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Age estimation of billfishes (*Kajikia* spp.) using fin spine cross-sections: the need for an international code of practice

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Abstract – Fin spine ageing is the most common technique used to estimate age and growth parameters of large pelagic billfishes from the families Istiophoridae and Xiphiidae. The most suitable methods for processing and interpreting these calcified structures for age estimation have not been clearly defined. Methodological differences between unvalidated ageing studies are of particular concern for highly migratory species because multiple researchers in different regions of the world may conduct age estimates on the same species or stock. This review provides a critical overview of the methods used in previous fin spine ageing studies on billfishes and provides recommendations towards the development of a standardized protocol for estimating the age of striped marlin, *Kajikia audax* and white marlin, *Kajikia albida*. Three on-going fin spine ageing studies from Australia, Hawaii, and Florida are used to illustrate some of the considerations and difficulties encountered when developing an ageing protocol for highly migratory fish species. Particular areas of concern that may influence age and growth estimates included differences in fin spine selection, sectioning methods, criteria for identifying and measuring annuli, distinguishing false annuli, validation procedures, identification of the first annulus, and methods used to replace annuli lost due to vascularization of the fin spine core.

Key words: Age determination / Sclerochronology / Growth / Population dynamics / Marlin / Tuna longline fisheries

Résumé – Déterminer l'âge à partir des rayons épineux des nageoires est la technique la plus commune pour déterminer l'âge et les paramètres de croissance des grands poissons pélagiques à rostre de la famille des Istiophoridés et Xiphiidés, marlins, voiliers et espadons. Les méthodes les plus adaptées pour interpréter et traiter ces pièces osseuses pour l'estimation de l'âge n'ont pas été clairement définies. Des différences méthodologiques entre des études non-valides d'âge prêtent à conséquence pour ces grands migrateurs car de nombreux chercheurs de différentes régions du monde peuvent faire des déterminations d'âge d'une même espèce et d'un même stock. Cette synthèse présente une analyse critique des méthodes utilisées antérieurement dans les études sur la détermination de l'âge à partir des rayons des nageoires de marlins et elle fournit des recommandations afin de développer un protocole standard pour déterminer l'âge du marlin rayé, *Kajikia audax* (ex. *Tetrapturus audax*), et du marlin blanc, *Kajikia albida* (ex. *Tetrapturus albidus*). Trois études en cours, en Australie, Hawaï et Floride, sont utilisées pour illustrer certaines des considérations et difficultés rencontrées lors du développement d'un protocole pour déterminer l'âge des poissons grands migrateurs. Les points pouvant influencer l'estimation de l'âge et de la croissance comprennent, en particulier, les différences dans la sélection des rayons d'une nageoire, les méthodes de coupe fine, les critères d'identification et de mesure des annuli, la distinction des faux annuli et dans la validation des procédures, l'identification du premier annulus et des méthodes utilisées pour remplacer des annulus perdus, dus à la vascularisation du centre du rayon de la nageoire.

1 Introduction

Fin spines and soft rays have been used to estimate the age structure and growth rate of a wide variety of freshwater and marine fishes. These calcified structures have been used in place of other hard parts for age estimation as they can generally be extracted and processed quickly and easily (Beamish 1981). Fin spines are also used due to demands for non-lethal or non-destructive ageing methods for at-risk or commercially valuable fish species (Metcalf and Swearer 2005). Fishes commonly aged using fin spines have included but are

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not limited to sturgeon (Acipenseridae), catfish (Ictaluridae), dogfish (Squalidae), tuna (Scombridae), and billfishes (Istiophoridae and Xiphiidae).

Eleven species of marlin, sailfish, and spearfish (Istiophoridae) and one swordfish species, *Xiphias gladius* (Xiphiidae) make up the group known as the "billfishes". Striped, *Kajikia audax* and white marlin, *Kajikia albida* were former members of the genus *Tetrapturus* but have recently been placed in the genus *Kajikia* (see Collette et al. 2006). Striped marlin inhabit the Indo-Pacific Oceans while white marlin are endemic to the Atlantic Ocean. Although they inhabit 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 retained as incidental catch in small-scale artisanal fisheries and in commercial longline fisheries that target large-bodied tuna species. A recent stock assessment of white marlin indicated that the species is overfished (Anon. 2003) while the status of striped marlin in the Pacific Ocean appears to be more stable but is considered uncertain in most areas (Hinton and Maunder 2004; Bromhead et al. 2004; Langley et al. 2006). The reliability of stock assessments for billfishes has been limited by a lack of information pertaining to biological parameters such as size-at-age and annual growth rate.

