SCRS/2008/177

A protocol for age estimation of striped and white marlin (Kajikia spp.) using fin spine cross-sections

Richard Keller Kopf[1], Katherine Drew[2], and Robert L. Humphreys Jr.[3]

SUMMARY

This manuscript gives a description of the methods used to age white and striped marlin using fin spine cross-sections. Aging techniques were detailed in order to standardize billfish aging methodologies so that future growth models from different regions would be comparable. This preliminary work was based on on-going age and growth studies of white marlin and striped marlin being conducted at Charles Sturt University in Australia, University of Miami in Florida, and the NOAA Fisheries Service, Pacific Islands Fisheries Science Center in Honolulu.

1. Charles Sturt University, School Animal and Veterinary Sciences, Boorooma St. Wagga Wagga NSW 2678, Australia. rkopf@csu.edu.au

2. University of Miami RSMAS, Division of Marine Biology and Fisheries, 4600 Rickenbacker Causeway Miami, FL 33149, U.S.A. kdrew@rsmas.miami.edu

3. NOAA Fisheries Service, Pacific Islands Fisheries Science Center, Aiea Heights Research Facility, 99-193 Aiea Heights Drive, Suite 417, Aiea, Hawaii 96701 U.S.A. Robert.Humphreys@noaa.gov

Introduction

Striped and white marlin, former members of the genus *Tetrapturus* (*audax* and *albidus* respectively) have recently been placed in the genus *Kajikia* (Collette et al. 2007). Striped marlin inhabit the Indo-Pacific Oceans while white marlin are generally restricted to the Atlantic Ocean. Although they are found in different ocean basins, white and striped marlin are very similar morphologically (Nakamura, 1985) and genetically (Graves and McDowell, 2003). Both species are migratory, occurring throughout tropical, sub-tropical, and temperate latitudes. They are popular gamefish and are targeted in small-scale artisanal fisheries and striped marlin are the most valuable Istiophorid billfish caught in commercial longline fisheries (ICCAT 2003, Hinton and Maunder 2004, Bromhead et al. 2005).

A recent stock assessment of white marlin indicated that the species is significantly overfished (ICCAT 2003). An assessment of striped marlin in the eastern Pacific Ocean suggests that the stock is being exploited at near-optimum levels (Hinton and Maunder 2004) while the status is uncertain in other areas of the Pacific (Langley et al 2006). Most stock assessments of billfishes have a high degree of uncertainty in their estimates, due in large part to a lack of information pertaining to biological parameters, particularly size-at-age.

Validated age and growth models could reduce this uncertainty and increase the effectiveness of management strategies. However, billfishes present unique challenges in age and growth determination due to their large body-size and solitary nature inhabiting the open ocean. Collection of adequate sample sizes and recapture of tagged individuals is rare (Radtke and Shepherd 1991, Holland 2003) and calcified hard parts are challenging to interpret (Radtke 1983). Despite these difficulties, growth models have been developed for several species, including swordfish (Ehrhardt 1992, Tserpes and Tsimenides 1995, Sun, *et al*. 2002, DeMartini et al. 2007), sailfish (Hedgepeth and Jolley Jr. 1983, Chiang, *et al*. 2004; Hoolihan 2007), black marlin (Speare 2003), and striped marlin (Melo-Barrera, *et al*. 2003; Kopf et al. 2005). Research has also been conducted on otoliths, vertebrae, and fin spines for use in aging blue and white marlin (Prince, et al. 1984, Prince et al. 1991, Hill, et al. 1989). These studies have found significant relationships between body length and the radius and calcified hard parts, particularly fin spines.

Although spines have proven to be useful aging structures, several problems have been identified which hinder age estimation. Most notably, the center of the fin spines is composed of vascularized tissue (Yatomi 1989; Drew et al. 2006). As spines grow, the core of vascularized tissue grows and obscures early formed increments. If unaccounted for, vascularization of the fin spine core can result in significant age underestimates and overestimates of growth. The level of difficulty in interpreting sections is exacerbated further by a high degree of variability in increment clarity and differing patterns of presumed annual and false band formation. Furthermore, the annual periodicity of annuli formation in fin spines has not been confidently validated. Despite these drawbacks, however, fin spines are the most feasible calcified structure for estimating age and growth parameters of billfishes and are the most commonly used structure to estimate biological parameters for stock assessment.

While previous research has increased our understanding of the age and growth parameters of billfish, they often provide limited details of fin spine preparation and interpretation. Therefore, there have been a variety of different methods used to derive age estimates and many estimates diverge widely from one another. This is of particular concern to migratory species such as the billfishes because individual stocks may be studied by multiple different international researchers. This manuscript provides a standardized template for estimating the age of striped and white marlin using fin spine cross-sections.

Fin spine preparation and observation

Morphology and selection

Previous age and growth studies on billfishes have used the terms fin spines, fin rays, and spiny fin rays to describe the structures of the first dorsal and anal fins (Davie & Hall 1990; Young et al. 2003; DeMartini et al. 2007). The term spine is used throughout the present manuscript to describe all fin supporting elements in the first dorsal (D) and anal (A) fins. Anatomically, D1-4 and A1-2 are solid cortical bone while the more caudal elements appear to be rays that branch distally.

