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Validating Otolith Annuli for Annual Age Determination of Common Carp

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Abstract.—Common carp *Cyprinus carpio* are an important pest species in Australia, yet little is known regarding their age and growth there. We examined otolith sections of common carp to validate their utility for age determination. For the 1999 year-class in Hut Lake near Barmah, we confirmed the absolute age at first annulus formation as age 1 by repeated sampling of a discrete young-of-year cohort. We confirmed the annual periodicity of annulus formation for common carp in a mark–recapture experiment when 19 recaptured adult common carp (from an original stocking of 141 fish marked by injection with oxytetracycline [OTC]) showed visible fluorescent marks on their otoliths. Time at liberty for these fish ranged from 6 to 25 months, and their ages on recapture ranged from 3 to 14 years. Increment counts outside the OTC mark agreed completely with time at liberty. We calculated precision estimates on age determinations as the average percent error (APE) and estimated the coefficient of variation (CV = $-0.15 + 1.41$APE) between readers and for each of two readers over time. Precision was assessed by rereading subsamples. The APE was less than 5% and CV was less than 8% in all cases. We conclude that examination of thin otolith sections is a suitable method for the determination of annual age estimates for common carp age 0–14.

Previous studies of the age and growth of common carp *Cyprinus carpio* in Australia used scales, otoliths, and cleithra to estimate annual and daily age (Vilizzi 1998; Vilizzi and Walker 1999a; Vilizzi et al. 1998). Otoliths offer several benefits over scales in aging studies. Lack of seasonal resorption, resorption at the scale edge, irregular growth, or periodic erosion can all cause problems in the interpretation of scale annuli (Johal et al. 1984; Carlos 1990; Casselman 1990). Therefore, we chose otoliths to determine age in current studies of the population biology of wild common carp in Victoria.

Validation of daily annulus periodicity in common carp was achieved using laboratory-reared, chemically marked larvae up to 5 weeks old (Vilizzi 1998). However, the validation of annual age determination has been limited to analysis of marginal increment formation in fish age 1–15 (Vilizzi and Walker 1999a). Campana (2001) was critical of marginal increment analysis as a validation method and considered absolute age to be the true goal of validation studies, although he conceded that this often was impossible. He recommended that if absolute age could not be determined, then two steps were necessary for annual age validation: (1) determination of the age of first annulus formation, and (2) verification that annulus formation has annual periodicity throughout the age range of interest.

Our study is part of a broader analysis of common carp population biology around Victoria that relies on age estimates of large sample sizes for the calculation of age at maturity, growth, and mortality rates. For this project, examining thin sections of otoliths was the preferred method for aging; we believed, however, that a greater certainty regarding the validation of common carp age determination from otolith sections was needed (Morison et al. 1998). Previous common carp aging studies using otoliths (either whole or sectioned) have reported poor precision (Vilizzi et al. 1998). A lack of precision in age determination can indicate problems in the inherent periodicity or clarity of increments within a structure, or it may simply reflect the level of experience of the readers. We used experienced otolith readers to

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minimize one possible source of poor precision. Our threefold purpose was to validate the age at formation of the first annulus, to determine the periodicity of annulus formation in a range of age-classes under natural conditions, and to quantify the precision of the age estimates produced.

Methods

We collected otoliths from fresh or frozen common carp using the ''up through the gills method'' (Secor et al. 1991). The cyprinid family is unusual because the asterisci are the largest of the three pairs of otoliths. Hence, we used asterisci to facilitate extraction and preparation.

Otoliths were embedded in polyester resin blocks with their primordia aligned. Serial sections (~0.4 mm) were cut through the area of the primordia using a modified gem-cutting saw and mounted on microscope slides under coverslips using more polyester resin. For age determination, we viewed the slides under a microscope using transmitted light. Details of the preparation and age determination process are given in Anderson et al. (1992a, 1992b) and Morison et al. (1998).