Billfishes present unique challenges that have prevented direct validation of age and growth estimates in most species. Techniques commonly applied to validate age estimates in other species of fish, such as tag-and-recapture, or captive rearing, have not been feasible for billfishes (Radtke and Sheppard 1991; Holland 2003; Ortiz et al. 2003). The difficulties of ageing billfish are related to logistical complications associated with sampling large-bodied, rare event species, that are widely distributed throughout the open ocean (Holland 2003). Collecting samples is expensive and funding for research has been limited because billfishes are less valuable than commercially important tuna species.

There are also unique technical challenges associated with interpreting growth increment formation in calcified hard parts of billfishes. Fin spines are the most feasible calcified structures to use for age estimation of billfishes due the small size of their otoliths (Radtke 1983), and because other bones such as vertebrae do not appear to grow in proportion to body length (Hill et al. 1989). The center of fin spines is composed of vascularized tissue that can affect the visibility of early formed annuli (Yatomi 1990; Drew et al. 2006). The level of difficulty in interpreting fin spine sections is exacerbated further by a high degree of variability in annulus clarity and by differing patterns of presumed annual and false increments. Due to the challenges of interpreting and validating fin spine age estimates, there remains considerable uncertainty in the key age and growth parameters required for stock assessment of most species of billfish.

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, *Istiophorus platypterus* (Hedgepeth and Jolley 1983; Chiang et al. 2004; Hoolihan 2006), black marlin, *Makaira indica* (Speare 2003), and striped marlin (Skillman and Yong 1976; Melo-Barrera et al. 2003; Kopf et al. 2005). Age estimates have also been made on blue marlin, *Makaira nigricans* (Hill et al. 1989; Wilson et al. 1991) but growth models have been restricted to juveniles (Prince et al. 1991) due to the difficulties of ageing older fish.

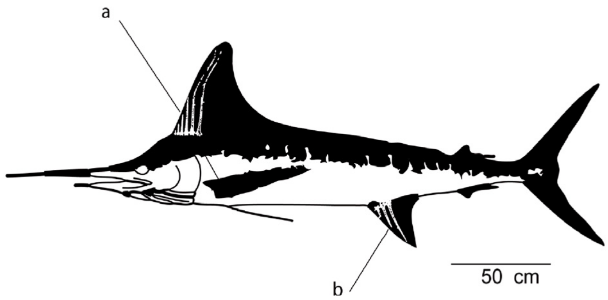

Fig. 1. Diagram of a striped marlin, *Kajikia audax* showing the position of first dorsal fin spines one through six (a) and first anal fin spines one through three (b).

This manuscript reviews methods used to estimate the age of billfishes using fin spine cross-sections. Specific recommendations relevant to striped and white marlin are provided so that future growth models from different regions can be compared. The importance of standardizing ageing methodologies for highly migratory fishes is highlighted and some of the difficulties in undertaking such a venture are discussed. The present review was restricted to billfishes due to the species-specific nature of interpreting fin spine sections for age estimation. However, the methodological framework may be of interest to fin spine ageing studies on other groups of teleost (Santiago and Arrizabalaga 2005; Santamaria et al. 2009) or chondrichthyan fishes (Cailliet et al. 2006; Clarke and Irvine 2006; Tovar-Avila et al. 2008) that are distributed over broad spatial areas or are studied by multiple ageing laboratories. Terminology and other issues relevant to age determination of large pelagic fishes are reviewed in the proceedings of an international ageing workshop convened and edited by Prince and Pulos (1983).

