

## Validation of age and growth estimates using thin otolith sections from the purple wrasse, *Notolabrus fucicola*

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**Abstract.** The age of *Notolabrus fucicola* from the east and southeast coasts of Tasmania was estimated from counts of opaque (transmitted light) growth zones in thin transverse sections of sagittal otoliths. The position of the first annulus was validated through the otolith radii of known-age juveniles. Marginal increment analysis was performed by examining the growing edge of otoliths over 2 years. This showed that opaque zones were formed annually and were consistently deposited from late September to early December (spring season), which is coincident with annual increases in somatic growth and water temperature during this season. A criterion was developed to ensure accurate age estimation for individuals sampled during the time of year when deposition of opaque material was incomplete or not apparent. The maximum age estimated was 20 years and the relationship between fork length and age was described by the von Bertalanffy growth function ( $L_{\infty} = 368$  mm,  $K = 0.116$  and  $t_0 = -1.87$  for males and  $L_{\infty} = 385.7$  mm,  $K = 0.109$  and  $t_0 = -1.96$  for females). The clarity of increment structure and rigorous validation protocol that was adopted minimised the risks associated with using marginal increment analysis to validate the periodicity of increment formation and it provided robust age and growth estimates for *N. fucicola* in Tasmanian waters.

**Extra keywords:** Australia, Labridae, marginal increment analysis, von Bertalanffy.

### Introduction

The interpretation of periodic increment structure in fish bony parts is a fundamental tool in estimating age structure, growth and mortality of fish populations. Otoliths are often used for this purpose because they incorporate periodic and incremental structure on a daily, seasonal and/or annual basis. The most common approaches used to examine otolith increment structure involve examination of whole otoliths (usually immersed in a liquid medium) (e.g. Hyndes *et al.* 1992), 'broken and burnt' otoliths (whereby the otolith is broken transversely through the primordium and heated to accentuate the increment structure) (e.g. Francis *et al.* 1992) or examination of thin sections sliced through the primordial region (e.g. Beamish 1979). Thin transverse otolith sections generally improve the detection of increment structure (Collins *et al.* 1989), particularly in older fish for which age estimates derived from unsectioned otoliths can underestimate age (Beamish 1979; Campana 1984; Collins *et al.* 1989; Hyndes *et al.* 1992; Ferreira and Russ 1994; Treasurer 1994).

Within age and growth research, there has been a history of inadequate attention to validation (Beamish and McFarlane 1983), with recent attention given to the neglected distinction between validation of the periodicity of increment formation and that of the accuracy of age estimates (Campana 2001). A

commonly used method of validating the periodicity of increment formation is marginal increment analysis (MIA), which utilises apparent temporal trends in the formation of increments on the growing edge of the otolith. Campana (2001) criticised MIA because of the subjectivity of interpretation of the otolith growing edge and because, unlike methods that measure absolute age such as known-age marked release studies (e.g. Beamish and McFarlane 2000) and bomb radio-carbon analyses (Kalish 1993), MIA does not allow the accuracy of age estimates to be calculated. Yet, Campana (2001) conceded that MIA can be applied validly provided that samples are randomised before examination, a minimum of two annual cycles are examined, cycles are determined using a statistical test, age classes are validated independently and the timing of formation of the first increment is determined.

The wrasse, *Notolabrus fucicola*, is a reef-associated labrid that is distributed throughout the temperate waters of southeastern Australia and New Zealand (Russell 1988; Gommon *et al.* 1994; Edgar 1997; Denny and Schiel 2001). As a dominant micropredator, *N. fucicola* plays a significant role in trophic dynamics of the temperate reefs in their range (Denny and Schiel 2001). *Notolabrus fucicola* are a significant component of a live fishery in Tasmania (Lyle and Hodgson 2001) and information on age and growth is required to manage the fishery and examine the impact

of fishing pressure. The age and growth of *N. fucicola* in Tasmanian waters has been estimated previously using counts of opaque zones from whole otoliths and tag–recapture methods, although sample sizes were small (58 and 82, respectively) (Barrett 1995). In addition, otolith increment periodicity was not validated, timing of the formation of annuli and the precision of age estimates were not examined and criteria for interpretation of the otolith margin were not defined.

The aims of the present study were to generate age estimates for *N. fucicola* using counts of opaque growth zones in thin transverse otolith sections and to address issues of the validation of their accuracy and precision. The first annulus was validated through measurement of the radii of otoliths from young-of-the-year specimens. A criterion for interpretation of the leading growth edge of otoliths was developed to accurately estimate age, taking into account the most recent year of growth. The timing and periodicity of opaque growth zone formation was validated by marginal increment analysis. Age data were used to estimate von Bertalanffy growth parameters for both males and females.

## Materials and methods

### Sampling

A total of 2826 purple wrasse (*Notolabrus fucicola*) were sampled from four sites on the east and southeast coasts of Tasmania between July 1999 and January 2001 (Point Bailey, Eaglehawk Neck, Trumpeter Bay, and Hen and Chicken Rocks) (Fig. 1). Samples were collected monthly from all sites up until January 2000, after which sampling was conducted on a 3-monthly basis. Larger fish (>130 mm fork length (FL)) were caught using baited fish traps set on coastal rocky reefs in less than 5 m of water. Smaller fish (<130 mm FL) were not vulnerable to the fish traps, so newly settled fish aged 0+ were caught from artificial habitats deployed inshore and at similar depths to the traps (Welsford 2003b). Fish aged 1+ were also collected with rotenone from a sub-tidal rock pool adjacent to the Point Bailey site.

