Journal of Fish Biology (2005) **66,** 1740–1747
doi:10.1111/j.1095-8649.2005.00704.x, available online at http://www.blackwell-synergy.com

Non-destructive ageing in *Notolabrus tetricus* using dorsal spines with an emphasis on the benefits for protected, endangered and fished species

S. J. Metcalf* and S. E. Swearer

Zoology Department, The University of Melbourne, Parkville, Victoria, 3010 Australia

(Received 12 May 2004, Accepted 20 January 2005)

Age estimates from clipped dorsal spines were compared to otoliths and scales in the blue throat wrasse *Notolabrus tetricus*. Dorsal spines provided accurate, non-destructive ageing and are recommended in favour of destructive methods for conservation purposes.

Key words: age estimation; dorsal spines; non-destructive; otolith.

A variety of methods have been used to age fishes yet researchers have largely utilized scales and otoliths due, respectively, to their ease of collection and accuracy (Carlander, 1987; Heidinger & Clodfelter, 1987). The use of these methods, however, is not acceptable in all circumstances. Tropical fish otoliths, for example, are often difficult to read due to problems differentiating between the opaque and translucent zones that form increments. Furthermore, the use of otoliths requires the fishes to be killed, thereby removing potentially valuable stock from the population. Alternative structures such as fin spines, vertebrae, opercular bones and cleithra have also been used to age fishes with success (Chilton & Bilton, 1986; Beamish & McFarlane, 1987; Graynoth, 1996). Each ageing method has associated problems, such as the occasional loss of first annuli in fin spines (Graynoth, 1996) and indeterminate growth of vertebrae causing age to be underestimated (Beamish & McFarlane, 1987). Yet, alternative ageing techniques that are non-destructive and accurate can be an extremely valuable resource when studying fishery stocks and endangered species.

As it is necessary for fisheries managers to correctly age stocks to ensure their sustainability, non-destructive ageing techniques can protect fisheries from economic loss by retaining all individuals. Methods that do not cause mortality, such as dorsal spine clipping, can also support larger sample sizes which, in turn, increases the power of the results (Worthington *et al*., 1995). Importantly, non-destructive ageing methods can be used to study population demographics in rare and endangered fish species without pushing them closer to extinction.

*Author to whom correspondence should be addressed at present address: School of Zoology, The University of Tasmania, Sandy Bay, Hobart, 7005 Australia. Tel.: +61 3 6226 1826; fax: +61 3 6226 2745; email: metcalfs@utas.edu.au

The response of resident fish species to protection can also be investigated in marine parks while ensuring no fish are killed. This could be particularly useful when marine parks are first implemented to gauge the benefits of the protection to exploited and rare species.

Collins & Smith (1996) found clipping the dorsal spine of Atlantic sturgeon *Acepenser oxyrinchus oxyrinchus* Mitchill to be non-deleterious. This suggests that clipped dorsal spines could be an effective tool in the investigation of the population age structure of protected and valuable species. Sectioned dorsal spines have been found to be as accurate as otoliths when ageing chinook salmon *Oncorhynchus tshawytscha* (Walbaum) (Chilton & Bilton, 1986), white sucker *Catostomus commensonii* (Lacepède) (Beamish & Harvey, 1969) and Pacific cod *Gadus macrocephalus* Tilesius (Lai *et al.*, 1987). Ease of sampling and cost efficiency have also been suggested as benefits of the dorsal spine method for ageing (Conand *et al.*, 1995; Sun *et al.*, 2001). While the use of fin spines in ageing is not uncommon (Shirvell, 1981; Chilton & Bilton, 1986; Kocovsky & Carline, 2000; Ihde & Chittenden, 2002) this method has often been overlooked in favour of the widely used otolith method. Given the potential benefits, dorsal spine ageing should be looked at in more detail as a non-destructive ageing technique, particularly as very few studies have investigated the effects of spine clipping on survival.

The blue throat wrasse *Notolabrus tetricus* (Richardson), is a common resident on temperate reefs throughout south-eastern Australia (Russell, 1988). Blue throat wrasse are subject to both commercial and recreational fishing in Victoria with the minimum legal size of capture at 27 cm fork length (L_F). As *N. tetricus* are protogynous, with sex change usually occurring between 27 and 33 cm L_F, fished populations undergo the selective removal of dominant males due to their larger size.

In November 2003, the Government of Victoria, Australia, established a network of marine parks and sanctuaries to protect and conserve the marine environment. The purpose of this study was therefore to develop a non-destructive ageing technique which could be utilized to assess the response of blue throat wrasse populations to the protection and to fishing.

