Determining the most suitable method of otolith preparation for estimating the age of tigerfish, *Hydrocynus vittatus* in the Pongolapoort Dam, South Africa

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This study compares sectioned and whole lapillus and asterisci otoliths as suitable structures for ageing tigerfish, *Hydrocynus vittatus*. Fifty tigerfish were collected from the Pongolapoort Dam, KwaZulu-Natal, South Africa. Growth zone counts on sectioned lapilli showed the greatest percentage agreement (52%) and highest precision (APE = 4.77%, CV = 6.20%). Growth zone counts were symmetrically distributed between structures (Bowker's tests $P > 0.125$). Between-reader analysis also showed sectioned lapilli to have the highest percentage agreement (68%) and best precision (APE = 5.55%, CV = 7.85%).

Key words: age, asterisci, lapilli, otolith, tigerfish.

Tigerfish, *Hydrocynus vittatus* Castelnau 1861, is a sought-after sport angling species in southern Africa (Smit *et al.* 2009) so understanding the biology of these fish is important to manage fisheries. According to Campana (2001), fish age is one of the most influential biological variables forming the foundation for productivity, growth and mortality studies. There is, however, little published literature on ageing techniques for tigerfish. Because several structures are available for use in ageing studies (scales, vertebrae, fin rays and opercula; Campana 2001), it is imperative to determine the best available methods for each species as ageing error can affect accuracy (closeness of age estimate to the true value) and precision (reproducibility of repeated measurements; Kalish *et al.* 1995). Correct technique eliminates errors in age-based assessments of growth and mortality rates and allows proper species management (Kimura *et al.* 2006).

Although sectioned otoliths are considered the most appropriate structures for age and growth estimates in tropical fishes (Beamish & McFarlane 1987), Gerber *et al.* (2009) is the only study that has considered tigerfish otoliths (lapilli). Asterisci have never been assessed for their possible use in tigerfish ageing.

Otolith shape is multifarious and can be species- (Secor *et al.* 1991) and/or population-specific (Messieh 1972) because shape specificity is due to deposition differences in the crystaline material making up the otolith (Bingel 1981). In tigerfish, lapilli are small, oblong and dense with smooth edges, a longer rostrum and obvious excisural notch. The asterisci are larger, round-oval and thinner with serrated edges and have a small, thin excisural notch separating the antirostrum and rostrum of almost equal lengths (Fig. 1a,b). The higher density of the lapilli, compared to asterisci, makes this structure thicker and less fragile (Assis 2005).

The aim of this study was to assess the suitability of lapilli and asterisci otoliths as potential ageing structures for tigerfish. As the rate of growth zone formation has never been validated for tigerfish, the accurate age cannot currently be estimated based on growth zone counts. In such cases information on the structure that yields the most precise estimate of the number of growth zones is still important (e.g. Filmalter *et al.* 2009) as future validation studies can then focus on that structure.

The Pongolapoort Dam (27°25′15″S 32°04′15″E) is situated on the Phongolo River in the subtropical region of northern KwaZulu-Natal (Heeg & Breen 1982). Built in the early 1970s for the specific purpose of irrigation, Pongolapoort Dam has a gross capacity of 2500×10^6 m^3, making it the third largest impoundment (by volume) in South Africa (Rossouw 1985). Forty three female and

