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Age and Growth of Murray Cod, *Maccullochella peelii* (Perciformes: Percichthyidae), in the Lower Murray–Darling Basin, Australia, from Thin-Sectioned Otoliths

John R. Anderson [A], *A. K. Morison* [B] *and D. J. Ray*

Kaiela Fisheries Research Station, Department of Conservation and Natural Resources, PO Box 1226, Shepparton, Vic. 3630, Australia.
[A] Present address: Faculty of Resource Science and Management, University of New England, Northern Rivers Campus, PO Box 157, Lismore, NSW 2480, Australia.
[B] Present address: Central Ageing Facility, Marine Science Laboratories, Department of Conservation and Natural Resources, PO Box 114, Queenscliff, Vic. 3225, Australia.

Abstract

Transverse thin sections (0·5 mm thick) of sagittal otoliths from 290 Murray cod up to 1400 mm in total length and 47·3 kg in weight were used to establish the age and growth of cod in the lower Murray–Darling Basin, including comparisons of recent (1986–91) and past (1949–51) growth rates and growth in different waters. The maximum estimated age was 48 years. Quantitative and qualitative analysis of the seasonal changes in otolith marginal increments showed that annuli in fish of all ages were laid down each spring, and 1 October was assigned as the birthday. The thin-sectioning method was validated by comparing age estimates for 55 Murray cod from Lake Charlegrark (age 0–21 years), which had been validated by using burnt and polished half-otoliths. The new method had an accuracy of 96·4% and it offers major advantages in ease of preparation, reading, and batch-handling of large numbers of otoliths. The precision of the method, estimated as an average error for four readers, was 5·4% (3·0% after ignoring discrepancies in relation to annuli on otolith edges). There was a linear relationship between otolith weight and fish age and an exponential relationship between otolith weight and fish length. Both otolith length and otolith width reached an asymptote at about 15 years, when fish length also approached its maximum. However, otolith thickness continued to increase throughout the life of the fish and, after about 15 years, contributed most to the increase in otolith weight. This confirmed that otoliths continued to grow in thickness and that annuli were laid down throughout life, and that cod could be aged reliably to the maximum age. The annulus pattern is very clear and distinct, and the reading techniques are fully described, including recognition of 'larval' and 'false' rings. Various differences were found in the growth rates, and the length–weight relationships for males and females, for cod caught in 1986–91 and those caught in 1949–51, and various subpopulations are discussed. The von Bertalanffy growth parameters (all individuals combined) were estimated at $L_\infty = 1202$ mm, $k = 0·108$ and $t_0 = -0·832$. The availability of a reliable ageing method provides the first opportunity to determine year of birth and thus to examine the age structure of populations and to effectively manage cod populations that have declined in abundance.

Introduction

An accurate and reliable knowledge of the age structure of a fish population, which is relatively easily obtained, is one of the fundamental criteria necessary to manage a fishery or a population of a species under threat. The Murray cod, which occurs naturally throughout the Murray–Darling Basin, is Australia's and one of the world's largest freshwater fish (Rowland 1989). It is regarded as a symbol for the Murray River itself, and it is the premier target species for inland recreational fishing based on endemic species in New South Wales and Victoria, primarily because of its large size. Whitley (1955) stated that Murray cod grow to 6 ft (1·8 m) and 182 lb (83 kg). There is a newspaper report of a cod of 113·6 kg

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(250 lb) being caught in the Barwon River near Walgett (New South Wales) in 1902 (Noble 1955), but the report depended on memory rather than direct substantiation and remains doubtful. Cod up to 40 kg are still caught in the lower Murray–Darling Basin (Rowland 1989).

The decline of the inland commercial fishery largely based on Murray cod (Rowland 1989), and the general decline in the species' abundance to the level where its future conservation is now threatened, highlight the need to gather the information required to effectively manage the species. The commercial fishery based on Murray cod peaked in 1918 but had declined to an unprofitable level by the mid-1930s (Rowland 1989), and the substantially reduced commercial fishery now depends on other species (Rowland 1985).

The abundance of cod in southern New South Wales has probably remained at a stable but low level over the last 25 years (Rowland 1989). In Victoria, since 1970, Murray cod have undergone a marginal reduction in their natural geographic range but have declined markedly in abundance (Cadwallader and Gooley 1984). Murray cod are now classified as a vulnerable species in Victoria (that is, a species that has undergone a substantial reduction in range and abundance and that is likely to become an endangered or threatened species if current trends continue) (Koehn and Morison 1990). The taking of cod is now banned in South Australian waters.

Previous attempts to manage the species and to understand and reverse the causes of its decline have been hampered by the lack of a reliable ageing technique to establish the age structure of populations and to compare growth rates and recruitment in different areas and in different years. Previous attempts to establish a link between the occurrence of dominant size classes and changes in the flooding regime have been based on length-frequency analysis, and on the use of commercial catch records as a measure of relative abundance, despite the problems with using such data (Reynolds 1976). The major initial strategy to improve the conservation status of Murray cod and to increase their numbers has been based on artificial breeding, rearing and restocking. In the absence of a reliable batch-marking technique for stocked juveniles, age determination is the only method currently available for stock identification and for monitoring the outcome of stockings. Only when the stocked populations have been shown to reproduce naturally and reliably can the establishment of viable self-sustaining populations be confirmed and the conservation status of the species be improved. Again, the availability of a reliable and verified ageing technique is essential for monitoring recruitment from stocked and natural populations and thereby for effective management of the species.

Various attempts to age Murray cod have been made since Llewellyn (1966) concluded that Murray cod were perhaps the most difficult of any of the larger native freshwater fish to age. Lake (1967) used whole otoliths to provide unvalidated estimates that a Murray cod of about 37 kg was about 15 years old and that cod become sexually mature when they are about 4 years old. Jones (1974) made some preliminary comments on the ageing of cod from otoliths, but he examined only 26 specimens. Rowland (1985) used whole sagittal otoliths and operculae to age 330 Murray cod, with a maximum total length of 1270 mm and weight of 40 kg, caught from six rivers and two impoundments in southern New South Wales. The method was validated by using known-age fish up to 3 years old as well as seasonal changes in marginal increments and the type of marginal edge material (opaque or hyaline) for pooled age/classes. The oldest estimated age he obtained was 34 years (1250 mm, 36 kg). Reading whole otoliths was found to be unreliable for cod larger than 800 mm (about 8 years old). Operculae were used for larger fish (Rowland 1985). Gooley (1992) used broken, burnt and polished half-sections of sagittal otoliths from 231 Murray cod from an isolated population in Lake Charlegrark, south-western Victoria, to provide a validated method for Murray cod up to 11 years old. The method was validated by using known-age fish up to 4 years old, seasonal changes in marginal increments, and modal progression of strong year classes in Lake Charlegrark. The oldest estimated age was 21 years.

The aim of the present work was to determine the accuracy and precision of the use of thin otolith sections for ageing Murray cod in the lower Murray–Darling Basin. Techniques using thin sections of otoliths are potentially more accurate, more precise and more reliable for older fish and are potentially more efficient because large batches of samples can be processed simultaneously and no staining, burning or other treatments are required. Otoliths have generally been shown to be the most reliable method for ageing fish compared with the use of other methods (Erickson 1983; Sharp and Bernard 1988; Casselman 1990). Annuli in otoliths of temperate-zone fishes have been shown to be generally related to seasonal changes in water temperature and concomitant changes in the growth rate of fish (Williams and Bedford 1974; Begenal and Tesch 1978; Taubert and Tranquilli 1982; Jearld 1983; Perry and Tranquilli 1984). Schramm (1989) provided experimental verification for the method by showing that, in bluegills (*Lepomis macrochirus*), the translucent bands, counted as annuli in otolith sections, were formed in response to cyclic temperature changes. Thin-section techniques have been shown to be more reliable than the use of whole otoliths, which are generally difficult to read, particularly with older and larger fish (Beamish 1979*a*; Harris 1985), or the use of broken, burnt and polished half-sections (Beamish and Chilton 1982; Perry and Tranquilli 1984). Beamish (1979) suggested that the examination of sections of otoliths should become a routine approach to any attempt to age fish from otoliths.

We also aimed to provide an adequate description of a standard method applicable to most, if not all, native fish in the Murray–Darling Basin. Anderson *et al.* (1992) have validated the method for golden perch (*Macquaria ambigua*), and the method also has potential for trout cod (*Maccullochella macquariensis*), Macquarie perch (*Macquaria australasica*) and silver perch (*Bidyanus bidyanus*), and for freshwater catfish (*Tandanus tandanus*) (modified technique of Davis 1977, using dorsal spines) (Anderson 1992). We aimed to provide a complete description of the preparation, sectioning and reading of the otoliths so that other research groups in the Murray–Darling Basin could confidently age cod by the same techniques, provided that verified material, or known-age material, was available. The need for an exchange of material between research groups to verify age estimates and to ensure consistency and accuracy in ageing has been discussed generally by Boehlert and Yoklavich (1984), and by Anderson (1992) for native fish in the Murray–Darling Basin.

Establishing the accuracy of the method and developing reliable reading techniques depended on the provision of validated material from Lake Charlegrark (Gooley 1992) and the provision of material from a wide range of sites in the lower Murray–Darling Basin. Many of the otoliths were provided by anglers, and the emphasis generally was on the larger specimens to try to establish the estimated maximum age of cod and a reliable growth curve. Thanks to the foresight of early fisheries officers from New South Wales, we had access to a historical collection of 151 Murray cod otoliths, collected by Langtry during his surveys of the Murray River and its tributaries in 1949–51 (Cadwallader 1977), when commercial catches were higher than they have been for the last 25 years. This provided a unique opportunity to compare modern and historical growth rates. Comparisons of growth rates and length–weight relationships for various subsamples of cod collected from various areas throughout the lower Murray–Darling Basin have been used to provide a preliminary assessment of the variability in growth and body form in different areas.

Materials and Methods

Source of Fish

There were four major sources of Murray cod used in this study: a subsample of 55 specimens from Lake Charlegrark (part of the sample used by Gooley 1992), a historical collection of 151 cod caught by drum-net and gill-nets throughout the lower Murray River and its tributaries by Langtry in 1949–51 (Cadwallader 1977), 49 cod caught during surveys in Victoria by staff of the Kaiela Fisheries Research

Station, and 40 cod caught by anglers and donated for the study or located by investigating newspaper and other reports of large cod being caught. Both the historical and the recent samples included fish from the lower Murray–Darling Basin, including the Murray River and its Victorian and southern New South Wales tributaries, generally south of latitude 33°S, and the endorheic Wimmera River catchment and associated lakes (Green and Taylors Lakes). Further details on localities and dates of capture are provided in the Appendix.

Data on total length, weight and sex were available for most, but not all, of the donated fish. Both sagittal otoliths of each fish were removed with tweezers from the exposed sacculi after the gill arches had been removed and the ventral outer covering of the sacculi had been cut away with a knife. The terminology used to describe the otolith and its features are those used by Blacker (1974) and Summerfelt and Hall (1987), and the terms 'anterior', 'posterior', 'dorsal', 'ventral', 'proximal' (='internal') and 'distal' (='external') refer to the position on the otolith relative to the original orientation of the otolith in the fish (see Fig. 1). The simpler term 'medial groove' has been used instead of 'sulcus acusticus'.

The otoliths were wiped clean and stored in envelopes or glass jars. The total length (span length—anterior to posterior) and width (dorsal-ventral) of each otolith was measured with dial callipers as described by Anderson *et al.* (1992). Because an otolith appeared to become more curved as it increased in size, the length measured as a span may have underestimated the actual length increase along the edge of the otolith. Otoliths were then weighed to the nearest 0·01 g. Generally, the left otolith was taken for sectioning unless it had been damaged in some way. It was considered unlikely that this would introduce any errors because other studies have shown that it is extremely rare for there to be different ring patterns on the two sagittal otoliths from the same fish (Williams and Bedford 1974).