Previous research by Barrett (1995) validated the use of whole otoliths as an ageing structure for *N. tetricus* through tag-recapture studies in fish up to the age of 12 years, the maximum age for this species. Otolith increments were found to be laid down annually and have therefore been used to provide an accurate method of age determination in the current study. *Notolabrus tetricus* also possess 20 dorsal spines that act as fin supports which have not been assessed for annual banding. The specific objectives of this study were, therefore, to validate dorsal spines as an ageing technique for the blue throat wrasse by comparing age estimates from otoliths and dorsal spines and to investigate wrasse survival after spine clipping.

This study took place inside Port Phillip Bay, Victoria, Australia with most collection sites within 1–2 km of Port Phillip Heads (38°30′ S; 144°62′ E). Fifty one blue throat wrasse were collected for age estimation and validation using box traps with mixed bait, including sea urchins and prawns.

Validation of the use of dorsal spines was undertaken through comparison with age estimates from otoliths and scales, which are generally accepted as ageing structures. Dorsal spines were selected in preference to anal or pectoral fin spines because they are larger and have provided more accurate age readings for fishes in the past (Conand *et al.*, 1995). Clove oil was used to euthanase the blue throat wrasse prior to the removal of the second dorsal spine, sagittal otoliths and scales.

Whole otoliths were studied under a dissecting microscope and aged similarly to Barrett (1995). Two scales were removed posterior to the pectoral fins to reduce the likelihood of selecting damaged or worn scales. The scales were then mounted using Aquamount on slides and examined under a compound microscope. The second dorsal spines were cut below the basal node and all remaining flesh was removed using a 3% bleach solution. The spines were then embedded in clear casting resin, sectioned into 2 mm blocks using a Gemnasta high speed saw and polished using wet and dry sandpaper. Ageing of the spine sections was undertaken with a dissecting microscope under transmitted light (Sullivan, 1977). Age estimates were taken both from the base and half-way up the length of the spine (called spine top), approximately where it emerged from the flesh. Estimates from spine base and spine top were compared to determine if age can be estimated accurately through the removal of the spine where it emerges from the flesh on live fishes (*i.e.* determining age without causing mortality). During sample preparation two spine bases and one spine top were damaged and could not be aged. All otoliths, scales and spine sections were randomly read three times on non-consecutive days to reduce observer bias. The median reading was used as the age estimate in analyses to ensure the integer form was retained.

The median age (years) estimates from spine base, spine top and scales were compared to otolith age estimates using linear regression. The regression was forced through zero and 95% CI were used to assess if the slope significantly deviated from one. ANOVA was used to investigate differences in the relationship between otoliths and alternative ageing methods. The within-observer error rates were calculated following the method of Beamish & Fournier (1981) for each ageing technique.

The survival rate of blue throat wrasse subsequent to spine clipping was investigated in the laboratory. Fourteen wrasse were captured and anaesthetized using clove oil in 70% ethanol. Each fish was tagged for individual identification using non-toxic paint injected anterior to the caudal fin. The second dorsal spine was clipped from seven experimental fish where it emerged from the flesh. In order to clip the spine, the fin membrane was cut along both sides between the first and third dorsal spines. The fish were held in two 1000 l tanks connected by the same seawater system with three experimental and four control fish in one tank and four experimental and three control fish in the other. All factors other than spine removal were held constant between the experimental and control fish. The survival of the blue throat wrasse was monitored for 4 weeks after removal from the field.

Spine sections were found to have distinct opaque and translucent bands similar to annual bands observed in the otoliths (Fig. 1). The distance between bands on spine sections became smaller as distance from the core increased. This was only noticeable in fish >5 years and did not cause difficulty in age estimation. Scale increments did not show variation in spacing that is usually used to age fishes by this method (Shepherd & Hobbs, 1985).