2 Fin spine selection

Fin spines used for age estimation of billfishes have usually been sampled from the leading edge of the first dorsal (D1-6) or first anal fin (A1-3) (Fig. 1). There are approximately 34-46 spines in the first dorsal fin and 12-18 in the first anal fins of white and striped marlin (Nakamura 1985; Davie 1990). Previous fin spine ageing studies on billfishes have used the terms fin spines, fin rays, or spiny fin rays to describe these calcified structures (Davie and Hall 1990; Young and Drake 2004; DeMartini et al. 2007). In order to maintain consistency, the term spine is used throughout the present manuscript to describe all fin-supporting elements. However, in striped marlin, D1-4 and A1-2 appear to be true spines and are solid cortical bone, supported by individual pterygiophores, while the more caudal elements are rays that branch distally (Davie 1990). A similar pattern of stout spines at the leading edge of fins followed by branching rays also appears to occur in swordfish (Uchiyama et al. 1998).

It is important that the same fin spine and section level along the spine be used to estimate individual ages of fish. Inconsistent collection of spines or section levels can prevent the application of validation techniques such as marginal increment analysis (Hoolihan 2006). Using different spines or section levels may also complicate the interpretation of annuli since the morphological appearance of sections may differ. The appearance and location of annuli may vary between or within bones (including spines) of the individual fish (Panfili et al. 2002). Variation in the internal and external morphology in fin spines of billfishes has led researchers to select particular spines that presumably facilitate age estimation. However, researchers have selected a variety of different spines as the most "suitable" for age estimation (Davie and Hall 1990; Melo-barrera et al. 2003).

Suitable spines should show the largest readable area relative to the vascularized area and show the greatest number of clearly observable annuli (Panfili et al. 2002). Swordfish have typically been aged using A2 (Berkley and Houde 1983; Sun et al. 2002; DeMartini et al. 2007) while Istiophorid billfishes have been aged using a variety of dorsal and anal fin spines including D3-D6 to A2-A3 (Hill et al. 1989; Davie and Hall 1990; Speare 2003). Speare (2003) found no differences in age estimates between A3 and D3 in black marlin and Hill et al. (1989) found no differences between A2 and D6 of blue marlin. However, annulus counts in swordfish sometimes varied between D1-3 and A1-3 (Uchiyama et al. 1998) and between different levels along spines (DeMartini et al. 2007). Prince et al. (1986) noted that the amount of vascularization increased from the first to the last spine of the first dorsal fin in a tagged-and-recaptured sailfish. Further comparisons of annulus counts and precision are needed to determine the most suitable fin spine for age estimation or whether various fin spines differ.

3 Fin spine preparation and sectioning

Entire portions of dorsal or anal fins have been sampled in the field so that after cleaning in the laboratory specific spines can be removed (Berkley and Houde 1983; DeMartini et al. 2007). Other studies have sampled only individual spines in the field (Hoolihan 2006) but experience with striped marlin suggests that this method may result in incorrect spines being sampled. As many studies make sections relative to the width of the condyle base (Chiang et al. 2004), or length of the spine (Hill et al. 1989), it may be important to extract the entire spine including the base that lies beneath the skin. However, cutting into the skin may not be permitted if fish are sold commercially (Hoolihan 2006) and it contradicts the less invasive objective of using fin spines rather than other calcified hard-parts.

Spines are usually stored in plastic bags at -20 °C and then heated in warm or boiling water to remove excess tissue (Chiang et al. 2004; Sun et al. 2002). Boiling fin spines to remove excess tissue has been used on sailfish (Chiang et al. 2004) and swordfish (Sun et al. 2002). However, boiling fin spine sections of striped marlin appeared to reduce annulus clarity and therefore only water less than 70 °C was used to clean spines. Excess material surrounding the spines can be removed with scissors, scalpels, and plastic bristle brushes but care should be taken to prevent scraping the margin of the spine. After spines are cleaned of all tissue, they can be air dried in paper envelops at room temperature (Hoolihan 2006) or in an oven at approximately 50 °C (Uchiyama et al. 1998). Berkley and Houde (1983) found that fin spine sections from swordfish lost clarity if they were allowed to dry and thus stored sections in 5% formalin. Mounting fin spine sections on microscope slides is a good long-term storage solution for sections but many studies simply store sections in vials (Davie and Hall 1990). More research regarding the suitability of different methods of preservation and their effects on annulus clarity would be useful.