### Laboratory procedures

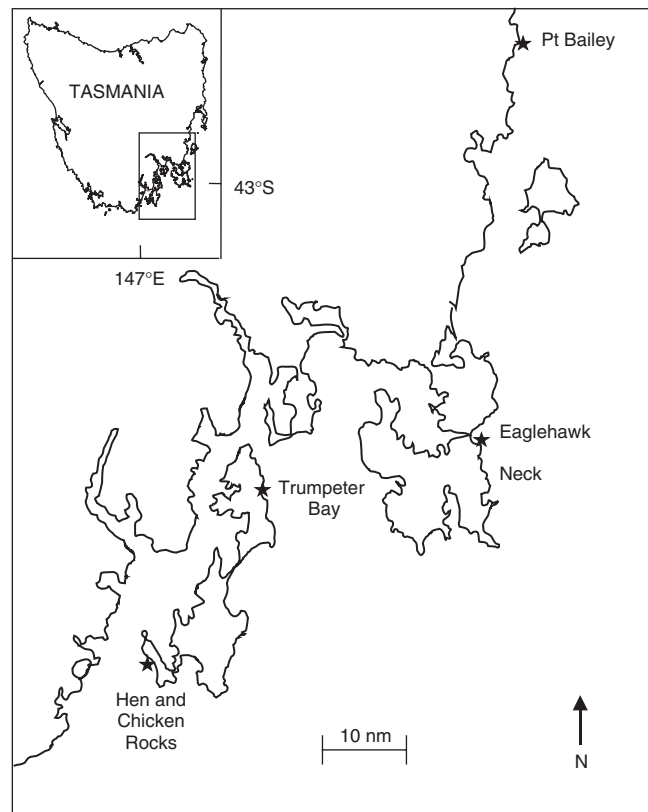
Each fish was sexed and its FL (to the nearest millimetre) and total weight (to the nearest gram) recorded. Gonads were removed and weighed (to the nearest 0.1 g) from mature fish. A gonadosomatic index (GSI) was calculated:

$$\text{GSI} = 100 \times W_{\text{g}} / W_{\text{s}}$$

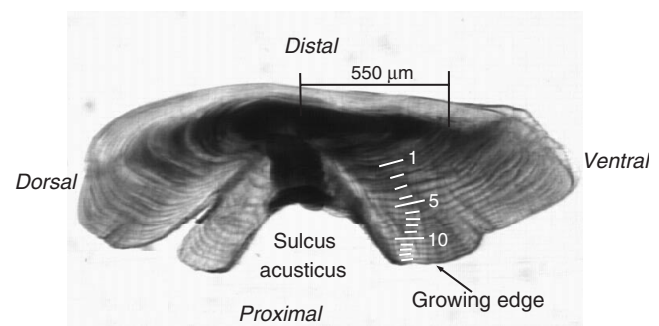
whereby  $W_{\text{g}}$  is the gonad weight and  $W_{\text{s}}$  is the somatic fish weight. The timing of peak spawning was inferred from the point at which annual peak GSI values commenced their decline. This time of peak spawning was established as the birth date for age estimations. Sagittal otoliths (hereafter referred to as 'otoliths') were removed, cleaned, dried and, if intact, weighed (to the nearest milligram).

### Terminology

The terminology used in the present paper follows Kalish *et al.* (1995). The term 'translucent zone' refers to a zone that appears brighter under transmitted light relative to an adjacent 'opaque zone', which appears darker under transmitted light. The term 'annulus' refers to an opaque zone that is continuous across the proximal region (ventral to the sulcus acusticus) of the otolith section and is the structure enumerated to estimate age in years (Fig. 2). The birth date used was October 1. The term 'otolith growing edge' refers to the outer edge of the otolith section at the proximal surface, ventral to the sulcus acusticus. The term



**Fig. 1.** Location of sampling sites for *Notolabrus fucicola* on the east and southeast coasts of Tasmania, Australia.



**Fig. 2.** Photomicrograph (transmitted light) of a transverse otolith section from *Notolabrus fucicola*. The scale bar indicates the distance from the primordium to the first annulus (determined from examination of the otoliths of young-of-the-year). Opaque zones are marked and age was estimated at 14.9 years (sample was collected 24th August and the assigned birth date was 1st October), with a wide marginal increment.

'marginal increment' refers to the translucent region beyond the last identifiable opaque zone out to the otolith growing edge. The term otolith 'core' refers to the uniformly opaque zone inside and concentric to the first clear translucent zone, and concentric to the 'primordium', which refers to the small area of initial otolith growth.