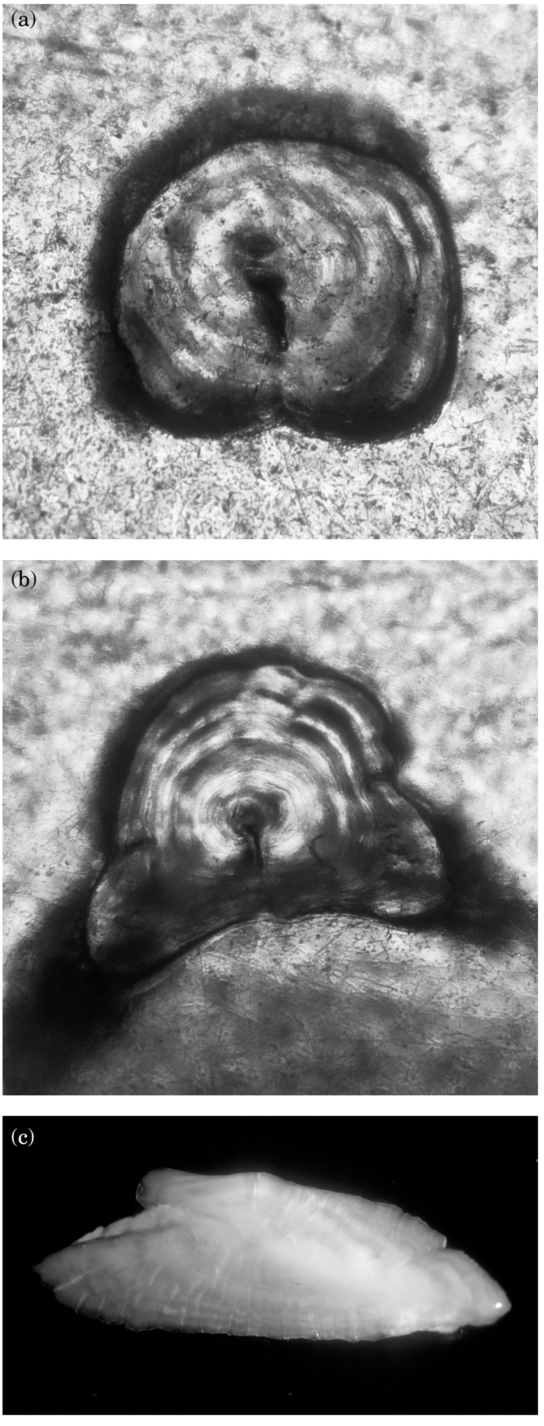

FIG. 1. Photographs showing the similarity between age increments from a 4 year-old *Notolabrus tetricus*. The four annual increments are shown in the (a) spine top section (×40), (b) spine base section (×28) and (c) otolith (×20). The photographs were taken during water immersion.

A strong relationship was found in median age readings from otoliths when related to both spine base and spine top sections using linear regression (Fig. 2; spine base, $F_{1,48}$, $P < 0{\cdot}001$, adjusted $r^2 = 0{\cdot}855$; spine top, $F_{1,49}$, $P < 0{\cdot}001$, adjusted $r^2 = 0{\cdot}878$). The slope of the regression relating otolith and spine age estimates was not significantly different from 1 (spine base 95% CI = 0·913–1·057, and spine top 95% CI = 0·986–1·122), indicating that age estimates from spine top are identical to ages from otoliths. Since increments in otoliths have been shown to form annually (Barrett, 1995), it was assumed that the growth rings observed in spine sections are also formed annually. In contrast to spines, there was a very poor relationship between otolith and scale readings ($F_{1,50}$, $P = 0{\cdot}364$, adjusted $r^2 = 0{\cdot}017$, 95% CI = 2·274–3·529), indicating that scales were not an accurate method for ageing *N. tetricus*. The within-observer error rates were calculated for each method and when compared to estimates in the literature, such as 19 and 23% average error in bighead carp *Aristichthys nobilis* (Richardson) (Nuevo *et al.*, 2004), reader precision is acceptable when using clipped spines [otoliths 9·75, scales 8·75, spine base 17 and spine top (clipped spines) 7% error rates].

All control and experimental fish survived the duration of this experiment ($n = 14$). Both spine-clipped and control fish quickly resumed swimming, feeding and behaving aggressively following handling. After 4 weeks, all experimental fish had re-grown the fin membrane that was cut during spine clipping.

