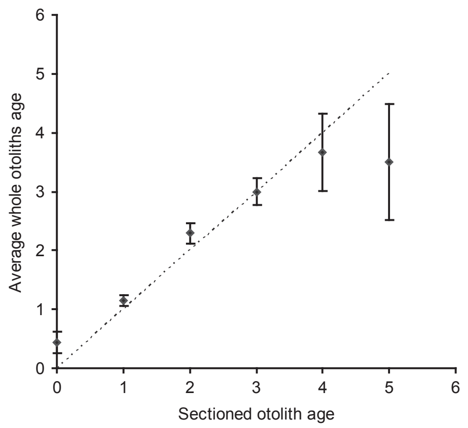

Fig. 5. Age bias plot for difference between final ages for whole and sectioned otoliths. X axis = age estimates, Y axis = average age estimates, error bars = 95% confidence interval about the average age estimate for Y axis. Dashed line = an expected 1:1 aging ratio about which deviation of the 95% confidence interval shows significant aging bias.

but these varied within fish and between structures. Our initial examination identified that whole otoliths provided greater repeatability in age estimates than either dorsal spines or scales and similarly, less ageing bias between reads compared to scales.

Significantly different results between age readings, ageing bias and a low readability for scales suggest they are not suitable for ageing *L. argus*. While growth increments were present in most scales, they were generally of poor resolution and difficult to interpret. Also, age estimates in scales were constantly larger than those found for whole otoliths and dorsal spines. It is possible that the sub-annular growth increments observed in otoliths and dorsal spines may have been interpreted as annuli in scales due to difficulties in their interpretation. In various fish species the scales have been found to form false increments or check marks more easily than otoliths, which can be caused by environmental and human induced stresses (Machias et al., 1998). In other studies, scales have been also shown to underestimate the ages of long-lived fish (>10 years), due to grouping of growth increments towards the outer margin of scales (Beamish and McFarlane, 1987). This was not observed in *L. argus*, probably due to the low age counts observed. Staples (1970) found that scales of another triglid, *Chelidonichthys kumu*, showed no regular patterns of growth increments and were therefore rejected as age indicators. However, the general consensus is that while scales are an inferior structure in comparison to otoliths (Beamish and McFarlane, 1987; Sipe and Chittenden, 2001; Maceina et al., 2007), the efficient removal, simple preparation process and non-destructive sampling continues to make scales a desirable and suitable structure from which to determine age for some fast growing, short lived species.

Paired comparisons of whole otoliths and dorsal spines showed that there was no significant difference between age estimates for *L. argus*. Dorsal spines were also found to provide repeatable age counts and low between-reader ageing biases. Despite this, dorsal spines were found to overestimate the age of younger fish and underestimate the age of older fish compared to whole otoliths. This concurs with the results from similar studies on other species, including spotted sea trout, *Cynoscion nebulosus* and walleye, *Stizostedion vitreum* (Ihde and Chittenden, 2002; Isermann et al., 2003). Dorsal spines also showed a lower range of age estimates in comparison to whole otoliths. These results suggested that either growth increments are naturally variable between structures or that one structure is gaining or losing growth increments due to an unidentified process. Dorsal spines of *L. argus* have a hollow vascular core and it was hypothesised that this vascular core increases in diameter as a fish grows, resulting in loss of an observable growth increment.

The vascular core radius of dorsal spines increased with fork length, with a similar result found for the relationship between the first growth increment radius and fork length. This increasing relationship with the first growth ring was not found with otoliths, and suggests that there is potential for the first growth increment in older individuals to be masked due to the hollowing of the vascular core. We also suggest that the hollow vascular core resulted in the overestimation of age of younger fish due to the absence of the visible core found in the otoliths, which made it difficult to determine the location of the first growth increment. Age estimates based on dorsal spines and fin ray ring counts have been shown to result in an underestimation of the true age for many species of fish, including brown trout *Salmo trutta* (Graynoth, 1996), sailfish *Istiophorus platypterus* (Chiang et al., 2004), brown bullhead *Ameiurus nebulosus* (Maceina and Sammons, 2006), and blue fish *Pomatomus saltatrix* (Sipe and Chittenden, 2002). In contrast, studies comparing bony structures in other fish species including blue throat wrasse *Notolabrus tetricus* (Metcalf and Swearer, 2005), and Pacific cod *Gadus macrocephalus* (Lai et al., 1987) have found dorsal spines to provide more or equally accurate and reliable age estimates than the otoliths. Due to the increased potential for agers to misidentify the first growth increment