### Otolith preparation and interpretation

One otolith from each adult was mounted in a block of polyester casting resin and sectioned transversely through the primordium, to a

thickness of 250–300  $\mu\text{m}$ , with a lapidary diamond saw. Sections were mounted on a glass slide with polyester resin. Each mounted section was examined with a stereomicroscope at  $\times 25$  magnification using transmitted light to count opaque growth zones and to categorise the otolith proximal growing edge (ventral to the sulcus acusticus) as having either a wide, intermediate or narrow translucent margin (relative to the width of the next inner translucent zone). Mounted sections were also viewed with a compound microscope at higher magnification ( $\times 100$ ) using transmitted light for more detailed examination of the otolith growing edge.

Otoliths were rejected if the increment structure was optically unclear or confusing, or if the primordium could not be identified. Otoliths were read with no reference to fish length, weight or date sampled.

#### Precision of age estimates

The primary reader (Reader A) examined all otoliths ( $n = 2826$ ) and then re-read the first 1011. A secondary reader experienced in examination of transverse sagittal otolith sections (Reader B), read a random subsample of 342 otoliths. The precision of age estimates between and within readers was calculated using the index of average percent error (APE) (Beamish and Fournier 1981) and examined visually using age-bias plots (Campana 2001). The age frequency distributions generated by the second and third readings were tested for age bias against the age frequency distribution generated by the initial reading, using a G-test for goodness of fit (Sokal and Rohlf 1995).

#### Validation of first annual increment

To confirm the position of the first annual increment, the maximum transverse radii of whole sagittae from fish aged 0+ and 1+ were measured to the nearest micrometre, using image analysis software, and plotted against month sampled. These radii were then compared with the radii of the inner opaque growth zones in the transverse plane of sectioned otoliths from older fish, which were measured using image analysis software.

#### Validation of the periodicity and timing of opaque zone formation

The periodicity of opaque zone formation was determined by analysing the temporal pattern of the marginal increment using  $\times 100$  magnification. Indices of completion (C) were calculated for a subsample of 1575 transverse sectioned otoliths, using the formula:

$$C = W_n / W_{n-1}$$

whereby  $W_n$  is the width of the marginal increment (distance from the otolith growing edge to the centre of the outermost complete opaque zone) and  $W_{n-1}$  is the width of the previous complete increment (distance from the centre of the outermost complete opaque zone to the centre of the second from outermost opaque zone) (Tanaka *et al.* 1981). An opaque zone was considered complete if translucent material was detectable between it and the otolith edge. The subsample included every fish, up to a maximum of 30 per age class, per month sampled. Widths were measured on digitised images to the nearest micrometre using image analysis software, and were taken from the proximal growing edge, ventral to the sulcus acusticus (Fig. 2). Analysis of variance on arcsine-transformed indices of completion was used to test for significant differences between indices of completion by month sampled (Davis and West 1992). Mean marginal increments were examined separately for otoliths with two to 12 opaque zones and pooled for otoliths with more than 12 opaque zones, owing to the small sample sizes of older fish.

The timing of opaque zone formation was determined by examining the same subset of 1575 otolith sections for the presence or absence of opaque material on the same growing edge, also at  $\times 100$  magnification using transmitted light.

#### Criterion for edge interpretation

The lower magnification ( $\times 25$ ) used for the counts of opaque zones for age estimation necessitated the development of a criterion to ensure the inclusion of newly deposited opaque zones in age estimates for individuals collected around the time of opaque zone formation. The criterion was based on the marginal increment width category (wide, intermediate or narrow at  $\times 25$  magnification) and the timing of opaque zone formation (derived from marginal increment analysis of samples at  $\times 100$  magnification).

#### Growth modelling

Ages assigned were a function of the number of opaque zones (adjacent and ventral to the sulcus acusticus) counted from, and including, the first annual zone, the edge interpretation criterion and the time of sampling with respect to the arbitrary birth date. The von Bertalanffy growth function was fitted to individual lengths at age of males and females using least-squares estimation procedures. The von Bertalanffy equation is

$$L_t = L_\infty [1 - e^{-K(t-t_0)}]$$

whereby  $L_t$  is the mean length at age  $t$  (years) and  $L_\infty$  is the mean asymptotic length (Francis 1995). Analysis of residual sum of squares (Chen *et al.* 1992) was used to test for differences in growth between males and females.