Otolith Preparation and Sectioning

The techniques for preparing, measuring, embedding, sectioning, mounting and reading cod otoliths were identical to those described by Anderson *et al.* (1992) for golden perch otoliths. Beamish (1979*a*, 1979*b*) suggested that burning otolith sections could be used to improve resolution, but this was not found to be necessary with Murray cod otoliths. No staining techniques were employed, and no other treatments were used. A selection of one to five sections, each about 0·4–0·6 mm thick, that passed through or close to the primordium were mounted on glass slides in polyester resin and covered with a glass cover slip.

Reading Techniques

Reading the age of fish from patterns on scales or otoliths is not a simple and unequivocal process (Pannella 1974). Lack of consistency in the use of terms and confusion in the application of the same terms to whole and sectioned otoliths under different lighting conditions has increased the need for clear and precise use of terms. The reversal of the *appearance* of opaque and translucent bands as 'light' and 'dark' bands in transmitted and reflected light has caused confusion in the recognition and use of the terms 'hyaline' and 'opaque' bands (Jensen 1965; Blacker 1974; Liew 1974; Pannella 1974; Beamish 1979*a*, 1979*b*). We have adopted the following terminology adapted from the definitions of Summerfelt and Hall (1987) for 'annulus' (concentric mark used to age fish), 'opaque band' (=dark band) and 'translucent band' (=light band) when thin sections are viewed with transmitted light. The term 'annulus' is hereafter used to refer to an annual mark or band corresponding to the transition or boundary between slower winter growth (opaque=dark band) and faster summer growth (translucent=light band) on the otolith sections. In the present study an annulus was usually terminated by a more-or-less distinct continuous narrow dark line or band across the proximal face of the otolith on both the dorsal and the ventral side of the medial groove, often immediately followed by a narrow clear area. A true annulus was often clearly distinguished because it extended as a band across part of the medial groove itself. Figs 1 and 2 show the distinctive alternate light (translucent) and dark (opaque) bands seen on Murray cod otolith sections under transmitted light. The banding pattern remained relatively easy to read even with very large and old fish (Fig. 2). Examination of otolith sections from Murray cod caught at different times of the year showed that the narrow opaque bands corresponded to the period of transition from the slow growth in winter to the faster growth in spring and early summer. The techniques for distinguishing between 'true' and 'false' annuli, and for recognizing 'larval bands', were generally similar to those described by Anderson *et al.* (1992) for golden perch, but there were important differences that need to be understood if correct counts of annuli and estimated ages are to be obtained. The true annuli were distinguishable as narrow dark bands extending down both the dorsal and the ventral sides of the medial groove. Often, the annuli had the appearance of being composed of densely packed finer circuli, particularly on the extreme outside of the proximal face of the otolith. Liew (1974) describes the banding pattern seen on transverse otolith sections as being derived from a series of concentric lamellae, which themselves consist of light and dark rings of varying thickness. This was similar to the pattern seen on Murray cod otolith sections. This feature was used as the first sign that a new annulus had formed on the edge, along with the presence of a narrow discernible edge of translucent material along the inner edge distal to the narrow dark line of the annulus itself.

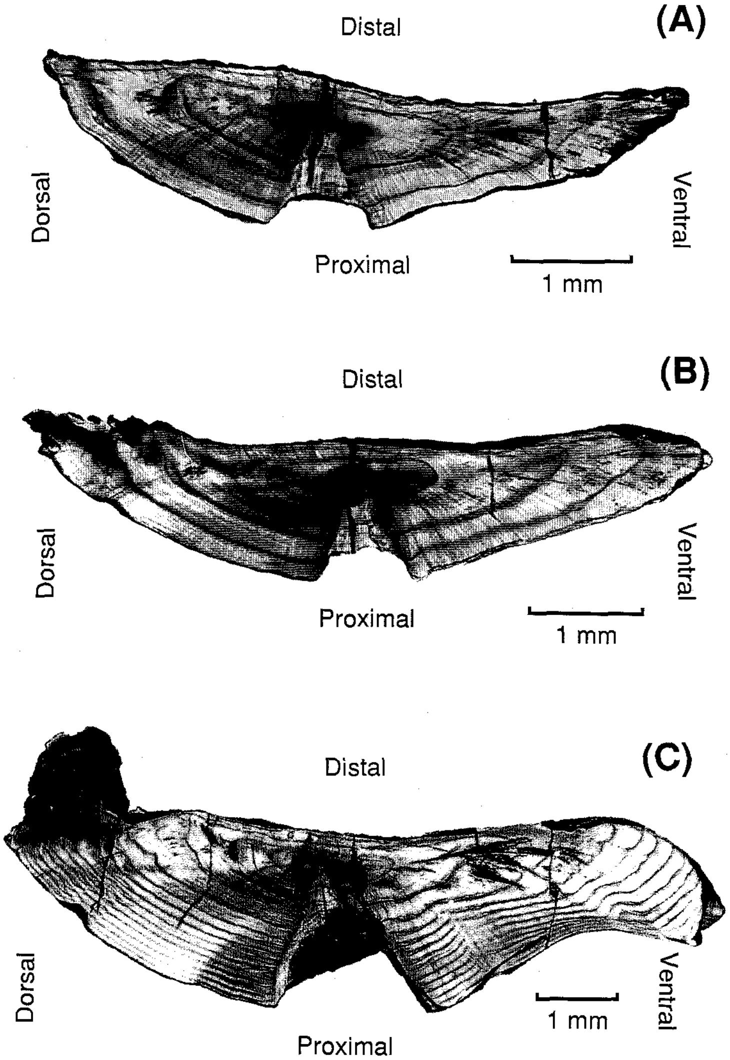

Fig. 1. Thin sections of *Maccullochella peelii* otoliths prepared by sectioning through the nucleus and photographed with transmitted light. Otoliths are from (*A*) a fish aged 2+ years; (*B*) a fish aged 4+ years, showing a new annulus just formed; and (*C*) a fish aged 13 years.

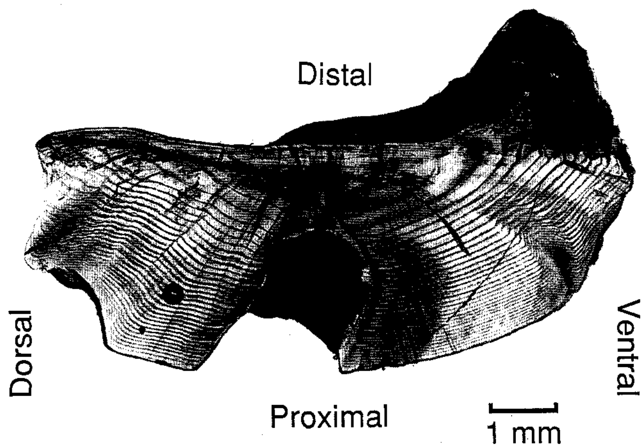

Fig. 2. Thin section of a *Maccullochella peelii* otolith prepared by sectioning through the nucleus and photographed with transmitted light. Otolith is from a fish estimated to be 34+ years old.

The reading techniques were evolved over a period of time by a process of calibration and verification (in the sense of Anderson 1992), using a known-age Murray cod from Lake Charlegrark. The availability of this reference material was crucial for verification and calibration of the reading techniques. Thin sections provide a great deal of detailed structure and pattern that can easily lead to misinterpretation and counting errors, particularly for the first three or four true annuli when 'false rings' and the 'larval ring' cause problems (Fig. 2). Each reader examined validated sections and unknown material successively until he was confident he could reliably age the fish. Rereading of the validated material was used to resolve disputes in the technique and to 'recalibrate' as necessary, particularly in the recognition of 'larval' and 'false' rings. All final readings (including those from Lake Charlegrark) were done without the reader having any information about the size of fish or the locality and date of capture. Williams and Bedford (1974) refer to the biases introduced when readers know the length of fish, and other biases arise when set increments are used to delineate true annuli from larval rings and false annuli. Although some differences in interpretation between readers remained, particularly in the recognition of annuli at the edge of otoliths, a reasonably consistent reading technique was developed by this means. The primordium was visible on many sections as an opaque area, often surrounded by a 'larval' check discernible as a faint band not extending far beyond the start of the medial groove and often coalescing with the first true annulus along the distal side of the section (Fig. 2). 'False' annuli, often found between the first three 'true' annuli, were distinguished as being visible only on one side of the medial groove, not forming a complete band around the distal face of the section, and having a fainter appearance. It was often necessary to examine several sections of the same otolith to distinguish these 'false' annuli and to separate the first 'true' annulus from the 'larval' check. Annuli were more easily counted beyond the first three or four as few, if any, 'false' annuli were apparent.

All otoliths were read independently by four readers. As well as counting the annuli, each reader was asked to classify each otolith according to the relative size of the marginal increment and whether an annulus was counted at the edge of the otolith. A three-element code was employed with C being used when an annulus was counted on the edge and the marginal increment was estimated to be less than 25% of the width of the last complete increment, N being used when the marginal increment was more than 75% of the last complete increment but no annulus was counted on the edge, and Z being used for other otoliths. This provided a qualitative method for assessing the seasonal changes in marginal increments to confirm that the annuli counted were truly laid down annually, and for detecting bands on the edge for verification of age estimates between readers (Anderson *et al.* 1992). This generally corresponded with the terminology and edge classification (summer and winter and opaque and translucent edges) recommended by Jensen (1965). Each reader also measured the annulus 'diameter', for the first annulus, as the maximum dorso-lateral span of the annulus across each section, without necessarily passing through the primordium. This 'diameter' was used as a measure of increments between bands, in preference to the 'radius' as the distance from the focus of the otolith to the annulus (Summerfelt and Hall 1987), because of the difficulty in always establishing the location of the primordium in every section. No consistent direction for measuring annulus increments could be established, and 'diameter' measurements were not considered reliable beyond the third annulus, because no annuli were visible in the dorso-ventral axis for larger cod. In older cod, the preferred path for counting annuli was down the proximal axis along or beside the edge of the medial groove. The preferred path was curved rather than linear in older cod, which made consistent measurement of increments difficult or impossible.

One reader measured the otolith thickness (maximum proximal-to-distal distance on the section, generally to the edge of the medial groove) and the otolith width (maximum dorso-ventral distance on the section) with a micrometer. This reader also measured the diameters of the first three annuli, which completed the series of otolith dimension measurements made on whole and sectioned otoliths.

Verification

A verification procedure was used to ensure consistency in age estimates. Age estimates were verified only when all four readers agreed or when a majority of readers agreed and the difference between readers was confined to whether there was an annulus on the outer edge of the section (Anderson *et al.* 1992). Many disputes concerning annuli at the edge were automatically resolved because of the adjustments made in relation to the date of capture and the designated birthday (discussed below). Disputed otoliths were generally re-examined by all four readers (one or two readers if the dispute concerned an annulus at the edge) without the readers knowing any details of the previous estimates until the counts were verified by a majority of readers. Unverified otoliths were discarded from further analysis. Only 10 otoliths could not be verified in this way, and most of the disputes concerned whether an annulus was present on the edge or whether the first annulus counted was a true annulus or the larval 'check'.

Periodicity of Annulus Formation

Marginal-increment indices, expressed as percentages of the last complete increment, were calculated for the first three annuli by taking the total-width measurement less the diameter of the distal annulus and dividing it by the difference between the diameters of the two outermost bands. For otoliths with only one annulus, the total-width measurement was divided by the diameter of the first annulus and expressed as a percentage. The marginal-increment measurements and the qualitative C, N and Z edge-classification technique were used to verify that the annuli were indeed produced each year, and to assign a 'birthday' for cod and so to derive standardized age estimates in decimal years (e.g. 2·37 years).

