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## Age and growth of the common carp, *Cyprinus carpio*, in the River Murray, Australia: validation, consistency of age interpretation, and growth models

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### Synopsis

The present study validates age estimates from a suite of calcified structures (scales, opercular bones and otoliths), assesses the consistency of age interpretations and evaluates growth models in common carp from the lower Murray River, Australia. Marginal increment analysis was used to validate annulus counts, with attention to the 'edge interpretation problem'. The formation of annuli occurred annually after pooling annulus groups, and in carp 4 and 5 years old. The regular alternation of opaque and translucent zones in opercular bones and whole otoliths of younger and older carp was also suggestive of annual periodicity. From systematic comparisons, the use of both opercular bones and whole otoliths in routine age determinations is recommended. Six growth models, including the von Bertalanffy growth function (VBGF) and five polynomial curves were tested to describe growth in length. A log-log quadratic function, by virtue of its precision, and the VBGF, with wider applicability and more biological realism, were chosen.

### Introduction

There are virtually no supporting data on the age and growth of common carp, *Cyprinus carpio* L., in Australia. Jones (1974) and Hume et al.<sup>1</sup> investigated methods for interpreting the ages of carp in the Murray-Darling Basin, but encountered problems related to variable annual growth rates between individuals and protracted spawning periods. In south-eastern Australia, concern over the spread of common carp and their impact on rivers and wetlands has encouraged researchers to seek methods

for control (Brumley 1991, Harris 1995). Population age data are helpful to assess the suitability of control methods (Roberts & Tilzey 1997), and to allow determination of recruitment and year-class strength (cf. Mann 1991).

Various methods for assessing the reliability of fish age interpretations have been developed in the last 20 years (Campana et al. 1995), and an indication of the consistency of estimates now is essential. Percent agreement ( $PAG_{i,j}$  = fish/structure), a simple measure of precision, has been superseded by measures like the average percent error ( $APE_i$ ; Beamish & Fournier 1981), the coefficient of variation ( $V_i$ ; Chang 1982) and the index of precision ( $D_i$ ; Chang 1982), all independent of the ages of the fish. There are also graphical methods (e.g. the age bias plot of Campana et al. 1995) to indicate linear and

<sup>1</sup> Hume, D.J., A.R. Fletcher & A.K. Morison. 1983. Carp program – final report. Report No. 10, Arthur Rylah Institute for Environmental Research, Fisheries and Wildlife Division, Ministry for Conservation, Victoria. 214 pp.

non-linear age estimation bias, another key to consistent interpretations. Calcified structures like scales, otoliths, flat bones, spines and vertebrae often have been used separately to interpret the age of fish, and other studies have compared suites of structures to corroborate age interpretations or resolve problems with identification of structure-dependent periodic patterns (e.g., Maraldo & MacCrimmon 1979, Erickson 1983, Sharp & Bernard 1988). In cyprinids, scales and, to a lesser extent, opercular bones are widely used (Mann 1991), and otoliths (especially lapilli) also have been employed successfully (e.g., Backe-Hansen 1982, Mills 1988, Mina 1989, O'Maoileidigh & Bracken 1989). In common carp there are several potential 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.

Modelling fish growth is an important outcome of studies designed to interpret the age profiles of stocks from their calcified structures. Thus, it is often of interest to compare the growth rates of fish stocks from different geographical areas, or of males and females within the same stock, or to describe year-to-year variation in growth as a response to changing abiotic and biotic factors (e.g., Weatherley & Gill 1987). However, no general agreement exists amongst fish and fisheries biologists as to which mathematical model would best describe growth (reviews in Ricker 1979, Moreau 1987).

The present study is designed to (1) validate the accuracy of age assessments, (2) evaluate the consistency of age interpretations, and (3) model growth in a stock of common carp from the River Murray in South Australia.

## Materials and methods

### Terminology

In the present study the terms *calcified structure* or *structure* are used interchangeably, and the generic

term *otolith* refers to the asteriscus (lagenar otolith). The term *annulus group* (for fish with the same number of annuli on a structure) applies to the validation study, whereas *age group* is reserved to an annulus group where formal validation is deemed to have been achieved. *Interpreter* is used as synonymous with and is preferred to *ager* or *reader*, and the same applies to *interpretable* and *uninterpretable* with respect to *readable* and *unreadable*, respectively. Similarly *annulus count* or, *count* is equivalent to *annulus reading* or *reading*, and *interpreting the age* of a fish is preferred to *ageing* a fish. Finally *estimated* age instead of *age* is employed in referring to growth models; this is determined by the presence of both validated and unvalidated annulus groups in the data set/model combinations.

### Validation

*Sampling and preparation.* – In total 603 common carp were collected by gill-netting in backwaters of the River Murray in South Australia, using monofilament nets of 20, 50, 75, 100 and 150 mm stretched mesh. Most fish ( $n = 519$ ) were obtained from monthly samples at Gurra Lakes, near Berri (34°18' S, 140°41' E), between July 1994 and July 1995. Small samples ( $n = 35$ ) were taken from Sunnyside, near Murray Bridge (34°01' S, 138°16' E), and from Swan Reach (34°34' S, 139°36' E) ( $n = 49$ ) between October 1993 and March 1994.

From 6–10 scales were removed from the antero-medial region of the body immediately above the lateral line, where normal scales usually are found in carp (Bagenal & Tesch 1978). The scales were cleaned of mucus and stored in paper envelopes. From 4–8 nonregenerated scales per fish, when possible, were mounted between glass slides after soaking briefly in 20% sodium hypochlorite and rinsing in distilled water.

Opercular bones were excised following McConnell (1952) and stored in water for a few weeks. This provided near-complete maceration of the attached tissue, so that later 5–10 min immersion in undiluted sodium hypochlorite and rinsing in water was sufficient preparation for analysis.

Asterisci<sup>2</sup> were recovered by the 'open-the-hatch' method, cleaned by soaking for a few minutes in 5% sodium hypochlorite, then rinsed in distilled water and 90% ethanol, following Secor et al. (1991). The reasons for choosing asterisci over lapilli or sagittae as indicators of age in carp were explained in a previous paper (Vilizzi & Walker 1995).

*Annulus identification.* – There are differences in the criteria for identification and counting of annuli on the various calcified structures. Techniques for carp scales and opercular bones are well known, but there is little information for otoliths; these are emphasised below.

The criteria of Chugunova (1963) were followed for scales, which were inspected with a dissecting microscope (10–20X) under transmitted light. An annulus is taken as a transition between two uninterrupted zones of closely- and widely-spaced circuli, with anastomosis as an essential criterion for identification. The scale radius and distance of each annulus from the focus (eye-piece units: EPU) were measured along the antero-ventral axis using an ocular micrometer (15X). The outer circuli between the last-formed annulus and the anterior margin were also counted. Scales from mirror carp (1.2% of the entire sample) could not be interpreted for age, contrary to Das & Fotadar (1965).

The methods of McConnell (1952) and English (1952) were adopted for interpretation of opercular bones. In this case an annulus is taken as a sharp transition between a translucent and an opaque zone, normally a continuous narrow ridge on the proximal (concave) surface. Annuli were counted by eye under transmitted light; their visibility usually improved after wetting with water. For older fish, observation of the distal surface under transmitted light sometimes was useful in determining the position of the first and, occasionally, the second

annulus, normally obliterated by ossification around the fulcrum. The opercular radius, taken as the distance from the centre of the fulcrum to the midpoint of the posterior margin of the bone, and the distance of each annulus from the fulcrum (along the same radius) were measured with dial callipers ( $\pm 0.05$  mm).

Wichers (1976) and Hume et al.<sup>1</sup> reported failed attempts to interpret otoliths for age, but did not indicate which pair was used. In apparently the only two studies where common carp otoliths have been used successfully (Raina 1987, Pinilla et al. 1992), neither the otoliths nor the criteria were specified. The morphology of carp asterisci invalidates the use of standard terminology (e.g., Jensen 1965, Pannella 1980), and Berinkey's (1956) scheme is used here. Whole otoliths are best viewed immersed in water, with the distal side facing the observer and the ventral margin up, using a dissecting microscope (20–40X) and a dark background (reflected oblique light). Under these conditions an annulus is seen as a transition between a translucent (dark) and an opaque (light) zone. Annuli were counted on the distal side of the otolith, along the antero-dorsal region, at the level of the dorsal spike, a projection analogous to the antirostrum of the non-otophysan teleost sagitta (Pannella 1980). When fewer than 5–6 annuli were present they generally could be followed through to the posterior and ventral side, an important feature that distinguishes true from false annuli (*pseudoannuli*), which appeared as discontinuous transitions. When more annuli were present this criterion could not be applied because of their crowding towards the ventral region. Increments (paired alternate opaque and translucent zones taken to represent one year of growth) often were composed of an irregular number of pseudoannuli. Three morphological classes of otoliths can be defined to aid interpretation of changes in zone patterns (the same features, with minor variations, apply to opercular bones):

- Class 1: all increments include an opaque and a translucent zone, without pseudoannuli (Figure 1a);
- Class 2: increments as above but lacking a sharp transition between translucent and opaque zones, although annuli are recognisable (Figure 1b);

<sup>2</sup> In carp, as in all otophysan fish (ostariophysii sensu Fink & Fink 1981), the pars inferior of the inner ear is distinguished by the presence of the Weberian apparatus, a series of ossicles connecting the swimbladder to the inner ear (Fay & Popper 1980). These are responsible for the peculiar morphology of the sagittae and asterisci, a point often overlooked and responsible for some terminological confusion (e.g., Fagade 1980, Landau et al. 1988, Mina 1989; see also Vilizzi & Walker 1995).

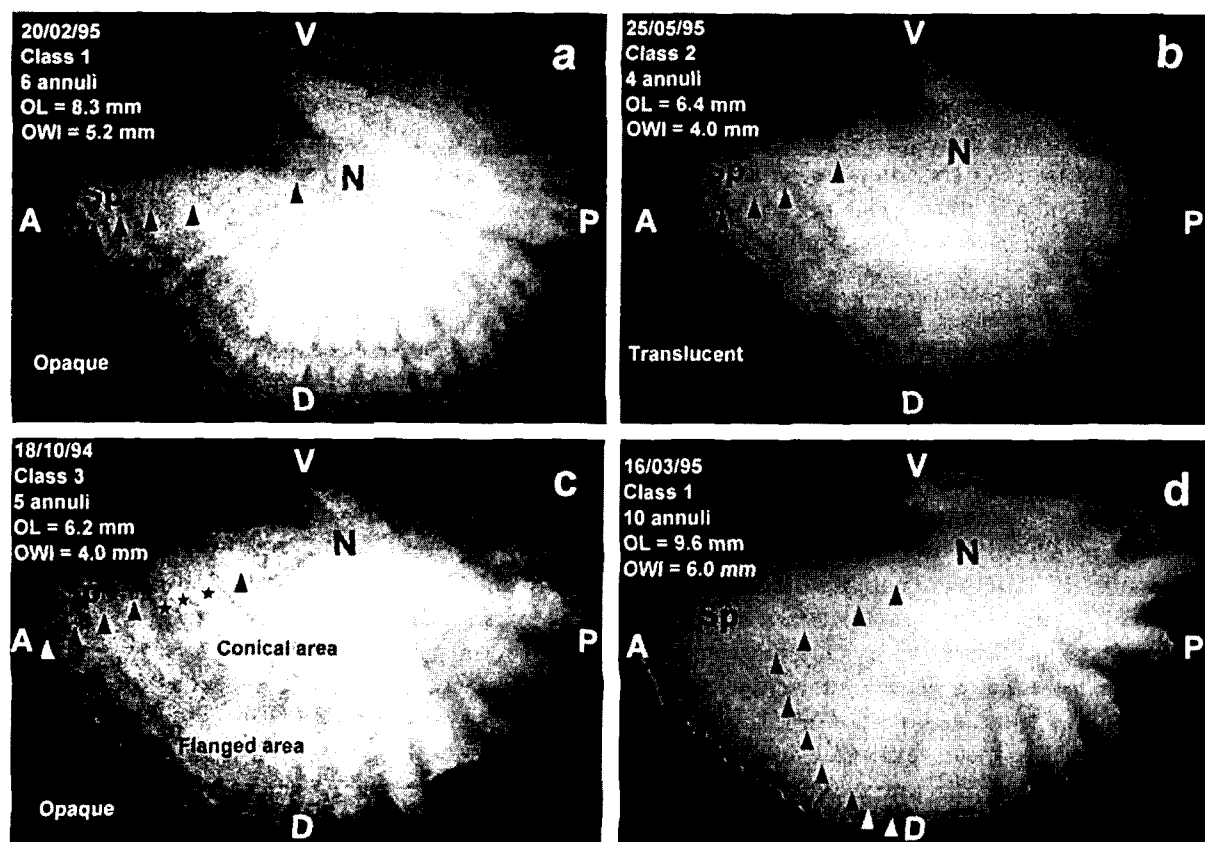


Figure 1. Morphological classes of carp otoliths (asterisci) defined to aid interpretation of changes in zone patterns. Under reflected oblique light on a dark background an annulus is a transition between a translucent (dark) and an opaque (light) zone, here indicated with a triangle. False annuli (pseudoannuli) are marked by an asterisk. The edge type (i.e. opaque or translucent), the date of capture of the fish and the number of annuli are reported. (Sp = antero-dorsal spike; A = anterior edge; P = posterior edge; D = dorsal edge; V = ventral edge; N = nucleus; OL = otolith length; OWI = otolith width). a – Class 1 (6 annuli), opaque edge, summer; b – class 2 (4 annuli), translucent edge, late autumn; c – class 3 (5 annuli), newly-formed opaque zone, spring; d – class 1 (10 annuli).

- Class 3: pseudoannuli present in the second increment. The region between the nucleus and the second annulus is often conical, or at least protruding, and further annuli, when present, are located in a flanged area (Figure 1c).

In fish with more than 6–7 annuli further deposition of calcium carbonate on the dorsal side of the otolith causes increased curvature. Under these conditions the translucent and opaque zones are closely spaced and alternate regularly, assuming a tiered arrangement (Figure 1d).

When possible the left otolith of each pair was embedded in clear polyester resin in 8-mm thick blocks (about 62 mm<sup>3</sup>), each containing 10–14 otoliths (depending on their size) with their nuclei carefully aligned. Three successive transverse sec-

tions (0.4–0.5 mm) were cut through the nucleus using a low-speed lapidary saw (Bedford 1983, Augustine & Kenchington 1987). Sections were polished with 1200-grit carborundum paper to enhance the visibility of the zones. The sections were examined under the same lighting conditions used for analysis of whole otoliths (transmitted light proved unsatisfactory), and the morphological classes described above were applied. A measure of otolith depth could not be obtained from the sections, as calcium carbonate deposition in older fish was almost exclusively limited to the dorsal side of the otolith, and did not cause a corresponding increase in overall depth. Attempts to find a reliable measure of dorsal curvature were unsuccessful.