## Results

#### Otolith structure

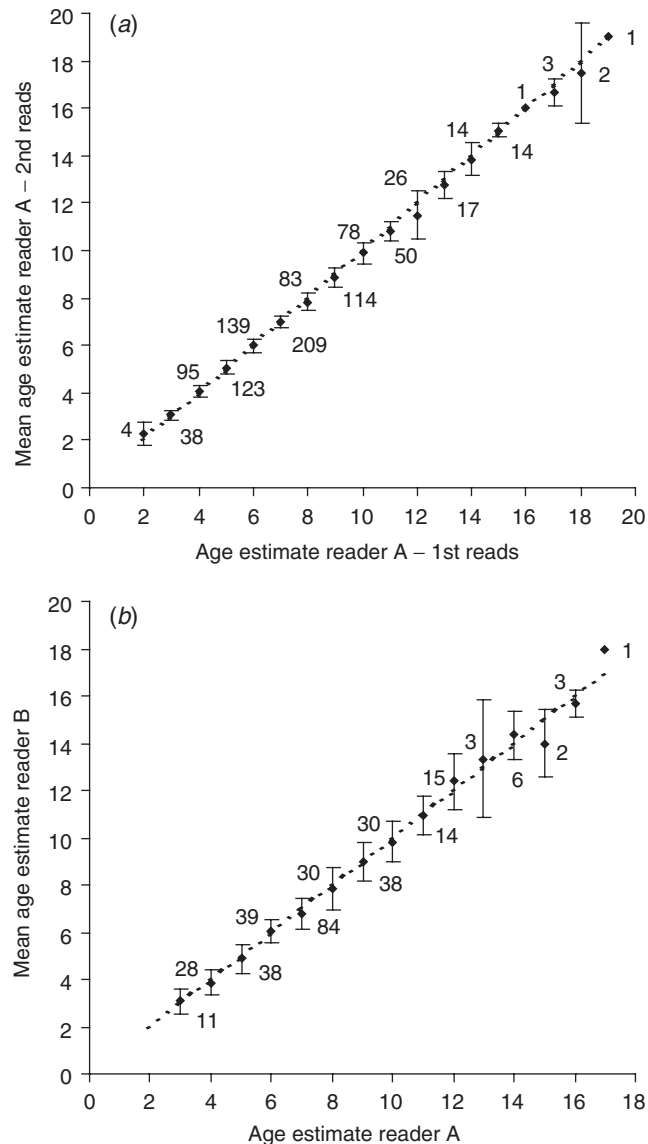
The transverse, sagittal otolith sections from *Notolabrus fucicola* generally showed alternating bands of translucent and opaque material radiating from a uniformly opaque inner zone when viewed with transmitted light (Fig. 2). The two innermost opaque zones (immediately exterior to the uniformly opaque inner zone) were consistently broader than subsequent opaque zones (Fig. 2). Of the 2826 otoliths read, 2% were rejected because of unclear increment structure resulting from poor sectioning.

#### Designation of a birth date

Mean GSIs for both males and females peaked at around 4% in early October, indicating a peak in spawning activity in October, which concurred with studies of the reproductive biology of *N. fucicola* in Tasmania (Barrett 1995) and New Zealand waters (Denny and Schiel 2002). Back-calculated birth dates from validated daily increment counts in juvenile *N. fucicola* also suggested that October–November is the peak of spawning activity (Welsford 2003a). To reflect this pattern, a birth date of 1st October was applied when estimating age, although spawning activity shows a broad duration, with individuals found in spawning condition and back-calculated spawning dates from as early as August and as late as January within a year.

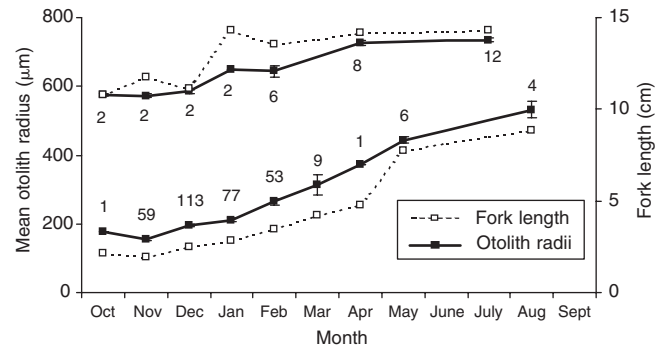
#### Precision

The APE for the within reader comparison for the primary reader (Reader A) was 0.8%, with 89% of readings in agreement and a maximum difference of three zones, indicating a very high mean level of agreement. Age-bias graphs showed

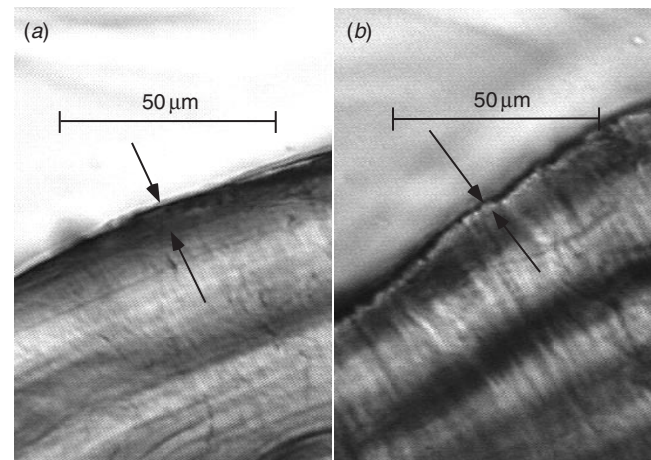


**Fig. 3.** Age-bias plots of mean age estimates compared to the initial reading by the primary reader (a) within reader bias and (b) between reader bias. Value labels are sample size (when <30) and error bars are standard deviation. Dotted line indicates equal ages.

a high level of agreement between readings (Fig. 3). There was no age bias in the residuals, as evident from the symmetrical spread of residuals around age-bias plots (Fig. 3). No significant difference was detected between the age frequencies generated from the first and second reads using a G-test for goodness of fit ( $G = 4.3$ , d.f. = 13,  $\chi^2_{0.05} = 22.36$ ). All of the largest discrepancies (three zones) and 60% of two zone discrepancies were from the first 300 otoliths read. Otoliths with a difference of greater than one zone were read a third time by Reader A. The third readings, when compared with the second readings, yielded no discrepancies of greater than one zone. Age estimates were derived from readings made by



**Fig. 4.** Monthly progression of mean otolith radius and mean fork length of 0+ and 1-year-old *Notolabrus fucicola*. Value labels indicate sample size and error bars indicate standard error.