The measurement of the diameter of the first annulus counted by each reader, and the edge-classification system (C, N or Z), provided an effective method for resolving most discrepancies between readers that involved disagreement over the first and the last ring to be regarded as an annulus and counted.

Designation of a Birthday

It was convenient to link the assigned birthday to the formation of the new annulus, rather than using a calendar age or some other date not related to when the new annulus formed each year. There was some variation in the actual date on which the new band was completed and was counted in individual fish, and therefore some adjustments to the annulus counts were required for fish caught in the months immediately before and after the assigned birthday. The first day of October was initially assigned as the birthday for reading purposes. Fish that had formed an annulus prior to the assigned birthday (classified as C by a majority of readers, i.e. CCC_) had their annulus count reduced by one. Fish that had not formed an annulus when caught after the birthday (classified as NNN_) had their annulus count increased by one. Ages were then calculated in relation to the adjusted annulus counts and the date of capture after the assigned birthday.

Validation and Accuracy

The use of thin otolith sections was validated by establishing the accuracy of the method compared with estimates obtained by Gooley (1992) from burnt and polished half-sections. The second otolith of each pair taken from 55 cod from Lake Charlegrark (Gooley 1992) was made available for thin-sectioning. The ages of the subsample ranged from 0 to 21 years and included replicates of known-age fish from 0 to 4 years old (38 fish). All of the otolith thin sections were read independently by all four readers without the readers knowing details of the ages, lengths or times of capture of the fish. Determining accuracy in this way obviously depended on whether the results obtained by the two methods were expected to be identical and whether the age estimates obtained independently from the two otoliths from the same fish were expected to be the same. It has been shown that the two methods generally produce almost identical age estimates (e.g. Beamish 1979*a*). Although no comparisons were made for Murray cod, it was considered very unlikely for there to be different ring patterns on the two otoliths from the same fish (Williams and Bedford 1974).

Precision

The precision of the estimates among readers was established by using the method of Beamish and Fournier (1981). This method is based on calculating the average absolute deviation from the mean age obtained by the readers, expressed as a function of the arithmetic mean age, for each age estimate. The estimated average error produced has the property that differneces in age estimates for younger fish will contribute more to the estimated total-precision measure than will similar errors for older fish. Other models have been suggested (e.g. Chang 1982), but the method of Beamish and Fournier (1981) is the more common method, and its use allowed direct comparison with other studies using thin-sectioning techniques.

$$\text{Average percentage error} = \frac{1}{N}\sum_{j=1}^{N}\left[\frac{1}{R}\sum_{i=1}^{R}\frac{|X_{ij}-X_j|}{X_j}\right],$$

where N is the number of fish aged, R is the number of times fish are aged, X_{ij} is the ith determination for the jth fish, and X_j is the average estimated age of the jth fish.

Growth Rates, Length–Weight Relationships, and Statistics

von Bertalanffy growth curves and length–weight relationships were determined with the nonlinear curve-fitting methods of the SYSTAT package, using least-squares estimation as the loss function and utilizing the models

$$\text{Fish total length (mm)} = L_\infty\{1-\exp[-k(\text{age}-t_0)]\},$$

where L_∞ is the theoretical asymptotic total length, t_0 is the hypothetical age when L is zero, and k is the growth coefficient indicating the rate at which fish total length approaches L_∞, and

$$\text{Fish weight (kg)} = a\times 10^{-5}(\text{fish total length})^b,$$

where a is a constant and b is the exponent of the relationship.

Growth rates and length–weight relationships for different subgroups of the total data set, and for cod from Lake Charlegrark, were compared by using an F-ratio test of the residual sum of squares between the subgroups pair and the combined data for the two subgroups (Zar 1984).

The other curve and regression programs of the SYSTAT package were used to describe the general relationships between various otolith dimensions, age, and fish total length. The curve-fitting routines were used primarily to derive lines and curves of best fit and general trends rather than to establish causal relationships.

The term 'estimated age' is used in presenting the results, rather than 'age' or 'annuli', which, strictly speaking, can be used only when the method has been validated to the maximum age of the fish. The use of otoliths for ageing Murray cod has been validated only to 11 years by Gooley (1992). Provided that the results of the thin-section method are proven accurate in relation to his estimates, validated ages can be claimed only to the same limit. The use of the term 'estimated age' avoids the problems of unjustified extrapolation and allows for ages of more than 11 years to be verified once the validated age range is extended.

Results

Sex Differences

Both the growth curves and the length–weight relationships for male and female Murray cod showed significant differences (Tables 1 and 2). However, the differences were small

Table 1. Values (±1 standard error) for the parameters of the von Bertalanffy growth function and for the length and age of Murray cod in various data sets, and tests of significance between some sets

The parameter values and the residual sums of squares for nonlinear regressions were obtained with the SYSTAT package. The growth function and the significance tests are explained in the text

Sample	Number of fish	L_∞ (mm)	k	t_0 (years)	Length (mm) Mean	Length (mm) Min.	Length (mm) Max.	Age (years) Mean	Age (years) Min.	Age (years) Max.	Residual sum of squares	Test between data sets
Males	100	1058·3 ±47·5	0·156 ±0·014	−0·514 ±0·070	622·58 ±20·51	155	1240	6·31 ±0·50	1·53	37·03	782557·440	Males/females: $F_{3,179}=5\cdot928$ ($0\cdot002>P>0\cdot001$)
Females	85	1041·9 ±122·1	0·130 ±0·044	−0·703 ±0·964	636·09 ±21·08	161	1150	7·76 ±0·57	1·53	39·15	889332·471	
Males + females	185	1064·1 ±53·5	0·131 ±0·021	−0·999 ±0·478	628·79 ±14·69	155	1240	6·98 ±0·38	1·53	39·15	1838003·432	
Entire sample[A]	290	1202·8 ±24·1	0·108 ±0·007	−0·832 ±0·235	644·27 ±16·28	45	1400	8·48 ±0·48	0·46	48·62	2625252·038	
Entire sample, no L. Charlegrark	235	1262·7 ±37·0	0·091 ±0·010	−1·838 ±0·524	701·03 ±16·11	133	1400	9·30 ±0·55	1·34	48·62	1703848·124	L. Charlegrark/lower Murray–Darling Basin: $F_{3,445}=196\cdot653$ ($P<0\cdot0005$)
L. Charlegrark[B]	231	694·6 ±18·3	0·234 ±0·091	0·174 ±0·091	447·24 ±12·31	45	940	5·98 ±0·25	0·46	21·91	789516·119	
L. Charlegrark + entire sample	451	1253·8 ±46·4	0·079 ±0·009	−1·411 ±0·429	571·04 ±11·71	45	1400	7·65 ±0·31	0·46	48·62	5798950·899	
Recent collection, no L. Charlegrark	69	1252·5 ±22·8	0·111 ±0·007	−0·565 ±0·250	780·65 ±40·68	133	1400	13·32 ±1·29	1·34	48·62	376778·113	Historical/recent: $F_{3,214}=7\cdot876$ ($P<0\cdot0005$)
All of historical collection	151	1206·1 ±63·3	0·085 ±0·010	−3·016 ±0·420	664·64 ±13·47	289	1230	7·10 ±0·38	2·08	40·17	1157643·913	
Upstream of Yarrawonga	45	1213·9 ±88·8	0·134 ±0·027	−1·019 ±0·494	695·40 ±27·16	419	1041	5·88 ±0·45	2·85	13·91	69951·467	Historical upstream/downstream of Yarrawonga: $F_{3,145}=27\cdot385$ ($P<0\cdot0005$)
Downstream of Yarrawonga	106	1243·1 ±94·8	0·069 ±0·014	−3·849 ±1·061	651·59 ±15·24	289	1230	7·62 ±0·51	2·08	40·17	668998·786	

[A] Includes a sample of 55 cod from Lake Charlegrark. [B] Data from Lake Charlegrark provided by Gooley (personal communication).

Table 2. Values (±standard error) for the parameters of the length–weight function and for the weight of Murray cod in various data sets, and tests of significance between some sets

The parameter values and the residual sums of squares for nonlinear regressions were obtained with the SYSTAT package. The length–weight function and the significance tests are explained in the text

Sample	Number of fish	$a \times 10^4$	b	Weight (kg) Mean	Minimum	Maximum	Residual sum of squares	Test between data sets
Males	93	0·070 ± 0·033	3·151 ± 0·072	6·250 ± 0·731	0·051	38·101	181841000	Males/females: $F_{2,156} = 7{\cdot}529$ $(0{\cdot}001 > P > 0{\cdot}0005)$
Females	67	0·006 ± 0·003	3·507 ± 0·073	7·019 ± 0·907	0·052	37·500	78513900	
Males + females	160	0·036 ± 0·013	3·250 ± 0·053	6·572 ± 0·569	0·051	38·101	285489000	
Entire sample[A]	257	0·360 ± 0·098	2·910 ± 0·039	8·689 ± 0·657	0·001	47·250	1650610000	
Entire sample, no L. Charlegrark	208	0·429 ± 0·072	2·884 ± 0·024	10·107 ± 0·75	0·031	47·250	1453775000	L. Charlegrark/all lower Murray: $F_{2,434} = 7{\cdot}701$ $(0{\cdot}001 > P > 0{\cdot}0005)$
L. Charlegrark[B]	230	0·098 ± 0·021	3·085 ± 0·032	2·237 ± 0·145	0·001	14·100	24918560	
L. Charlegrark + entire sample	438	0·217 ± 0·014	2·981 ± 0·010	5·975 ± 0·410	0·001	47·250	1531173000	
Recent collection, no L. Charlegrark	82	1·492 ± 1·433	2·705 ± 0·136	14·126 ± 1·484	0·031	46·000	1126732000	Historical/recent: $F_{2,204} = 15{\cdot}833$ $(P < 0{\cdot}0005)$
All of historical collection	126	0·015 ± 0·004	3·381 ± 0·042	7·492 ± 0·694	0·964	47·250	131695500	
Upstream of Yarrawonga	45	0·070 ± 0·045	3·163 ± 0·095	8·415 ± 1·001	1·644	25·401	43884600	Historical upstream/downstream of Yarrawonga: $F_{2,122} = 11{\cdot}628$ $(P < 0{\cdot}0005)$
Downstream of Yarrawonga	81	0·008 ± 0·002	3·474 ± 0·037	6·979 ± 0·925	0·964	47·250	66726360	

[A] Includes a subsample of 49 cod from Lake Charlegrark. [B] Data from Lake Charlegrark provided by Gooley (personal communication).

(Fig. 3) and were considered unlikely to produce major differences in the analyses. The biological significance of the sex differences is considered further in the Discussion. The data for males and females were combined with those for the fish that had not been sexed for all subsequent analyses.