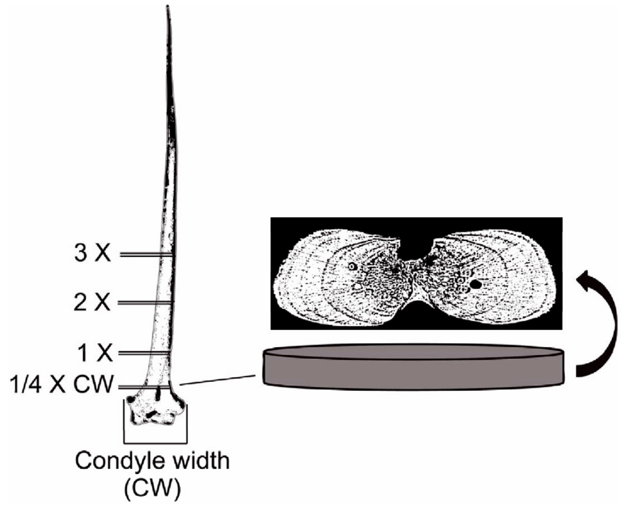

Fig. 2. Diagram of first dorsal fin spine four from a striped marlin, *Kajikia audax* showing the level of transverse sections between 1/4 X and 3 X maximum condyle width (CW). An example of a transverse section from 1/4 CW is displayed on the right side of the illustration.

Sections are usually cut using a low speed (approximately 1600 RPM) lapidary saw fitted with a diamond edged wafering blade. Spines can be sectioned dry (Davie and Hall 1990) or imbedded in clear casting resin (Hoolihan 2006). Embedding spines ensures that sections remain intact and is more costly and time consuming than dry cutting. Section thickness usually ranges from 0.30–1.0 mm (Sun et al. 2002; Speare 2003) but sections between 0.30 and 0.60 mm thickness were optimal for striped marlin. Cutting sections less than 0.50 mm is difficult with dry spines since sections tend to warp. Thinner transverse spine sections allow detail to be observed but may also expose a greater number of false annuli. Grinding and polishing individual sections to appropriate thickness may be required and heating sections as been shown to improve annulus clarity (Hoolihan 2006).

Most studies have found that the lower levels of the tallest or widest spines (Hill et al. 1989; Uchiyama et al. 1998; Speare 2003) are most suitable for age estimation. In order to standardize sectioning methods some studies on billfishes (Ehrhardt 1992; Chiang et al. 2004) have sectioned spines at a level relative to the width of the condyle (Fig. 2). Previous studies have also used relative proportions of fin spine length (Hill et al. 1989; Speare 2003), external reference points (DeMartini et al. 2007), or no standardization procedures at all (Davie and Hall 1990; Hoolihan 2006). The base of dorsal fin spine four, between 1/4 and one condyle width, appears to be the most suitable area for age estimation of striped marlin (Fig. 3). Sections removed from levels above one condyle width tended to show fewer annuli than sections taken from 1/4 or one condyle width. Since the morphology of spines changes with height, removal of sequential sections can bias measurements associated with the width of fin spine sections. It is recommended that at least some form of standardization be applied so that sections are removed from the same relative level.

4 Characteristics of the annulus

Growth marks that form in bones such as vertebrae, cleithra or fin spines are generally referred to as annuli (Panfili et al. 2002). The term annulus does not refer to a yearly formation, rather it is the Latin word for ring. Therefore, annulus counts do not always relate to yearly age. Prior to using annulus counts for age determination, the periodicity of annulus formation must be validated (see Validation). Annuli in fin spine sections of billfishes have been presumed to have an annual periodicity and have previously been used to estimate the age of many species of billfish (Hill et al. 1989; Speare 2003; Melo-Barrera et al. 2003; Hoolihan 2006; DeMartini et al. 2007). However, the interpretation of annuli for age determination is not straightforward and is complicated by the presence of non-age related bands (false increments) and vascularization of the fin spine core (see Vascularization).