The distance of each annulus from the nucleus

and the distance from the nucleus to the edge (*radius*) were measured ( $\pm 0.005$  mm) using a dissecting microscope linked via camera to a desktop computer and image analysis software. In whole otoliths, measurements were taken along an imaginary line parallel to the ventral edge of the antero-dorsal spike. In sectioned otoliths, the annuli were measured along a line connecting the nucleus to the mid-point of the dorsal margin. When there was consistent curvature on the dorsal side (in sectioned otoliths from older fish) measurements were taken along two lines, the break being where the change in curvature occurred. No attempt was made to evaluate the bias of these measurements.

*Data analysis.* – Marginal increment analysis (MIA) was used to validate counts of annuli. This is a two-step procedure consisting of marginal increment ratio analysis (MIRA) and edge type analysis (ETA) (Haas & Recksiek 1995). The ‘edge interpretation problem’ (Francis et al. 1992) was also addressed.

MIRA calculation of the marginal increment ratio (MIR) in all calcified structures was based on the formula:

$$\text{MIR} = [(CE - CLA) / (CLA - CSA)] \times 100,$$

where: CE = distance from centre of origin (C: focus in scales, fulcrum in opercular bones, nucleus in otoliths) to the outer edge (E) (thus, CE is equivalent to the radius); CLA = distance from centre of origin to the last-formed annulus (LA); CSA = distance from centre of origin to the second last-formed annulus (SA). When only one annulus is present the denominator in the formula is the distance of the annulus from the centre of origin (CLA).

For ETA initially the margins of opercular bones, whole and sectioned otoliths were recorded simply as opaque or translucent (opaque-translucent OT analysis or OTA). However, to identify more precisely the edge in the calcified structures, a modification of a technique proposed by Anderson et al. (1992a, b) was used (CZN analysis or CZNA): from the MIR value the last-formed annulus was judged as ‘C’ (close), ‘Z’ (other) or ‘N’ (now due). For scales and opercular bones the edge was scored C if

MIR  $\leq 25\%$ , Z if  $25\% < \text{MIR} < 75\%$ , and N if MIR  $\geq 75\%$ . In whole and sectioned otoliths it was not possible to detect a newly-formed opaque zone when its width was less than 10–40% of the previous increment, and the edge was scored C if MIR  $\leq 50\%$ , Z if  $50\% < \text{MIR} < 75\%$ , and N if MIR  $\geq 75\%$ .

Values for MIR (all structures), number of outer circuli (scales), OT (opercular bones and otoliths), and CZN (all structures) were plotted against month of collection for pooled annulus groups and for annulus groups 4 and 5, where sufficient sample sizes were available. For each of the resulting time series the corresponding autocorrelation coefficients ( $r_k$ ) were then calculated and plotted as a correlogram against the number of lags ( $k$ ). Tests of significance for departure from white noise were at  $\alpha = 0.05$ . In the evaluation of the correlograms more emphasis was given to the overall pattern of the data than to the precise numerical values of individual  $r_k$  (Diggle 1990). Finally, frequency distributions of MIR values for each month and structure examined were plotted for pooled annulus groups.

#### *Consistency of age interpretation*

*Experimental layout.* – Two interpreters (A and B) counted annuli on the structures under study. Annulus counts were made initially by interpreter A (LV) on 471 scales, 468 opercular bones, 563 whole otoliths and 546 sectioned otoliths. The overall interpretability of each structure was also evaluated in this first count. Whenever a pattern (closely- and widely-spaced circuli in scales or alternating opaque and translucent zones in opercular bones and otoliths) could not be consistently identified the preparation was scored as *uninterpretable*. All *interpretable* structures, except sectioned otoliths, were then examined a second time by interpreter A, whereas uninterpretable structures were discarded, with small numbers of opercular bones and whole otoliths ( $n = 5$ ) from 0+ fish, lacking annuli. This was two months after the first count, and involved 380 scales, 440 opercular bones and 417 whole otoliths. Finally a single count by interpreter B was made on the sample of interpretable scales, opercular bones and whole otoliths examined by interpreter

er A. Both interpreters in all counts had no indication of the date of capture, length or weight of the fish.

**Data analysis.** – The interpretability of the structures, the visibility of annuli (especially the first and second) and the bias and precision of annulus counts were determined. As sectioned otoliths provided annulus counts comparable to those from whole otoliths throughout the range of annuli enumerated, they were eventually discarded. Subsequent counts were on scales, opercular bones and whole otoliths (i.e. second count by interpreter A; single count by interpreter B).

The within-interpreter A bias and precision of counts on all structures were analysed by statistical and graphical methods. For each pair of replicate counts on each structure this included (1) an age frequency table<sup>3</sup>, (2) an age bias plot, (3) the index of average percent error (IAPE) and the mean coefficient of variation (V) for pooled annulus counts, (4) IAPE, the mean coefficient of percent agreement (PAG) and an index of percent bias (Kimura & Lyons 1991), and (5) parameter estimates for the von Bertalanffy growth function (VBGF). PAG, IAPE and V were obtained by averaging  $PAG_i$ ,  $APE_i$  and  $V_i$ , respectively, across preparations of the same type (Campana et al. 1995). Following Kimura & Lyons (1991), the nominal age (= number of annuli) used to compare replicate annulus counts by interpreter A on the same structure at each annulus group was the rounded mean. Further, in comparing annulus counts between structures, and between counts by interpreters A and B on scales, opercular bones and whole otoliths, the coefficient of variation, an age frequency table and an age bias plot were used. VBGFs were fitted to length-at-annulus data from the annulus counts by interpreters A and B on the three structures. The first annulus count by interpreter A for scales and opercular bones was arbitrarily used in comparisons between interpreters A and B for the corresponding structures. For whole otoliths the second count by interpreter A was instead used, as this was considered to be more reliable (see Discussion).

The reproducibility of replicate counts for opercular bones and whole otoliths was tested by two-way nonparametric ANOVA (Zar 1984) to determine whether a significant interaction occurred between morphological class and number of annuli counted. The factors were the type of annulus pattern on the structure (classes 1–3, as defined above) and the combined annulus groups (arbitrarily: I = 1–3 annuli, II = 4–7 annuli, III  $\geq$  8 annuli). The response variable was the coefficient of variation V, based on three replicate counts (the two counts from interpreter A and the only count from interpreter B).

### Growth models

**Sample measurement** – Fish were measured for fork length (FL,  $\pm$  1 mm) (hereafter 'length') and total weight (W,  $\pm$  1.0 g). Fish with macroscopically differentiated gonads were sexed; others were classified as juveniles. The length (OL) and width (OW) of both otoliths were measured with dial callipers ( $\pm$  0.05 mm) and the weight (OW,  $\pm$  0.1 mg) recorded with a digital balance after drying at 50 °C for 24 h (Secor et al. 1991).

*Table 1.* Computation of the adjustment in annulus counts based on a nominal birth-date of October 1 for each annual cohort of common carp in the lower Murray River, Australia. Annuli = number of annuli counted on a calcified structure; MIR = marginal increment ratio.

Month of capture	Annuli	MIR (%)	Estimated age (yr)
January	$\leq 3$		Annuli
	$\geq 4$	$\geq 75$	Annuli + 1
February	$\leq 4$		Annuli
	$\geq 5$	$\geq 75$	Annuli + 1
March–July August–September			Annuli
		$\leq 25^a$	Annuli – 1
		$\leq 50^b$	Annuli – 1
October		$\geq 75$	Annuli + 1
November	1		1
	$\geq 2$	$\geq 75$	Annuli + 1
December	$\leq 2$		Annuli
	$\geq 3$	$\geq 75$	Annuli + 1

<sup>a</sup> Scales and opercular bones.

<sup>b</sup> Whole and sectioned otoliths.

<sup>3</sup> Available upon request from the senior author.

*Designation of a 'birth-date' and ages adjustment.* – October 1 was taken as the nominal birth-date of each annual cohort of carp in the sampled stock. This choice was dictated primarily by the time of formation of new annuli in the calcified structures when values for MIR approach a minimum. Further, carp in the lower Murray generally have a major spawning event in October (L. Vilizzi unpublished).

Setting a common date in this way has implications for ages estimated from annulus counts, as an adjustment is required whenever an annulus is formed before or after the designated birth-date. Three factors must be taken into account, namely, (1) the month of capture, (2) the corresponding MIR value, and (3) the number of annuli counted on each structure (equivalent to the estimated age of the fish) (Table 1). If, regardless of the number of annuli, a fish was caught in August or September and a new annulus was already forming (hence  $\text{MIR} \leq 25\%$  for scales and opercular bones, or  $\text{MIR} \leq 50\%$  for otoliths), its estimated age was decreased by one year. If, again regardless of the number of annuli, a fish was caught in October (the nominal month of birth) and the corresponding  $\text{MIR} \geq 75\%$  (i.e. a new annulus was due), its estimated age was increased by one year. For fish caught between 1 November and 28 February a third factor, namely the number of annuli counted on the corresponding structures, comes into play. Thus, if a fish with more than one annulus was about to form a new annulus in November (i.e.  $\text{MIR} \geq 75\%$ ), its estimated age was incremented by one year. The same adjustment was made for fish with structures having more than 2, 3 or 4 annuli, captured in December, January and February respectively, with  $\text{MIR} \geq 75\%$ . No adjustment was needed for fish captured between March and July.

*Data analysis.* – For modelling purposes, (estimated) age was expressed as a decimal according to:

$$\text{age (as integer)} + (\text{days from October 1 to date of capture}) / 365.$$

Six growth models, including the VBGF, four quadratic functions with combinations of log-trans-

formed and decimal variables, and a cubic function were employed to describe growth in length, following Chen et al. (1992). Growth in weight was described using the Beverton and Holt's adaptation of the VBGF for length (Ricker 1975). Estimated ages from whole otoliths, sectioned otoliths, opercular bones and scales, in this order, were used in modelling somatic growth. This choice was dictated by the better visibility of the first annuli in the otoliths (see Discussion). Otolith growth was studied by linear and non-linear functions (Table 2), based on estimated ages from whole otoliths.

The six length-at-age models were fitted to the four data sets from examination of calcified structures. Linear models for males and females were compared for goodness of fit ( $r^2$ ) by means of a randomised blocks ANOVA, with the growth function as the fixed effect and the structures as blocks (Zar 1984). Bonferroni-corrected pairwise comparisons were then made between the resulting growth models, and VBGFs for each sex were compared by a modified procedure for analysis of the residual sum of squares (ARSS), following Chen et al. (1992).

For all VBGFs, starting values for  $FL_{\infty}$ ,  $W_{\infty}$  (for body growth),  $OL_{\infty}$  and  $OWI_{\infty}$  (for otolith growth),  $K$  and  $t_0$  were obtained by the Ford-Walford method (Kimura 1980). In fitting the VBGF to the length- and weight-at-age data for males and females the  $t_0$  value of the VBGF for the combined sexes, including juveniles, was set as a constraint in the model. This was found to provide more realistic parameter estimates.

A multiple linear regression model was employed in an attempt to estimate age from otolith and body morphometrics in males and females.  $OW$ ,  $OL$ ,  $OWI$ ,  $FL$  and  $W$ , their quadratic and cubic terms, and the interactions  $OW*OL$ ,  $OW*OWI$ ,  $OL*OWI$ ,  $FL*W$  were included in the model, with the estimated whole otolith age as the dependent variable. Models were fitted by stepwise regression, using  $\alpha = 0.10$  for both entry and exit criteria.

The Anderson-Darling statistic was employed as a test for normality (Snedecor & Cochran 1989), and homoscedasticity was tested by a variance ratio test or Bartlett's test, when applicable (Sokal & Rohlf 1981). When the assumptions of normality and homoscedasticity were not satisfied, equivalent



non-parametric tests were used. Finally, residuals from all linear and non-linear regressions were plotted against fitted values of the dependent variable to check that assumptions of independence of errors, zero mean, constant variance and normal distribution were met.

## Results

### Validation

*Marginal increment ratio analysis (MIRA).* – If a new annulus is formed once a year and the time of formation is strongly seasonal, a dip (minimal MIR values) should be evident during the period of annulus formation. Conversely, peak MIR values should be apparent during the period of little or no growth. After pooling annulus groups, a dip was evident in October–December. This was visible for all structures examined and was generally supported by the corresponding correlograms, with the possible exception for sectioned otoliths. The time of annulus formation appeared to be October/November for scales and opercular bones, and November/December for otoliths (Figures 2, 3).

A major drawback with pooling annulus groups is

that variations in timing of annulus deposition among age classes may obscure the seasonal pattern of annual increment development (Williams & Bedford 1974). Thus, a separate analysis for each annulus group is necessary if the range of validated ages is to be determined. When MIR values for all calcified structures were plotted against time for annulus groups 4 and 5 a consistent seasonal pattern was revealed. This was also evident upon examination of the corresponding correlograms (Figures 2, 3).

*Outer circuli counts of scales.* – In pooled annulus groups the number of circuli beyond the last-formed annulus was minimal in November. This pattern was still recognised when annulus groups 4 and 5 were plotted separately. In all cases a consistent pattern was also evident in the correlograms (Figure 4).

*Edge type analysis (ETA: OTA and CZNA).* – Although a higher percentage of opercular bones with an opaque margin was recorded for pooled annulus groups and annulus groups 4 and 5 during spring and summer, opaque edges were still common in July/August (Figure 4). In annulus groups 1 and 2 (not shown) all opercular bones were scored as translucent, probably reflecting the overall thinness of the

Table 2. Mathematical functions evaluated in describing the somatic and otolith growth of carp in the lower River Murray, Australia. a, b, a<sub>1</sub>, b<sub>1</sub>, c<sub>1</sub>, d<sub>1</sub> = parameters in the model; t = time expressed as a decimal; FL, W, OL, OWL, OW = fork length, total length, otolith length, otolith width, and otolith weight; FL<sub>t</sub>, W<sub>t</sub>, OL<sub>t</sub>, OWL<sub>t</sub>, OW<sub>t</sub> = fork length, total weight, otolith length, otolith width and otolith weight, respectively, at time t; FL<sub>∞</sub>, W<sub>∞</sub>, OL<sub>∞</sub>, OWL<sub>∞</sub> = asymptotic fork length, total weight, otolith length and otolith weight, respectively; K = Brody coefficient of growth; t<sub>0</sub> = theoretical time at which the length of the fish was 0 (Ricker 1975); b = exponent from length-weight relationship. Logarithms to the base 10 in LDQF, LLOF, DLOF, and to the base e in LDLF.

