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Interpretability and precision of annulus counts for calcified structures in carp, *Cyprinus carpio* L.

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With 2 tables in the text

Abstract: Scales, opercular bones and whole otoliths of carp from the River Murray, Australia, were analysed for interpretability and reproducibility of annulus counts by three interpreters of disparate experience. Interpretability was least for scales and highest for opercular bones, and annulus counts from scales and opercular bones were more precise than those from otoliths. However, these differences were not statistically significant. Relatively high values of the index of average percent error (IAPE) and of the mean coefficient of variation (V) confirmed that age interpretation in carp is difficult and requires extensive training and experience.

Introduction

In the last 20 years diverse methods have been developed for determining the ages of fish from their calcified structures, like scales, otoliths, flat bones, vertebrae and fin rays (Campana et al. 1995), and indications of the reliability of estimates now are mandatory in age-growth studies. Thus, percent agreement, a simple measure of precision, has been superseded by measures like the average percent error (APE_j: Beamish & Fournier 1981; j = fish/structure), the coefficient of variation (V_j: Chang 1982) and the index of precision (D_j: Chang 1982). All are independent of the age of the fish.

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Individual calcified structures may be used to interpret the age of fish, and suites of structures may be employed to corroborate age interpretations or resolve problems with identification of periodic patterns (e.g. MARALDO & MACCRIMMON 1979, ERICKSON 1983, SHARP & BERNARD 1988). In cyprinids, scales and to a lesser extent opercular bones are used widely (MANN 1991), and otoliths (especially lapilli) also have been employed (e.g. BACKE-HANSEN 1982, MILLS 1988, MINA 1989, O'MAOILEIDIGH & BRACKEN 1989). In carp *Cyprinus carpio* L. there are several potentially suitable structures (VILIZZI & WALKER 1995). For example, ENGLISH (1952) and REHDER (1959) found scales and opercular bones to be good indicators of age and growth, and WICHERS (1976) and BISHAI & LABIB (1978) judged fin rays and vertebrae, respectively, to be most convenient and reliable. No one method, however, has proved consistently effective.

In Australia, appropriate methods for studies of the dynamics of natural populations are needed, where there is concern over the spread of carp and its impact on rivers and wetlands (BRUMLEY 1991, HARRIS 1995, ROBERTS & TILZEY 1997). To this end, the present paper evaluates the interpretability and reproducibility (precision) of annulus counts by several interpreters for a suite of calcified structures, obtained in the course of work on the age and growth of carp in the River Murray, South Australia. This material was a subset of a sample analysed for bias and precision by the senior author and another interpreter. The data here complement those presented elsewhere (VILIZZI & WALKER 1998), and are a necessary part of validation of the methods described therein.

Materials and methods

Scales, opercular bones and whole otoliths (asterisci) were obtained from carp 1+ and older, sampled monthly from July 1994 to July 1995 at Gurra Lakes, on the River Murray near Berri, South Australia (34° 18′ S, 140° 41′ E). Otolith sections, intended to facilitate detection of annuli in whole otoliths, were discarded, as no general improvement in interpretability was achieved. Details on sample collection and preparation, criteria for annulus identification, and terminology are provided by VILIZZI & WALKER (1998) (hereafter 'the main study').

The experiment involved three interpreters: B (TJ), C (DMcG) and D (VT). Interpreters B and D had some prior experience and C was well experienced, but none was familiar with carp. All were given a one-day familiarisation course to ensure a consistent approach. In the first part of the experiment, the interpreters were asked to count annuli on 90 preparations of 30 scales, 30 opercular bones and 30 whole otoliths. They were given the same 90 preparations, chosen randomly from triplets from 362 fish, and were aware of the possible presence of uninterpretable structures (see below). Each preparation was randomly assigned a number from 1–30 so that there was no numerical correspondence between structures of the same triplet; indeed, the interpreters did not know that there were triplets from the same fish. They were asked to complete their counts in one day, independently of one another. They had no information about the date of capture, length or weight of the fish. The second part of the experiment was identical, except that the three interpreters performed their analyses at different times from one another (1–2 months after the first count), and B and D relied on image analysis to enumerate annuli on whole otoliths. All structures scored as uninterpretable by B, C and D on the first occasion were discarded for the second count.

The results were compared with data from the senior author (hereafter, Interpreter A), who examined the same preparations as part of the main study. Evaluation of bias was not possible due to small sample sizes. The interpretability of a structure was based on the following criterion: whenever a pattern (alternating closely- and widely-spaced circuli in scales; opaque and translucent zones in opercular bones and otoliths) could be consistently identified the preparation was scored as **interpretable,** otherwise it was rejected as **uninterpretable**. Within-interpreter (B, C and D) and between-interpreter (A–B, A–C and A–D) precision for each preparation were measured by APE_j and V_j, which were then averaged across preparations of the same type of structure to obtain an index of average percent error (IAPE) and a mean coefficient of variation (V), respectively. The index of precision D_j was not calculated, as this is identical to APE_j whenever two counts are made on the same preparation. Finally, within-interpreter reproducibility was based on the two (first and second) replicate counts by all interpreters, and between-interpreter reproducibility on the first count by B, C and D, and one count by A.

Results

Given annulus counts by B, C and D, the average interpretability of scales was lower than for opercular bones and whole otoliths, and the same was true when data from A were included (Table 1). However, Friedman's test showed these differences to be not statistically significant (respectively, $\chi^2 = 2.167$, $P = 0.338$, df = 2; $\chi^2 = 3.875$, $P = 0.144$, df = 3). In particular, D enumerated annuli on all structures, as did C for opercular bones and otoliths. Fewer scales

Table 1. Percentages and corresponding numbers (in parentheses) of interpretable calcified structures from carp in the lower River Murray, Australia. Sample size in parentheses.