Description of otolith sections.—Transversely sectioned common carp otoliths are complex in both morphological characteristics and in annulus structure. The primordial regions of otoliths are relatively opaque, which can reduce the clarity and definition of the presence of the first annulus. Interpretation of annuli is easier toward the edge of otoliths of large common carp because the translucent edge region reveals a regular pattern of repeating light and dark bands. Before we assigned ages to samples, both a primary (CG) and a secondary reader (KKG) became familiar with the morphology and annuli structure of the otolith. Both readers were experienced with the interpretation of increments in otoliths from a wide range of species. We studied a sample of approximately 100 otoliths from carp of various sizes to establish interpretation criteria and used terminology following the glossary for otolith studies (Secor et al. 1995). Production of an annual increment was defined as the completion of an opaque zone following a translucent zone, so that the annulus used to denote each year was the completion of an opaque zone. Readers measured samples using the image analysis software Optimate and marked annuli ''onscreen'' along a transect from the primordium to the edge of the ventral lobe (Figure 1). We extracted the distance between each annulus for the first 10 annuli. Even though the ventral lobe was used to provide a consistent measurement axis, the entire preparation was used to assist in interpretation. To avoid biasing age determinations, otolith readers were not informed of the collection dates of the common carp specimens.

First annulus formation.—Juvenile carp were sampled 8 times in 12 months using fyke nets (5-m single wing, 10-mm mesh bag) from Hut Lake, a wetland in the Barmah Forest associated with the Murray River (latitude −35.9120, longitude 144.9931). We confirmed age at formation of the first otolith annulus in samples taken from a juvenile cohort that was observed in length-frequency distributions.

Annuli periodicity.—The frequency with which common carp deposit otolith annuli was determined using the mark–recapture of chemically marked fish. The technique uses a fluorescent mark ''time stamp'' produced by administering a fluorochrome chemical to the fish (Kobayashi et al. 1964).

Field crews captured 141 common carp from local Victorian waterways. During January, February, and June 1999, we electrofished carp from five locations: (1) Meggitts Lagoon (n = 12); (2) Elliotts Lagoon (n = 46); (3) Lake Mokoan (n = 2); (4) Lake Eildon (n = 68); and (5) Lake Nagambie (n = 13). We maintained the carp in laboratory tanks for 2 d to observe any injuries or mortality resulting from capture. The adult common carp were anesthetized by immersion in a 1 : 3,000 solution of tricaine methanesulfonate (MS-222). We measured the fork length (LCF, mm) and total weight (g) of each fish. All fish were of unknown age and ranged from 200 to 700 mm LCF and 150–8250 g. Laboratory staff then gave each fish an intraperitoneal injection of oxytetracycline hydrochloride (OTC; as Terramycin/MA [injectable solution]) at a dose of 50 mg/kg body mass, and a numbered dart tag (Hallprint) placed into the dorsal muscle just below the dorsal spine.

We injected batches of common carp in January, February, and June 1999. We gave tagged and injected fish a salt bath (NaCl) the day after the treatment to reduce the likelihood of subsequent infections, and observed the fish for a week to ensure that treatment and handling caused no short-term adverse affects. OTC-marked common carp were stocked into a permanent lagoon system (Elliott's Lagoon) on the Goulburn River floodplain (latitude −37.2470, longitude 145.8050) which had an existing, reproducing, wild stock of common carp.

Field crews seined about 2000 young-of-year common carp in February from Hut Lake and