The appearance and location of annuli may vary between or within bones (including spines) of the individual fish (Figure 1; Panfili et al 2001). Variation in internal and external morphology in spines of billfishes has led researchers to select particular spines which presumably facilitate age estimation. Davie & Hall (1989) used D3 and Melo-Barrera (2003) used D4 for striped marlin, while other studies on billfishes have used D6 (Hill et al. 1989), A2 (DeMartini et al. 2007), or A3 (Speare 2003). Speare (2003) found no differences in age estimates between A3 and D3 in black marlin but Uchiyama et al (1998) found that annuli counts in swordfish varied between D1-3 and A1-3. Although spine selection is important, it is equally important to consistently use the same structure in order to avoid unknown bias.

The most suitable fin spines for white and striped marlin in the present study were A3 and D4-5, respectively. Suitable spines were selected based on a large readable area relative to vascularization and showed the greatest number of clearly observable bands (Figure 1; Panfili et al 2001). Size of the vascularized area in spines of striped marlin generally decreased moving caudally in anal and dorsal spines. Spines A1 and D1-2 of striped marlin often exhibited conspicuously low band clarity or were totally unreadable.

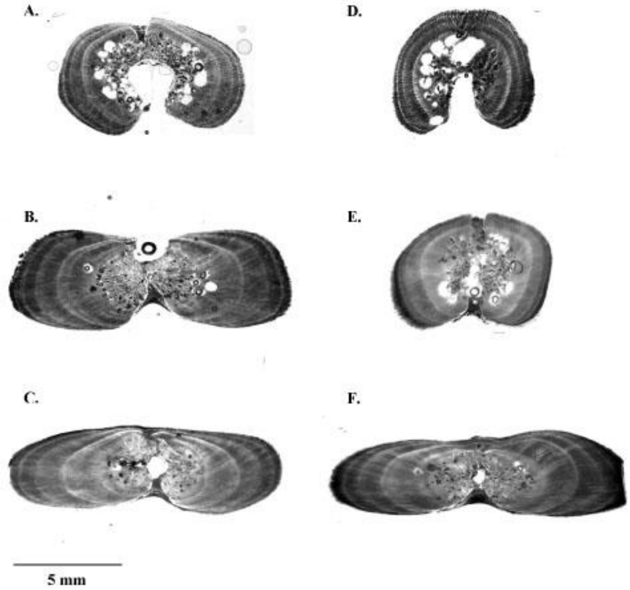

Figure 1. Spine sections cut at distance of ¼ the width of the condyle from dorsal fin spines 3 (A), 4(B), 5(C) and anal fin spines 1 (D), 2 (E), 3 (F) from a 28kg striped marlin. Note differences in the size of the central vascular core region and variation in annuli clarity and readable area.

Extraction

Fin spines can be removed using a robust knife by cutting along the length of the spine toward the condyle. Severing the ligaments which attach each condyle to the body simplifies the extraction process. It is preferable to keep fin spines intact to ensure they are kept in sequential order so that the fin spine number can be determined. As the present study made sections relative to the condyle width, it was also important to extract the entire spine including the base which was below the skin.

Spines should be stored in plastic bags at -20C or below. It is preferable to freeze spines as soon as possible after extraction. Repeated thawing or extended freezer times (beyond 12 months) may affect band quality. Spines can be thawed and heated in warm water (<70degC) but boiling reduces band clarity. Heating for 20-30 minutes loosens fat and skin which surrounded the spines. Excess material surrounding the spines can be removed with scissors, scalpels, and plastic bristle brushes. When cleaning with sharp tools, care should be taken to prevent scraping the outer edge of the fin spine. After spines are cleaned of all tissue they should be air dried in an oven at 50-60deg. C for no more than 24 hrs.

Sectioning and mounting

After a fin spine is selected, the position where the section is taken must be determined using consistent criteria. Sections for age estimation in the present manuscript were cut at distances relative to the maximum width of the condyle (Figure 2). Previous studies have also used relative proportions of fin spine length (Hill et al. 1989, Speare 2003) or external reference points (DeMartini et al. 2007). The highest number of bands and lowest percent vascularization in the present study was observed within one condyle width of the base of the spine (Figure 3). Additionally, sections from the base of spines showed wider increments which that were easier to resolve, particularly at the edge and near the focus.

Sections can be cut using a low speed (approximately 1600 RPM) lapidary saw fitted with a diamond edged wafering blade. Spines can be sectioned dry or imbedded in clear casting resin. Imbedding spines ensures that sections remain enact, particularly the edge/margin, but is more costly and time consuming than dry cutting. Cutting sections less than 0.30mm is difficult with dry spines since sections tend to warp. Thinner transverse spine sections allow greater detail to be observed in increments but appear to expose a greater number of false increments. Optimal thickness of sections appears to be between 0.30 and 0.60mm but varies depending on the individual fish. Grinding and polishing individual sections to appropriate thickness may be required. Since the morphology of the spines changes with height, it is recommended that no more than two sequential sections are removed from any one level (eg. ¼ or ½ condyle width).

Figure 2. Diagram of dorsal fin ray number four from a striped marlin showing sectioned regions between ¼ and 1X maximum condyle width.

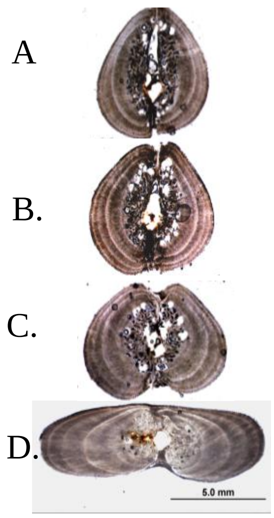

Figure 3. Dorsal fin spine number five from a 28kg striped marlin showing morphological variation between sections cut at distance of 3X (A), 2X (B) , 1X (C), and ¼ (D) the maximum width of the condyle.