Type	Abbreviation	Mathematical function
Decimal-decimal quadratic (FL)	FL DDQF	$FL_t = a_1 + b_1 t + c_1 t^2$
Log-decimal quadratic (FL)	FL LDQF	$FL_t = a_1 + b_1 \text{Log}(t + 1) + c_1 \text{Log}(t + 1)^2$
Log-log quadratic (FL)	FL LLQF	$\text{Log}(FL_t) = a_1 + b_1 \text{Log}(t + 1) + c_1 \text{Log}(t + 1)^2$
Decimal-log quadratic (FL)	FL DLOF	$\text{Log}(FL_t) = a_1 + b_1 t + c_1 t^2$
Decimal-decimal cubic (FL)	FL DDCF	$FL_t = a_1 + b_1 t + c_1 t^2 + d_1 t^3$
von Bertalanffy (FL)	FL VBGF	$FL_t = FL_{\infty} (1 - \exp(-K(t - t_0)))$
von Bertalanffy (W)	W VBGF <sup>b</sup>	$W_t = W_{\infty} (1 - \exp(-K(t - t_0)))^b$
von Bertalanffy (OL)	OL VBGF	$OL_t = OL_{\infty} (1 - \exp(-K(t - t_0)))$
Ln-decimal linear (OL)	OL LDLF	$OL_t = a_1 + b_1 \ln(t)$
von Bertalanffy (OWI)	OWI VBGF	$OWI_t = OWI_{\infty} (1 - \exp(-K(t - t_0)))$
Ln-decimal linear (OWI)	OWI LDLF	$OWI_t = a_1 + b_1 \ln(t)$
Decimal-decimal linear (OW)	OW DDLF	$OW_t = a_1 + b_1 t$
Length-weight relationship	FLWR	$W = a FL^b$

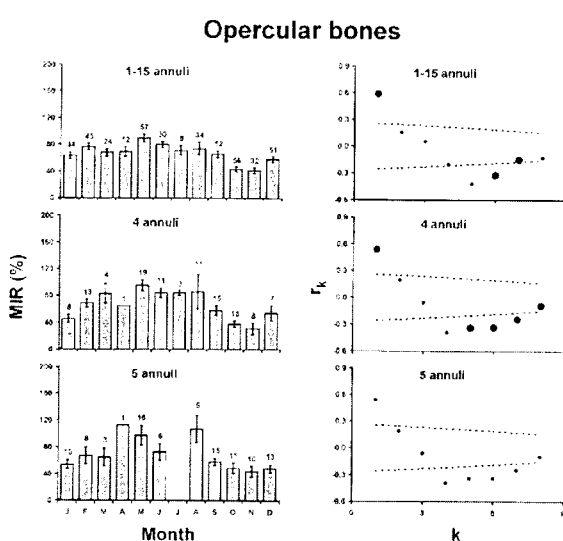
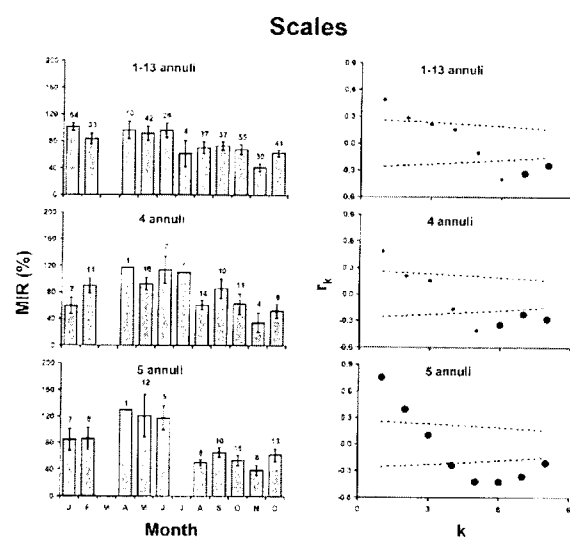


Figure 2. Mean monthly values ( $\pm$  SE) of the marginal increment ratio (MIR), expressed as a percentage of the previous increment, in scales and opercular bones of carp from the lower Murray River, Australia. Sample sizes are indicated. Data for pooled annulus groups and for annulus groups 4 and 5 are shown. For each time plot the corresponding correlogram is given. ( $r_k$  = autocorrelation coefficient;  $k$  = lag number). Broken lines are white noise standard errors. Larger circles indicate significant  $r_k$  values ( $\alpha = 0.05$ ).

bone when less than three annuli were present. For the pooled annulus groups the percentage of whole otoliths with an opaque edge consistently increased from October to December and decreased from January to March. When annulus groups were examined separately, the percentage of whole otoliths

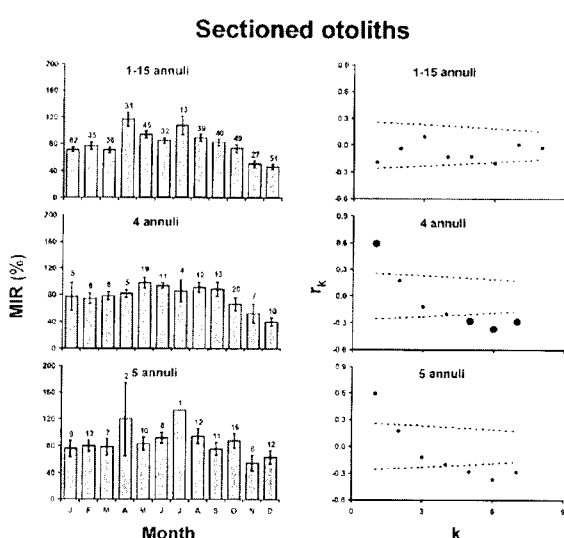
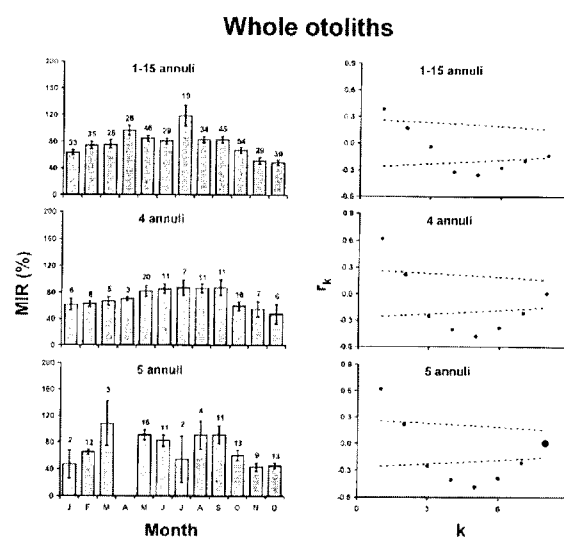


Figure 3. Mean monthly values ( $\pm$  SE) of the marginal increment ratio (MIR), expressed as a percentage of the previous increment, in whole and sectioned otoliths of carp from the lower Murray River, Australia. Sample sizes are indicated. Data for pooled annulus groups and for annulus groups 4 and 5 are shown. For each time plot the corresponding correlogram is given. ( $r_k$  = autocorrelation coefficient;  $k$  = lag number). Broken lines are white noise standard errors. Larger circles indicate significant  $r_k$  values ( $\alpha = 0.05$ ).

with an opaque edge was still higher in late spring/early summer in annulus groups 4 and 5. In annulus group 1 (not shown) translucent edges occurred almost year-round, again because of the rapid development and relatively small width of the first opaque zone. Curiously, no consistent pattern was

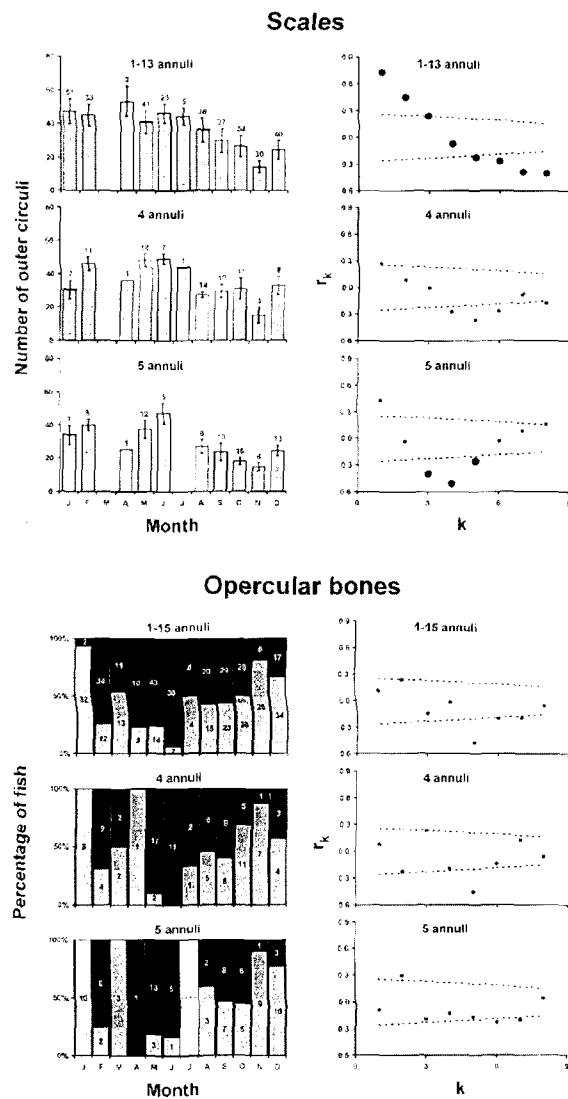


Figure 4. Number ( $\pm$  SE) of scale circuli beyond the last-formed annulus, and percentage of opercular bones with an opaque (gray bars) or translucent (black bars) margin (under transmitted light) in carp from the lower Murray River, Australia. Sample sizes are indicated. Data for pooled annulus groups and for annulus groups 4 and 5 are shown. For each time plot the corresponding correlogram is given. ( $r_k$  = autocorrelation coefficient;  $k$  = lag number). Broken lines are white noise standard errors. Larger circles indicate significant  $r_k$  values ( $\alpha = 0.05$ ).

identified in sectioned otoliths: opaque zones in the proximity of the margin were present in most annulus groups throughout the year. Upon visual evaluation of the time plots, a consistent pattern in the correlograms was identified only for whole otoliths (Figure 5).

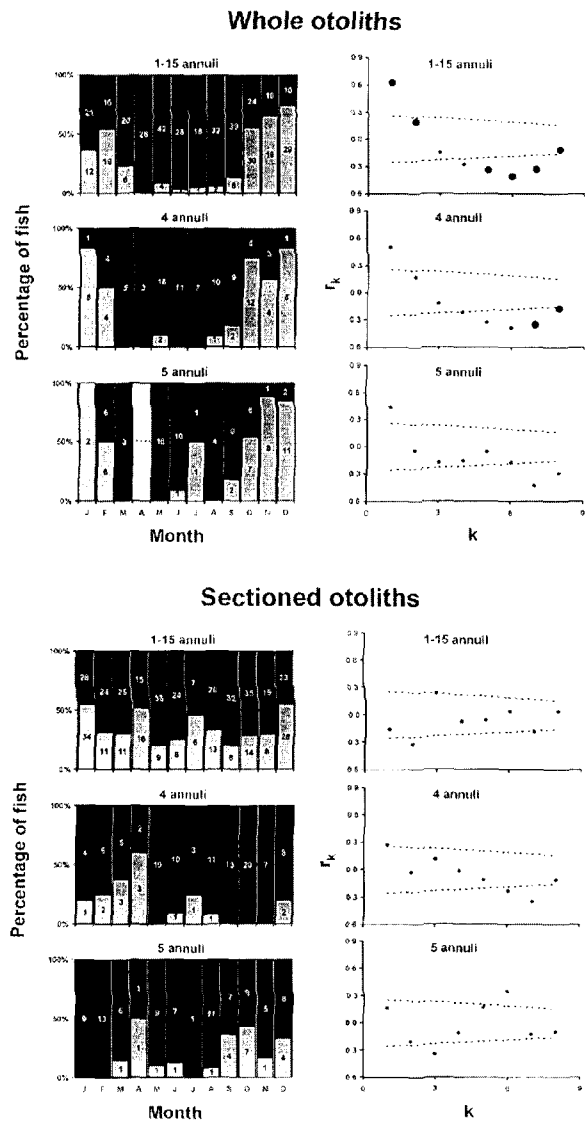


Figure 5. Percentage of whole and sectioned otoliths with an opaque (gray bars) or translucent (black bars) margin (under reflected light) in carp from the lower Murray River, Australia. Sample sizes are indicated. Data for pooled annulus groups and for annulus groups 4 and 5 are shown. For each time plot the corresponding correlogram is given. ( $r_k$  = autocorrelation coefficient;  $k$  = lag number). Broken lines are white noise standard errors. Larger circles indicate significant  $r_k$  values ( $\alpha = 0.05$ ).

In pooled annulus groups small marginal increments (type C edges) predominated in October/November in scales and opercular bones, and in October–January in whole and sectioned otoliths. Edge type C otoliths were also found, but in very low proportions, throughout autumn and winter.