A complete annulus consists of wide opaque zone (fast growth) followed by a narrow translucent zone (slow growth) when viewed under reflected light (Berkley and Houde 1983; Fablet 2006). The appearance of growth zones is reversed when sections are viewed under transmitted light. Annuli presumed to form on a yearly basis are continuous around the circumference of fin spine sections (Berkley and Houde 1983) and do not show breaks or partially formed checks (DeMartini et al. 2007). In practice, however, it is difficult to apply general criteria (see Fablet 2006) to derive "age" estimates from annulus counts. Due to the interpretative skills required to derive accurate age estimates from calcified hard-parts, the process of counting annuli is generally referred to as reading. The size, shape, and clarity of annuli may vary between individual fish, populations, species, and between different methods of preparation.

To help interpret inconsistencies in counting annuli, we recommend the use of a color-coding system (Fig. 4). 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 assignment of final age estimates. Green annuli should be clear and undisputable bands (Fig. 4) that meet all functional criteria while yellow annuli also meet functional criteria but are less obvious. Yellow annuli were usually indistinct but were otherwise similar in appearance to green annuli counted in fin spine sections of striped and white marlin (Fig. 4).

5 Identification of false increments

Sub-annual or false increments that do not meet the criteria of an annulus have been reported in fin spine sections of Istiophorid billfishes (Hedgepeth and Jolly 1983; Hill et al. 1989; Speare 2003; Chiang et al. 2004) and swordfish (Berkley and Houde 1983; Sun et al. 2002). A qualitative comparison of images from fin spine sections of billfishes suggests that false increment formation may be a more common feature in Istiophorid billfishes (Hill et al. 1989) compared to swordfish (Berkley and Houde 1983; Sun et al. 2002). The discrimination of false increments from true annuli has the potential to be a large source of error between ageing studies. Observations from fin spine sections of striped marlin indicated that if every possible increment was counted as an annulus then age estimates could be doubled or in some cases tripled. The only directly validated study for Istiophorid billishes (Speare 2003) suggested that false-increments were commonly observed in fin spine sections of black marlin. Speare (2003) noted that false increments did not display translucent zones that extended into the anterior and posterior region of the section.

Criteria used for identifying false increments have essentially been the reverse of characteristics used to define annuli. False annuli have been reported as partial segments that do not extend around the cranial and caudal margins of fin spine sections (Berkley and Houde 1983; Speare 2003). False annuli were sometimes, but not always, thinner and less distinct than annuli color-coded green in fin spine sections of striped marlin (Fig. 4).

The most common type of false increment reported in fin spine sections of billfishes are doublet or triplet bands that form adjacent to a presumed annulus (Fig. 5). Hill et al. (1989) observed doublet or triplet bands in fin spine sections of blue marlin and referred to them as multiple rings. Doublet and triplet bands were identified in white and striped marlin according to the criteria developed by Cayre and Diouf (1983) and assumptions based on the growth pattern of billfishes. The growth rate of fish naturally declines each year, except in years of unusual growth. It is important to note, however, that the distance between annuli will vary depending on the fin spine and section level. On average, the width of an annulus nearer to the focus (R1) should be wider than the next annulus closer to the outer edge (R2). We assumed that growth of billfishes would not increase by more than 25% compared to any previous year. Therefore, a doublet or triplet increment was identified when R1/R2 was less than 0.75. If there was a presumed annulus within a doublet or triplet band, the clearest increment that best fitted the growth pattern of adjacent annuli was used for measurements.

6 Edge type classification

Edge type classification assigns a state of completion to the outermost annulus. Similar to the marginal increment ratio, the percentage of edge type classifications can be plotted monthly or seasonally to estimate the timing and periodicity of annulus formation (Young and Drake 2004). Classification of the edge type of sections also has important implications for placing fish in particular age classes and is dependent on