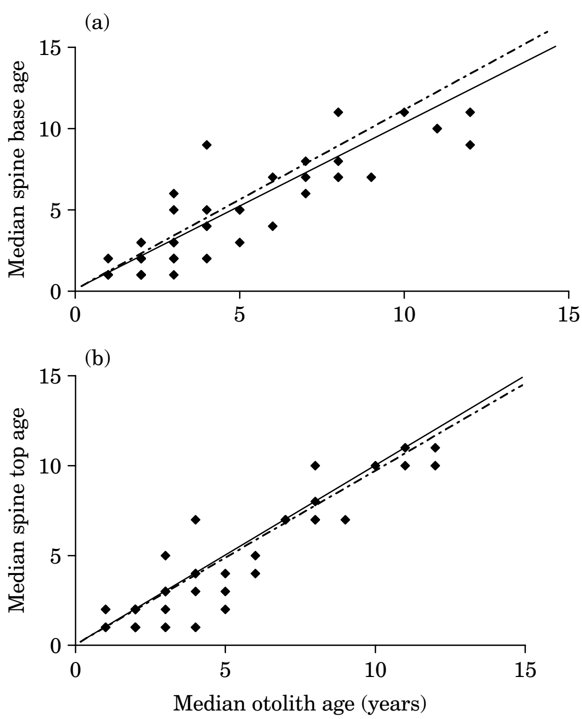

FIG. 2. Scatterplots showing the relationship between *Notolabrus tetricus* otolith age estimates and (a) spine base and (b) spine top. The regressions for spine ageing (---) are shown to display the similarity of the slopes to a slope of 1 (—, no difference between otolith and alternative ageing estimates): (a) $y = 1{\cdot}015x$ and (b) $y = 0{\cdot}949x$.

The relationship between *N. tetricus* otolith and spine age was not significantly different, indicating that spine sections are a viable alternative to otolith ageing. In addition, the high reader precision rates for clipped spines supports the use of this method. The ability to use clipped spine sections gives blue throat wrasse fisheries and marine park management a method of ageing that is beneficial for a number of reasons in addition to being non-destructive. Dissections are not necessary, increasing the ease of sampling (Holden & Meadows, 1962; Tserpes & Tsimenides, 1995), heightening the efficiency of age determination and reducing costs. Larger sample sizes are also possible without compromising stock protection (Worthington *et al.*, 1995) and this increases the statistical power of the results.

A reduction in the accuracy of spine age estimates as a result of annulus loss has been observed in adult striped bass *Morone saxatilis* (Walbaum) and brown trout *Salmo trutta* L. (Welch *et al.*, 1993; Graynoth, 1996). The loss of annuli was not observed in older *N. tetricus* for the age range (1–12 years) investigated in this study, contributing to the high level of agreement between spine age estimates and otoliths. Narrowing of increments towards the outer edge of spine sections was found in older blue throat wrasse, however, this did not hamper age estimation. As the age range investigated encompasses approximately the entire range of ages for the species, it is concluded that the use of spine top sections, as a non-destructive method of age determination is acceptable. This conclusion is further supported through the acceptance and use of dorsal spines as an ageing technique with correlations to the validated method as low as 68% (Shirvell, 1981; Lai *et al.*, 1987).

The 100% survival rate of both the experimental and control wrasse supports Collins & Smith's (1996) assertion that fin spine removal is non-destructive. The clipping of fin spines has also proved to be non-destructive in the white sucker (Beamish & Harvey, 1969), rainbow trout *Oncorhynchus mykiss* (Walbaum) (Faragher, 1992) and shortnose *Acepenser brevirostrum* Lesueur and Atlantic sturgeon (Collins & Smith, 1996). The regeneration of the fin membrane also supports this hypothesis and confirms that the ageing of blue throat wrasse through dorsal spines can be used as a non-destructive method. Yet, conditions in the laboratory cannot adequately mimic the natural environment and recommendations based solely on a laboratory study are risky. A large-scale tag-recapture study would be effective in monitoring survival and in the direct validation of annular increment formation in dorsal spines. The examination of predation and foraging levels should be undertaken in the field to conclusively ensure this method is non-destructive. In addition, the successful use of dorsal spines to age a range of species would increase the significance of these results.

This study has confirmed dorsal spines to be an accurate, non-destructive and useful ageing technique for the blue throat wrasse. The study also indicates that ageing of clipped dorsal spines may prove to be a successful method of estimating the population demographics of both protected and exploited temperate reef fishes.

We would like to thank R. Francis and R. Watson for many hours spent in the field as well as three anonymous reviewers for their useful comments.

References

Barrett, N. S. (1995). Aspects of the biology and ecology of six temperate reef fishes (Families Labridae and Monacanthidae). PhD Thesis, University of Tasmania, Tasmania, Australia.

Beamish, R. J. & Harvey, H. H. (1969). Age determination in white sucker. *Journal of the Fisheries Research Board of Canada* **26,** 633–638.

Beamish, R. J. & Fournier, D. A. (1981). A method for comparing the precision of a set of age determinations. *Canadian Journal of Fisheries and Aquatic Sciences* **38,** 982–983.

Beamish, R. J. & McFarlane, G. A. (1987). Current trends in age determination methodology. In *The Age and Growth of Fish* (Summerfelt, R. C. & Hall, G. E., eds), pp. 15–42. Ames, IA: Iowa State University Press.