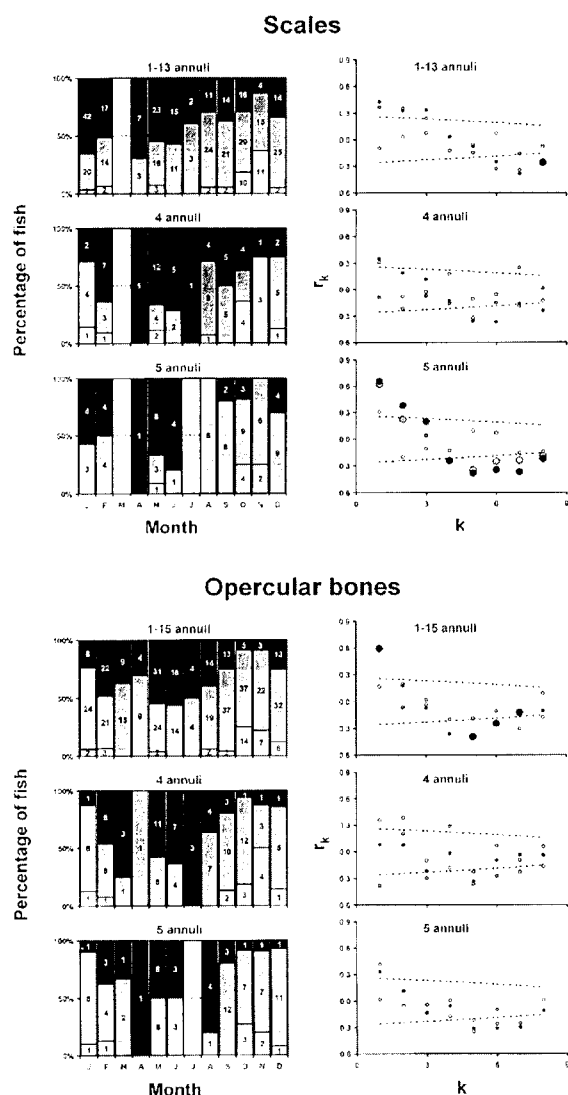


Figure 6. Percentage of scales and opercular bones scored as C (MIR  $\leq 25\%$ ) (white bars), Z (25% < MIR < 75%) (gray bars) or N (MIR  $\geq 75\%$ ) (black bars) in carp from the lower Murray River, Australia. Sample sizes are indicated. Data for pooled annulus groups and for annulus groups 4 and 5 are shown. For each time plot the corresponding correlogram is shown ( $r_k$  = autocorrelation coefficient;  $k$  = lag number). Broken lines are white noise standard errors. Larger circles (colour corresponding to bars) indicate significant  $r_k$  values ( $\alpha = 0.05$ ).

This 'noise' probably stemmed from the criterion adopted for determination of edge type categories. Preliminary trials based on the criteria for scales and opercular bones were inconclusive for otoliths, and the correction applied here is preferable even if there are some mismatches. Well-developed an-

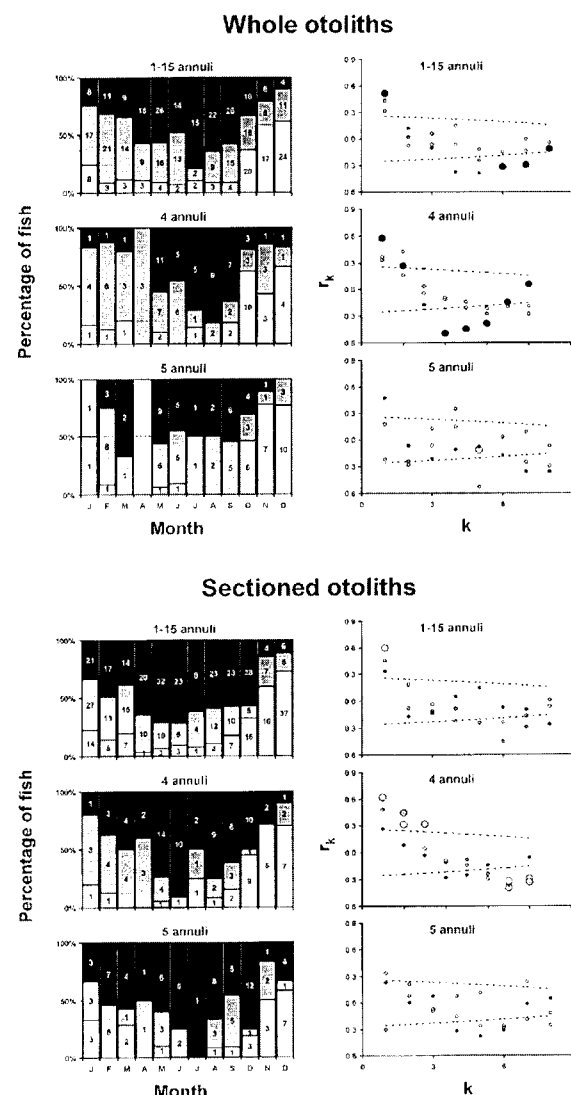


Figure 7. Percentage of whole and sectioned otoliths scored as C (MIR  $\leq 50\%$ ) (white bars), Z (50% < MIR < 75%) (gray bars) or N (MIR  $\geq 75\%$ ) (black bars) in carp from the lower Murray River, Australia. Sample sizes are indicated. Data for pooled annulus groups and for annulus groups 4 and 5 are shown. For each time plot the corresponding correlogram is shown ( $r_k$  = autocorrelation coefficient;  $k$  = lag number). Broken lines are white noise standard errors. Larger dots (colour corresponding to bars) indicate significant  $r_k$  values ( $\alpha = 0.05$ ).

nual increments (type N) became progressively more common from January to July in scales, February to August in opercular bones, and February to September in otoliths (whole and sectioned). A consistent pattern suggesting new annulus formation at the beginning of the growing season was

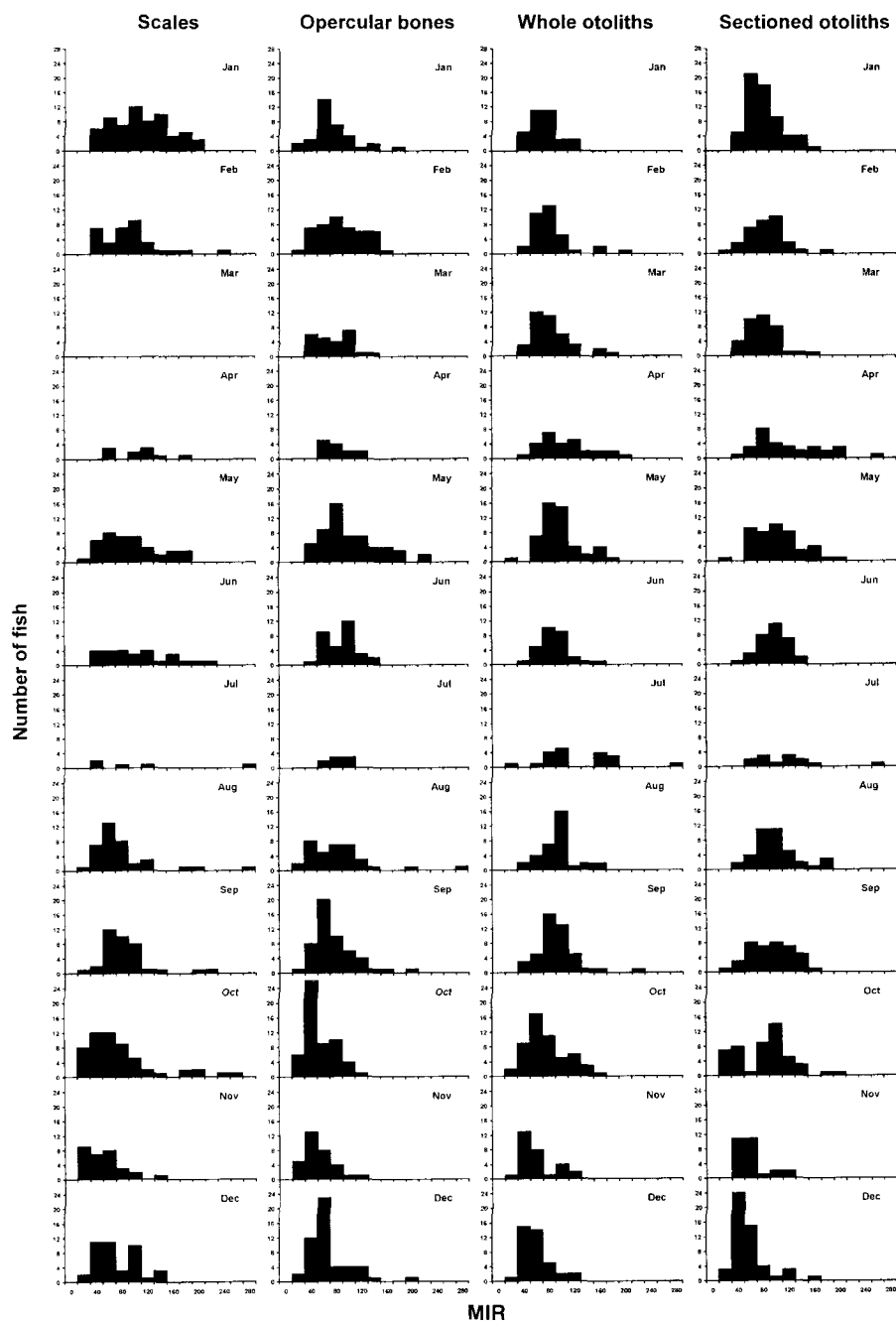


Figure 8. Marginal increment ratio (MIR) frequency distributions for four calcified structures of carp from the lower Murray River, Australia. Monthly data are for pooled annulus groups.

identified in annulus groups 4 and 5 for all structures. These observations were supported by the corresponding correlograms (Figures 6, 7). In annulus group 1 (not shown), the rapid development of the second-year increment, compounded with

the relative thinness of the associated zone of closely-spaced circuli (in scales) or of the opaque zone (in opercular bones and otoliths), probably was the reason for the low numbers of edge type C structures recorded in October–December. However

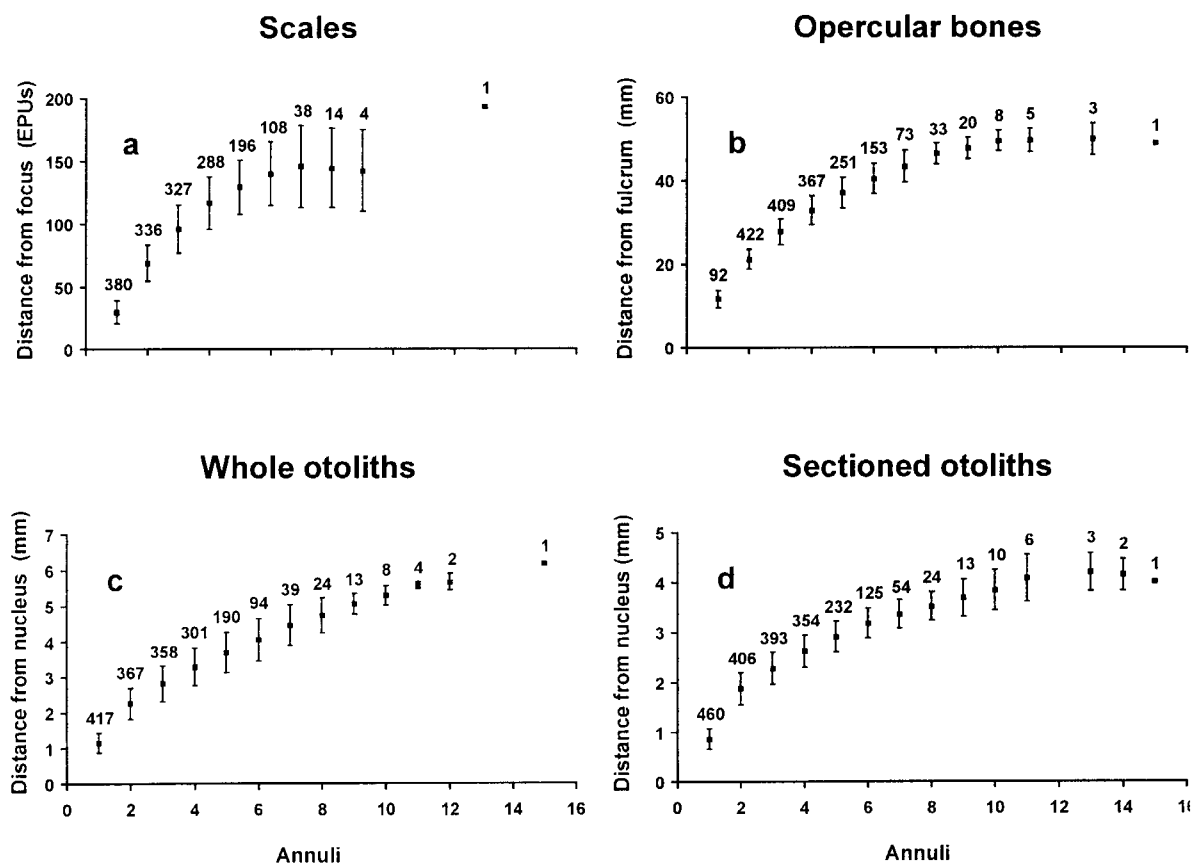


Figure 9. Mean annulus distance ( $\pm$  SD) from the centre of origin (focus in scales, fulcrum in opercular bones, nucleus in otoliths) in carp from the lower Murray River, Australia. Sample sizes are indicated. Annulus counts are based on the corresponding structure (EPUs = eye-piece units: a – scales; b – opercular bones; c – whole otoliths; d – sectioned otoliths).

no definite conclusions can be drawn given the small sample sizes.

*MIR frequency distributions.* – MIR modes for all structures moved across the range of values during the year, with lower values in October–January and higher in June–August. In scales, however, these values were generally spread over a wider range than those for opercular bones and whole and sectioned otoliths (Figure 8).

*Annulus distance.* – In scales standard deviations for the distance from the centre of origin were higher than those recorded for the other structures, where they remained reasonably constant across all annulus groups. The lower number of radii measured in opercular bones up to the first annulus was

an indication of the difficulties encountered in its identification (Figures 9a–d).

#### Consistency of age interpretation

*Comparison of structures: interpretability.* – Interpretability was not independent of the type of structure ( $\chi^2 = 50.28$ ,  $p < 0.001$ ): scales and opercular bones, respectively, provided the lowest ( $n = 380$ , 80.7%) and highest ( $n = 444$ , 94.9%) number of interpretable preparations. Whole and sectioned otoliths had similar interpretability ( $n = 492$ , 87.4% and  $n = 465$ , 85.2%, respectively), suggesting that no real improvement was achieved by sectioning. In most scales recorded as uninterpretable some annuli could not be recognised because of partial or complete resorption. In other cases no clear pattern

of alternating zones of widely- and closely-spaced circuli could be seen, often because of strong irregularities in the arrangement of the circuli. Although opercular bones showed the highest interpretability, these results could be misleading, because the position of the first and, sometimes, the second annulus could not be determined consistently. In opercular bones with less than three annuli the thinness of the bone usually inhibited the interpretation of pseudoannuli often present during the first two years of growth. When 3–6 annuli were present the first annulus, when visible, appeared at the transition between a translucent zone at the level of the fulcrum and a more opaque zone marking the inception of the second year of growth. In opercular bones with more than six annuli ossification at the level of the fulcrum completely obscured the first and, occasionally, the second annulus. As noted, whole and sectioned otoliths had similar interpretability, midway between that of scales and opercular bones. Faded, narrow, discontinuous and randomly intersecting opaque zones in a translucent matrix were typical of uninterpretable whole otoliths, and sectioning did not improve their visibility. In all interpretable whole and sectioned otoliths the first annulus was always identifiable, unlike that in scales and opercular bones.