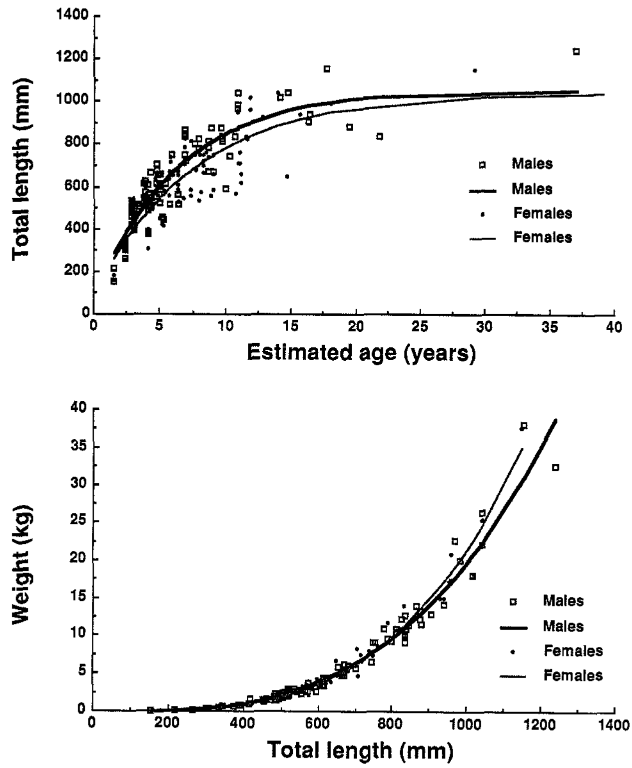

Fig. 3. von Bertalanffy growth-in-length curves and length–weight curves for male and female Murray cod in the total data set. Values for the parameters of the functions used to determine the curves are shown in Tables 1 and 2.

Seasonal Pattern of Annulus Formation

For Murray cod 1–3 years old, the mean monthly marginal-increment index, expressed as a percentage of the last complete increment, was lowest in October and highest in August (Fig. 4). For the entire sample of cod, combining all age groups, the majority of cod caught between the second week of January and the first week of May were classified as ZZZ_ (i.e. as neither CCC_ nor NNN_) (Fig. 5). During the winter months, from the second week of June to the first week of October, most of the fish were classified as NNN_ (summer edge more than 75% of the last complete increment) because the summer edge was relatively broad and had completely formed. Fish with a new annulus on the edge (CCC_) were first caught in September and continued to be classified as having a relatively narrow translucent edge (less than 25% of the last complete increment) until the end of March. Most cod taken from the second week of October until the second week of January had an annulus on the edge, with up to 70% of the sample in any one fortnightly period being classified in this way. Only a minority of fish were classified as having a narrow summer edge after December. This pattern was consistent with the new annulus forming during the winter

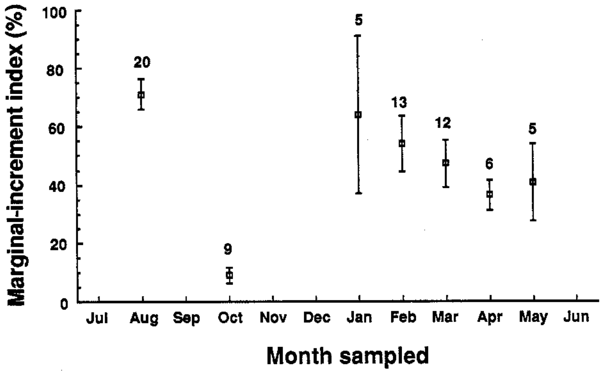

Fig. 4. Mean monthly values (±1 standard error) of the marginal-increment index (expressed as a percentage of the last complete increment) for Murray cod aged 1–3 years. The number of cod included in each sample is shown above each data point.

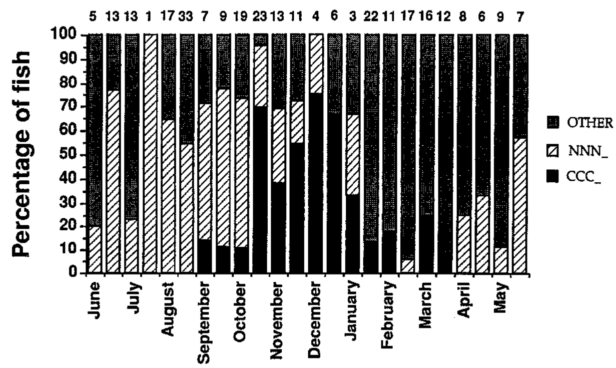

Fig. 5. Percentage of Murray cod of all ages caught in each fortnightly period and classified by a majority of otolith readers as CCC_ (having an annulus on the otolith edge and a marginal increment less than 25% of the last complete increment), NNN_ (not having an annulus on the edge but having a marginal increment more than 75% of the last complete increment), or Other (other than CCC_ or NNN_). The number of cod included in each sampling period is shown above each bar.

months and becoming recognizable as completely formed in the spring. In the southern Murray–Darling Basin, July is generally the coldest month of the year, with minimum water temperatures. The criteria used to distinguish a newly completed annulus on the edge were therefore linked with the transition from slow winter growth to the faster spring growth associated with the increased water temperatures in September and October. The relatively long time over which new annuli were counted on the edge (from September to March) was partially due to the lack of a precise distinction between the C and Z classifications. However, some individuals, particularly older fish, had very narrow translucent summer bands as late as March, and this confirmed that the annulus was formed over a relatively long period of time for individual cod. Given the range of sites and the range of years over which cod were collected, considerable variation in the time of annulus formation would be expected.

Pooling of all of the various age groups may produce erroneous results and could obscure changes in annulus formation for older fish. It was therefore important to demonstrate that annuli were consistently laid down each spring for individual age groups. Fig. 6 shows the seasonal pattern of annulus formation for various individual age groups. There was considerable variation in the month when the new annulus was first seen and the month when the frequency of otoliths with a new annulus reached a maximum in terms of the percentage of fish sampled. Although the number of fish sampled in some months was too small to draw definite conclusions, the majority of fish with new annuli present occurred during October–March for all age groups, and no new annuli were counted during winter (Arpil–July). This confirmed a consistent trend for annuli to be produced each year, generally beginning in October and continuing to be classified as newly formed until February–March.

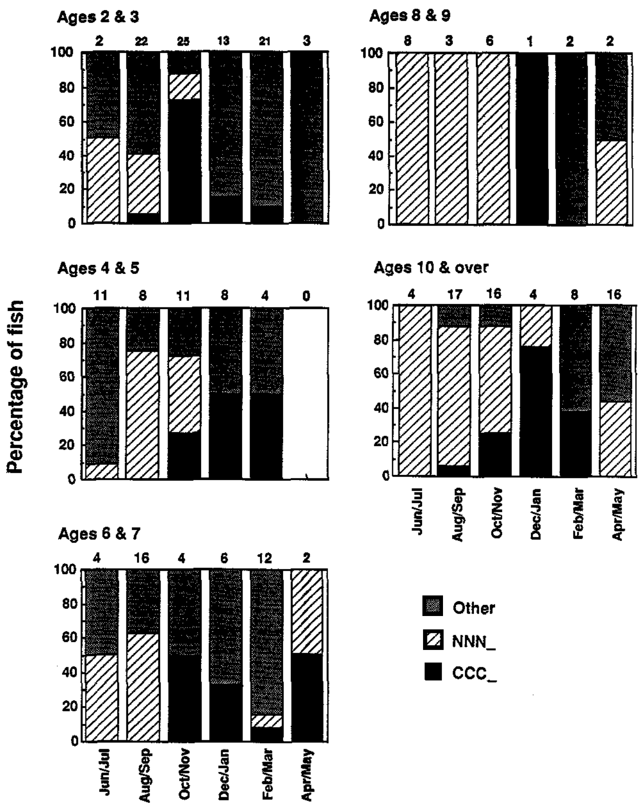

Fig. 6. Percentage of Murray cod in various age classes caught in each bimonthly period and classified by a majority of otolith readers as CCC_ (having an annulus on the otolith edge and a marginal increment less than 25% of the last complete increment), NNN_ (not having an annulus on the edge but having a marginal increment more than 75% of the last complete increment), or Other (other than CCC_ or NNN_). The number of cod included in each sampling period is shown above each bar.

The new annulus appeared to form later in the year for fish more than 10 years old (December–January) than for fish 2–3 years old (October–November) (Fig. 6). This may have been an artefact caused by the narrowing of the increments in older fish, particularly in the width of the summer (translucent) bands, and the associated difficulty in recognizing when new annuli should be counted on the edge. The numbers of fish were inadequate for further analysis.

The mean annulus diameters for fish aged 1, 2 or 3 years showed significiant differences between ages (Fig. 7) but no significant differences between the historical and recent collections. There was, however, considerable overlap in the range of diameters, reflecting differences in the relative growth rates of individual otoliths. The significant differences between the mean diameters at various ages confirmed that the bands counted as annuli were of a relatively consistent size, which would be expected if the bands were produced over a similar time period and the counting was consistent. Major overlaps in the mean diameters would have suggested that false bands were being counted and that there were inconsistencies in the readings.

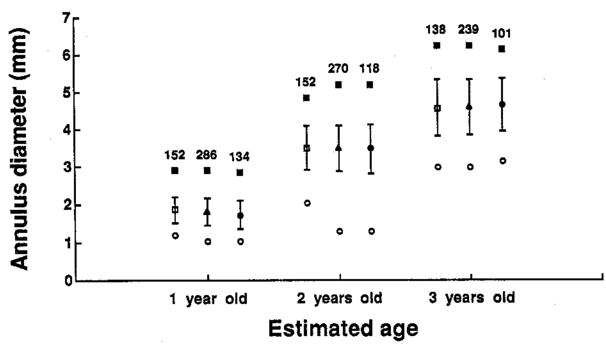

Fig. 7. Mean annulus diameters (±1 standard deviation) for 1-, 2- and 3-year-old Murray cod from (▲) the total sample, (□) the historical collection, and (●) the recent collection. The (■) maximum (○) minimum diameters, and the number of cod included, are shown for each sample.

Designation of a Birthday

The designation of a birthday for Murray cod was based on the date when the new annulus was recognizable as being completely formed on the edge of the otolith and would begin to be counted in most fish. Few cod were found to have new annuli in September, and although only 10% of fish caught in the first week of October had new annuli, this had increased to 70% by the second week of October and remained relatively high during November and December. The first of October was therefore assigned as the birthday for Murray cod. The first of the month was chosen as being more convenient for making age adjustments than a date later in the month, and also as being more conservative, allowing for an expected earlier annulus formation in warmer areas in the upper Murray–Darling Basin. Choosing the first of October also minimized the negative age adjustments required for cod that had formed an annulus prior to the designated birthday. From the birthday and the date of capture, the estimated age of each fish was calculated in decimal years. The assigned birthday coincided with the presumed natural spawning period for cod in the wild, which is from October to November (Cadwallader and Gooley 1984; Rowland 1985). This meant that the year of birth could also be calculated. Age estimates were standardized by adjusting the annulus counts, using the edge-classification system (C, N and Z) to determine the adjustment required (+1 for otoliths sampled after the birthday and classified as N, −1 for otoliths sampled before the birthday and classified as C).

Accuracy

The accuracy of the method, determined by using thin sections of otoliths from the subsample of 55 cod of 'known' age from Lake Charlegrark (aged by Gooley 1992), was estimated at 96·4%. The only two errors involved discrepancies of 1 year—one error was for a fish known to be 3 years old and read as 4 years old (an extra ring was counted on the edge), and the other was for a fish aged 17 years and read as 16 years. The use of otoliths to age cod up to 11 years had been validated by Gooley (1992). The relatively high accuracy of our readings of second otoliths from the same cod from Lake Charlegrark provided validation of the thin-section method. The clarity and consistency of the banding pattern for fish older than 11 years, and the confirmation that older fish showed a consistent seasonal pattern of annulus development, suggested that the ageing technique could be reliable for older fish, potentially to the maximum estimated age.

Precision

The average error for four readers was 5·4% ($n=266$). All readings were done independently, and the precision estimates were made by using the first readings (that is, before additional readings were made as part of the verification procedure). This average error decreased to 3·0% after the elimination of errors caused by inconsistencies in reading annuli close to the otolith edge for fish caught in spring. The relatively high precision between readers was attributed to the clarity of the banding pattern, the success of the 'calibration' procedures adopted that used known-age fish, and the relative consistency between readers in recognizing 'true' annuli.