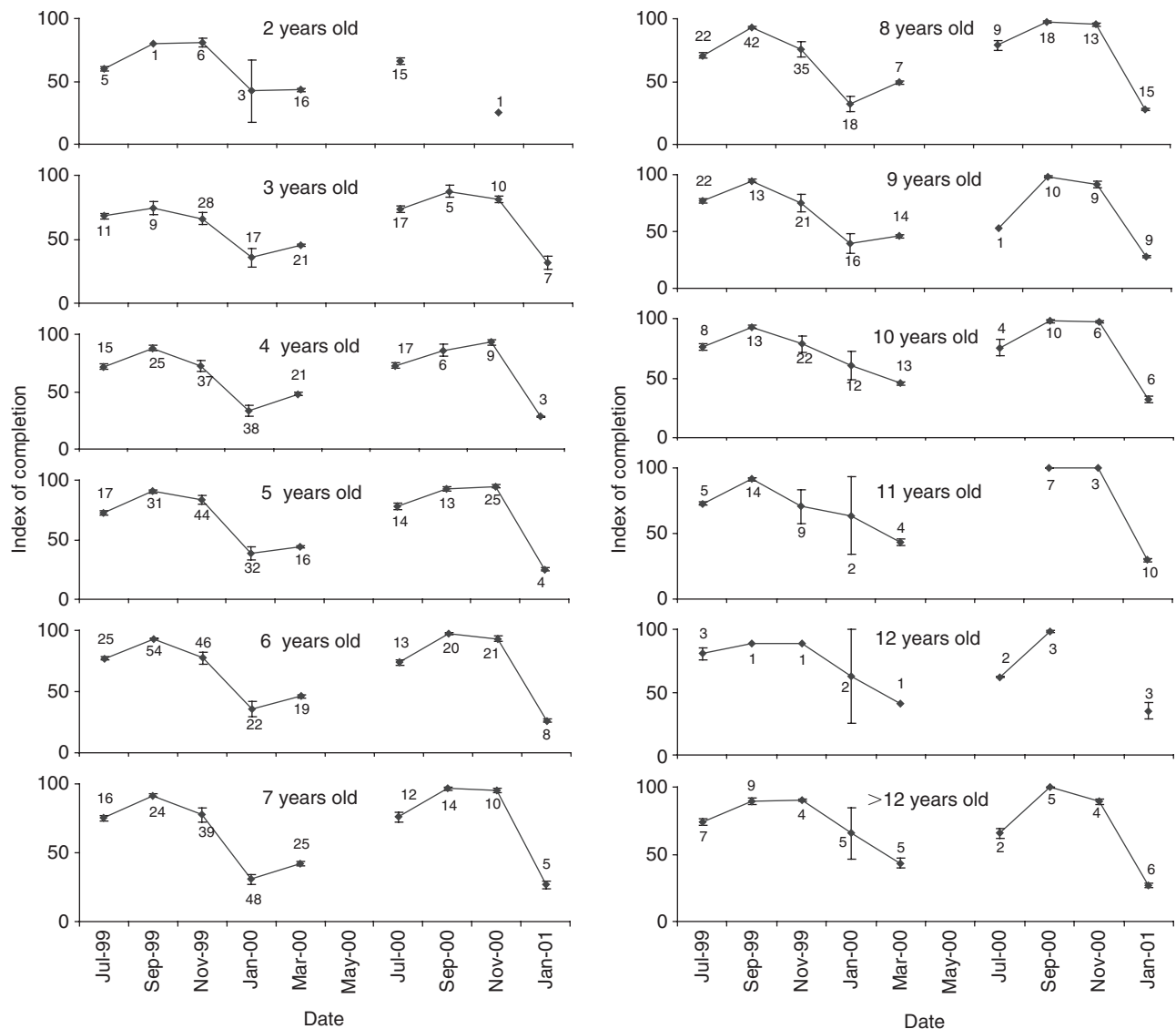
**Fig. 5.** Photomicrographs of the proximal otolith growth edge (ventral to the sulcus acusticus) showing a partially deposited opaque zone (between arrows in section a, sampled in November) and a narrow translucent margin (between arrows in section b, sampled in February).

Reader A and, in the case of the first 1011 otoliths, from the second readings made by Reader A.

The APE for the between-reader comparison was 2.6% with 67% agreement and a maximum difference of three counts. Age-bias graphs showed a high level of agreement between readers with the exception of a small number of specimens above 12 years of age (Fig. 3). There was no age bias visible in the residuals and no significant difference was detected between the age frequencies generated from the reads of the primary and secondary readers, using a G-test for goodness of fit ( $G = 12.4$ , d.f. = 11,  $\chi^2_{0.05} = 19.7$ ).