Carlander, K. D. (1987). A history of scale age and growth studies of North American freshwater fish. In *The Age and Growth of Fish* (Summerfelt, R. C. & Hall, G. E., eds), pp. 3–14. Ames, IA: Iowa State University Press.

Chilton, D. E. & Bilton, H. T. (1986). New method for ageing chinook salmon (*Onchorhynchus tshawytscha*) using dorsal fin rays, and evidence of its validity. *Canadian Journal of Fisheries and Aquatic Sciences* **43,** 1588–1594.

Collins, M. R. & Smith, T. I. J. (1996). Sturgeon fin ray removal is nondeleterious. *North American Journal of Fisheries Management* **16,** 939–941.

Conand, F., Camara, S. B. & Domain, F. (1995). Age and growth of three species of Ariidae (Siluriformes) in coastal waters of Guinea. *Bulletin of Marine Science* **56,** 58–67.

Faragher, R. A. (1992). Growth and age validation of Rainbow Trout, *Oncorhynchus mykiss* (Walbaum), in Lake Eucumbene, New South Wales. *Australian Journal of Marine and Freshwater Research* **43,** 1033–1042.

Graynoth, E. (1996). Determination of the age of brown and rainbow trout in a range of New Zealand lakes. *Marine and Freshwater Research* **47,** 749–756.

Heidinger, R. C. & Clodfelter, K. (1987). Validity of the otolith for determining age and growth of walleye, striped bass and smallmouth bass in power plant cooling ponds. In *The Age and Growth of Fish* (Summerfelt, R. C. & Hall, G. E., eds), pp. 241–251. Ames, IA: Iowa State University Press.

Holden, M. J. & Meadows, P. S. (1962). The structure of the spine of the spur dogfish (*Squalus acanthias* L.) and its use for age determination. *Journal of the Marine Biological Association of the United Kingdom* **42,** 179–197.

Ihde, T. F. & Chittenden, M. E. , Jr. (2002). Comparison of calcified structures for ageing spotted seatrout. *Transactions of the American Fisheries Society* **131,** 634–642.

Kocovsky, P. M. & Carline, R. F. (2000). A comparison of methods for estimating ages of unexploited walleyes. *North American Journal of Fisheries Management* **20,** 1044–1048.

Lai, H., Gunderson, D. R. & Lee Low, L. (1987). Age determination of pacific cod, *Gadus macrocephalus*, using five ageing methods. *Fishery Bulletin* **85,** 713–722.

Nuevo, M., Sheehan, R. J. & Heidinger, R, C. (2004). Accuracy and precision of age determination techniques for Mississippi River bighead carp *Hypophthalmichthys nobilis* (Richardson 1845) using pectoral spines and scales. *Archiv fur Hydrobiologie* **160,** 45–56.

Russell, B. C. (1988). Revision of the Labrid fish genus *Psuedolabrus* and allied genera. *Records of the Australian Museum* **9,** 1–76.

Shepherd, S. A. & Hobbs, L. J. (1985). Age and growth of the blue-throated wrasse *Pseudolabrus tetricus*. *Transactions of the Royal Society of South Australia* **109,** 177–178.

Shirvell, C. S. (1981). Validity of fin-rays ageing for brown trout. *Journal of Fish Biology* **18,** 377–383.

Sullivan, K. J. (1977). Age and growth of the elephant fish *Callorhinchus milii* (Elasmobranchii: Callorhynchidae). *New Zealand Journal of Marine and Freshwater Research* **11,** 745–753.

Sun, C., Huang, C. & Yeh, S. (2001). Age and growth of the big-eye tuna, *Thunnus obesus*, in the western Pacific Ocean. *Fishery Bulletin* **99,** 502–509.

Tserpes, G. P. & Tsimenides, N. (1995). Determination of age and growth of swordfish, *Xiphias gladius* L., 1758, in the eastern Mediterranean using anal-fin spines. *Fishery Bulletin* **93,** 594–602.

Welch, T. J., van den Avyle, M. J., Betsill, R. K. & Driebe, E. M. (1993). Precision and relative accuracy of striped bass age estimates from otoliths, scales, and anal fin rays and spines. *North American Journal of Fisheries Management* **13,** 616–620.

Worthington, D. G., Fowler, A. J. & Doherty, P. J. (1995). Determining the most efficient method of age determination for estimating the age structure of a fish population. *Canadian Journal of Fisheries and Aquatic Sciences* **52,** 2320–2326.