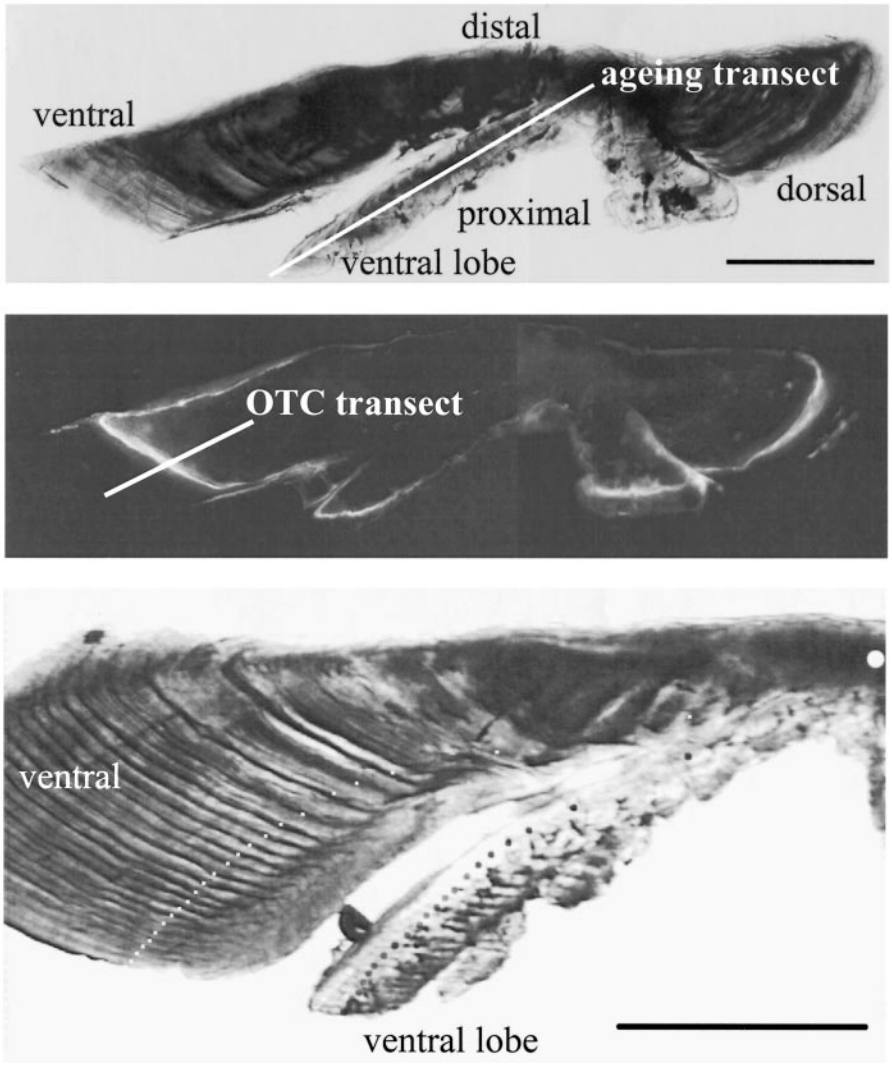

FIGURE 1.—Photomicrograph using transmitted light showing (top) the orientation of a thin section common carp otolith and the transect used for counts of annuli, scale bar = 300 μm; (center) the same otolith section under incident ultraviolet light showing an oxytetracycline (OTC) mark and the transect used for measuring its position ; and (bottom) detail of the increment structure on a clear specimen as a guide to interpretation showing annual increments on two transects. Black dots indicate where annuli were marked and measured. White dots indicate an alternative aging plane used to help in the interpretation. A large white dot indicates the position of the primordium. Scale bar = 1 mm.

transferred them to laboratory tanks. The daily mortality rate of these juveniles was initially high so we maintained them on artificial diets for 3 months until mortalities had stabilized.

We marked the small fish by immersion in a buffered OTC solution (0.5 g/l) for 12 h (McFarlane and Beamish 1987). A single exposure to OTC was carried out in two batches—one in March, the other in June. Following marking, we also stocked about 1000 young-of-year common carp (which were not externally marked due to their small size) into the Elliott lagoon system.

Initial sampling to confirm mark status.—During June 1999, 6 months after OTC marking and the release of the first batch of common carp, we recaptured and sacrificed two adults. We sectioned, mounted, and photographed their otoliths under ultraviolet light to verify that adequate marking

had occurred. An obvious fluorescent mark was observed near the otolith edge of both specimens.

Sampling 1 and 2 years after marking.—During January and February 2000, and February 2001, we caught 45 and 40 common carp, respectively, from Elliott's Lagoon using electrofishing, gill nets, angling, and a seine. We identified 10 of these common carp as being OTC-marked fish by their tags, but tag loss appeared high. We tentatively identified nine other common carp as OTC-marked fish by the presence of tagging scars. To be certain, we collected the otoliths from the entire sample in both years.