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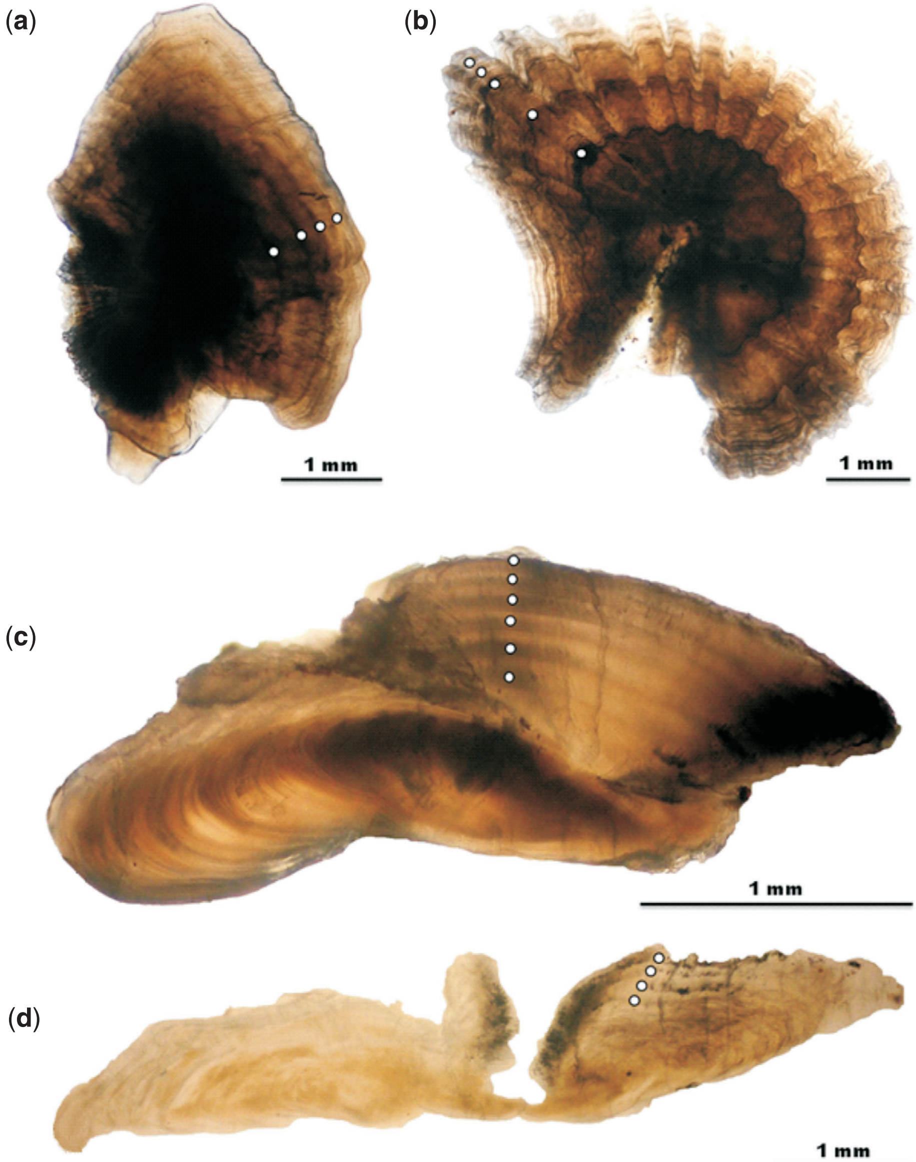

Fig. 1. Micrographs of (**a**) whole lapilli, (**b**) whole asterisci, (**c**) sectioned lapilli and (**d**) sectioned asterisci otoliths from the tigerfish of Pongolapoort Dam. White dots represent single growth zone counts.

seven male tigerfish were collected by angling in July (n = 11) and September (n = 27) 2009 and in April (n = 12) 2010. Fish were sacrificed by severing the spinal cord. Lapilli and asterisci were immediately removed, cleaned, air-dried and stored in 1.5 ml Eppendorf tubes. Owing to the lack of male specimens, sexes were pooled.

Whole lapilli (Fig. 1a) and asterisci (Fig. 1b) were immersed in methyl salicylate for enhancement of clarity (Winker *et al.* 2010). For sectioning, otoliths were prepared following standard techniques (Gerber *et al.* 2009). Whole otoliths were embedded, and sectioned using a double-bladed diamond-edged saw. Sections were mounted onto microscope slides using DPX mountant. Growth zone counts were obtained using a stereo-microscope under both incident and transmitted light. Alternating opaque and hyaline zones encircling the entire otolith were considered valid growth zones; the outer edge was not accepted as a growth zone

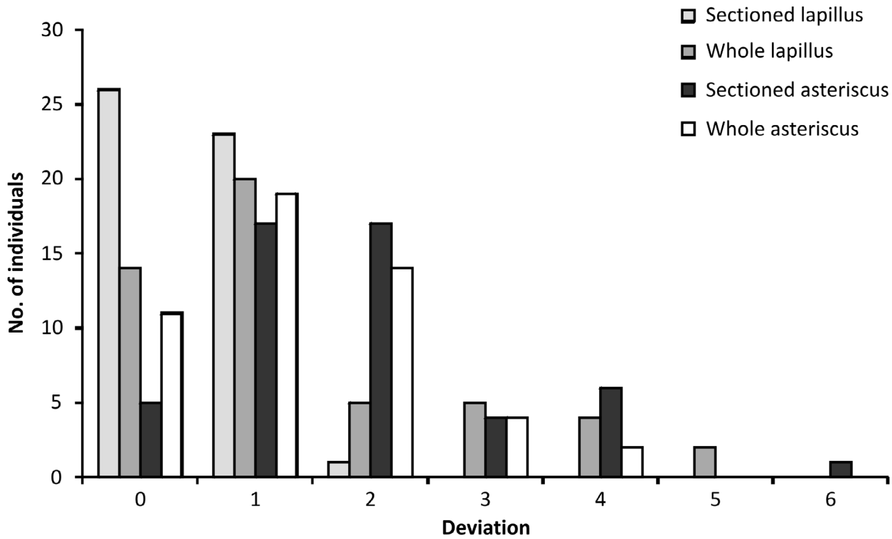

Fig. 2. Deviations of three replicate growth zone counts obtained from the primary reader from all four ageing techniques tested (whole lapilli, whole asterisci, sectioned lapilli and sectioned asterisci).