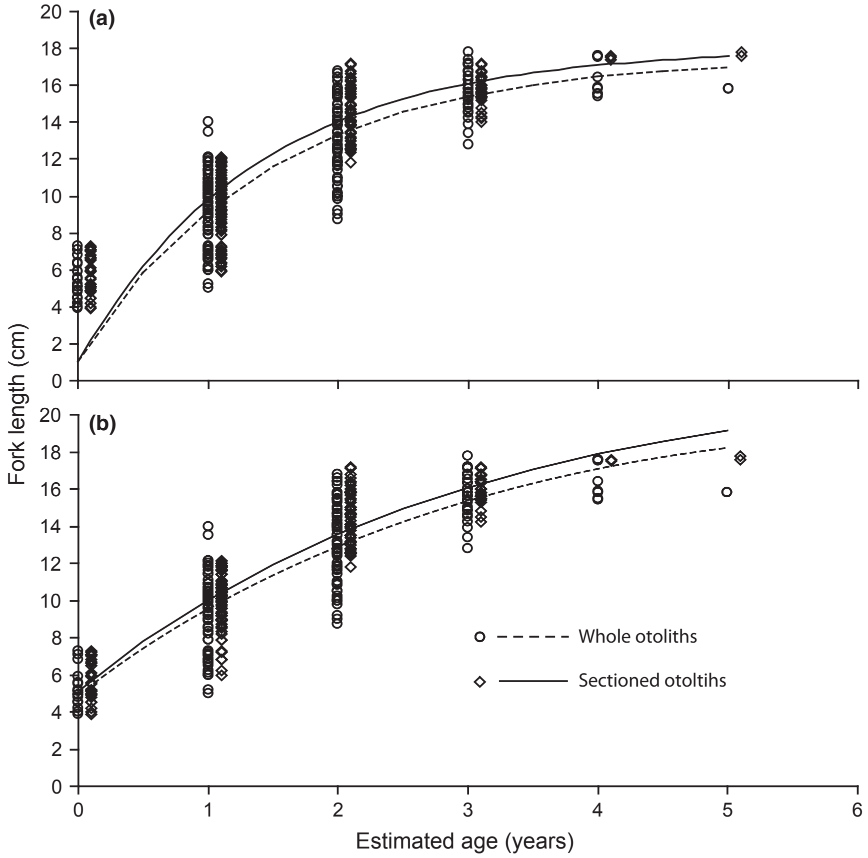

Fig. 6. Length at age estimates and von Bertalanffy growth curves derived for whole and sectioned otoliths. (a) Growth curve estimates determined with age 0 constrained to size at settlement (FL = 1 cm). (b) Growth curve estimates determined with age 0 not constrained. Sectioned otolith age estimates offset by +0.1 to clearly display age estimates. Growth curves have not been offset.

in dorsal spines and because of the ability for dorsal spines to lose their first growth increment, this is not recommended as a suitable structure from which to determine *L. argus* age.

Whole otoliths did not display any evidence suggesting loss of growth increments or underestimation of growth increment counts when compared to dorsal spines and scales. Therefore, whole otoliths were initially used to estimate the age for *L. argus*. Previous studies on triglids have also utilised otoliths to estimate age (Staples, 1970; Booth, 1997; Colloca et al., 1997, 2003; Ismen et al., 2004). However, caution must be exercised when using whole otoliths to determine the age of fish. Firstly, the resolution of growth increments in thick otoliths can often be poor (Stevens et al., 2005); secondly, it is suggested that areas of pigment can be visible in whole otoliths which are not visible in sectioned otoliths (Fowler and Short, 1998); and thirdly, reflected light used to examine whole otoliths can cause a halo effect around the edge of the otolith which can make edge determination difficult. Thus, sectioned otoliths are more often used to estimate age than whole otoliths.

Comparison of 25% re-reads for sectioned and whole otoliths showed that there was no significant ageing bias between reads for either method and that repeatability and coefficients of variance were also similar. This result does not conclude that both structures are suitable for age estimation, as repeatable non-bias age readings can still be inaccurate (Campana, 2001). Sectioned otoliths were found, however, to have much clearer growth increments and were easier to interpret than whole otoliths, which is consistent with previous studies (Ferreira and Russ, 1994; Stevens et al., 2005). Comparison of final ages for sectioned and whole otoliths revealed that an ageing bias was evident between the two methods. Whole otoliths were found to overestimate younger fish and underestimate older fish in comparison to the sectioned otoliths. Similarly, Booth (1997) found that, for other triglids, maximum ages obtained from whole otoliths were also found to be lower than ages obtained from the sectioned otolith.

The von Bertalanffy growth curves derived from sectioned and whole otoliths showed similar trends, but were statistically significantly different for the constrained and non-constrained growth models. The parameters L_{inf} and K for both models were lower for whole otoliths than for sectioned otoliths. The constrained growth curves used in this study gave more realistic estimates of L_{inf}, with values approaching the maximum size of fish captured in this study. While age data is a crucial component of stock assessments (Campana, 2001; Campana and Thorrold, 2001; Reeves, 2003), it is likely that errors between the two methods would not result in changes to stock assessment or management advice for this species due to the young maximum ages. Nevertheless, because of the increased clarity of growth increments obtained for sectioned otoliths, especially towards the outer margins, it is recommended that sectioned otoliths be utilised for further biological studies on this small Triglidae.

This study showed that for *L. argus*, sectioned otoliths were more suitable to determine age estimates than whole otoliths, dorsal spines and scales. Sectioned otoliths provided better clarity for ease of reading without loss of any growth increments. Whole otoliths and dorsal spines are potentially useful structures from which ageing can be determined for *L. argus,* however caution need be taken when interpreting ages derived from these structures due to ageing biases. Scales were considered unsuitable due to high levels of between reading ageing bias, between structure ageing bias, low precision and poor readability for this species.

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