*Comparison of structures.* – In comparing the four structures for bias all possible pairwise combinations were examined. The corresponding values for IAPE and V are shown in Table 3. As expected, counts from whole and sectioned otoliths were

Table 3. Reproducibility of annulus counts based on all pairwise comparisons of four calcified structures in carp from the lower Murray River, Australia. IAPE = index of average percent error; V = mean coefficient of variation; Sc = scale; Op = opercular bone; WOt = whole otolith; SOt = sectioned otolith; A1 = first count by interpreter A; A2 = second count by interpreter A.

Calcified structure	n	IAPE (%)	V (%)
OpA1 / ScA1	323	8.38	11.85
WotA2 / ScA1	297	8.94	12.65
SOt / ScA1	324	8.45	11.95
WotA2 / OpA1	337	9.30	13.15
SOt / OpA1	362	10.57	14.94
SOt / WotA2	357	7.01	9.92

more precise than those from other combinations. The least reproducibility was seen in comparison between opercular bones and sectioned otoliths.

Bias was also studied for each of the six possible combinations of structures. In all these comparisons, the wider deviations from equivalence at higher annulus counts may be simply an artefact of small sample sizes.

- *Opercular bones vs. scales.* There was good agreement up to eight annuli. Opercular bones under-estimated scale annulus counts when the scales had more than eight annuli. One scale with six annuli accompanied an opercular bone with 15 annuli (Figure 10a).
- *Whole otoliths vs. scales.* Little bias was detected between whole otoliths and scales up to five annuli, but scale annulus counts thereafter increased disproportionately (Figure 10b).
- *Sectioned otoliths vs. scales.* The pattern was similar to that for scales and whole otoliths. There was a tendency for counts to be inflated at three scale annuli and under-estimated at 7–8 scale annuli. Four sectioned otoliths with more than 10 annuli corresponded to scales with 6–7 annuli (Figure 10c).
- *Whole otoliths vs. opercular bones.* Interpretations were broadly similar, but with some striking deviations. Opercular bones under-estimated otolith annulus counts of 3 annuli, and slightly over-estimated them at six and nine annuli. Two whole otoliths with 12 and 15 annuli corresponded to opercular bones with 6 and 7 annuli, respectively (Figure 10d).
- *Sectioned otoliths vs. opercular bones.* Annulus counts from opercular bones were under-estimated when 2–4 sectioned otolith annuli occurred, and over-estimated beyond five annuli. No bias was apparent when only five annuli were present. One sectioned otolith with 14 annuli corresponded to an opercular bone with only seven annuli (Figure 10e).
- *Sectioned otoliths vs. whole otoliths.* Sectioned otoliths over-estimated whole otolith counts at 2–4 annuli, and there were wide deviations at nine annuli. The caveat regarding small sample sizes should not be forgotten (Figure 10f).

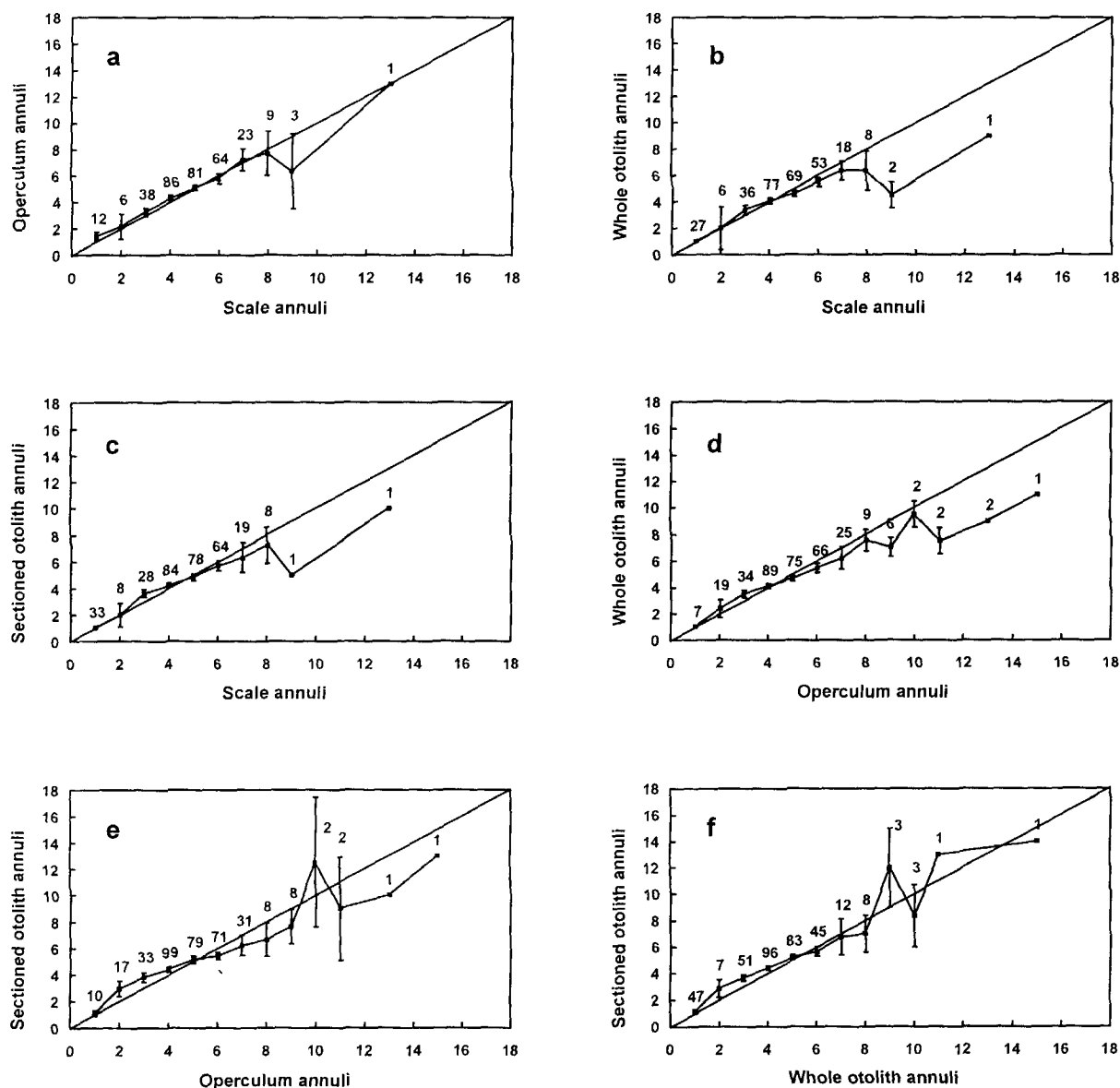


Figure 10. Age bias plots for all pairwise comparisons between four calcified structures from carp in the lower Murray River, Australia. The sample size and 95% confidence limits at each annulus group and the 1 : 1 equivalence line are indicated. Annulus counts from scales and opercular bones are based on the first count by interpreter A, those from whole otoliths on the second count and those from sectioned otoliths on the single count by the same interpreter (a – opercular bones and scales; b – whole otoliths and scales; c – sectioned otoliths and scales; d – whole otoliths and opercular bones; e – sectioned otoliths and opercular bones; f – sectioned otoliths and whole otoliths).

*Consistency by interpreter A.* – Upon fitting of a VBGF to the length-at-annulus data sets for each calcified structure (Table 4), an *F*-test showed no significant differences among the curves ( $p > 0.05$ ), suggesting that annulus counts from the different structures were equally effective in describing growth. As no difference in the visibility of annuli

was apparent in sectioned otoliths, subsequent discussion of the consistency of age interpretations is based on data from scales, opercular bones and whole otoliths.

Replicated annulus counts by interpreter A had similar reproducibility, indicated by values of *V* (Table 5). Age bias plots showed that in his second



Table 4. Comparison of length-at-annulus von Bertalanffy growth functions for carp in the lower Murray River (Australia), based on annulus counts from different calcified structures and interpreters. Values ( $\pm$  asymptotic SE) for the parameters in each model and tests of significance are reported. FL <sub>$\infty$</sub>  = asymptotic fork length; K = Brody coefficient; t<sub>0</sub> = estimated time at which the length of the fish was 0 (Ricker 1975). F values are based on an analysis of residual sum of squares (ARSS).

Group	<i>n</i>	FL <sub><math>\infty</math></sub> (mm)	K (yr <sup>-1</sup> )	t <sub>0</sub> (yr)	F
<b>All calcified structures</b>					
Scale (interpreter A, first count)	380	595.6 $\pm$ 14.1	0.413 $\pm$ 0.040	0.410 $\pm$ 0.105	1.736 ns ( <i>p</i> = 0.076)
Opercular bone (interpreter A, first count)	440	663.8 $\pm$ 18.0	0.276 $\pm$ 0.029	-0.173 $\pm$ 0.225	
Whole otolith (interpreter A, second count)	417	644.1 $\pm$ 21.4	0.305 $\pm$ 0.034	0.020 $\pm$ 0.159	
Sectioned otolith (interpreter A, single count)	460	629.9 $\pm$ 20.1	0.313 $\pm$ 0.035	0.065 $\pm$ 0.151	
<b>Scale</b>					
interpreter A (first count)	380	595.6 $\pm$ 14.1	0.413 $\pm$ 0.040	0.410 $\pm$ 0.105	2.239 ns ( <i>p</i> = 0.082)
interpreter A (second count)	380	642.9 $\pm$ 18.5	0.306 $\pm$ 0.029	0.210 $\pm$ 0.117	
<b>Opercular bone</b>					
interpreter A (first count)	440	663.8 $\pm$ 18.0	0.276 $\pm$ 0.029	-0.173 $\pm$ 0.225	0.393 ns ( <i>p</i> = 0.758)
interpreter A (second count)	440	663.1 $\pm$ 18.6	0.270 $\pm$ 0.029	-0.186 $\pm$ 0.240	
<b>Whole otolith</b>					
interpreter A (first count)	417	569.2 $\pm$ 12.6	0.486 $\pm$ 0.056	0.371 $\pm$ 0.136	3.795 ( <i>p</i> = 0.010)
interpreter A (second count)	417	644.1 $\pm$ 21.4	0.305 $\pm$ 0.034	0.020 $\pm$ 0.159	
<b>Scale</b>					
interpreter A (first count)	380	595.6 $\pm$ 14.1	0.413 $\pm$ 0.040	0.410 $\pm$ 0.105	9.148 ( <i>p</i> = 6.0E-06)
interpreter B (single count)	380	647.2 $\pm$ 20.6	0.277 $\pm$ 0.027	0.211 $\pm$ 0.133	
<b>Opercular bone</b>					
interpreter A (first count)	440	663.8 $\pm$ 18.0	0.276 $\pm$ 0.029	-0.173 $\pm$ 0.225	0.148 ns ( <i>p</i> = 0.931)
interpreter B (single count)	440	665.5 $\pm$ 20.6	0.280 $\pm$ 0.032	-0.043 $\pm$ 0.236	
<b>Whole otolith</b>					
interpreter A (second count)	417	644.1 $\pm$ 21.4	0.305 $\pm$ 0.034	0.371 $\pm$ 0.136	2.636 ns ( <i>p</i> = 0.050)
interpreter B (single count)	417	683.0 $\pm$ 24.2	0.250 $\pm$ 0.026	-0.049 $\pm$ 0.155	

count interpreter A under-estimated annulus counts (relative to the first count) when more than seven annuli were present in scales and whole otoliths and more than 10 annuli in opercular bones (the counts for opercular bones therefore were con-

sistent for a greater range of annulus counts than for scales and whole otoliths). In whole otoliths the confidence limits were wider over seven annuli than for scales and opercular bones, and there was a tendency toward over-estimation in the second count when two annuli were enumerated in the first count (Figures 11a–c).

The bias and precision of counts by interpreter A were assessed for each structure at each annulus count (counts were averaged over the two replicates). In all structures PAG fell below 50% when more than six annuli were present. For scales and opercular bones V was 10–20% at all annulus counts, and in whole otoliths it was over 25% at two annuli and near 25% at eight annuli (Table 6).

Comparisons of VBGF models fitted to length-at-annulus data showed no significant differences for the first and second counts on scales and opercular bones (*p* > 0.05), but significant differences for whole otoliths (Table 4).

Table 5. Reproducibility of annulus counts within interpreter A and between interpreters A and B based on three calcified structures from carp in the lower River Murray, Australia. IAPE = index of average percent error; V = mean coefficient of variation.