(Brouwer & Griffiths 2004). The term 'growth zone' is used throughout this manuscript as the growth zone deposition rate for tigerfish has not yet been validated.

Sectioned and whole lapilli and asterisci otoliths were read independently. To determine increment readability, three replicate growth zone counts were made by one reader, for each of the four structures tested (between-structure analysis). Counts were made in random order at weekly intervals. The modes of these three readings were adopted as the final growth zone count for each specimen. To evaluate repeatability of counts, a second reader also read each otolith (between-reader analysis).

Following methods previously employed to assess for precision between hard structures (Campana 2001; Gerber *et al.* 2009), between-reader and between-structure comparisons were done by means of age-bias plots (Campana *et al.* 1995), average percentage error (APE; Beamish & Fournier 1981) and coefficient of variation (CV; Campana 2001). For the between-reader analysis, the mode of three growth zone counts by the primary reader were used to compare with the once-off reading by the second reader.

Linear regression plots (Egger *et al.* 2004) tested for linear relationships between comparisons of the various ageing techniques (sectioned lapilli, whole lapilli, sectioned asterisci and whole asterisci). Bowker's tests (Hoenig *et al.* 1995) were used to test the symmetrical distribution of the data around the agreed growth zone counts to determine if significant differences exist between structures (Taylor & Weyl 2012). This was done by comparing the more precise otolith (sectioned lapilli) growth zone estimates to that of the other three ageing techniques (whole lapilli, sectioned asterisci and whole asterisci).

Deviations of three replicate growth zone counts obtained from the primary reader for all four ageing techniques (whole lapilli, whole asterisci, sectioned lapilli and sectioned asterisci) showed sectioned lapilli had the highest agreement (52%) between replicate growth zone readings (Fig. 2). Furthermore, 46% of the readings only varied by one growth zone count. Sectioned asterisci had the highest divergence with 90% of readings differing by 1–6 growth zone counts. Thus, whole asterisci rendered poor repeatability. Processing was also complicated by the thin and delicate nature of the asterici and 73% of the otoliths shattered during sectioning. Grinding (Egger *et al.* 2004) the asterisci could possibly have rendered better results. Age-bias plots (Fig. 3) indicate that growth zones were more apparent in lapilli. Growth zone counts on this structure always exceeded those from asterisci regardless of whether the reading was conducted on whole or sectioned samples. On comparison (Table 1), sectioned lapilli also yielded more precise (CV = 6.20%, APE = 4.77%) estimates of growth zone number than sectioned asterisci (CV = 19.68%, APE = 14.77%). As lapilli precision was within precision limits set by Campana (2001;

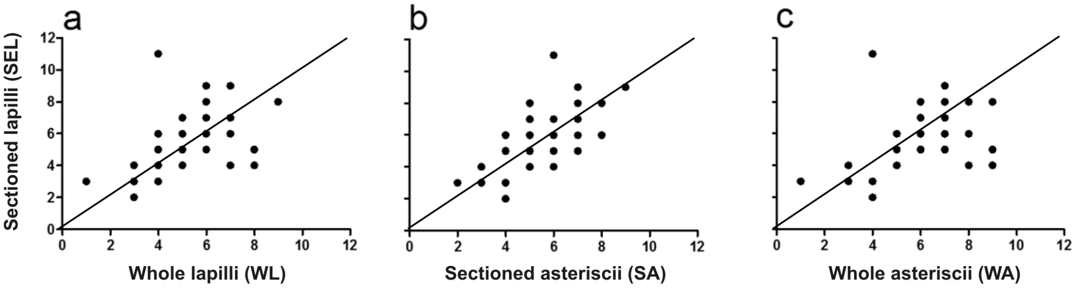

Fig. 3. Plots comparing data obtained using the various ageing methods: growth zones obtained from sectioned lapilli *vs* (**a**) whole lapilli, (**b**) sectioned asterisci and (**c**) whole asterisci. Solid lines indicate 1:1 relationship.

Table 1. Average percent error (APE) and coefficients of variation (CV) for the between-structure and between-reader analyses; *n* = number of samples.