OTC mark analysis.—We prepared otolith thin sections 2 years after the initial marking and release of the OTC-treated common carp. We assessed the presence of OTC marks by examination for fluorescence under a Leitz Laborlux compound microscope fitted with a 100-W incident ultraviolet light source, and a Lietz I2 filter block (exciting filter 450–490 nm) to suit the fluorescent properties of OTC (Birk 1984). Where present, the OTC mark appeared as a bright yellow band. Increment measurements from the primordium to the otolith edge were not suitable due to the low magnification required and the difficulty of replicating the exact transect when aging the fish. Therefore, we used fluorescent light to determine the position of the OTC by measuring the distance between the mark and the otolith edge on the proximal-ventral side. We used this measurement to determine the position of the OTC mark in samples viewed using transmitted light. Under transmitted light, the position of an OTC mark can appear as a growth discontinuity within the otolith, probably reflecting the stress of the capture and marking process. Once we located the position of the OTC mark under transmitted light, we made a more accurate measurement from the mark to the edge using a compound microscope at 40× magnification (Figure 1). We took all measurements along a transect perpendicular to the increments and measured the distance between the otolith edge and the outer edge of each opaque increment outside the OTC mark. We measured up to four increments and calculated the number of increments present after the OTC mark.

Precision of age determination.—We used otoliths from a total of 6,110 common carp from 15 locations around Victoria for age determination over a 3-year period. The primary reader (CG) determined 5,257, and a secondary reader (KKG) determined 853. To assess drift in precision over time, about 20% of the samples were reread by the primary (n = 1,068) and secondary (n = 230) readers. We used an age bias plot to assess the level of bias between readers (Campana et al. 1995). This type of graph plots the mean age determination of reader 1 (with 95% confidence intervals) corresponding to the age categories determined by reader 2. The equivalence line (reader 1 = reader 2) is also plotted. The confidence intervals are not used for statistical comparisons but allow for an informed interpretation of any difference between the observations and the equivalence line. Confidence intervals overlapping the equivalence line indicate no detectable bias between the readers.

For the statistical comparison of age determination consistency both within and between readers, we calculated bias-corrected, mean average percent error (APE; Beamish and Fournier 1981) and 95% confidence intervals on the mean using a bootstrapping procedure (Efron and Tibshirani 1993) suggested in Morison (1998). We calculated APE as

$$\mathrm{APE}_j = 100\% \times \frac{1}{R}\sum_{i=1}^{R}\frac{|x_{ij} - x_j|}{x_j},$$

where X_{ij} is the ith age determination of the jth fish, X_j is the mean age estimate of the jth fish, and R is the number age determinations for each fish. We used APE to estimate the coefficient of variation, another commonly used measure of precision, using the linear relationship CV = −0.15 + 1.41APE to allow comparisons with the published literature (Campana 2001).

Results and Discussion

First Annulus Formation

The first annual increment began to form by 30 November 1999 and was almost complete by 21 December 1999. The length-frequency distribution and age determination for juvenile common carp sampled from Hut Lake (Figure 2) showed predominantly age-0 fish. The only fish that showed a single annulus (age 1) were greater than 200 mm LCF. By June 1999, the sample length-frequency distribution suggested that the age-0 cohort had a modal length of ~85 mm LCF, and again we observed some age 1 fish (>120 mm LCF). By August and September 1999, only age-0 fish remained in the sample, ranging in size from 70 to 160 mm LCF. By November 1999, 30% of the fish sampled showed their first increment. By December 1999, the first annulus was observed on 92% of the individuals within the sample. In January 2000, in