Growth Rates and Length–Weight Relationships

Fig. 8 shows the relationships between length, weight and estimated age for the combined data set, which includes the sample of 55 cod from Lake Charlegrark. The inclusion of these fish was justified by the stated objective of establishing age and growth relationships for the entire lower Murray–Darling Basin. Most of the results presented below relate to the combined data set, but information is also presented on the data set excluding the Lake Charlegrark sample and various other subsamples of the data for comparative purposes. Values for the parameters of the von Bertalanffy growth functions and the length–weight relationships are shown in Tables 1 and 2. Estimated ages ranged up to 48·6 years (1280 mm in total length, 31·8 kg in weight), total length up to 1400 mm (46·0 kg in weight), and weight up to 47·3 kg. The cod used for the study included 15 individuals that were estimated to be more than 25 years old and that were at or near the hypothetical maximum total length of 1263 mm (excluding the Lake Charlegrark sample). There was considerable variation in length-at-age. One cod with an estimated age of 22 years was about the same length (800 mm) as another estimated to be only 7 years old. Likewise, one cod estimated to be 40 years old was about the same length (1150 mm) as another estimated to be 11 years old. Weights were even more variable, and the variance in weights of fish at the same total length or estimated age increased substantially with length and age (Fig. 8). Cod at any given estimated age had about a four- to fivefold range of weights. Similarly, cod weighing about 10 kg were estimated to be from 7 to 12 years old, and cod weighing about 30 kg were estimated to be between 18 and 48 years old. Yearly average rates of weight gain ranged from 0·43 kg year^{-1} (10-kg cod estimated to be 23 years old) to 2·00 kg year^{-1} (48-kg cod estimated to be 24 years old). The estimated weight for a fish at the hypothetical maximum total length of 1263 mm was about 35 kg.

Parameter values of the growth functions and length–weight relationships for various subgroups of the data and for all the cod studied from Lake Charlegrark (data provided by Gooley from his study) are shown in Tables 1 and 2. Fig. 9 compares the growth rate and the length–weight relationship for cod from Lake Charlegrark with those for cod from other areas. The von Bertalanffy growth function for Lake Charlegrark fish shows a very poor

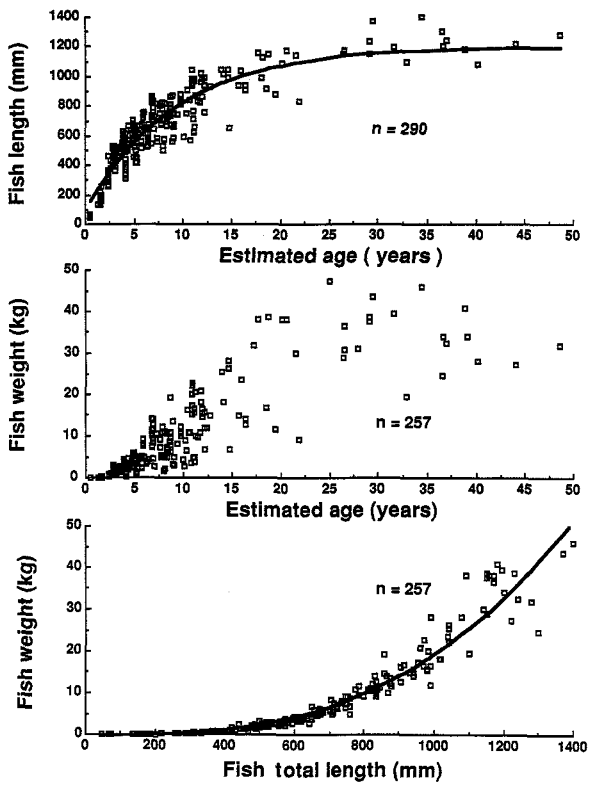

Fig. 8. Relationships between fish total length, weight and age for the total Murray cod sample. Values for the parameters of the functions used to determine the curves are shown in Tables 1 and 2. The number of cod included in each relationship is shown in each graph.

fit for data points in the upper part of the range of values. The L_∞ and k values from this function are probably not valid descriptors of the growth of this group of Murray cod and cannot be used reliably to compare growth between this group and cod sampled from other sites. Murray cod from Lake Charlegrark had a different body form. The estimated growth rate was significantly slower in Lake Charlegrark (Fig. 9, Table 1), and the von Bertalanffy growth function produced a much smaller hypothetical maximum length ($L_\infty = 695$ mm) at a younger age (about 11–12 years) but at a faster rate ($k = 0{\cdot}234$). Cod from Lake Charlegrark were also significantly heavier at a given total length (Fig. 9, Table 2), with the exponent (b) being 3·085, compared with 2·884 for cod from elsewhere. Despite the poor fit of the von Bertalanffy growth function to the cod from Lake Charlegrark, particularly the four cod more than 15 years old (as discussed by Gooley 1992), even these four heavier fish were shorter than cod of similar estimated age from elsewhere (Fig. 9). Also, the mean lengths-at-age for cod aged 2–11 years from Lake Charlegrark were significantly smaller (about 20%) than those for cod from elsewhere (Fig. 10), which confirmed the slower growth rate in Lake Charlegrark.

Cod in the historical collection, caught 30–40 years ago (from 1949 to 1957), had significantly slower growth rates and were heavier for a given total length than cod caught recently (1986–91) (Fig. 11, Tables 1 and 2). Length-at-age was highly variable for cod in the historical collection compared with that for recently caught cod, with a large number

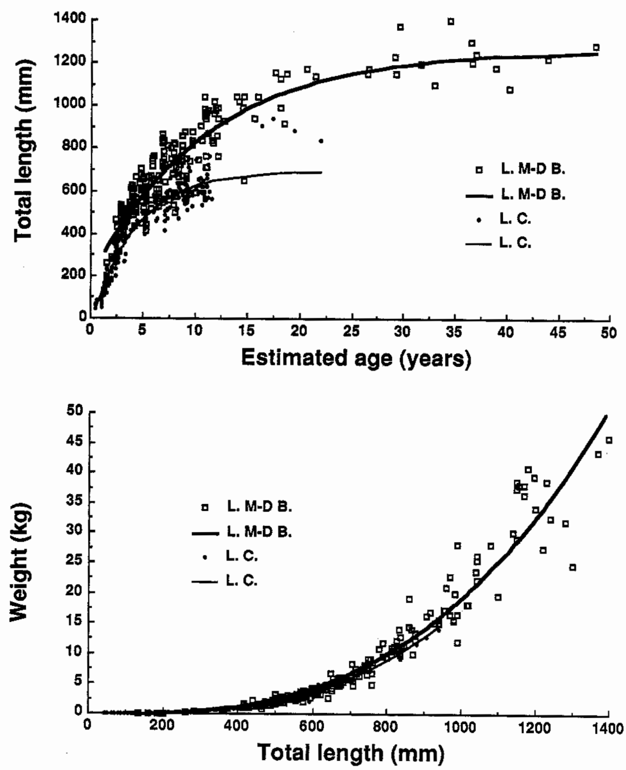

Fig. 9. von Bertalanffy growth-in-length curves and length–weight curves for Murray cod from the lower Murray–Darling Basin (LM-DB) and Lake Charlegrark (LC). Values for the parameters of the functions used to determine the curves are shown in Tables 1 and 2.

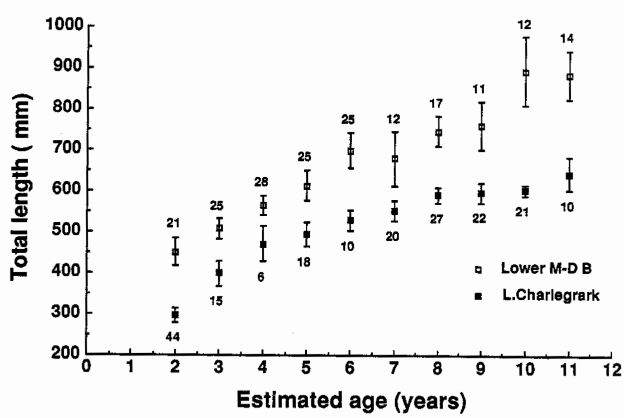

Fig. 10. Mean lengths-at-age (±95% confidence intervals) for Murray cod from the lower Murray–Darling Basin (LM-DB) and Lake Charlegrark (LC). The number of cod included at each age is shown above and below each data point.

of fish 5–15 years old from the historical collection being much shorter than those caught as part of the present study (Fig. 11). However, the fastest-growing fish in the historical collection had similar lengths-at-age as recently caught cod over the same age range. The higher variability in growth rates for cod in the historical collection was the most notable feature. In order to resolve some of this variability, the historical collection was divided into two subgroups. One group, caught upstream of Yarrawonga, consisted of 45 fish caught in the Ovens River and in Lake Mulwala immediately upstream of Yarrawonga Weir. The other group was composed of the remainder of the historical collection, caught from a variety of sources in the Murray River, downstream of Yarrawonga Weir, and its tributaries. Fig. 12 shows the growth curves and length–weight relationships for the two groups, which were significantly different (Tables 1 and 2). Cod caught upstream of Yarrawonga, ranging from 3 to 13 years old, appeared to be longer at a given age, and generally heavier for a given length, than the remainder of the cod (Fig. 12, Tables 1 and 2). The sample sizes were too small for more detailed statistical comparisons.

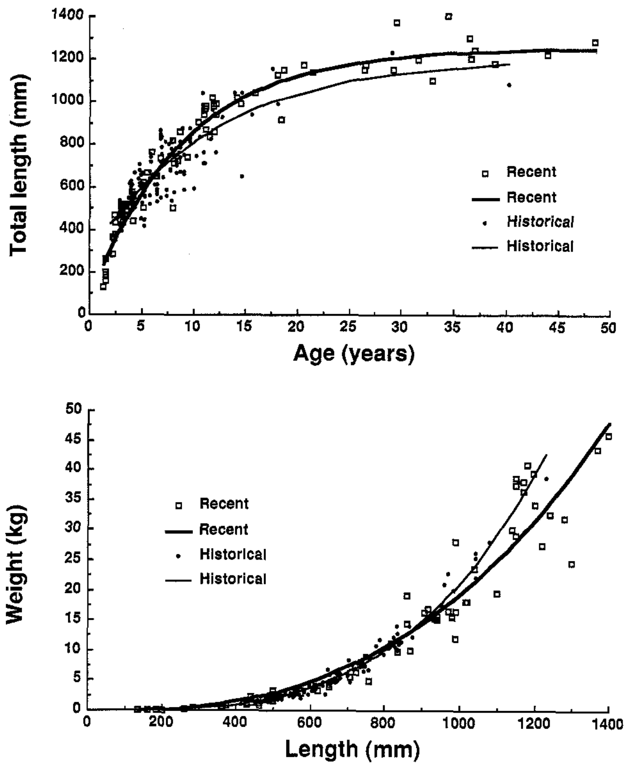

Fig. 11. von Bertalanffy growth-in-length curves and length–weight curves for Murray cod in the historical and recent collections. Values for the parameters of the functions used to determine the curves are shown in Tables 1 and 2.

Otolith Dimensions

Figs 13 and 14 show the relationships between the various otolith dimensions and fish total length and estimated age. von Bertalanffy growth functions were fitted to the relationships between otolith length and age and between otolith width and age (Fig. 13). Various other regression lines were fitted to the other relationships to establish trends and reasonable correlations rather than to necessarily establish causal relationships.