#### Validation of first annual increment

The first three opaque zones exterior to the uniformly opaque inner zone had mean radii of 365 μm (s.d. = 25 μm,  $n = 100$ ), 546 μm (s.d. = 33 μm,  $n = 100$ ) and 691 μm (s.d. = 41 μm,  $n = 100$ ), respectively (Fig. 2). Large numbers of young-of-the-year fish were first captured in artificial habitats in



**Fig. 6.** Bimonthly trends in indices of completion for sagittal otoliths of *Notolabrus fucicola*. Value labels are sample size and error bars indicate standard error.

November. In March, fish aged 0+ had a mean transverse total otolith radius of 314  $\mu\text{m}$  (s.d. = 86  $\mu\text{m}$ ,  $n = 9$ ), and by May a mean of 444  $\mu\text{m}$  (s.d. = 23  $\mu\text{m}$ ,  $n = 6$ ) (Fig. 4). Young-of-the-year fish sampled in the following October (at around 1 year of age), had a mean transverse otolith radius of 574  $\mu\text{m}$  (s.d. = 6  $\mu\text{m}$ ,  $n = 2$ ) (Fig. 4). This corresponds with the location of the second opaque zone in otoliths of adult fish, indicating that the second opaque zone corresponds with the first birthday.

#### Validation of the periodicity of increment formation

The higher magnification ( $\times 100$ ) used for edge interpretation provided sufficient clarity for the measurement of the width of the translucent margin on the otolith growing edge

(Fig. 5). Because there were no changes in the appearance of increments after age 8 years (mean increment width 29.6  $\mu\text{m}$ , s.d. = 6.5  $\mu\text{m}$ ,  $n = 200$ ) and owing to low sample sizes, otoliths with more than 12 opaque zones were pooled for marginal increment analysis. Mean indices of completion (C) peaked around October for both years of sampling and for all age classes up to 12 opaque zones, and for pooled age classes of greater than 12 years old (Fig. 6). The one-way ANOVA of arcsine square-root transformed C values by month showed significant differences ( $F_{8,184} = 92.3$ ,  $P < 0.001$ ) in all age classes. Post hoc tests (Tukey's HSD) showed that otoliths collected in February (the annual minima) were significantly different from those collected in August, September, October, November and December ( $P < 0.05$ ).



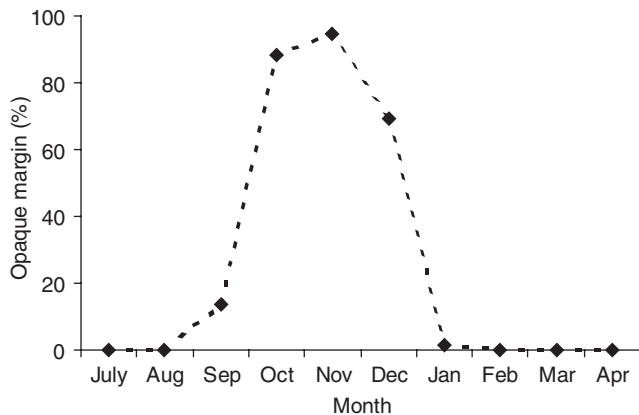


Fig. 7. Percentage of otoliths with an opaque zone on the growing edge according to month sampled.

#### Timing of opaque zone formation

The  $\times 100$  magnification used for the marginal increment analyses provided adequate resolution for the detection of opaque material on the otolith growing edge (Fig. 5). Opaque material was absent on the otolith growing edge of samples collected in January–August and was detectable in 13.8% of pooled age classes from samples collected in late September, 88.2% from October, 94.7% from November and 69.3% from samples collected early December (Fig. 7). This indicates that for the period sampled, opaque zones were deposited on the sagittal otolith growing edge of *N. fucicola* from around September to December.

#### Criterion for edge interpretation

By contrast with the higher magnification used for edge analysis, opaque material was not detectable on the otolith growing edge under the lower magnification ( $\times 25$ ) used for counting opaque zones, until translucent material at the otolith growing edge provided sufficient contrast to indicate the presence of recently formed opaque material. Newly formed opaque zones, despite being visible at  $\times 100$  magnification in samples collected September–December, were undetectable at  $\times 25$  magnification in November samples (all otoliths having a wide translucent margin), and were first detectable in December samples (with a small proportion of otoliths having a narrow translucent margin). Recently formed opaque zones were not detected in all otoliths until April (whereby every otolith had a visible translucent margin). Thus, it was necessary to develop a criterion to ensure that recently formed opaque zones were included in the opaque zone counts taken at  $\times 25$  magnification. For samples collected between October 1st (the designated birth date and near the onset of opaque zone formation) and April 1st (the time at which all newly formed opaque zones were detected), otoliths with a wide margin (when viewed under  $\times 25$  magnification) were inferred to have an undetected opaque zone