Calcified structure	Within interpreter A			Between interpreters A and B		
	<i>n</i>	IAPE (%)	V (%)	<i>n</i>	IAPE (%)	V (%)
Scale	380	5.61	7.94	380	9.15	12.94
Opercular bone	440	5.32	7.52	440	6.99	9.89
Whole otolith	417	5.64	7.97	417	6.23	8.80

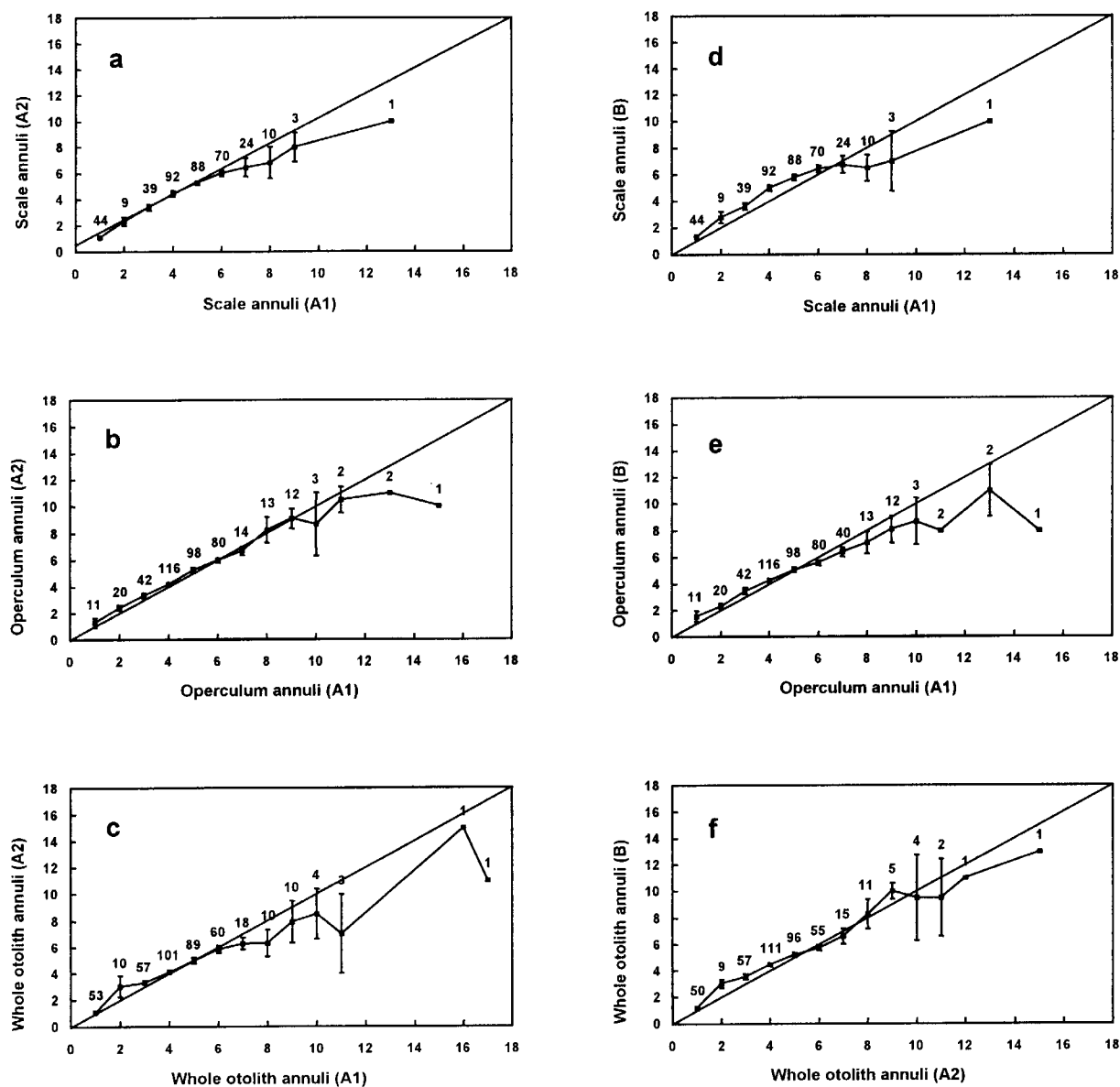


Figure 11. Age bias plots for pairwise comparisons between replicate annulus counts from the same and from different interpreters based on three calcified structures from carp in the lower Murray River, Australia. The sample size and 95% confidence limits at each annulus group and the 1:1 equivalence line are indicated (A1 = first count by interpreter A; A2 = second count by interpreter A; B = single count by interpreter B; a – second and first count of scales by interpreter A; b – second and first count of opercular bones by interpreter A; c – second and first count of whole otoliths by interpreter A; d – single count by interpreter B and first count by interpreter A of scales; e – single count by interpreter B and first count by interpreter B of opercular bones; f – single count by interpreter B and second count by interpreter A of whole otoliths).

**Consistency by interpreters A and B.** – The reproducibility of counts between interpreters A and B (Table 5) was least for scales, better for opercular bones and best for whole otoliths. The age bias plot for scales showed that interpreter B over-estimated

counts by interpreter A below six annuli and under-estimated counts above 8 annuli (Figure 11d). Interpreter B also under-estimated counts for opercular bones when more than six annuli were counted by interpreter A (Figure 11e). However, there was

close agreement between the two from 1–6 annuli. Counts on whole otoliths agreed up to eight annuli, although interpreter A over-estimated when 2–3 annuli were present. The overall precision of counts when more than 10 annuli were present was higher in whole otoliths than in scales and opercular bones (Figure 11f).

VBGFs fitted to age estimates by interpreter A (first count for scales and opercular bones, and second count for whole otoliths) and interpreter B showed significantly different curves ( $p < 0.001$ ) for scales, but no significant differences between the curves for opercular bones and whole otoliths (Table 4).

*Comparison of opercular bones and whole otoliths by morphological classes.* – No significant interaction occurred between morphological class and structure in the reproducibility of interpretations (measured by V) for opercular bones and whole otoliths ( $p = 0.143$ ). However, Friedman's tests showed that in opercular bones the reproducibility between annulus groups was significantly different

( $p < 0.01$ ), although there were no such differences between morphological classes. Post hoc Tukey-type pairwise comparisons indicated no significant differences in the coefficients of variation for the combined annulus groups I and II, and I and III, although groups II and III did have different values. In whole otoliths, reproducibility did not differ between either morphological class or combined annulus group.

#### Growth models

In total 539 carp were successfully interpreted for age, including 380 specimens from scales, 440 from opercular bones, 417 from whole otoliths and 460 from sectioned otoliths. Summary statistics for length- and weight-at-age of juveniles, males and females are shown in Table 7.

*Comparison of length-at-age functions.* – The VBGF and the five polynomial functions fitted to each of the four length-at-age data sets resulted in a

Table 6. Bias and precision of annulus counts by interpreter A at each annulus group based on three calcified structures from carp in the lower Murray River, Australia. ScA1 = first count of scales; ScA2 = second count of scales; OpA1 = first count of opercular bones; OpA2 = second count of opercular bones; WOtA1 = first count of whole otoliths; WOtA2 = second count of whole otoliths. Precision is measured as the mean percent agreement (PAG) and the mean coefficient of variation (V), while bias is calculated as the difference between the two counts on each structure, averaged over preparations with the same mean number of annuli. The nominal age (= annuli) is the rounded mean.

Annuli	ScA1 / ScA2				OpA1 / OpA2				WOtA1 / WOtA2			
	n	PAG (%)	V (%)	Bias (%)	n	PAG (%)	V (%)	Bias (%)	n	PAG (%)	V (%)	Bias (%)
1	41	100.0	0.0	0.0	9	100.0	0.0	0.0	49	100.0	0.0	0.0
2	9	66.7	15.7	-16.7	14	85.7	8.4	-10.7	8	37.5	29.5	-18.8
3	31	77.4	7.6	3.2	38	71.1	8.7	-3.5	45	86.7	4.2	-2.2
4	73	75.3	7.0	-2.4	101	75.2	5.6	-0.2	102	69.6	7.5	-2.2
5	93	58.1	8.3	-3.2	97	53.6	8.3	-2.1	96	56.3	8.0	2.5
6	81	49.4	8.9	-2.7	92	53.3	7.3	-3.3	64	48.4	8.7	2.1
7	35	20.0	13.9	-6.1	52	34.6	9.0	-2.5	27	18.5	14.7	10.6
8	13	38.5	9.2	-8.7	12	41.7	5.5	-1.0	12	25.0	21.3	-2.1
9	3	33.3	5.5	0.0	14	28.6	9.3	3.2	6	50.0	5.4	7.4
10					4	0.0	18.0	-25.0	5	40.0	8.8	8.0
11					4	25.0	8.4	-6.8	1	0.0	20.2	-27.3
12	1	0.0	18.4	25.0	2	0.0	11.8	16.7				
13					1	0.0	28.3	38.5				
14									1	0.0	30.3	42.9
15												
16									1	0.0	4.6	6.3

total of 24 coefficients of determination. The lowest value ( $r^2 = 0.622$ ) was recorded for a DDQF fitted to age estimates based on opercular bones, and the highest value ( $r^2 = 0.807$ ) was for a LLQF fitted to scale-based age estimates. A randomised blocks ANOVA indicated significant differences in the mean  $r^2$  values for growth functions ( $F = 86.226$ ,  $p < 0.001$ ). Bonferroni-adjusted pairwise comparisons between growth functions showed significant differences between the LLQF and other functions, with the first providing best goodness of fit. Conversely, the DDQF resulted in lower  $r^2$  values compared to other models. No significant differences occurred among  $r^2$  values for the VBGF and the other three functions (DLQF, LDQF, DDCF). The

LLQF, by virtue of its precision, and the VBGF, with wider applicability and more biological realism (Moreau 1987), were chosen to model growth in length.

*Comparison of growth between sexes.* – Growth in length between males and females was significantly different (Table 8, Figures 12a, b). Preliminary fits of the VBGF to length-at-age data for males and females resulted in unrealistic estimates for the model parameters, especially  $FL_{\infty}$  and  $t_0$ , and the  $t_0$  value calculated for the whole sample (males, females, juveniles) was used to estimate the values of  $FL_{\infty}$  and  $K$  for each sex. Growth rates, indicated by  $K$ , were similar between the sexes, but the asymp-

Table 7. Summary statistics for fork length (FL) and total weight (W) of juvenile, male and female carp from the lower Murray River, Australia. The estimated ages of the fish are based on annulus counts from whole otoliths, sectioned otoliths, opercular bones and scales, in this order.

Estimated age (yr)	n	FL (mm)				W (g)			
		Mean	± SE	Min	Max	Mean	± SE	Min	Max
Juveniles									
1	63	207.4	6.9	96	341	213.8	21.8	19	817
2	5	292.4	24.2	230	373	474.2	117.3	205	850
Males									
1	2	358.0	13.0	345	371	843.5	95.5	748	939
2	7	368.0	24.4	268	464	1001.1	180.9	409	1730
3	27	436.4	17.4	268	600	1825.5	201.2	341	4222
4	56	448.6	9.9	259	602	1837.7	117.8	312	3791
5	56	500.9	9.5	319	656	2458.0	129.8	541	4956
6	31	516.3	13.7	317	638	2756.6	193.2	690	4620
7	21	566.1	14.2	417	678	3454.9	224.6	1214	5180
8	6	606.3	20.0	520	654	4035.3	399.8	2521	5514
9	2	628.5	10.5	618	639	4287.5	272.5	4015	4560
10	1	643				4483			
11	2	643.0	6.0	637	649	4956.5	343.5	4613	5300
12	1	577				3365			
Females									
2	4	388.5	28.6	308	435	1194.5	230.1	582	1611
3	26	433.3	12.0	336	555	1658.7	135.3	706	3390
4	77	510.5	6.9	356	623	2560.7	96.9	788	4468
5	60	540.3	9.7	352	641	3096.9	146.3	830	5000
6	48	554.2	10.2	383	655	3364.3	171.5	1051	5340
7	20	581.1	17.3	365	681	3941.1	298.0	904	6880
8	12	605.7	18.3	491	685	4023.8	300.6	1918	5320
9	3	656.0	22.6	629	701	5232.0	745.0	4140	6656
10	6	645.8	10.6	604	682	4627.8	203.9	3925	5160
11	2	668.0	20.0	648	688	5329.5	910.5	4419	6240
15	1	686				5540			

Table 8. Models describing the somatic growth of male and female carp in the lower Murray River, Australia. Values ( $\pm$  (asymptotic) SE) for the parameters in linear and non-linear models, and tests of significance between the sexes are reported. The residual sum of squares (RSS) and the value and corresponding probability for the  $F$ -ratio test are also indicated for all comparisons of growth curves between sexes. When no significant differences at a probability level  $\alpha = 0.05$  were detected the model for the combined sexes is given. Acronyms and symbols as in Table 2.

Statistics	Males + Females	Males	Females
<b>FL VBGF</b>			
$n$	471	212	259
$FL_{\infty}$ (mm) <sup>a</sup>	$625.4 \pm 13.6$	$600.3 \pm 21.7$	$639.2 \pm 15.9$
$K$ (yr <sup>-1</sup> ) <sup>a</sup>	$0.344 \pm 0.021$	$0.346 \pm 0.035$	$0.353 \pm 0.026$
$t_0$ (yr) <sup>a,b</sup>	$0.174 \pm 0.116$	$0.174 \pm 0.116$	$0.174 \pm 0.116$
$r^2$	0.336	0.318	0.350
RSS	2519257.149	1193324.483	1180721.550
DF	469	210	257
$F$	9.522 ( $p = 4.9E - 07$ )		
Model		$FL_t = 600(1 - \exp(-0.346(t - 0.174)))$	$FL_t = 639(1 - \exp(-0.353(t - 0.174)))$
<b>FL LLQF</b>			
$n$	471	212	259
$a_1$	$2.196 \pm 0.095$	$2.357 \pm 0.125$	$2.007 \pm 0.153$
$b_1$	$0.835 \pm 0.233$	$0.371 \pm 0.320$	$1.348 \pm 0.365$
$c_1$	$-0.250 \pm 0.143$	$0.046 \pm 0.204$	$-0.567 \pm 0.216$
$r^2$	0.335	0.340	0.339
RSS	2.059	1.033	0.889
DF	468	209	256
$F$	16.573 ( $p = 1.1E - 07$ )		
Model		$\text{Log}(FL_t) = 2.357 + 0.371(\text{Log}(t + 1)) + 0.046(\text{Log}(t + 1))^2$	$\text{Log}(FL_t) = 2.007 + 1.348(\text{Log}(t + 1)) - 0.567(\text{Log}(t + 1))^2$
<b>FLWR</b>			
$n$	484	217	267
$a^d$	$4.600E - 08 \pm 9.000E - 09$	$4.800E - 08 \pm 1.200E - 08$	$4.600E - 08 \pm 1.300E - 08$
$b^d$	$2.856 \pm 0.030$	$2.848 \pm 0.040$	$2.857 \pm 0.045$
$r^2$	0.963	0.970	0.955
RSS	29.202	9.385	19.803
DF	482	215	265
$F$	0.108 ns ( $p = 0.897$ )		
Model	$W = 4.600E - 08FL^{2.856}$	$W = 4.800E - 08FL^{2.848}$	$W = 4.600E - 08FL^{2.857}$
<b>W VBGF<sup>b</sup></b>			
$n$	471	212	259
$W_{\infty}$ (kg) <sup>d</sup>	$4.994 \pm 0.288$	$4.749 \pm 0.447$	$5.123 \pm 0.335$
$K$ (yr <sup>-1</sup> ) <sup>d</sup>	$0.297 \pm 0.017$	$0.279 \pm 0.026$	$0.314 \pm 0.022$
$t_0$ (yr) <sup>a,b</sup>	$-0.242 \pm 0.370$	$-0.242 \pm 0.370$	$-0.242 \pm 0.370$
$b^d$	$2.856 \pm 0.030$	$2.848 \pm 0.040$	$2.857 \pm 0.045$
$r^2$	0.336	0.350	0.318
RSS	502.994	195.127	275.018
DF	469	210	257
$F$	10.703 ( $p = 7.4E - 09$ )		
Model		$W_t = 4.749(1 - \exp(-0.279(t + 0.242)))^{2.848}$	$W_t = 5.123(1 - \exp(-0.314(t + 0.242)))^{2.857}$

<sup>a</sup> Asymptotic SE.

<sup>b</sup> Calculated from a model including the pooled sexes and juveniles.