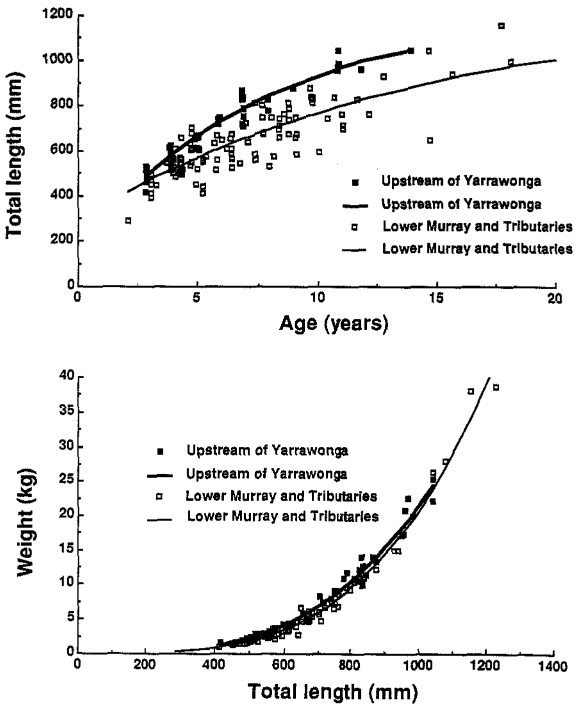

Fig. 12. von Bertalanffy growth-in-length curves and length–weight curves for Murray cod from the historical collection, caught upstream of Yarrawonga (in Lake Mulwala and the lower Ovens River) and in the lower Murray River and its tributaries. Values for the parameters of the functions used to determine the curves are shown in Tables 1 and 2.

The von Bertalanffy growth functions for otolith length and width (Figs 13 and 14) were very similar to that for fish total length (Fig. 8), with the rates of growth in otolith length and width, and in fish total length, declining rapidly after about 15–20 years of age. Second-order quadratic curves explained 93% and 94·5% of the variation in, respectively, otolith length and otolith width with fish total length (Fig. 14), further confirming that the growth in these otolith dimensions was highly correlated with fish total length. However, otolith thickness continued to increase with age, and a piecewise (SYSTAT) regression, with a transition at about 6 years of age (Fig. 13), appeared to provide a reasonable fit to the data. A third-order polynomial was fitted to the relationship between otolith thickness and fish total length (Fig. 14). Otolith thickness increased more or less proportionally with fish total length up to a length of about 900 mm, then increased more rapidly. This transition corresponded to the age (about 12 years) when the rates of increase in otolith length and width, and in fish length, began to approach the asymptotes (Figs 8, 13 and 14).

Otolith weight was linearly related to estimated age (Fig. 13). The relationship between otolith weight and otolith thickness (Fig. 15) was resolved into two phases described by piecewise regression equations, with a transition at a fish total length of about 850 mm (corresponding to an age of about 8–10 years, which was the age at which the rates of increase in otolith length and width began to decline and approach the hypothetical asymptotes). The first of these two phases corresponded to the initial period when fish total length and otolith length and width were increasing rapidly, the second to the subsequent period when the rate of increase in fish total length had declined and the only increase in

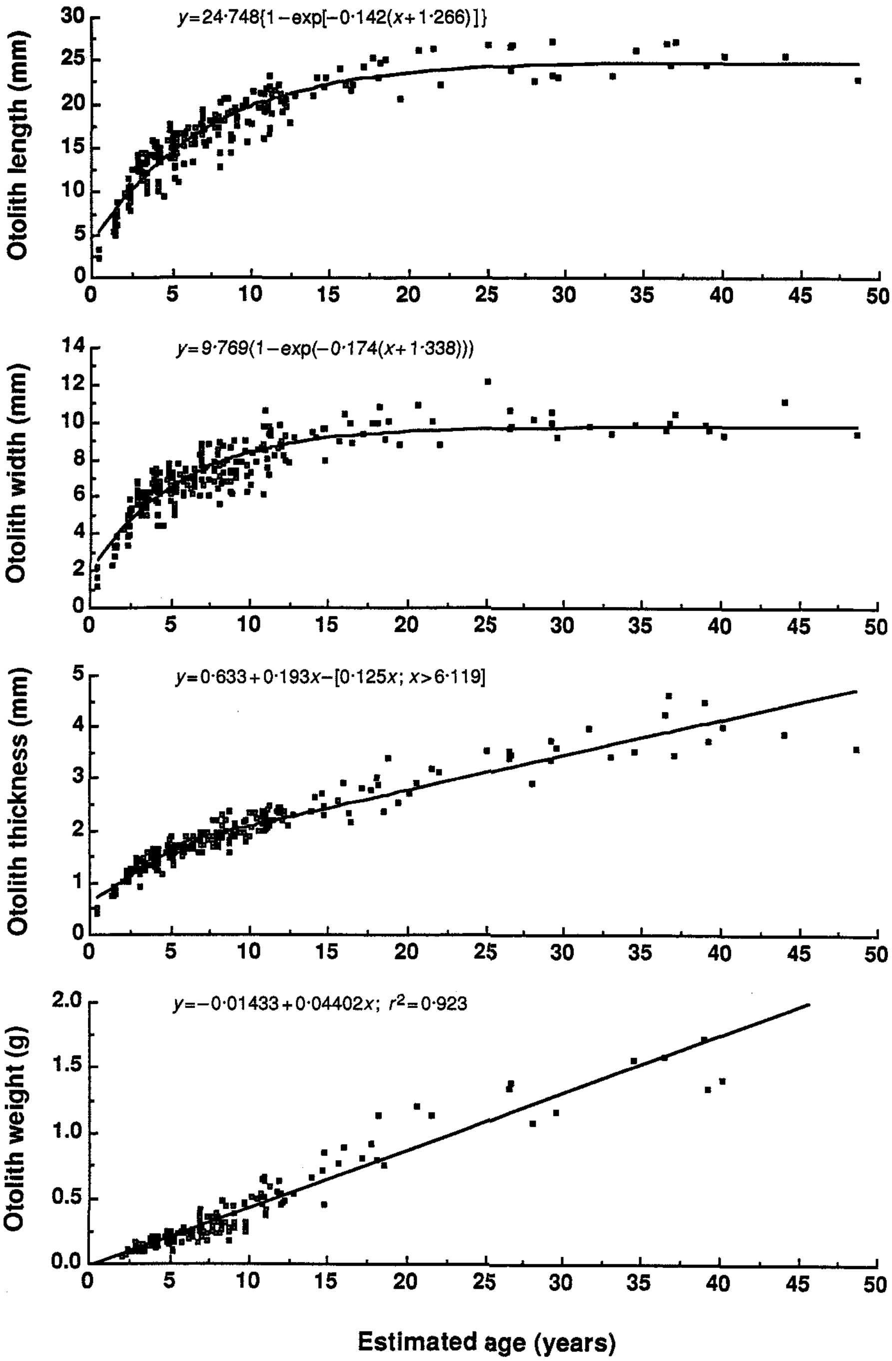

Fig. 13. Plots of otolith dimensions ($n=290$) and otolith weight ($n=192$) against estimated age for the entire Murray cod collection. The functions shown have been used to establish general trends only.

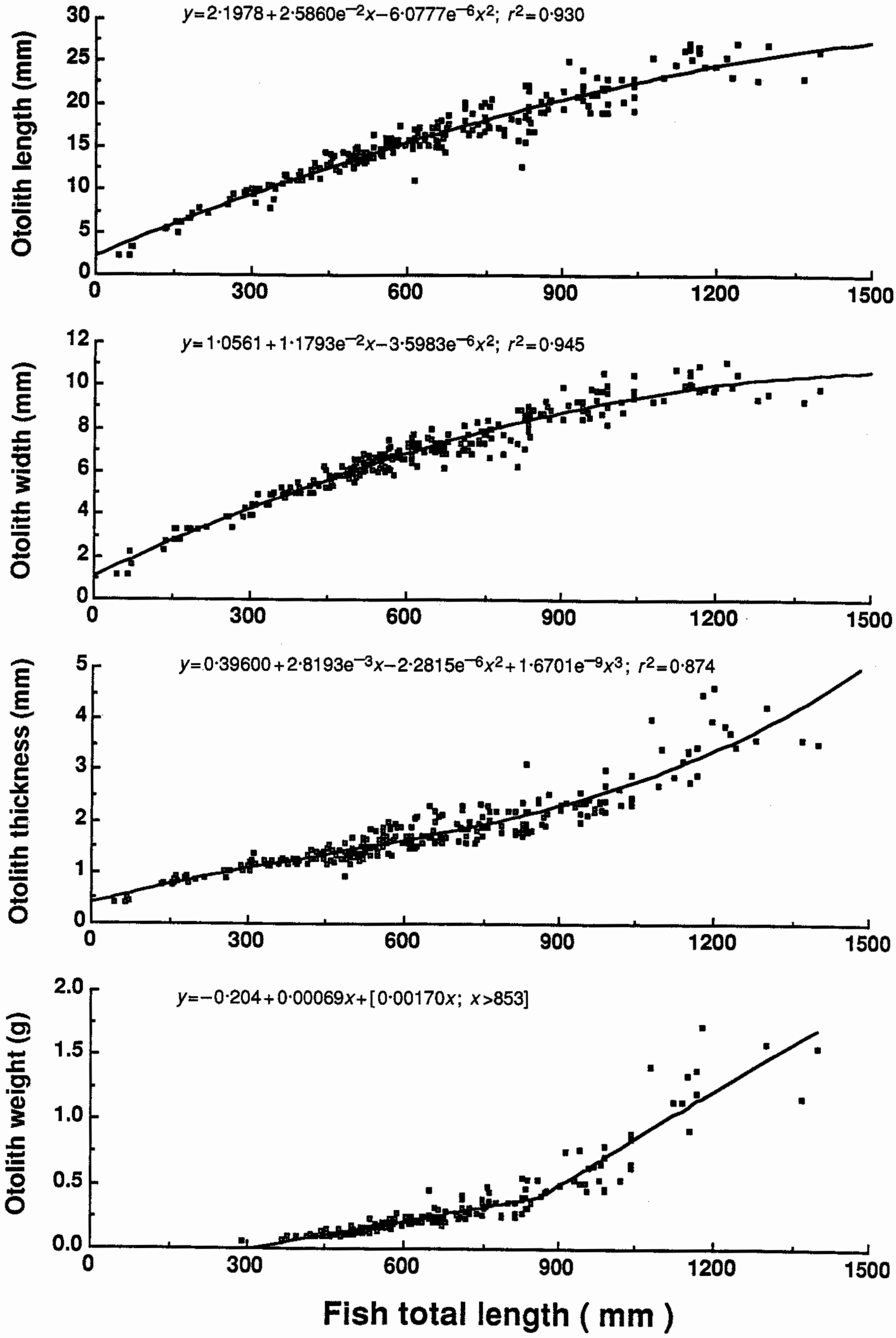

Fig. 14. Plots of otolith dimensions ($n = 290$) and otolith weight ($n = 192$) against fish total length for the entire Murray cod collection. The functions shown have been used to establish general trends only.