Observation

Prior to official readings all sections should be examined and the clearest section should be photographed using a digital microscope camera. Age readings should be conducted using the same magnification, light source (reflected or transmitted), and image resolution. Transmitted light illuminates the translucent growth bands (presumed annuli) white while reflected light gives the bands a dark appearance. If digital images are captured with transmitted light they can be inverted to give the same appearance as reflected light (Figure 4).

One image of the whole section and one image of the edge at a higher magnification should be saved. Additional images showing the ID numbers and scale bars should be saved separately. Sections photographed for conducting measurements should come from the same spine and relative level of the spine even if a different section was used for age readings. The increments observed on the age reading section can usually be transcribed to the measurement section. In conducting formal age readings, it is preferable to use digital images as the optical properties of the section can be fixed. Digital images are also easy to archive and share with researchers in different regions. Digital imaging programs such as Adobe Photoshop or Image J (free-online) have graphic enhancement and measuring tools that can facilitate examination of sections (Figure 4).

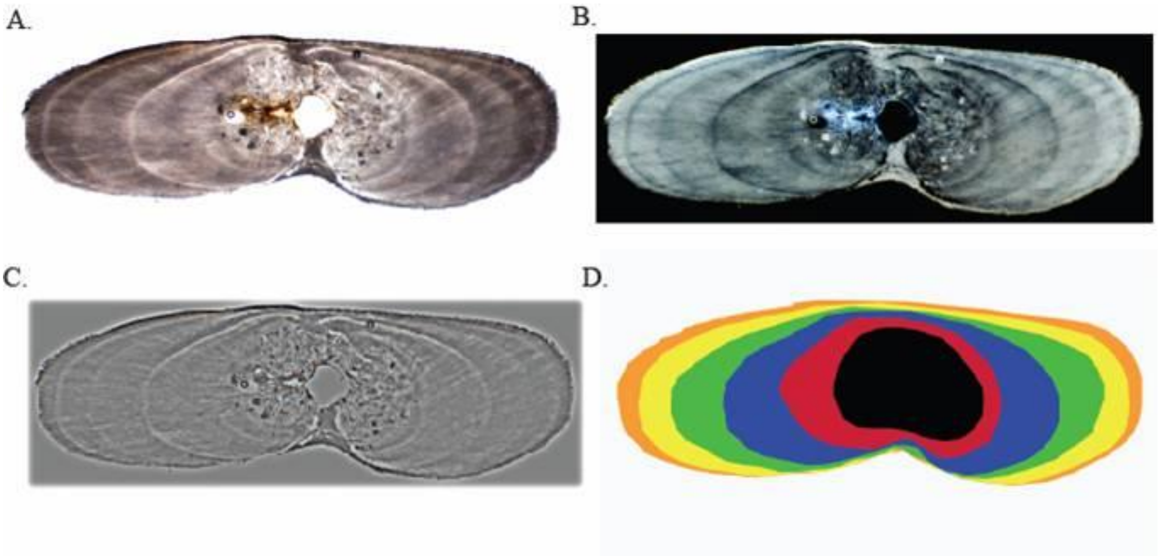

Figure 4. Examples of digital image modifications to improve annuli clarity or facilitate measurements of presumed annuli. Unmodified image under transmitted light (A), Inverted (B), Embossed (C), Digitized annuli (D).

Annuli classification

Annuli

We define a complete annulus to consist of a wide opaque zone (fast growth) followed by a narrow translucent zone (slow growth) (see Fablet 2006). These increments have previously been used to estimate the age of other billfish species (Hill et al. 1989; Speare 2003; Hoolihan 2006; DeMartini et al. 2007) but interpretation of annuli in billfishes is not straight forward. The primary difficulty in interpreting fin spines of billfishes is distinguishing true annuli from other non-age related bands (false annuli) and accounting for annuli lost due to vascularization (see Correcting for vascularization).

To help interpret inconsistencies in counting annuli, we recommend the use of a color-coding system (Figure 5). Digitally labeling annuli as green (obvious), yellow (less easily distinguished), or red (visible mark but does not meet the qualifications of an annulus) may help identify inconsistent interpretations between or within readers. Proportions of red, yellow, and green annuli may also be used to determine section readability scores and facilitate assigning final age estimates.

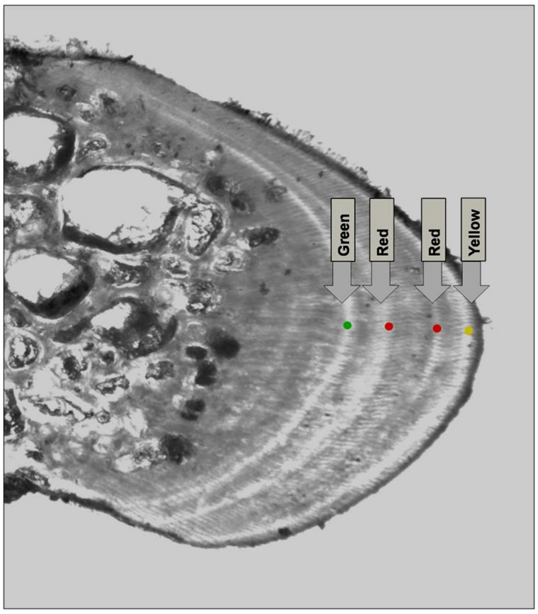

Figure 5. Diagram of a white marlin anal fin spine section showing color-coded annuli classification scheme.