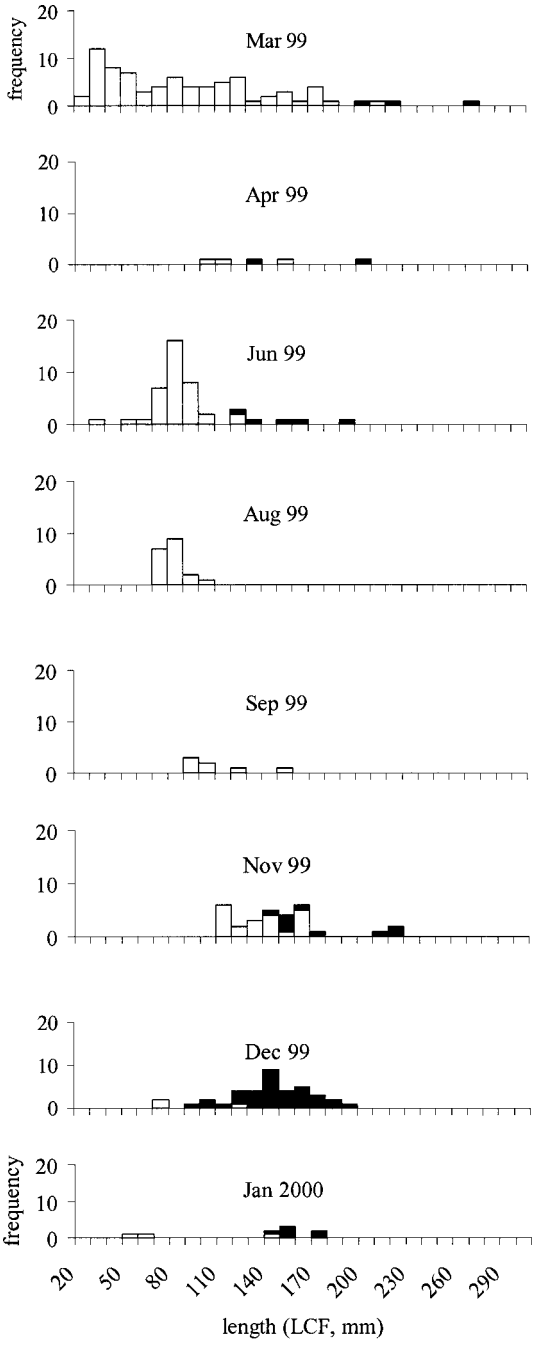

FIGURE 2.—Time series of length-frequency distributions for monthly samples of carp (<300 mm LCF) from Hut Lake in the Barmah Forest in 1999. Clear bars are age-0 common carp, and solid bars are age-1 common carp.

addition to the original cohort (now >90 mm LCF and age 1), the presence of a new age-0 cohort of fish less than 80 mm LCF was evident.

Our regular sampling of this juvenile cohort was equivalent to ''sampling length modes for age structure'' that is described as a viable method for validating the interpretation of annuli in young fish (Campana 2001). Studies of juvenile common carp growth and ontogeny, as well as the absence of any visible annuli on the otoliths of the 1999 juvenile cohort from Hut Lake, support our assumption that this cohort was composed of age-0 fish (Chakrabarti and Jana 1992; Szumiec 1997; Vilizzi 1998). Accepting this assumption, sampling length modes for age structure is essentially the validation of absolute age for age-1 common carp (one of the key requisites of age validation; Campana 2001).

Annuli Periodicity

We confirmed that annulus counts made from thin sections are a reliable indicator of age for common carp age 3–14 years. Two years after the initial marking procedure,examination of the otoliths from recaptured common carp showed OTC marks in eight that were tagged, six that had a tag scar, and five that had no tag or recognizable tag scar. None of the age-2 carp, potentially from the immersion experiment, had visible OTC marks. Common carp sacrificed 1 year after OTC injection all had a single increment outside the OTC mark. Common carp sacrificed 2 years after OTC injection had two increments outside the OTC mark (Table 1). The age range of these fish was 3–14 years.

Recovery of OTC-marked fish was most successful in adult fish (McFarlane and Beamish 1987). However, the lack of OTC marks detected in recaptured juvenile common carp that had been marked by immersion may not have been a failure of the marking process itself. Rather, the poor survival of stocked juveniles, the dilution of marked juveniles among natural recruits, or some combination of these factors may have been responsible. OTC immersion successfully marked common carp larvae from the Murray River in South Australia and was used to validate daily increment periodicity in common carp larvae up to 5 weeks old (Vilizzi 1998).