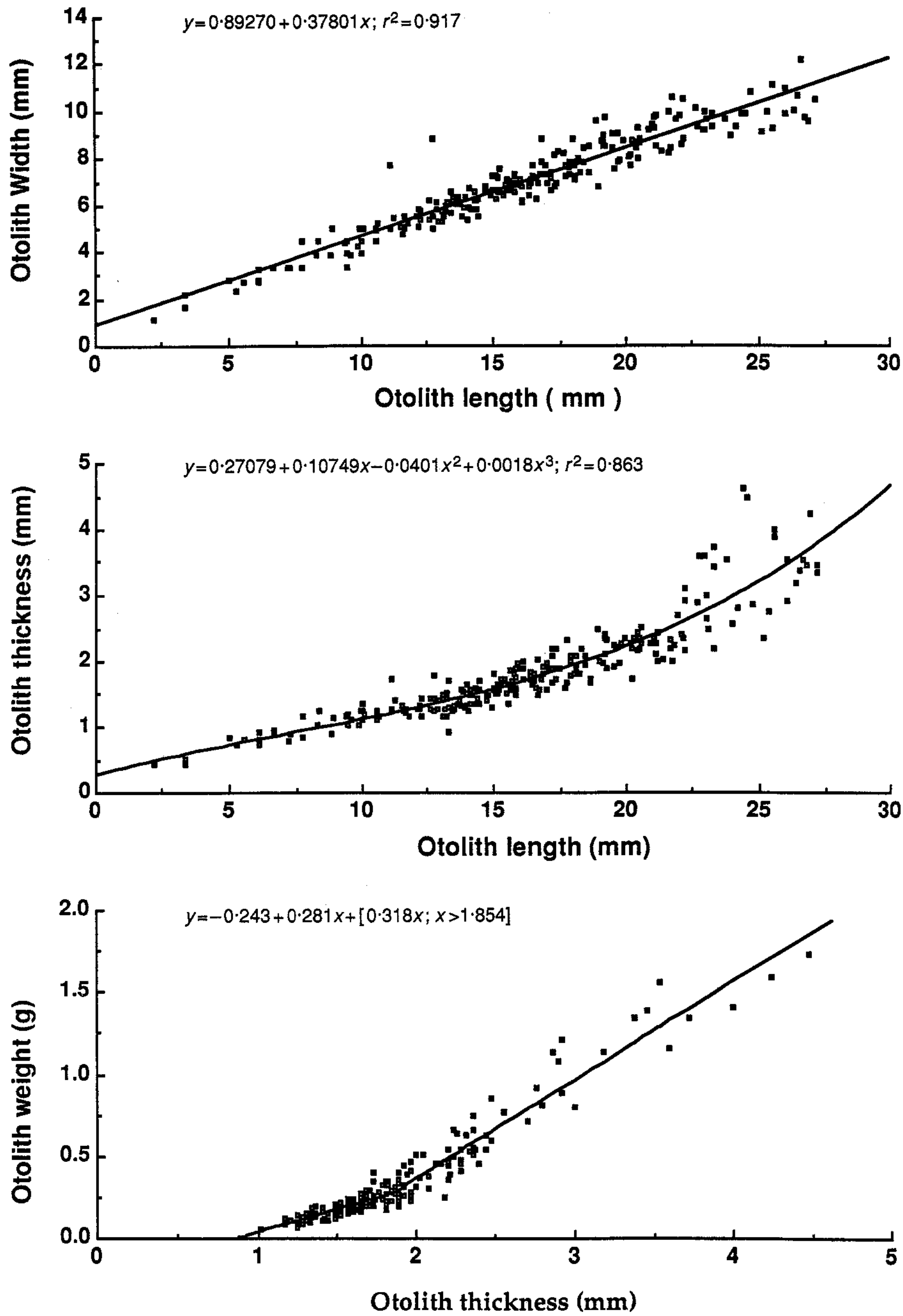

Fig. 15. Plots of otolith width and thickness against otolith length ($n=290$) and of otolith weight against otolith thickness ($n=192$) for the entire Murray cod collection. The functions shown have been used to establish general trends only.

otolith size was in thickness and weight. It was also apparent that, despite the consistency in the linear correlation between otolith weight and age, there were considerable changes in otolith dimensions that contributed to this increase in weight for cod of different ages.

Almost all of the increase in otolith weight for cod older than 10 years was attributed to the continuous laying down of material on the proximal face of the otolith (that is, to an increase in thickness) (Fig. 14). The demonstration that material continued to be laid down on the proximal face, which was where annuli were counted for older cod, was crucial to establishing the reliability of the ageing technique for older cod. The otolith continued to increase in thickness, apparently to the maximum age, well beyond the age when the other otolith dimensions and the fish total length had approached maximum values. The continuous increase in thickness also confirmed the general observations made during the reading of the otolith sections that annuli cease to be clearly distinguishable along the dorso-ventral axis at a relatively young age and that otoliths from older and larger cod are disproportionately thicker.

Discussion

Accuracy and Validation

The accuracy of the method (96·4%) determined by reading 55 otoliths (including those from known-age fish) from Lake Charlegrark was adequate and showed that the thin-sectioning method provided virtually identical estimates to those obtained by Gooley (1992) from broken, burnt and polished half-sections. The failure of the von Bertalanffy relationship to adequately describe the growth of cod in Lake Charlegrark does not necessarily mean that the use of thin otolith sections cannot be validly applied elsewhere throughout the Murray–Darling Basin, just as it similarly does not invalidate the use of the method for the Lake Charlegrark population. The present study generally supports the validation of the method. New annuli were shown to be formed in spring and summer for various age groups, including cod older than 10 years. Although the study cannot provide absolute justification for any extension of the validation of the use of otoliths to age cod beyond 11 years (Gooley 1992), there was no significant change in the pattern seen for older cod. There was therefore no evidence to reject the hypothesis that the method could be valid to the maximum estimated age of 48 years and potentially could be valid for even older cod (using the argument of Beamish 1979*b*). After the first three or four annuli in the dorso-ventral plane were counted, further annuli were read down either side of the medial groove (that is, in the plane of otolith thickness). It was shown that otoliths continued to increase in thickness and weight with age to the maximum age, thus confirming that the otolith continues to increase in size with age, which is a necessary condition for the method to be valid, especially given reasonable numbers of larger, older cod at or close to the hypothetical maximum total length. Further work is obviously needed to extend the absolute validation of the method for cod older than 11 years, using either conventional tagging techniques or chemical markers such as oxytetracycline.

Evaluation of the Method

The use of otolith thin sections was shown to provide a clear pattern of opaque and translucent bands that could be resolved into true annuli laid down and recognizable as newly completed bands each spring. The thin-sectioning method is readily adapted for dealing with large batches of otoliths, with 10 or more otoliths being sectioned together in a block. Given its efficiency and the good accuracy and precision, the technique should be accepted as the standard method for ageing Murray cod throughout the Murray–Darling Basin. Anderson *et al.* (1992) have validated the use of the technique for golden perch, and the thin-sectioning technique has also been shown to have potential for ageing trout cod, silver perch, catfish, Macquarie perch, and other species and therefore should be considered as the standard method for ageing these species.

The description of the reading method for Murray cod should enable other researchers to obtain reliable and consistent age estimates, provided that there is an exchange of otoliths between research groups and that the issues of verification, calibration and standardization of procedures (Anderson 1992) have been adequately dealt with. The availability of validated material is crucial to establishing the accuracy of the estimates and ensuring consistency between various research groups (Boehlert and Yoklavich 1984). Adoption of the otolith thin-sectioning method as a standard method would appear to be the crucial first step in alleviating the major deficiencies in not having reliable and consistent age-structure data for native fish throughout the Murray–Darling Basin as a tool for management.

Birthday

The designation of the first of October as the assigned birthday for Murray cod is identical to that used by Gooley (1992) on the basis of his estimated time of annulus formation in Lake Charlegrark, and to that assigned by Rowland (1985) for cod in the Gwydir River on the basis of the estimated biological birthday or spawning time. It is a month earlier than the 1 November birthday assigned by Rowland (1985) for cod in southern New South Wales. Given the earlier onset of warmer temperatures in the northern parts of the Murray–Darling Basin, and the earlier spawning times, it is suggested that 1 October be designated and applied to cod throughout the Murray–Darling Basin. This date also corresponds to the approximate natural spawning time of cod and so to the biological birthday (Cadwallader and Gooley 1984; Rowland 1985). It is also identical to that recommended by Anderson *et al.* (1992) for golden perch.

Precision

The precision of the method was very high, reflecting the clarity of the pattern on the otoliths. The average error of 5·4% compares well with that of 11·2% for blue grenadier (*Macruronus novaezelandiae*) obtained by Kenchington and Augustine (1987) from otolith thin sections prepared in an almost identical way, and with estimates ranging from 0·3 to 27·6% obtained in various other studies (quoted by Kenchington and Augustine 1987). Withell and Wankowski (1989) obtained average errors for two readers of 4·7% and 3·1% for pink ling (*Genypterus blacodes*) and gemfish (*Rexea solandri*), respectively, using identical sectioning and mounting methods.

The method of qualitatively classifying the width of the 'summer edge' (C, N and Z) worked well in establishing when the new annulus was laid down each year, it helped to identify and resolve discrepancies between readers as part of the verification procedure, and it was useful for adjusting annulus counts to calculate corrected decimal ages standardized to the designated birthday.

Otolith Dimensions

It has been established that there was a quadratic (almost linear) relationship between otolith length and width plotted against fish total length. Both otolith length and otolith width reached a maximum at an age of about 10–15 years, when the rate of increase in fish length had begun to decline. Otolith thickness was shown to continue to increase linearly with age, initially at a slower rate to about 5 years of age but then linearly at a faster rate to the maximum age. Otolith weight was linearly related to age.

Similar changes in otolith dimensions with age and fish length have been described for other species of fish (Blacker 1974; Beamish *1979a*, *1979b*; Perry and Tranquilli 1984; Boehlert 1985). Boehlert (1985) observed an increase in the thickness of redfish (*Sebastes diploproa*) otoliths with increasing age and a nearly linear relationship between otolith thickness and otolith weight. Although otolith weight and age were exponential functions of fish length, Boehlert (1985) also showed that the wide fluctuations in otolith weight at older ages correlated closely with variations in fish total length, and he therefore concluded that otolith weight alone was a relatively poor predictor of fish ages for the oldest fish and that otolith growth is a complex function of age as well as fish total length. This suggests that there may be uncoupling of the otolithic and somatic growth processes (Wright *et al.*

1990), such that individuals in populations experiencing slow somatic growth may have disproportionately large otoliths (Reznick *et al.* 1989; Secor and Dean 1989). Otoliths of many fish species have been shown to continue to grow despite the virtual cessation of somatic growth either on a seasonal basis, over a shorter period of days due to starvation or other factors, or in response to the decline in overall growth of fish with age (Krivobok and Shatunovskiy 1976; Pawson 1990; Wright *et al.* 1990; Fletcher 1991). Wright *et al.* (1990) discuss some of the theories that have been put forward to explain the observed so-called uncoupling between changes in otolith dimensions and weight and somatic growth measured as a change in fish length; however, more work is needed to explain this relationship. For Murray cod, the correlation between otolith weight and estimated age was very high ($r^2 = 0 \cdot 923$), and the inclusion of other otolith dimension measurements or of fish length or fish weight in complex multiregression models as proposed by Boehlert (1985) seemed unlikely to provide a better method of estimating age than the simple use of otolith weight itself.