Presumed annuli (green) should be clear and undisputable bands (Figure 6a) that meet functional criteria for defining annulus growth increments in calcified structures of other fishes (see Fablet 2006). These annuli extend around the full perimeter of both lobes of the spine and do not include partial or spit bands (Ehrhardt et al. 1996; Hill et at al. 1989). In practice, however, differences in preparation, preservation, and individual variation may affect clarity. Thus, true annuli may not meet the functional criteria and in this case are color coded yellow (Figure 6b). Incomplete yellow annuli usually do not connect at the lateral edge of the sections but were otherwise similar in appearance to green annuli. Yellow annuli were distinguished from false annuli (red) by the presence of a clear band initiated at the cranial and caudal margin of sections (Figure 6c).

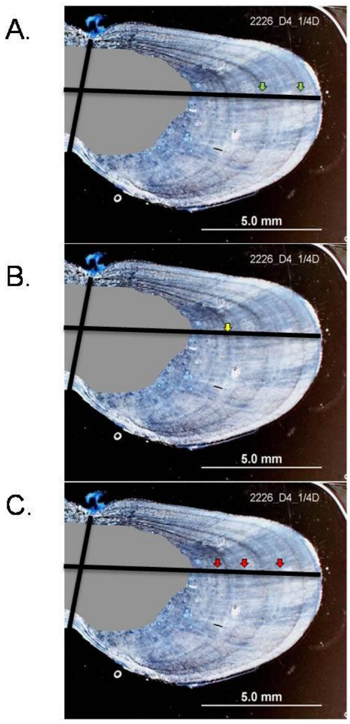

Figure 6. Diagram of a dorsal fin spine section from a striped marlin showing color-coded increment classification scheme green (A.), yellow (B.), and red (C.).

False annuli

Two types of false (red) annuli are commonly observed in fin spine sections of white and striped marlin. The first type is a doublet or triplet bands that form adjacent to presumed annulus (Figure 7a). Doublet and triplet bands can be identified according to the criteria developed by Cayre and Diouf (1983). Briefly, the growth rate of fish naturally declines each year, except in years of unusual individual growth. Therefore, the width of the first annulus increments nearest the focus (R1) should be wider than the next annulus closer to the outer edge (R2). We postulate that growth of billfishes would rarely increase by more than 25% compared to any previous year. Therefore, a doublet or triplet annulus was identified when R1/R2 was less than 75%. If there is a presumed annulus within a doublet or triplet band, the clearest increment that best fitted the growth pattern of adjacent annual annuli was used for measurements.

The second type of false (red) band is not necessarily adjacent to a presumed annulus and may occur throughout the fin spine section (Figure 6c). This type of false annulus is apparent in most sections but is widespread in 0-1yr old fish where there appears to be frequent growth checks during this rapid stage of growth (Figure 7b). False annuli were generally short segments at the lateral edge of spines and, unlike yellow annuli, were not usually present at both the cranial and caudal margins. These bands were also thinner and generally fainter than true annuli.

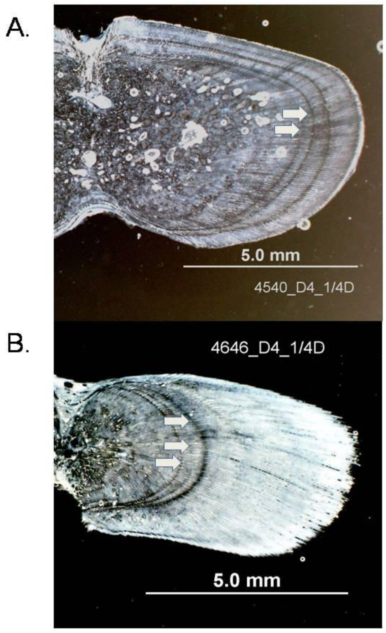

Figure 7. Diagram of a striped marlin fin spine section with arrows pointing to false double increments (A.) and sub-annual triplet increments in a juvenile (B.).

Edge type

Edge type classification assigns a state of completion to the outermost annulus. Similar to the marginal increment ratio (See *Marginal Increment Ratio*), the percentage of edge type classifications can be plotted monthly or seasonally to estimate the timing and periodicity of annulus formation. Furthermore, classification of the edge type of sections has important implications for placing fish in particular age classes (see *Final age estimates*). Many different types of edge classifications have been published (see Kimura et al. 2007) but we recommend the use of a three stage system (Pearson 1996) which simplifies interpretation of edge types and data analysis. In this system, the edge or periphery of spine sections are classified as either translucent, narrow opaque or wide opaque (Figure 8).

Translucent edge types were classified when the cranial and caudal margins of spines displayed a clearly formed band that was similar in thickness to other presumed annuli and it extended along greater than half of the perimeter (Figure 8a). Thin translucent bands not clearly observable at the cranial and caudal margin were recorded as opaque since they could not be distinguished from false bands. Opaque narrow types were classified when the edge was less than half of the previous increment width (Figure 8b). Opaque wide edges were classified when an opaque edge was greater than half the previous opaque area (Figure 8c).

Each edge type was given a confidence reading of unreadable to highly confident (1-4). Using samples that display a clearly discernable edge will facilitate an accurate assessment of the timing of annulus formation. However, being too conservative with rejection of samples/assignment of edge type may exclude sections where annuli formation is starting to occur. Edge types should be plotted as percentages by month or season. Peak percentages of translucent band formation should indicate the timing of increment formation which should be followed by narrow opaque peaks and subsequently by wide opaque peaks.