Interpreter	Scale	Opercular bone	Whole otolith
A	80.0 (24)	100.0 (30)	93.3 (28)
B	90.0 (27)	96.7 (29)	93.3 (28)
C	96.7 (29)	100.0 (30)	100.0 (30)
D	100.0 (30)	100.0 (30)	100.0 (30)
Average B–D	95.6	98.9	97.8
Average A–D	91.7	99.2	96.7

Table 2. Within-interpreter and between-interpreter reproducibility of annulus counts based on three calcified structures from carp in the lower River Murray, Australia. Values for the index of average percent error (IAPE), the coefficient of variation (V: in italics) and the number of preparations (in parentheses) are given for each calcified structure and count combination. The within-interpreter reproducibility is based on two replicate counts by all interpreters (X_1 and X_2), while the between-interpreter reproducibility is from the first count by Intepreters B, C and D (X_1) and one count by Interpreter A.

Within interpreters				Between interpreters			
Count	Scale	Opercular bone	Whole otolith	Count	Scale	Opercular bone	Whole otolith
B_1-B_2	10.79; *15.26* (27)	7.56; *10.70* (29)	12.54; *17.73* (28)	A-B_1	9.57; *13.53* (27)	10.52; *14.87* (29)	13.28; *18.78* (28)
C_1-C_2	9.19; *13.00* (29)	14.09; *19.92* (30)	12.81; *18.11* (30)	A-C_1	10.64; *15.04* (29)	10.99; *15.54* (30)	12.02; *17.00* (30)
D_1-D_2	7.37; *10.43* (30)	12.18; *17.22* (30)	11.63; *16.45* (30)	A-D_1	9.71; *10.41* (30)	10.68; *14.71* (30)	12.82; *18.13* (30)
Average	9.12; *12.90*	11.28; *15.95*	12.33; *17.43*	Average	9.97; *12.99*	10.73; *15.04*	12.71; *17.97*

were scored as interpretable by A, and the number of interpretable whole otoliths was the same for A and B. Only one of the six scales recorded as uninterpretable by Interpreter A was scored as such by B, and the only scale considered uninterpretable by C was also uninterpretable to B, but not to A. Only one of the two whole otoliths uninterpretable to A was also uninterpretable to B.

The average within-interpreter precision was lowest in otoliths, highest in scales, and intermediate in opercular bones. Similar results were obtained for the between-interpreter precision (Table 2). Again, Friedman's test for IAPE indicated no significant differences among structures for within- and between-interpreter reproducibility (respectively, $\chi^2 = 2.000$, $P = 0.368$, df = 2; $\chi^2 = 6.000$, $P = 0.050$, df = 2).

Discussion

The relative interpretability of scales, opercular bones and otoliths corroborated the findings of the main study. The lowest number of interpretable scales was indicative of the difficulties encountered by most interpreters in the recognition and enumeration of annuli in these structures. Resorption, a common process in carp scales (Ichikawa 1953, Carlander 1969), is a major impediment to reliable interpretations. The lowest rejection rate recorded for opercular bones relative to scales accords with other comparative studies (cf. English 1952, McConnell 1952, Rehder 1959), and the same is true of otoliths (Raina 1987, Pinilla et al. 1992).

The precision of annulus counts for the structures under scrutiny was also in agreement with the results of the main study. Although the higher reproducibility of counts for scales, even if not statistically significant, may at first seem unexpected, this was probably an effect of their lower interpretability, so

that difficult scales would be more easily rejected than opercular bones and whole otoliths. By discarding a higher number of structures as uninterpretable, it comes as a consequence that the remaining preparations must be easier to interpret, especially when a 'destructive' process like scale resorption prevents any attempt to discern a consistent pattern. Evaluation of within- and between-interpreter reproducibility resulted in relatively high values for IAPE (and V) compared to those reported in the literature. Although a standard measure of reproducibility in fish age and growth studies probably cannot be defined, values for IAPE below 10 % may be taken as an acceptable level of precision (Powers 1983). In the present study, IAPE ranged 7.4–14.1 %, with 13 out of 18 values above 10 %, hence consistently higher than the IAPE values reported for Murray cod (*Maccullochella peeli*, 3.0–5.4 %: Anderson et al. 1992 a) and golden perch (*Macquaria ambigua*, 3.9–5.6 %: Anderson et al. 1992 b) in the Murray-Darling Basin (MDB). This can be explained by noting that reproducibility here was dependent on the expertise gained over time by the individual interpreters. Thus, not only the counts by Interpreter A, who developed the age determination methodology for carp in the MDB, but also those by a second interpreter, who spent several weeks on the entire set of structures, proved consistently more precise than those from Interpreters B, C and D, ranging 5.3–9.2 % (Vilizzi & Walker 1998).

Interpretations of annulus patterns in carp scales, opercular bones and otoliths are not easy, and instruction and experience are required for precise assessments. Age determination programmes, involving training of interpreters and calibration (sensu Anderson et al. 1992 a) of their interpretations, are therefore encouraged as an essential component in monitoring the dynamics of carp populations in the MDB. Interpreters from various agencies undertaking routine age assessments could exchange collections of calcified structures (Williams & Bedford 1974, Kimura et al. 1979, Boehlert & Yoklavich 1984, Boehlert 1985), ensuring that the bias and precision of counts do not deteriorate over time (Campana et al. 1995).

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