All but one recaptured common carp with retained tags showed positive growth-in-length increments. Growth of fish in our study was typical of growth rates and variability observed previously in other common carp stocks (Lorenzen 1996;

TABLE 1.—Details of common carp observed with oxytetracycline (OTC) mark in otolith sections. For recaptured fish that had shed their tags, time at liberty can be estimated from the three injection dates; a = tag not present on recapture although a tag scar was.

Age determination (years)	Date injected	Date of recapture	Tag number on recapture	Time at liberty (months)	Number of annuli outside OTC mark
4	Jan 1999	Jan 2000	1053	12	1
5	Jan, Feb, or Jun 1999	Jan 2000	a	6–12	1
8	Jan, Feb, or Jun 1999	Jan 2000	a	6–12	1
10	Jan 1999	Jan 2000	1713	12	1
12	Jan, Feb, or Jun 1999	Jan 2000	a	6–12	1
14	Feb 1999	Jan 2000	1710	11	1
3	Jan, Feb, or Jun 1999	Feb 2000	a	7–13	1
4	Jan, Feb, or Jun 1999	Feb 2000	a	7–13	1
5	Jan, Feb, or Jun 1999	Feb 2000	a	7–13	1
7	Jan 1999	Feb 2000	0679	13	1
7	Feb 1999	Feb 2000	1703	12	1
10	Jan, Feb, or Jun 1999	Feb 2000	a	7–13	1
11	Jan, Feb, or Jun 1999	Feb 2000	a	7–13	1
5	Jan, Feb, or Jun 1999	Feb 2001	a	20–25	2
6	Jan 1999	Feb 2001	1077	25	2
7	Jan, Feb, or Jun 1999	Feb 2001	a	20–25	2
9	Jun 1999	Feb 2001	1825	20	2
10	Jan 1999	Feb 2001	1063	25	2
10	Jan, Feb, or Jun 1999	Feb 2001	a	20–25	2

Soller et al. 1965; Vilizzi and Walker 1999a, 1999b).

We could not be certain of including older common carp within the trial until we recovered the marked fish. However, we provide evidence that the periodicity of annulus formation in adult common carp age 3–14 years was annual. Furthermore, outer otolith increments in fish older than age 14 were similar to otolith increments in fish younger than age 14, suggesting that the process of increment formation is also annual in older common carp.

Precision of Age Determination

An age bias plot showed no systematic bias between primary and secondary readers in the reread samples ($n = 228$). The 95% confidence intervals on the mean age for the primary reader plotted against the age determinations of the secondary reader overlapped with the primary–secondary reader equivalence line.

Estimated, bias-corrected mean APE (95% confidence interval) for reread samples from the primary reader ($n = 1068$) was 4.56% (4.14–4.97%); for the secondary reader ($n = 230$), 4.04% (2.67–5.42%); and between readers ($n = 228$), 4.98% (4.18–5.79%). The estimate of mean CV for the reread samples from the primary reader was 6.41%; for the secondary reader, 5.55%; and between readers, 6.90%. The overlapping 95% confidence intervals for APE suggested that there was no difference in the precision of age determination between readers.

Our precision was better than that of other aging studies of common carp using otoliths. Mean APE values were reported for within (>12%) and between-reader (~ 6%) precision in whole common carp otoliths (Vilizzi 1998). Poor precision may be a result of variability in otolith structure, a lack of clarity of increments, or the skill of the reader. Variation between species and among the different calcified structures used for aging hinders the assigning of any target level of precision a priori (Campana 2001; Morison et al. 1998). However, expectations for APE values of 5% are typical across a variety of species and structures being aged (Morison 1998). Many aging studies yield a precision level of less than 5.5% for APE or less than 7.6% for CV (Campana 2001). Our levels of precision, both within and between readers, are therefore typical.

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