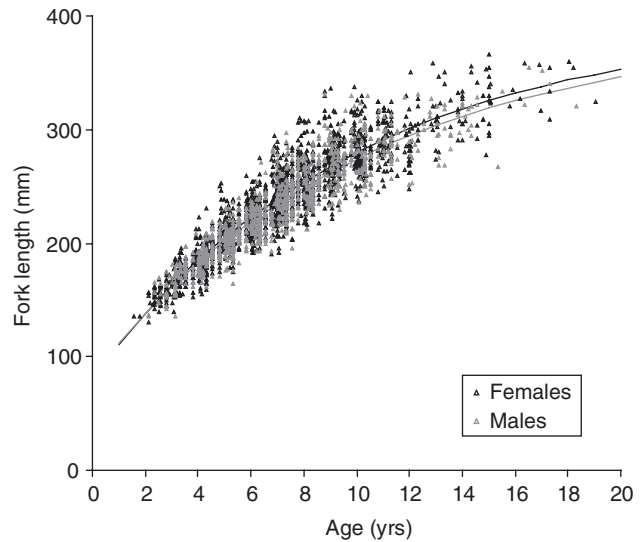


Fig. 8. Age-at-length estimates for *Notolabrus fucicola* (symbols) and von Bertalanffy growth functions (lines) fitted using least-squares estimation procedures.

recently formed at the otolith margin (and had 1 year added to their age estimate), whereas otoliths with a narrow translucent margin were deemed not to have an unseen opaque zone on their margin and were left unadjusted.

#### Growth modelling

The von Bertalanffy growth function was fitted separately to male and female length age data from all sites combined by least-squares regression (Fig. 8). Analysis of residual sum of squares (ARSS) (Chen *et al.* 1992) showed a significant difference between the male and female growth functions ( $F = 7.7$ , d.f. = 3, 3000,  $P < 0.01$ ). The von Bertalanffy parameters for the males were  $L_{\infty} = 368$  mm,  $K = 0.116$  and  $t_0 = -1.87$ , for females were  $L_{\infty} = 385.7$  mm,  $K = 0.109$  and  $t_0 = -1.96$  and for both genders pooled were  $L_{\infty} = 388.4$  mm,  $K = 0.104$  and  $t_0 = -2.13$ .

#### Discussion

The transverse sectioning of *Notolabrus fucicola* sagittal otoliths provided interpretable increment structure, with annual opaque zones readable across all age classes. The precision analyses demonstrated that thin sagittal sections provided very reproducible age estimates in *N. fucicola*, particularly with increasing reader experience. The clarity of increment structure in sectioned otoliths was consistent with the findings for other temperate species (Francis *et al.* 1992; Morison *et al.* 1998).

The close similarity between the mean transverse radius of sagittae from 1-year-old *N. fucicola* (574  $\mu\text{m}$ ) and that of the second opaque zone in transverse otolith sections from older specimens (546  $\mu\text{m}$ ) confirmed that the first opaque zone

(mean radius 365  $\mu\text{m}$ ) was deposited before the first birthday. Subannual macrostructure before the first annual zone has been reported in several other species (Kerstan 1995; Mann Lang and Buxton 1996; Karlou-Riga 2000). The presence of a clear subannual opaque band within the first year supports the contention that validation of an ageing methodology must include determination of the position of the first annulus (Francis *et al.* 1992; Campana 2001). Clearly, if this requirement was ignored in this species, fish would be over-aged by 1 year.

The analyses of the otolith growing edge indicated that opaque zones were deposited annually from approximately September to December, across all age classes and over both years sampled. This timing was consistent with other studies, suggesting that opaque zones are deposited in late austral spring and early summer (Choat and Axe 1996; Fowler and Short 1998; Cappo *et al.* 2000). Analysis of otolith radii suggested that the first annual opaque zone was deposited around October.

Francis *et al.* (1992) reported that *Pagrus auratus* opaque zones are only apparent on the margin after additional translucent material has been laid down, and stressed that it is important to distinguish between the time when opaque zones become apparent and the time at which they were formed. This is consistent with the finding in the present study, whereby under low ( $\times 25$ ) magnification opaque zones were first detected on the growing edge of otolith sections only after translucent material was deposited beyond them. Although opaque zones were detectable on the margin using higher magnification, the opaque zone counts in the present study were derived from analyses under low magnification and, thus, were subject to problems of interpretation of the margin for samples collected between the time of opaque zone formation and detection. Methods of dealing with this problem range from avoiding collecting samples around the time of annulus formation (Francis *et al.* 1992) to the generation of algorithms to infer the presence of unseen annuli on the margin (Francis *et al.* 1992; Fowler and Short 1998; Morison *et al.* 1998; Cappo *et al.* 2000). The criterion developed in the present study inferred the presence of unseen annuli on the basis of the margin width in the period between the beginning of October (the designated birth date) and the following April (when the opaque zone deposited in the preceding spring was detected in all otoliths). We are confident that this criterion clearly discriminated between otoliths with an unseen opaque zone on the margin (wide margin) and otoliths without (narrow margin). No otoliths during this period were detected with a medium margin width and samples were interpreted without reference to date sampled, so such inferences could be made without biases.

The validation of the periodicity of increment formation using marginal increment analysis (MIA) has been questioned recently primarily because of the inherent subjectivity in interpreting the otolith growing edge (Campana 2001).