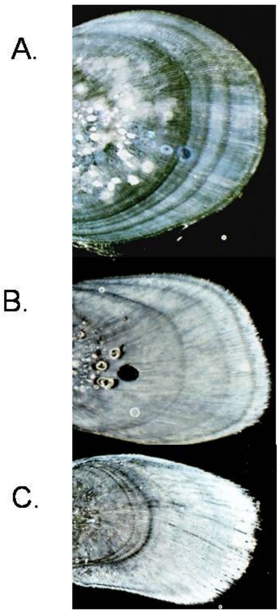

Figure 8. Fin spine section of striped marlin showing different edge types including translucent (A.), opaque narrow (B.), and opaque wide (C.)

Section measurements

Defining the focus and measurement axis

The central portion of fin spine sections is referred to as the focus and is the starting point for measurements and increment counts. Some studies (e.g., Ehrhardt 1992) have found the focus by tracing the striations in sections back to a single point of origin. This method may not be feasible when the angle of the striations changes between growth bands. Therefore, we recommend that the focus of symmetrical sections be determined by the intersection of the vertical and horizontal growth axes. The horizontal axis is also used as the counting path for readings and measurements. The vertical axis connects inflection points at the top and bottom of the section and the horizontal axis connects the outer most edges of each lobe (Figure 9). For asymmetrical fin spine sections or where the maximum width of the lobe extends downward different criteria are required. In this case, the vertical axis could still be defined as above but the horizontal axis should be determined for each lobe independently. A horizontal line from the outer most edge of each lobe should extend through the midpoint of the vertical axis.

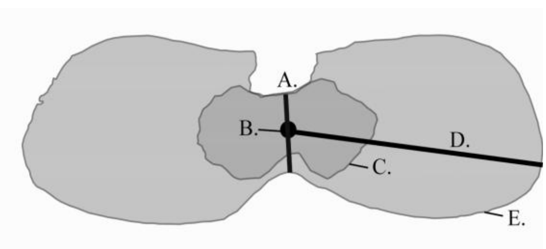

Figure 9. Measurements and notable features of a fin spine section displaying the vertical axis (A), focus (B), vascularized perimeter and surface area (C), counting path and horizontal axis (D), and the perimeter and total surface area of the section (E.).

Linear measurements and surface area

All linear distances should start at the focus and be measured along the horizontal axis used to define the focus. Measurements should include the spine radius, vascularized radius, and the radius of each increment (Figure 9). Distances should be measured where the horizontal axis (counting path) intersects each respective feature. Increment radius measurements should extend from the focus to the outer edge of translucent bands. To expedite marginal increment analysis, widths of the ultimate and penultimate band pair can be measured directly. Alternatively, increment widths can be calculated indirectly by subtracting increment radii measurements (See Marginal Increment Ratio).

Area measurements should include the vascularized area and total surface area of the section (Figure 9). The surface area of each increment can also be measured to compare with linear increment measurements. Surface area measurements can be calculated on digital images using the Image J software Plug-in, Area Calculator, available free-online. The vascularized and total surface area of the section should be traced at high enough resolution to follow smooth edges. Readable area is calculated by subtracting the vascularized area from the total surface area.

Statistical procedures

Marginal Increment Ratio

Annuli observed in fin spine sections are presumed to have a yearly periodicity which has been indirectly validated in some species of billfish through marginal increment analysis (Chiang et al 2004; DeMartini et al. 2007) and also by recapture oxytetracyline injected fish (Speare 2003). The marginal increment and edge type analysis can provide indirect validation of annuli periodicity by examining monthly or seasonal changes in the most recently formed increment (see Campana 2001). The margin or edge of sections extends from the perimeter of the section to the boarder of the previous annulus. The marginal increment ratio generates a percentage of completion while the edge type analysis provides a qualitative assessment of the status of the perimeter of the section.

We recommend use of the Marginal Increment Ratio (see Stephensen and Hall 2003) described by the equation $MIR = (R - r_n)/(r_n - r_{n-1})$, where R = ray radius, r_n = radius of the ultimate translucent band, and r_{n-1}= radius of the penultimate translucent band. The Marginal Increment Ratio calculates the percentage of annulus completed at the edge by comparing the widths of the margin to the previous increment. When the mean and standard error of the MIR is plotted each month over 12 months, the trend line should form one sinusoidal cycle if increment formation is annual. Conversely, if two increments are formed per year, two cycles may be observed. It is recommended that an appropriate statistical measure is used to test for differences in the mean MIR by month, season, or year. Due to the difficulties in sampling billfishes during all months of the year and relatively low sample sizes compared to other fishes, non-parametric approaches may provide more robust statistical comparisons. We recommend use of the Kruskall-Wallis one-way ANOVA on ranks to compare MIR (see Calliet et al. 2006).

Average Percent Error and Coefficient of Variation

The reproducibility of age estimates (precision) can be measured using the Average Percent Error (APE) and Coefficient of Variation (CV) (see Campana 2001). Both methods measure the difference between multiple age estimates of one fish to calculate error which is then averaged across the sample population. The CV provides a more conservative estimate of precision than APE but both are widely accepted methods. As the fin spines of billfish display a high degree of variability in annulus clarity, it is important to consider acceptability and rejection thresholds. There are no standard thresholds but reliable fin spine age estimates generally exhibit one reader error of less than 15% CV (Campana 2001). Average Percent Error (APE) and Coefficient of variation (CV) are described by the following equations:

$$APE_j = 100\% \times \frac{1}{R} \sum_{i=1}^{R} \frac{\left| X_{ij} - X_j \right|}{X_j}$$

$$CV_j = 100\% \times \frac{\sqrt{\sum_{i=1}^{R} \frac{(X_{ij} - X_j)^2}{R-1}}}{X_j}$$

Variable *X*ij is the *i*th age determination of the *j*th fish, *X*j is the mean age estimate. *X*j is the mean age estimate of the *j*th fish, and *R* is the number of times each fish is aged. APE_j and CV_j are precision estimates for the jth fish which is then averaged across the population.