		Between-structure		Between-reader	
Ageing method	*n*	APE (%)	CV (%)	APE (%)	CV (%)
Sectioned lapilli	50	4.77	6.20	5.55	7.85
Whole lapilli	50	10.87	14.52	12.78	18.07
Sectioned asterisci	50	14.77	19.68	17.46	24.69
Whole asterisci	50	9.77	12.97	11.62	16.44

APE < 5.5% and CV = 7.6%), they are therefore considered as acceptable structures for future use in tigerfish ageing studies. These results show a similar (although slightly higher), precision to that of Gerber *et al.* (2009) who reported APE = 5.81% and CV = 7.62% for sectioned lapilli from Okavango Delta tigerfish.

Bowkers tests (Hoenig *et al.* 1995), comparing the growth zone estimates of sectioned lapilli to the three other ageing methods, showed symmetrical distribution ($P > 0.125$) for all three comparisons; sectioned lapilli *vs* whole lapilli ($\chi^2 = 16.867$, d.f. = 15, $P = 0.327$), sectioned lapilli *vs* sectioned asterisci ($\chi^2 = 13.033$, d.f. = 15, $P = 0.600$) and sectioned lapilli *vs* whole asterisci ($\chi^2 = 25$, d.f. = 18, $P = 0.125$). Although all comparisons showed symmetrical distribution, the percentage of agreement between growth zone estimations was highest for sectioned lapilli *vs* whole lapilli (34%).

Between-reader disparity is an excellent indicator of the ageability of the structures being tested (Kimura & Lyons 1991). Less deviation shows higher agreeability of the structure and repeatability of readings. Reader variation also provides information about the readers themselves (Eklund *et al.* 2000). In this study reader one consistently counted one less growth zone in young fish (≤2 growth zones), using all four methods (Fig. 4). Variability in young fish may be attributed to the incorporation of dissimilar resolving criteria for early growth zones (Kimura & Lyons 1991). The between-reader analysis shows sectioned lapilli has the highest precision (CV = 7.85%, APE = 5.55%). These values are slightly above (APE = 0.05% and CV = 0.2%) the average values set by Campana (2001). The percentage agreement for readings in the between-reader analysis was 68%, 36%, 20% and 24% for sectioned lapilli, whole lapilli, sectioned asterisci and whole asterisci, respectively. Significant differences ($P < 0.05$) were only found between the primary and secondary reader when reading the whole lapilli ($P = 0.0067$).

For tigerfish ageing, sectioned lapilli show the highest percentage agreement, precision and repeatability in both the between-structure and between-reader analyses. Thus, sectioned lapilli are the superior structure for ageing this species. Tigerfish ageing still needs to be validated in order to prove the accuracy of the technique.

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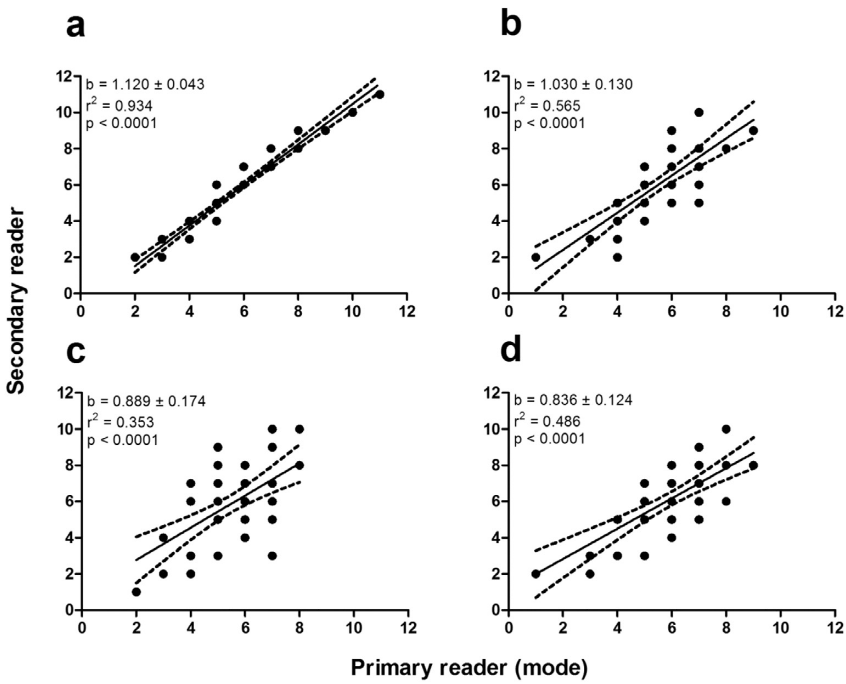

Fig. 4. Linear regressions depicting the between-reader analysis and 95% confidence bands for (**a**) sectioned lapilli, (**b**) whole lapilli, (**c**) sectioned asterisci and (**d**) whole asterisci otoliths; b = slope, r^2 = correlation, P = significance value.

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