In the present study, the use of higher magnification ( $\times 100$ ) for otolith edge analyses and the analysis and description of intra-specific and temporal variation in the timing of increment formation, in a temperate species that displays clear increment structure out to the otolith edge, minimised this subjectivity. Campana (2001) also warned that because MIA does not utilise a measure of absolute age, it does not allow the accuracy of age estimations to be calculated. We believe that the rigorous validation protocol suggested by Campana (2001) that we followed, and particularly the validation of increment periodicity for each year class from young-of-the-year (using cohort analysis) to 12-year-old fish (MIA), has provided robust age estimates in the absence of absolute ages.

The biological processes that govern the deposition of annuli in otoliths have been the source of considerable discussion (Beckman and Wilson 1995). Annulus deposition has been related to annual migrations (Tserpes and Tsimenides 1995; Franks *et al.* 1999) and timing of spawning seasons (Hostetter and Munroe 1993; Ferreira and Russ 1994). *N. fucicola* are site-attached and spawn from mid August to late January (Barrett 1995; Denny and Schiel 2002), with a peak of spawning activity in early October. As the timing of opaque zone formation is not consistent with the peak of spawning activity (although there is some overlap), and because opaque zones are formed at a similar time of the year in juveniles, it is unlikely that reproductive activity directly influences the timing of opaque zone formation. The hypothesis that the stimulus for opaque zone formation may be correlated with the same external factors that initiate spawning cycles (Newman *et al.* 1996) is not supported by the present study as spawning begins in August, at least 2 months before opaque zone deposition. There is also conflicting evidence on whether opaque zones are formed at times of slow or fast somatic growth (Wilson 1995), or in fact whether otolith growth has any direct relationship with somatic growth (Francis *et al.* 1993). The present study determined that opaque zones are deposited on the proximal edge of otoliths from September to early December. The peak growth rate for *N. fucicola* from the southeast coast of Tasmania, which was derived from individual growth trajectories obtained from tag release and recapture studies, occurs from November to January (Welsford 2003a). Thus, opaque zones are deposited just before and over the first month of the period of peak annual somatic growth. These findings support the hypothesis that opaque zones mark the transition from slow winter somatic growth to fast spring and summer somatic growth, rather than coinciding with periods of slow or fast somatic growth.

Otolith growth has also been linked with environmental events, particularly temperature variation (Lombarte and Lleonart 1993; Woodbury 1999). The timing of the opaque zone formation in *N. fucicola* is coincident with the annual increase in sea surface temperatures on the southeast

Tasmanian coast, from an annual mean minima of 12.6°C (s.d. = 0.44°C,  $n = 80$ ) in August and 12.7°C (s.d. = 0.54°C,  $n = 77$ ) in September, through to an annual mean of 15.2°C (s.d. = 0.88°C,  $n = 76$ ) in December. Thus, the timing of opaque zone formation in *N. fucicola* also supports the alternative hypothesis that otolith zonation is an independent physiological process responding to environmental stimuli (Fowler 1995) and, rather than being linked to somatic growth, may mark the onset of increasing water temperatures that precede the summer season in southeast Tasmanian waters.

The von Bertalanffy growth function adequately modelled the growth of *N. fucicola* over the age range of fish collected. High negative theoretical ages at zero length ( $t_0$ ) reflect the relatively fast growth rates of juveniles. Given the considerable variation of size at age, length is a poor predictor of age, especially in fish aged >5 years. Male and female growth rates were found to be significantly different, with males initially growing more quickly but approaching a lower asymptotic length. Yet, these differences are not considered to be biologically important, as samples for both sexes are clustered from 4- to 10-year-olds (less than 5% of fish from either sex were older than 10 years) and differences are small (no differences in mean length at age were greater than 2% for fish aged between 4 and 10 years).

The growth parameters estimated for both sexes combined ( $L_\infty = 38.8$  cm,  $K = 0.104$  and  $t_0 = -2.13$ ), were close to those estimated from counts of opaque zones in whole otoliths ( $L_\infty = 39.9$  cm,  $K = 0.12$  and  $t_0 = -2.36$ ) (Barrett 1995). Similar maximum ages were also encountered (20 and 17 years, respectively). Although the timing of opaque zone formation and unseen opaque material on the margin were not examined by Barrett (1995), he did not collect samples in the 'problem period' (i.e. from October to January) and, thus, avoided the necessity for such considerations.

In summary, we believe that the measures taken to validate the location of the first annuli and the timing of opaque zone formation, the attention given to interpretation of the otolith growth edge and the rigorous protocol adopted, have minimised the risks associated with using marginal increment analysis to validate the periodicity of increment formation and have provided robust age and growth estimates for *N. fucicola* in eastern and southeastern Tasmanian waters. This is especially the case given the clarity of the increment structure, the consistent timing of the formation of opaque zones across all age classes and years sampled and the large sample sizes used.

## Acknowledgments

We gratefully acknowledge the assistance of Dr Jeremy Lyle for his editorial assistance and Simon Willcox and Sean Tracey for their assistance with sampling and otolith processing. Our appreciation also goes to Neville Barrett for his constructive comments on the manuscript.

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Manuscript received 4 September 2002; revised and accepted 1 October 2003.