Three within laboratory age readings and one external reading are recommended to evaluate precision. Intra-laboratory and intra-reader comparisons will most often generate less precise age estimates and CV's of 10-15% are not uncommon for large pelagic fish (DeMartini et al. 2007). As vascularization has been reported to increase in larger fish, caution must be taken not to bias age estimates by rejecting a disproportionate number of large fish. Size/age specific bias in rejection rates should be tested statistically and accounted for using the appropriate measures.

Correcting for vascularization

Vascularization of the fin spine core is present to some degree in all fin spine sections of white and striped marlin (Figure 10). However, vascularization increases as fish grow (Figure 11) and therefore older individuals have a greater number of increments obscured than younger fish. Aging studies on large pelagic fishes have overcome the effects of vascularization of the fin spine core by using statistical replacement methods (Hill et al. 1989). In this technique, the radii of visible annuli are compared to the mean and 95% confidence intervals of annuli radii from smaller, younger fish that have not been affected by vascularization. The number missing annuli in older fish are then estimated based on the annuli radii statistics from smaller individuals. This technique has been used with blue marlin (Hill *et al.*, 1989), swordfish (Tserpes and Tsimenides, 1995), and sailfish (Chiang *et al.*, 2004). However, this method may not be appropriate for spines that exhibit extensive vascularization.

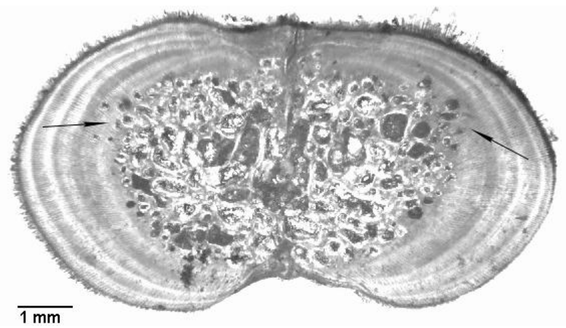

Figure 10. White marlin spine section with black arrows indicating where vascular tissue is beginning to obscure the innermost ring.

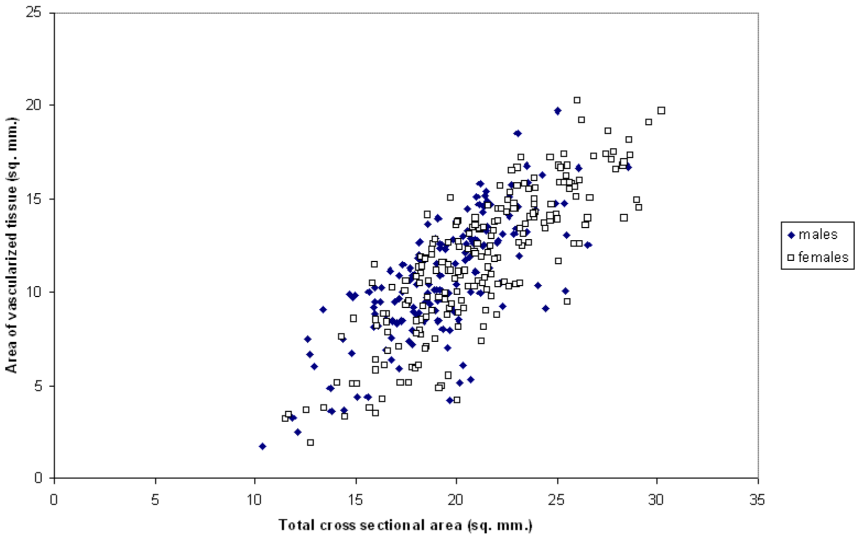

Figure 11. Area of vascularized tissue vs. cross-sectional area of a white marlin fin spine section.

One technique being explored is *k*-means clustering (MacQueen, 1967). This technique assigns an age estimate to each annulus based on its radii measurement. In fin spine sections, annulus radii are the observations and clusters represent ages. The center of the cluster is in effect the average annulus radius for that age. Since this technique is sensitive to starting values, the analysis should be seeded with realistic values of annulus radii calculated using the statistical replacement technique (Hill et al. 1989) on a sub-sample of spine sections covering the observed range of sizes. This technique relies on the same assumption as the statistical replacement technique described by Hill et al. (1989). That is, the annuli of particular age classes are formed at approximately the same distance from the center of the spine. Ensuring that sections are cut from the same spine and relative level is an important consideration for replacing increments lost due to vascularization (see *Sectioning and mounting).*

Assigning age estimates

Reading procedures

We recommend developing a library of reference sections of varying degrees of readability and reviewing this library before each reading session. Prior to readings, the focus, counting path (horizontal axis), and vascularized area should be marked on digital images using Image J or other imaging software (see *Measurements*). Blind readings should be conducted with new ID numbers and should not show any other uniquely identifiable characteristics.

The horizontal axis used to determine the focus should be used as a counting path during readings. Start at the focus and proceed toward the lateral edge. Mark increments along the counting path using the color-coded system (see *Annuli classification*). Annuli do not necessarily have to intersect the counting path to be counted but the estimated point of intersection should be marked. Be sure to save a separate, unedited, image for future readings. Record increment counts from inside the vascularized perimeter and outside the vascularized perimeter separately as this will facilitate increment replacement. When finished marking annuli on a section, classify the edge type using both the whole section image and high magnification edge image.