The strong correlation between otolith thickness and otolith weight, and the linear (or almost linear) relationship with age up to the maximum age of fish, has potential for age determination (Boehlert 1985; Reznick *et al.* 1989). Pawson (1990) suggested that the technique has limited application in ageing fish from wild populations of *Sardinella aurita* because of highly variable growth rates. Fig. 16 shows regressions between otolith weight and age for the lower Murray–Darling Basin sample and the Lake Charlegrark subsample, as well as the 99% confidence limits derived from the regression for the former sample. Fig. 16 also shows the residuals versus the estimated ages for these regressions. These data suggest that the error expected for estimated ages derived from the otolith weight measurements would be of the order of 1–2 years for a 99% confidence limit, and that an error of ±4 years would have accounted for all but 7 of the 220 fish aged over the entire age range. This suggests that quite reasonable estimated ages could be derived directly from the otolith weights, which may be useful as first approximations for estimating age. This would obviate the need for sectioning and reading. However, as shown in Fig. 16, the slope of the regression for the Lake Charlegrark cod (Gooley, personal communication) was significantly different, and the slower growing cod from Lake Charlegrark had lighter, not heavier, otoliths than those found elsewhere. This contrasts with the tendency in other species for slower growing individuals to have heavier and larger otoliths (Reznick *et al.* 1989; Secor and Dean 1989). The large difference in the regression relationships between otolith weight and fish age for cod from Lake Charlegrark and cod from elsewhere raises concern about the reliability of using otolith weight itself as a predictor of age. A separate relationship between otolith weight and age may need to be derived for each subpopulation, or each locality, in the Murray–Darling Basin.

Maximum Size and Age of Murray Cod

The maximum total length of 1400 mm and the maximum weight of 47·3 kg found for Murray cod in the present study were less than the maximum confirmed recorded values of 1800 mm and 83 kg (Whitley 1955) but were close to those recorded by Rowland (1985): a total length of 1270 mm (38·4 kg, estimated age 24 years) and a weight of 40 kg (1220 mm, estimated age 30 years). The maximum estimated age of 48 years (1280 mm and 32·5 kg) found in the present study considerably extends previous estimates and clearly establishes Murray cod as the longest living percichthyid in Australia. Gooley (1992) found a maximum estimated life span of 21 years for cod in Lake Charlegrark, and Rowland (1985) found 34 years for cod in southern New South Wales. The other Australian native percichthyids have estimated maximum ages of 22 years for Australian bass (*Macquaria novemaculeata*) (Harris 1985), 26 years for golden perch (*M. ambigua*) (reported by Rowland 1985), about 11 years for Macquarie perch (*M. australasica*) (Cadwallader 1984), and more than 6 years for nightfish (*Bostockia porosa*) (Pen and Potter 1990).

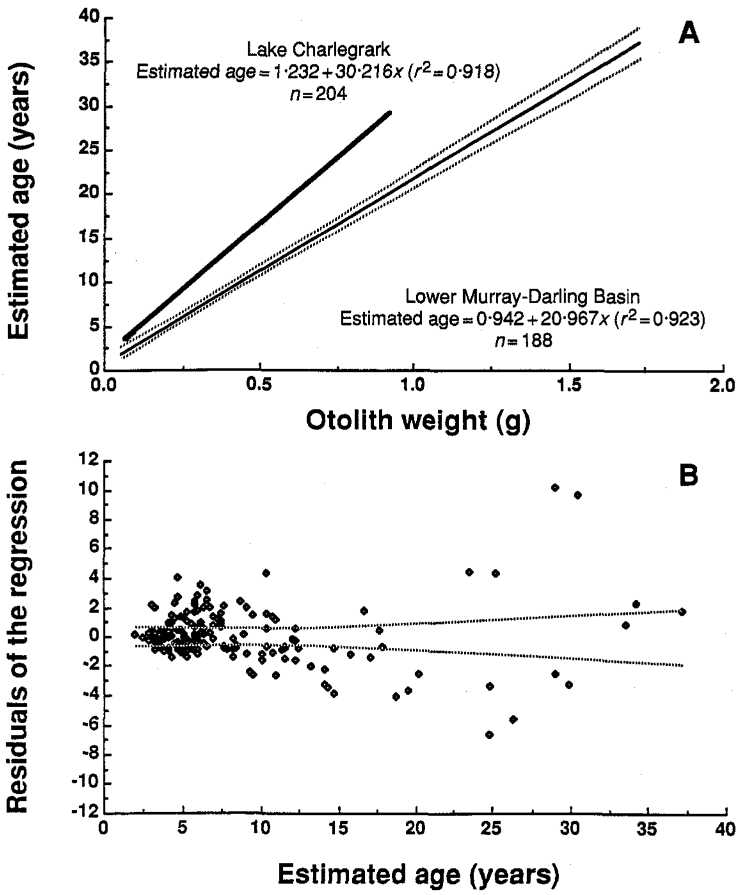

Fig. 16. (*A*) Comparison of the relationships between otolith weight and estimated age for populations of Murray cod from Lake Charlegrark and the lower Murray–Darling Basin. (*B*) Regression residuals versus estimated age for the population from the lower Murray–Darling Basin. The dotted lines show 99% confidence limits.

Growth Rates and Length–Weight Relationships

The growth rates and length–weight relationships for male and female cod were found to be statistically different. However, most of the subsample of 185 cod that had been sexed were from the historical collection, which has been shown to have highly variable growth rates. Although the ranges of lengths, weights and ages of male and female cod used for the comparison were very similar, the differences found were small for both the L_∞ and k parameters of the growth function and for the exponent (b) of the length–weight relationship. Neither Rowland (1985) nor Gooley (1992) found any significant difference in the growth rates of males and females. The larger average size of cod used in the present study may mean that sex-related differences become apparent only in larger and older fish. Further work is needed to confirm that there are biologically meaningful sex-related differences in the growth and length–weight relationships of Murray cod.

The von Bertalanffy growth function appeared to provide a reasonable fit to be data. The number of very large and old fish included in the sample provided some assurance that the growth parameters were reasonable estimates for the entire size range of Murray cod.

Hirschhorn (1974) has shown that L_∞ and k are inversely related and are very much affected by the maximum individual total length included in the analysis. The L_∞ value for the sample excluding the 55 cod from Lake Charlegrark was 1203 mm, and the corresponding k value was 0·091. These values were similar to the L_∞ value of 1369 mm and the k value of 0·061 obtained by Rowland (1985).

The higher k value of 0·234 and the smaller L_∞ value of 694·6 mm for the cod from Lake Charlegrark may not be meaningful because the von Bertalanffy growth function did not provide a good fit to the data and a higher k value would be expected given the smaller number of larger and older fish included in the sample and the smaller maximum length of 940 mm (Hirschhorn 1974). It has generally been shown that Murray cod from Lake Charlegrark have a different body form from that of cod sampled elsewhere. Cod from Lake Charlegrark were shown to be considerably shorter at the same age than cod from elsewhere, but they were also significantly lighter at a given length. Likewise, the otoliths from Lake Charlegrark cod were much lighter at a given age. These differences could not be explained easily by a simple reduction in growth rate (increase in length) due to the smallness and poor quality of the lacustrine habitat and the confinement and isolation of the population (Gooley 1992). Inbreeding and loss of genetic diversity in the original founder population introduced into Lake Charlegrark from the lower Murray River in 1955 may have produced the differences, but this also appears to be unlikely. The differences in body form may represent some form of stunting, but further work is needed to explain these differences and to determine their cause.

The significant differences in growth rates and length–weight relationships between the historical and recent collections appeared to be due to higher proportions of slower growing fish in the historical collection. The recent collection had a significantly higher growth rate, but the faster growing fish in the historical collection had comparable growth rates, so it is difficult to draw any definite conclusions because the cod in both samples were collected from a relatively wide range of localities and a broad range of habitats. The cod in the historical collection taken upstream of Yarrawonga were shown to have significantly faster growth rates ($L_\infty = 1213$ mm, $k = 0{\cdot}134$) than the remainder of the cod ($L_\infty = 1243$ mm, $k = 0{\cdot}069$). The growth rates and length–weight relationships of cod in the historical collection taken upstream of Yarrawonga were more comparable to those of recently caught cod, whereas those of cod caught downstream and in the tributaries were more comparable to those of cod from Lake Charlegrark (slower growth and heavier for a given length). Rowland (1985) also obtained a relatively low k estimate of 0·060, and he found that Murray cod from impoundments (Lake Mulwala and Lake Burrinjuck) were significantly heavier at a given length than cod taken from riverine habitats. Although the available data are too few for detailed statistical analysis of the differences between the subpopulations, the present study generally confirms the suggestion that cod populations from different habitats and different regions within the Murray–Darling Basin may have different body forms (e.g. Lake Charlegrark) or different growth patterns (e.g. the population upstream of Yarrawonga). Habitat-induced changes in growth rates have been demonstrated for other related species; for example, Harris (1985) found almost a twofold difference in growth rates of female Australian bass in gorge habitats ($k = 0{\cdot}21$) compared with those in tidal habitats ($k = 0{\cdot}12$). Differences in growth rates may be partially explained by the earlier spawning times of Murray cod in the northern part of its distribution, allowing for greater growth in the first year that could be retained in later years (Lake 1967). Greenberg and Brothers (1991) found a threefold increase in growth rates of young-of-the-year of *Etheostoma simoterum* from the highest-elevation sites to the lowest, associated with temperature differences of 3–4°C in spring and summer. The possibility of regional or local variation in cod growth rates should therefore be considered when population studies are undertaken for management purposes. Further detailed work is needed to study the variation in growth patterns of Murray cod throughout its distribution and the factors that influence growth rates and body form in different habitats.

The growth rate of Murray cod in terms of the estimated k values of 0·091 for the combined recent and historical collections and 0·111 for the recent collection alone is somewhat lower than those for other species in the Murray–Darling Basin. The estimated growth rates for other species are $k=0\cdot341$ for freshwater catfish (Davis 1977), $k=0\cdot454$ for golden perch (Anderson *et al.* 1992), and $k=0\cdot12$ to 0·22 for Australian bass (Harris 1985). The Macquarie perch growth rate appears to be similar to or somewhat faster than that of Australian bass (Harris 1985). These estimates do not necessarily imply that Murray cod has a lower aquacultural potential or a lower growth rate in the first few years of life because cod have a longer life span and a much larger maximum potential length and weight than the other species.

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Appendix. Collection localities and dates for the historical and recent collections of Murray cod

Locality	Sampling date	Number of fish
Historical collection		
Murray River, Loch 8	8.vii.54	10
Moira Lakes	14–19.vi.52	8
Murray River, Loch 15	4.x.49–1.iii.51	16
Murray River, Euston Weir	24.ix.49	2
Murray River, Morna woolshed	16–22.i.54	17
Murray River, Mildura	1.x.54	11
Murray River, Boundary Bend	10.x.49–4.vii.50	7
Murray River, Speiwa	2.vii.51	1
Murray River, Swan Hill	15.iv.49	1
Murray River, Bundalong	25.viii.53	4
Murray River, below Ovens River junction	24.viii.53	17
Murray River, at Ovens River junction	5.viii.53	16
Ovens River, between Bundalong and Peechebar	25–30.viii.53	6
Taylors Lake	19.vi.55	7
Edwards River, Old Marago	25–26.xii.50	3
Edwards River, Chasingh	17.xii.50	2
Murrumbidgee River	4.xi.49	1
Murrumbidgee River, Irrigation Canal	28–30.x.49	7
Murrumbidgee River, Hay	31.viii.49	2
Gunbower Creel	31.v.53	7
Wimmera River	30.x.51	1
Recent collection		
Loddon River	19.i.82–5.x.89	3
Edwards River	5.vi.86–3.xi.90	7
Murray River	19.x.79–26.iii.91	21
Broken River	24.ii.88–15.i.91	5
Taylors Lake	14.ii.88	1
Goulburn River	21.ix.85–1.iv.91	20
Wakool River	15.v.88	1
International Village, Shepparton	11.viii–6.ix.88	3
Billabong Creek, Jerilderie	20.ix.88	2
Delatite River	2.v.89–14.ii.90	5
Lake Charlegrark (validation)	22.ii.79–11.xi.90	55
Ovens River	21.xi.81–15.iii.91	11
Marma Lake	9.ix.81	1
Lake Googong (Australian Capital Territory)	3.vii.89	1
Darling River	1.v.88	1

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