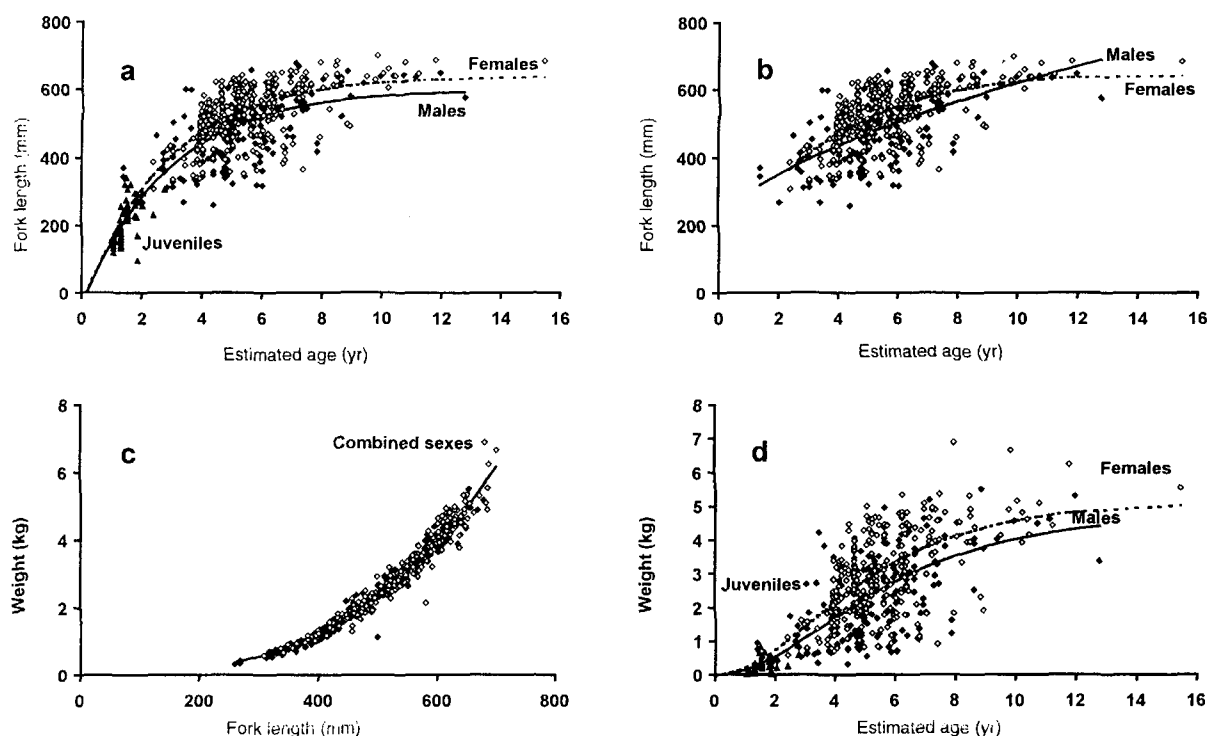


Figure 12. Somatic growth of male and female carp in the lower Murray River, Australia. Estimated ages are based on annulus counts from whole otoliths, sectioned otoliths, opercular bones and scales, in this order (filled circles = males; open circles = females; triangles = juveniles; a – growth in length, von Bertalanffy growth function (VBGF); b – growth in length, log-log quadratic function (LLOF); c – length-weight relationship; d – growth in weight, Beverton & Holt's (1957) modification of the VBGF). Parameters for all models in Table 8.

otic length reached by the females was higher than that for the males.

As no significant differences were apparent in the length-weight relationships for males and females (Table 8, Figure 12c), pooling was valid, with the pooled  $b$  being close to that for allometric growth (Ricker 1975). The  $b$  values for males and females, from the corresponding length-weight relationships, were used in modelling growth in weight with the modified VBGF (Table 8, Figure 12d). In VBGFs fitted to the length-at-age data the  $t_0$  values for males and females were calculated from a model including the pooled sexes and juveniles. The females showed a faster rate of growth in weight than males, and their asymptotic weight also was relatively high. Again, significant differences were evident in the growth in weight between sexes.

**Otolith growth.** – As for somatic growth, the growth of otoliths in length, width and weight was de-

scribed by both linear and non-linear models. Although there were no significant differences between the length and width of the right and left otoliths ( $Z = 0.089$ ,  $p = 0.929$ ;  $Z = 0.002$ ,  $p = 0.999$  for length and width, respectively), their weights were different ( $Z = 0.730$ ,  $p < 0.01$ ). This may have reflected difficulties encountered upon extraction, as the procedure may occasionally damage the otolith at the level of the *fossa acustica* (Berinkey 1956). However, a tentative 'sensitivity analysis', involving fitting of growth models to both right and left otoliths, resulted in no appreciable visual differences. Therefore, in further calculations the length, width and weight of the left otoliths were used, and their growth modelled using ages estimated from whole otoliths.

Two curves, namely a VBGF and a first-order linear model with log-transformed ages (LDLF), were fitted to the otolith length- and otolith width-at-age data (Figures 13a–d) for both sexes. The  $t_0$  values

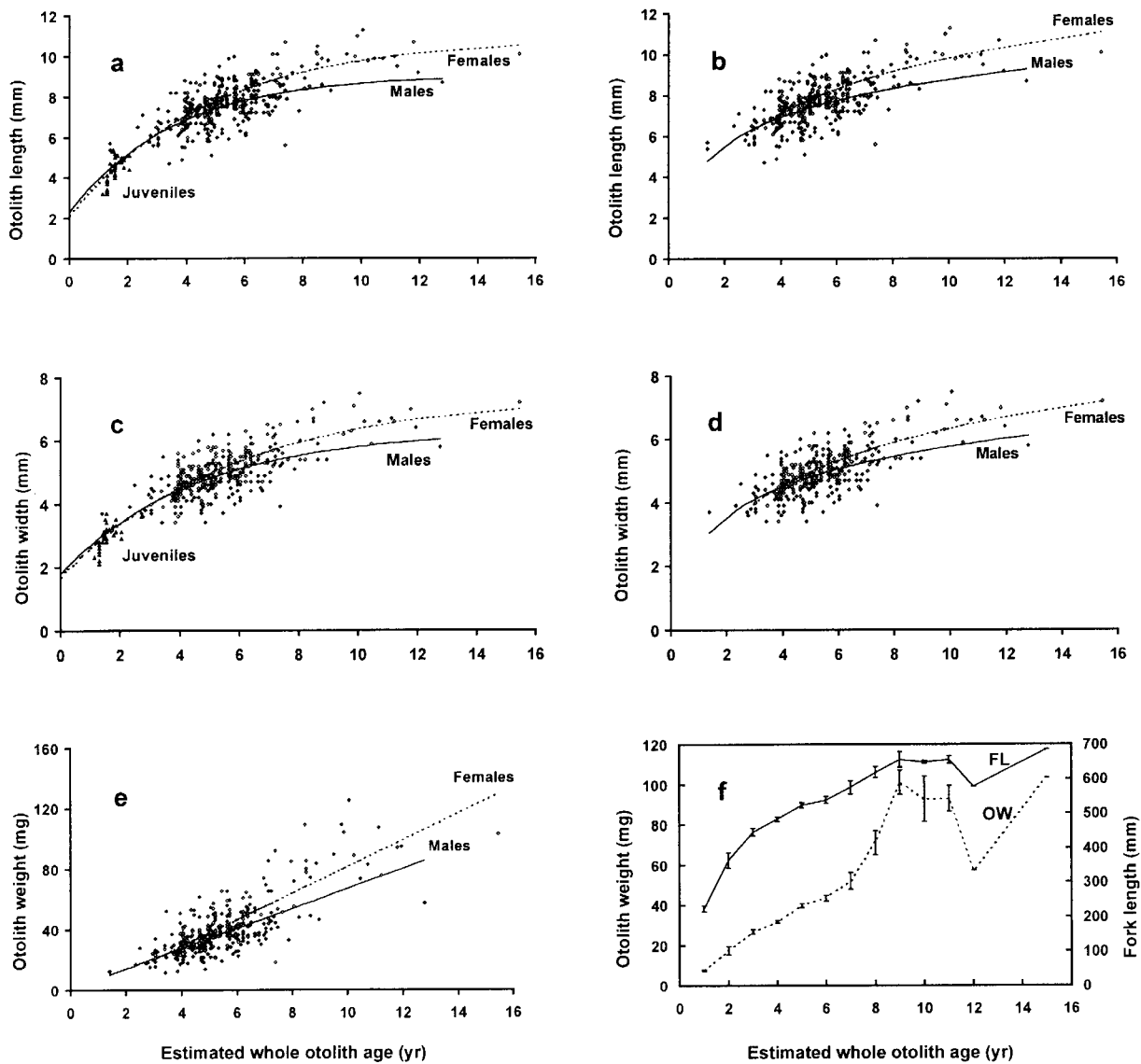


Figure 13. Otolith growth in male and female carp from the lower Murray River, Australia. Estimated ages are based on annulus counts from whole otoliths (filled circles = males; open circles = females; triangles = juveniles; a – growth in otolith length, von Bertalanffy growth function (VBGF); b – growth in otolith length, log-decimal linear regression function (LDLF); c – growth in otolith width, VBGF; d – growth in otolith width, LDLF; e – growth in otolith weight, decimal-decimal linear regression function (DDLRF); f – changes in fork length (FL) and otolith weight (OW) in each estimated age group). Error bars are  $\pm$  SE. Parameters for all models in Table 9.

for the VBGFs for males and females were calculated from VBGFs fitted to the entire sample, including the juveniles. Males had lower asymptotic values for OL and OWI than females, although they always showed a higher K (Table 9). In contrast, OW generally increased linearly with age, although only 55% (males) and 63% (females) of the variance was explained by the model (Figure 13e). Final-

ly, ARSS tests always indicated significant differences in otolith growth between males and females.

Correlation matrices for otolith morphometrics and age (Table 10) showed that in both sexes OW had the strongest linear relationship with age, followed in order by OWI, OL and FL. Changes in OW within the same estimated age group were also correlated to fluctuations in FL, especially at older ag-

Table 9. Models describing otolith growth in male and female carp in the lower Murray River, Australia. Values ( $\pm$  (asymptotic) SE) for the parameters in linear and non-linear models and tests of significance between the sexes are reported. The residual sum of squares and the value and corresponding probability for the  $F$ -ratio test are also indicated for all comparisons of growth curves between sexes. When no significant differences at a probability level  $\alpha = 0.05$  were detected the model for the combined sexes is given. Acronyms and symbols as in Table 2.

Statistics	Males + Females	Males	Females
<b>OL VBGF</b>			
$n$	353	164	189
$OL_t$ (mm) <sup>a</sup>	$10.109 \pm 0.265$	$9.068 \pm 0.308$	$10.868 \pm 0.372$
$K$ (yr <sup>-1</sup> ) <sup>a</sup>	$0.231 \pm 0.014$	$0.281 \pm 0.026$	$0.208 \pm 0.015$
$t_0$ (yr) <sup>a,b</sup>	$-1.307 \pm 0.225$	$-1.307 \pm 0.225$	$-1.307 \pm 0.225$
$r^2$	0.452	0.389	0.525
RSS	229.060	99.280	108.464
DF	351	162	187
$F$	11.937 ( $p = 1.9E - 07$ )		
Model		$OL_t = 9.1(1 - \exp(-0.281(t + 1.307)))$	$OL_t = 10.9(1 - \exp(-0.208(t + 1.307)))$
<b>OL LDLF</b>			
$n$	353	164	189
$a$	$3.556 \pm 0.243$	$4.132 \pm 0.312$	$3.009 \pm 0.347$
$b$	$2.508 \pm 0.145$	$2.013 \pm 0.189$	$2.952 \pm 0.204$
$r^2$	0.459	0.411	0.527
RSS	225.966	95.736	107.882
DF	351	162	187
$F$	19.152 ( $p = 1.6E - 11$ )		
Model		$OL_t = 4.132 + 2.013\ln(t)$	$OL_t = 3.009 + 2.952\ln(t)$
<b>OWI VBGF</b>			
$n$	353	164	189
$OWI_t$ (mm) <sup>a</sup>	$6.953 \pm 0.215$	$6.307 \pm 0.260$	$7.422 \pm 0.313$
$K$ (yr <sup>-1</sup> ) <sup>a</sup>	$0.187 \pm 0.012$	$0.220 \pm 0.021$	$0.169 \pm 0.014$
$t_0$ (yr) <sup>a,b</sup>	$-1.514 \pm 0.276$	$-1.514 \pm 0.276$	$-1.514 \pm 0.276$
$r^2$	0.483	0.435	0.529
RSS	91.981	40.554	46.850
DF	351	162	187
$F$	6.092 ( $p = 4.7E - 04$ )		
Model		$OWI_t = 6.3(1 - \exp(-0.220(t + 1.514)))$	$OWI_t = 7.4(1 - \exp(-0.169(t + 1.514)))$
<b>OWI LDLF</b>			
$n$	353	164	189
$a$	$2.200 \pm 0.155$	$2.571 \pm 0.202$	$1.814 \pm 0.227$
$b$	$1.608 \pm 0.093$	$1.390 \pm 0.122$	$1.962 \pm 0.134$
$r^2$	0.484	0.443	0.533
RSS	91.895	39.958	46.468
DF	351	162	187
$F$	11.042 ( $p = 6.1E - 07$ )		
Model		$OWI_t = 2.571 + 1.390\ln(t)$	$OWI_t = 1.814 + 1.962\ln(t)$
<b>OW DDLF</b>			
$n$	353	164	189
$a$	$-3.480 \pm 1.995$	$1.115 \pm 2.599$	$-6.369 \pm 2.837$
$b$	$7.895 \pm 0.351$	$6.588 \pm 0.468$	$8.781 \pm 0.489$
$r^2$	0.591	0.550	0.633
RSS	44219.751	16153.789	25189.618
DF	351	162	187
$F$	12.140 ( $p = 1.4E - 07$ )		
Model		$OW_t = 1.115 + 6.588t$	$OW_t = -6.369 + 8.781t$

<sup>a</sup> Asymptotic SE.

<sup>b</sup> Calculated from a model including the pooled sexes and juveniles.



Table 10. Correlation matrix for otolith morphometrics and age in carp from the lower Murray River, Australia. Estimated ages (Age) from annulus counts are based on whole otoliths. Acronyms as in Table 2.

Males/Females	Age	FL	OW	OL	OWI
Age	1.000	0.582	0.796	0.707	0.729
FL	<b>0.541</b>	1.000	0.769	0.852	0.824
OW	<b>0.742</b>	<b>0.805</b>	1.000	0.910	0.924
OL	<b>0.647</b>	<b>0.868</b>	<b>0.896</b>	1.000	0.934
OWI	<b>0.688</b>	<b>0.864</b>	<b>0.922</b>	<b>0.926</b>	1.000

es (Figure 13f). However, multiple linear regression models to estimate fish age from body and otolith morphometrics did not yield satisfactory results. For the males, a model including OW and the interaction term FL\*W described only 56% of the variation in age, and for females OW alone was retained in the model and accounted for only 63% of the total variance (Table 11).