A confidence reading should be assigned to the edge/margin and the entire section. The reading scale should include unreadable, readable, confident, or no doubt (1-4). A limit of approximately 2-3 minutes should be set for aging each section. All spines should be read at least three times by the primary reader with at least two weeks between each reading. It is also recommended that at least 10% of the total sample size is read by an experienced external reader. Prior to the third and final reading, discrepancies between the first two readings should be individually examined.

Final age estimates

Final age estimates of individual fish should consider the most appropriate annuli count, the number of annuli lost due to vascularization, and the edge type classification. The final age estimate of the fish can be described by the following equation $Age_{final} = Annuli_{spine} + Annuli_{vascular} + Edge_{1+,-or\ 0}$. $Age_{final,}$ is the final age estimate for the individual fish, $Annuli_{spine,}$ is the number of complete annuli observed outside the vascularized area, $Annuli_{vascular,}$ is the number of annuli estimated to have been lost due to vascularization, and $Edge_{1+\ or\ 0,}$ is the edge type classification.

The precision of age estimates (APE and CV) should decrease with progressive readings as the primary reader becomes more experienced. Therefore, the final reading (third) should provide the best estimate. Sections with an average percent error of greater than 15% over the three readings should be re-examined and discarded if a consensus age estimate cannot be reached. The number of annuli lost due to vascularization should be estimated using the methods described in the *Correcting for vascularization* section. Particular care should be taken to ensure that increments inside the vascularized perimeter are not counted twice. This type of age overestimation can be avoided by recording annuli inside and outside the vascularized perimeter separately or by not recording annuli inside the vascularized perimeter (see *Reading procedures*).

Accounting for the edge type allows individual fish to be placed into appropriate year classes. For example, even though a fish may only show one complete annulus (translucent and opaque zone), the individual may be closer to two year old than a one year old if it is near but has yet to reach the season of translucent zone formation. This fish should therefore be placed in the two year age cohort. If the radius of the first true annulus can be validated through an independent means such as examination of daily micro-increments then determination of the final age is relatively straight forward.

If the edge is opaque and wider than 50% of the previous complete increment (opaque wide), then 1 “year” should be added to the final estimate. No increments should be added if the edge type is translucent or if it is opaque and narrower than 50% of the previous increment. However, if the first true annulus has not been identified then more complicate edge type assignments are necessary (see Kimura et al. 2007). For example, if an individual fish was born in January 2007 and caught/sampled in March 2007 it should be classified as a one year old fish. However, if translucent zones form in March, a one year old fish may display two increments since it has experienced two seasons of translucent zone formation.

References

BROMHEAD, D., PEPPERELL, J., WISE, B., AND FINDLAY, J. 2005. Striped marlin: biology and fisheries. Final Report to the Australian Fisheries Management Authority and the Fisheries Research Fund. Bureau of Rural Sciences, Canberra, Australia.

CAMPANA, S.E. 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. Journal of Fish Biology 59: 197–242.

CAYRE, P. M., DIOUF, T. 1983. Estimating age and growth of little tunny, Euthynnus alleteratus, off the coast of Senegal using dorsal fin spines. Proceedings of the International Workshop on Age Determination of Oceanic Pelagic Fishes: Tunas, Billfish, and Sharks. NOAA Tech. Rep. 8:105-110.

CHIANG, W. C., C. L. Sun, S. Z. Yeh and W. C. Su. 2004. Age and growth of sailfish (Istiophorus platypterus) in waters off eastern Taiwan. Fishery Bulletin 102 (2): 251-63.

COLLETTE, B. B., J. MCDOWELL, and J. GRAVES. 2006. Phylogeny of recent billfishes (Xiphioidei). Bulletin of Marine Science. 79:455-468.

DAVIE, P. S., HALL, I. 1989. Potential of dorsal and anal spines and otoliths for assessing age structure of the recreational catch of striped marlin in New Zealand, p. 287-294. *In:* Proceedings of the Second International Billfish Symposium. R. H. Stroud (ed.), Kailua-Kona, Hawaii.

DAVIE, P. S. 1990. Pacific Marlins: Anatomy and Physiology. Simon Print, Palmerston North, New Zealand.

DEMARTINI, E.E., J.H. Uchiyama, R.L. Humphreys Jr, J.D. Sampaga, and H.A. Williams. 2007. Age and growth of swordfish (Xiphias gladius) caught by the Hawaii-based pelagic longline fishery. Fishery Bulletin 105(3) 356-367.

DREW, K., D.J. Die, and F. Arocha. 2006. Understanding vascularization in fin spines of white marlin (Tetrapturus albidus). Bulletin of MarineScience 79(3): 847-852.

EHRHARDT, N. M. 1992. Age and Growth of Swordfish, Xiphias-Gladius, in the Northwestern Atlantic. Bulletin of Marine Science 50 (2): 292-301.

FABLET, R. Semi-local extraction of ring structures in images of biological hard tissues: application to the Bayesian interpretation of fish otoliths for age and growth estimation. Canadian journal of fisheries and aquatic sciences/Journal canadien des sciences halieutiques et aquatiques. 63:1414-1428.

GRAVES, J. E. and J. R. McDowell. 2003. Stock structure of the world's istiophorid billfishes: a genetic perspective. Marine and Freshwater Research 54: 287-298.

HEDGEPETH, M. Y. and J. W. Jolley Jr. 1983. Age and growth of sailfish, Istiophorus platypterus, using cross section from the fourth dorsal spine. NOAA Tech Rep NMFS 8 131-35.

HILL, K. T., G. M. Cailliet and R. L. Radtke. 1989. A Comparative-Analysis of Growth Zones in 4 Calcified Structures of Pacific Blue Marlin, Makaira-Nigricans. Fishery Bulletin 87 (4): 829-43.

HINTON, M. G. and M.N. Maunder. 2004. Status of striped marlin in the eastern Pacific Ocean in 2002 and outlook for 2003-2004. Inter-American Tropical Tuna Commission Stock Assessment Report 4: 287-310.

HOLLAND, K.N. 2003. A perspective on billfish biological research and recommendations for the future. Marine & Freshwater Research 54(4): 343-347.

HOOLIHAN, J.P. 2006. Age and growth of Indo-Pacific sailfish, Istiophorus platypterus, from the Arabian Gulf. Fisheries Research 78(2-3): 218-226.

ICCAT. 2003. Report of the 2002 ICCAT white marlin stock assessment meeting. Col. Vol. Sci. Pap. ICCAT 55 (2): 350-452.

IMAGEJ. 2003. WAYNE, R. National Institutes of Health, USA. http://rsb.info.nih.gov/ij/Java1.31_03.

KIMURA, D. K., D. M. ANDERL, and B. J. GOETZ. 2007. Seasonal marginal growth on otoliths of seven Alaska groundfish speices support the existence of annual patterns. Alaska Fisheries Research Bulletin. 12:243-251.

KOPF, R.K., P.S. Davie, and J.C. Holdsworth. 2005. Size trends and population characteristics of striped marlin, Tetrapturus audax caught in the New Zealand recreational fishery. New Zealand Journal of Marine and Freshwater Research 39(5): 1145-1156.

LANGLEY, A., MOLONY, B., BROMHEAD, D., YOKAWA, K., WISE, B. 2006. Stock assessment of striped marlin (Tetrapturus audax) in the southwest Pacific Ocean, WCPFC-SC2-2006/SA WP-6. Western and Central Pacific Fisheries Commission, Manilla, Philippines.

MACQUEEN, J. 1967. Some Methods for classification and Analysis of Multivariate Observations. Proceedings of the Fifth Berkeley Symposium on Mathematical Statistics and Probability 1: 281-297.

MELO-BARRERA, F. N., R. Felix-Uraga and C. Quinonez-Velazquez. 2003. Growth and length-weight relationship of the striped marlin, Tetrapturus audax (Pisces: Istiophoridae), in Cabo San lucas, Baja California Sur, Mexico. Cienc Mar 29 (3): 305-13.

NAKAMURA, I. 1985. "FAO species catalogue Vol.5. Billfishes of the world: An annotated and illustrated catalogue of marlins, sailfishes, spearfishes and swordfishes known to date." FAO Fisheries Synopsis (125) Vo1. 5: 65 p.

PANFILI, J. and H. Troadec, eds. 2001. Manual of Fish Sclerochronology. IFREMER, Plouzané, France.

PEARSON, D.E. 1996. Timing of hyaline-zone formation as related to sex, location, and year of capture in otoliths of the widow rockfish, Sebastes entomelas. Fishery Bulletin 94(1): 190-197.

PRINCE, E. D., D. W. Lee, C. A. Wilson and J. M. Dean. 1984. Progress in estimating age of blue marlin, Makaira nigricans , and white marlin, Tetrapturus albidus , from the western Atlantic Ocean, Caribbean Sea, and Gulf of Mexico. Colle 20 (2): 435-47.

PRINCE, E. D., D. W. Lee, J. R. Zweifel and E. B. Brothers. 1991. Estimating Age and Growth of Young Atlantic Blue Marlin Makaira-Nigricans from Otolith Microstructure. Fishery Bulletin 89 (3): 441-59.

RADTKE, R. L. and B. S. Shepherd. 1991. Current Methodological Refinements for the Acquisition of Life-History Information in Fishes - Paradigms from Pan-Oceanic Billfishes. Comparative Biochemistry and Physiology a-Physiology 100 (2): 323-33.

SPEARE, P. 2003. Age and growth of black marlin, Makaira indica, in east coast Australian waters. Marine and Freshwater Research 54 (4): 307-14.

STEPHENSON, P. C., and N. G. HALL. 2003. Quantitative determination of the timing of otolith ring formation from marginal increments in four marine teleost species from northwestern Australia. Fishery Bulletin. 101:900-909.

SUN, C. L., S. P. Wang and S. Z. Yeh. 2002. Age and growth of the swordfish (Xiphias gladius L.) in the waters around Taiwan determined from anal-fin rays. Fishery Bulletin 100 (4): 822-35.

TSERPES, G. and N. Tsimenides. 1995. Determination of Age and Growth of Swordfish, Xiphias-Gladius L, 1758, in the Eastern Mediterranean Using Anal-Fin Spines. Fishery Bulletin 93 (3): 594-602.

UCHIYAMA, J. H., SKILLMAN, R. A., SAMPAGA, J. D., DEMARTINI, E. E. 1998. A Preliminary Assessment of the Use of Hard Parts to Age Central Pacific Swordfish, Xiphias gladius. NOAA Tech. Rep. 142:261-274.

YATOMI, H. 1989. A potential problem in estimating the ages of striped marlin using dorsal fin spines, p. 317-321. *In:* Proceedings of the Second International Billfish Symposium. R. H. Stroud (ed.), Kailua-Kona, Hawaii.

YOUNG, J. D., A. 2004. Age and growth of broadbill swordfish (Xiphias gladius) from Australian waters. CSIRO Marine Research, Hobart TAS.