## Discussion

### Validation

Although decreases in MIR in October–December were apparent for age groups 4 and 5 for all structures, the values were never less than 20–30% of the previous incremental zone, and were independent of the estimated ages of the fish and the structure considered. Variations in time of annulus deposition among fish of a different age class are to be expected as a result of differences in relative growth. However, the variability remaining when annulus groups were examined separately appears to be related to intrinsic and extrinsic factors linked to both ecophysiological variation among individual fish of any one age class and to the limitations of the age interpretation technique. Carp may spawn through most of the year in the lower Murray River (Jones 1974), possibly even at water temperatures below 18 °C, which is commonly regarded as a lower limit (Sarig 1966). Jones (1974) reported that young-of-the-year (YOY) carp were found in shallow swampy areas of the river throughout the year, probably as a result of protracted flooding in 1974

Table 11. Multiple linear regression models for estimation of age from body and otolith morphometrics in carp from the lower Murray River, Australia. Acronyms as in Table 2.

Variable	Coefficient	± SE	p
<b>Males (n = 164)</b>			
Constant	2.085	0.241	< 0.001
OW	0.107	0.011	< 0.001
FL*W	– 0.522E – 06	0.207E – 06	0.013 ns
Multiple $r^2 = 0.562$			
<b>Females (n = 189)</b>			
Constant	2.492	0.186	< 0.001
OW	0.072	0.004	< 0.001
Multiple $r^2 = 0.633$			

that may have increased the availability of spawning sites for adult carp and food for larvae and juveniles. Jones' contention was supported by scale morphology (the number and spacing of circuli around the focus) indicating five cohorts of YOY carp. Although in the lower Murray spawning conditions for carp may occur occasionally in winter, several confounding factors may prejudice the reliability of interpretations of scale morphology. Mann & Steinmetz (1985) showed that the relative widths of the first year of growth in scales of rudd, *Scardinius erythrophthalmus*, varied as a result of differences in individual growth rates within the same age class, the first annulus being further from the focus in fast-growing than in slow-growing individuals, and they attributed these variations to differences in microhabitat characteristics and growth rates between years. Another limitation to MIA became evident in the present study when reading otoliths with a newly-forming annulus along the edge, usually seen (under reflected light) as a narrow opaque zone within a translucent matrix, making the margin itself appear translucent. The new opaque zone usually is recognisable only when it attains 20–40% of the previous annual increment, so that an otolith with an incipient zone would be scored as having a translucent edge. This has been recorded for the otoliths of other cyprinids (Mina 1989), and clearly would affect determinations even after grinding or sectioning. Indeed, in the present study otolith sections provided no real advantage over whole otoliths in terms of the visibility of newly-formed opaque zones.

The absence of strong periodicity in annulus formation was confirmed by the ETA results, although the morphology of the calcified structures again seems to have had a confounding influence. In opercular bones, and especially in whole otoliths, the numbers of structures with an opaque edge, as expected, were greater in the growing season than in winter, and sectioning obscured these patterns. Although it has been argued that sectioned otoliths, generally considered superior to whole otoliths (e.g. Beamish 1979a, b), should enhance the visibility and resolution of zonation, especially in older individuals and perhaps those resuming their seasonal growth, this did not appear to be so for carp. A detailed analysis of the value and reliability of scales, opercular bones, and whole and sectioned otoliths for age determinations is discussed later. Here, it may be noted that otolith sectioning does obscure alternating translucent and opaque zones, especially near the edge, preventing identification of transitions in the same growth zones as the ones normally visible in whole otoliths.

A goal of the validation component of the present study was to identify a criterion to assist in interpretation of the edges of calcified structures. To this end the method of Anderson et al. (1992a, b) was modified to accommodate variation in the relative width and appearance of annual increments, especially the opaque zones in otoliths. The increased resolution is most evident because the presence of 'aberrant' opaque edges in fish sampled in late autumn and winter is suppressed by giving more emphasis to the relative width of the last annual increment than to its appearance. Another advantage of the proposed method is that it allows a margin of precision for the designated common 'birthdate', given that this may vary over the range of the species (see also Vilizzi & Walker 1998). The versatility of the method, a mathematical implementation to graphical representations of the edge interpretation problem (Francis et al. 1992), is further apparent when we postulate the following. In the unlikely case of a stock of fish where all age classes form an annulus at the same time (birth date), thus regardless of their age, no adjustment would be necessary, and the use of the annulus count corrections would simply have a null effect. Since variation in time of

annulus formation is likely to be present in most fish stocks, corrections are made whenever required.

As an alternative, qualitative approach to describing seasonal trends in scale morphology, the numbers of outer circuli provided a reasonably satisfactory indication of the annual formation of new annuli, although pronounced irregularities narrowed the range of age classes to which this method could be applied, especially in fish with more than 4–5 annuli.

By comparing MIRA and ETA the present study demonstrates that for the calcified structures examined the formation of annuli occurred annually in pooled annulus groups (this was also supported by MIR frequency distributions) and in carp 4 and 5 years old. Although extrapolations should be made cautiously, the regular alternation of zones observed in all structures of younger and older carp is also strongly suggestive of annual periodicity. Larger sample sizes may help to provide sufficient resolution for a better recognition of the timing of annulus formation in other age classes. Previously reported difficulties in determining the age profiles of wild carp stocks in Australia, due to protracted spawning conditions and a lack of a pronounced seasonality (Jones 1974, Hume et al.<sup>1</sup>), therefore can be overcome by methods designed to minimise the confounding effects of these factors on the formation of periodic patterns in scales, opercular bones and otoliths. More research is needed to extend the range of validated ages to the entire life span, possibly using long-term sampling and/or mark-and-recapture studies, and by radiometric analysis of otoliths (Fenton & Short 1992).

#### *Consistency of age interpretation*

Two notable and somewhat contradictory outcomes are that (1) growth curves based on counts from all structures equally described the length-at-age relationship, and that (2) some bias in annulus counts was detected in systematic comparisons of the structures. However, the biases were small over the annulus groups with large sample sizes, and the growth curves very similar. This may be the key to understanding the confounding effects that struc-

ture-specific factors could have had on interpretability and on the reproducibility of interpretations.

Scales, flat bones and otoliths grow at different rates relative to fish length. Casselman (1990) showed that in several fishes the otoliths and, to some extent, the cleithra continued to grow allometrically as the fish approached its asymptotic length, but that this was not the case for scales, which often underwent resorption and erosion. Indeed, scale resorption in carp is a major impediment to reliable interpretations of annulus patterns (Carlander 1987), and may be an effect of starvation (Ichikawa 1953). Thus, several authors (McConnell 1952, English 1952, Rehder 1959) have found opercular bones to be better indicators of age and growth in this species. Annulus counts from otoliths also should indicate the age of older fish more accurately than scales. Unfortunately, information on the use of carp otoliths is fragmentary (Vilizzi & Walker 1995), and only Raina (1987) and Pinilla et al. (1992) apparently did not experience difficulties in interpretation of these structures. Finally, sectioned otoliths generally are regarded as superior to whole otoliths, especially in estimating the ages of older fish (Beamish & McFarlane 1987), but this method has not previously been applied to carp.

Some of the results from the present study may appear to contradict findings reported in the literature. However, the perceived similarity of annulus counts based on whole and sectioned otoliths stems from the peculiar morphology of carp asterisci relative to the sagittae of non-otophysan fish (sensu Fink & Fink 1981), and this was the primary reason for rejection of otolith sections from further analyses. Patterns of calcium carbonate deposition in the otoliths of older fish were responsible for the slight under-estimation of annulus counts seen in comparisons with opercular bones. Later annuli in whole otoliths often were indistinct due to the overall translucency of the margin, and sectioning further disrupted patterns recognisable in the whole otoliths, magnifying the variations in the respective annulus counts. On the other hand, difficulties in discerning the first 1–2 annuli in opercular bones caused these structures to under-estimate annulus counts when compared to whole and sectioned otoliths. This problem did not emerge in interpreting

scales, even though the exact position of the first annulus often had to be estimated.

Evaluation of within- and between-interpreter reproducibility showed relatively high values for IAPE (or V), compared to those reported in the literature. Although a standard measure of the reproducibility of interpretations 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<sup>4</sup>). In the present study IAPE values for carp were higher than those reported for Murray cod, *Maccullochella peelii* (3.0–5.4%; Anderson et al. 1992a) and golden perch, *Macquaria ambigua* (3.9–5.6%; Anderson et al. 1992b) in the Murray-Darling Basin.

No attempt was made to reconcile different annulus counts from interpreters A and B, and growth modelling employed age estimates by interpreter A (the second count from whole otoliths, the only count from sectioned otoliths and the first counts from opercular bones and scales, in that order). Given the impossibility of knowing the *true* age of the fish, counts by interpreter A were taken to be the most precise. The choice of otoliths (whole and sectioned) for estimation of growth parameters was dictated primarily by the need to clearly identify the positions of the first and second annulus (this became too subjective in opercular bones and, to some extent, scales). On the other hand, as most otoliths from older fish could not be reliably aged (at least with the techniques used here), and would therefore be uninterpretable, the use of opercular bones would offset the variability in annulus counts seen in the age bias plots for otoliths.

Pseudoannuli (present in all structures during the second year of growth) and variations in annulus patterns with age did not appear to affect the reproducibility of age interpretations. In whole otoliths, however, the presence of pseudoannuli during the first 3–4 years (in class 3 preparations) probably was responsible for bias at older ages in the replicate counts by interpreter A. After this experience, the second count of whole otoliths was deemed more

<sup>4</sup> Powers, J.E. 1983. Some statistical characteristics of ageing data and their ramifications in population analysis of ocean pelagic fish. NOAA Technical Report NMFS 8: 19–24.

reliable than the first. The lack of bias between annulus counts on whole otoliths from interpreter A (second count) and interpreter B at all ages suggests that these problems were largely overcome. As a result, the classification of opercular bones and otoliths into arbitrary morphological classes was of real value in identifying patterns likely to confound age interpretations.

Interpretations of annulus patterns in carp scales, opercular bones and otoliths are not easy and instruction and experience are required for precise and unbiased assessments (see also Vilizzi et al. 1998). We recommend the use of both opercular bones and whole otoliths in routine age interpretations of carp in the Murray-Darling Basin. The use of opercular bones to age older fish (10+ years) should help to resolve difficulties in detection of later annuli in otoliths. Whole otoliths are recommended as a means to age carp up to 10 years, and are essential if the locations of the first 2–3 annuli are to be accurately determined. The visibility of otolith annuli is not improved by sectioning. Scales are less easily interpretable, but may be useful in less rigorous assessments of the age profiles of carp stocks (they may be the only option in examinations of historical collections). Scale samples do not harm the fish, and could be of value in tagging programs where the fish are injected with markers (McFarlane & Beamish 1987). In monitoring the dynamics of carp stocks researchers undertaking routine age assessments could exchange collections of calcified structures (Kimura et al. 1979, Boehlert & Yoklavich 1984, Boehlert 1985), ensuring that the bias and precision of counts do not deteriorate over time.

### *Growth models*

*Precision, realism and generality* are three key attributes that an ideal model should possess. Ricker (1979) referred goodness of fit and convenience as the only valid criteria in choosing a growth curve, and cites a number of papers concerning the mathematico-physiological theory of growth regulation. Moreau (1987) also addressed the problem of quality of fit and convenience. Chen et al. (1992, p. 1233) reported that 'advocates of the PFs [polynomial

functions] emphasize their mathematical simplicity rather than their biological usefulness'. In fact no model could satisfy all of the above requirements – a precise-realistic-general model inevitably would require trade-offs between each attribute. In the present study an integrated approach was adopted.

Several models have been proposed to describe growth in length or weight in fish (reviews in Ricker 1979, Moreau 1987). Chen et al. (1992, p. 1234) noted that comparisons among growth functions have seldom been made (but see Beckman et al. 1990), especially between the VBGF and polynomial functions, and recommended that 'before selecting the growth function for a growth data set, a systematic comparison should be done among growth functions being considered for use'. In keeping with this recommendation, the analyses here indicate that a parabola with log-transformation of both predictor and response variables (here referred to as LLQF, equivalent to PF3 of Chen et al. 1992) provided the best fit, and was chosen by virtue of its precision and ease of computation. However, the VBGF was retained as an alternative model to allow comparisons with published data and provide 'quasi-biological' parameters for describing growth. Chen et al. (1992) also suggested use of the VBGF in their study of six species, with the LLQF as an alternative linear function. In contrast, most comparative studies of the VBGF and polynomial models imply a mutually exclusive choice between the two approaches. Roff (1980; see also Knight 1968) proposed 'retirement' of the VBGF, urging fisheries biologists to rely exclusively on polynomial functions, but his call has gone largely unheeded (McDowall 1994).

When modelling growth in males and females no appreciable improvement in goodness of fit was gained from the LLQF rather than the VBGF, in contrast to the preliminary comparison of growth models. This is explained by considering that the lower portion of the growth curve, especially the VBGF, will be truncated in the absence of younger fish, resulting in an incomplete data set. In the present study no histological examinations of the gonads of immature fish were made, so that their sex remained unknown.

The maximum estimated ages for males and fe-

males in the present study were 12 and 15 years, respectively, and the females attained asymptotic lengths and weights greater than the males. The dearth of information on the age and growth of carp in Australia does not allow comparisons, except that Jones (1974) cited mean total lengths for 1-, 2- and 3-year old carp from the lower Murray River of 440, 630 and 720 mm respectively, consistently higher than those reported here.

Following the seminal study by Boehlert (1985), several researchers have investigated the possibility of using otolith weight as a means to estimate the age profiles of fish stocks (e.g. Worthington et al. 1995), and analyses of otolith morphometrics have become routine in age and growth studies of species other than common carp (Brothers 1987). In the present study the variation in age explained by otolith weight, even after inclusion of additional morphometric variables, was too low to warrant computation of an otolith weight vs. age relationship. This was not unexpected, as the peculiar morphology of carp asterisci may have been an additional source of variability, stemming from the uncoupling of otolith and somatic growth rates (Wright et al. 1990; but see Secor & Dean 1992). As a result, the otolith growth models reported here are intended only for descriptive purposes. The growth in length and weight of otoliths might be described interchangeably using a VBGF or linear function, as for somatic growth.

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