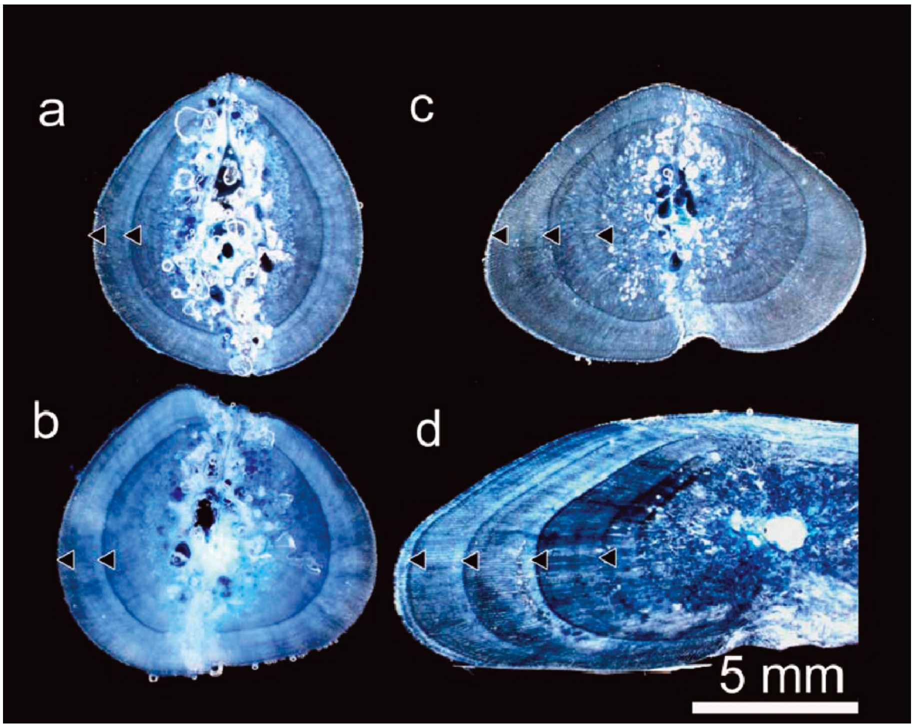

Fig. 3. Dorsal fin spine four from a striped marlin, *Kajikia audax* with black arrows indicating annulus counts from sections cut at different levels. Sections were made at four levels including: 3X (a), 2X (b) , 1X (c), and 1/4 X(d) the maximum condyle width (CW). The base (1/4 X CW) of the spine showed the greatest number of annuli compared to sections taken at higher levels between 2X and 3X CW.

the criteria used to define edges. 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.

The edge or periphery of spine sections are defined as either translucent, narrow opaque, or wide opaque (Fig. 6). The translucent edge type is defined when the cranial and caudal margins of spines display a clearly formed band that is similar in thickness to other presumed annuli and it extends along greater than half of the perimeter of the section (Fig. 6a). Thin translucent bands not clearly observable at the cranial and caudal margin should be recorded as opaque since they cannot be distinguished from false increments. When the margin is opaque and less than half as wide as the previous opaque band, the edge is classified as narrow opaque (Fig. 6b). When the width of an opaque margin is greater than half the width of the previous increment the edge is classified as wide opaque (Fig. 6c).

7 Fin spine section measurements

In billfishes the linear measurements of fin spine sections have typically included spine radius, vascularized radius, and the radius of each annulus (Fig. 7), which is also used to determine the marginal increment ratio. The central portion of the fin spine sections is referred to as the focus (Fig. 7b) and is the starting point for measurements and annulus counts. It is important to use objective criteria to identify the location of the focus since all linear measurements begin at this point. Some studies have identified the focus by tracing growth striations in sections back to a single point of origin (Ehrhardt 1992). However, this method may not be feasible when the angle of the striations changes between annuli.

We recommend that the focus of symmetrical sections be determined by the mid-point of a vertical line (Fig. 7a) and its intersection with the horizontal growth axes (Fig. 7d). The horizontal axis is also used as the counting path for readings and linear measurements. The vertical axis connects the inflection points at the top and bottom of the section and the horizontal axis connects the focus to the outer most edge of a lobe. For asymmetrical fin spine sections or where the maximum width of a lobe extends downward different criteria are required. If this occurs, the vertical axis could still be defined as above but the horizontal axis should be determined for each lobe independently.

With the increasing power of digital imageing programs, area measurements of fin spine sections have become more common and have primarily been used to determine the size of the readable area relative to the vascularized area (Drew et al. 2006). Two dimensional area measurements might also be more appropriate than linear measurements for describing the growth relationships between fin spine sections and body length or weight. Surface area measurements can be calculated on digital images using the Image J software Plug-in, Area Calculator which is available free-online (Rasband 1997) (Fig. 8).

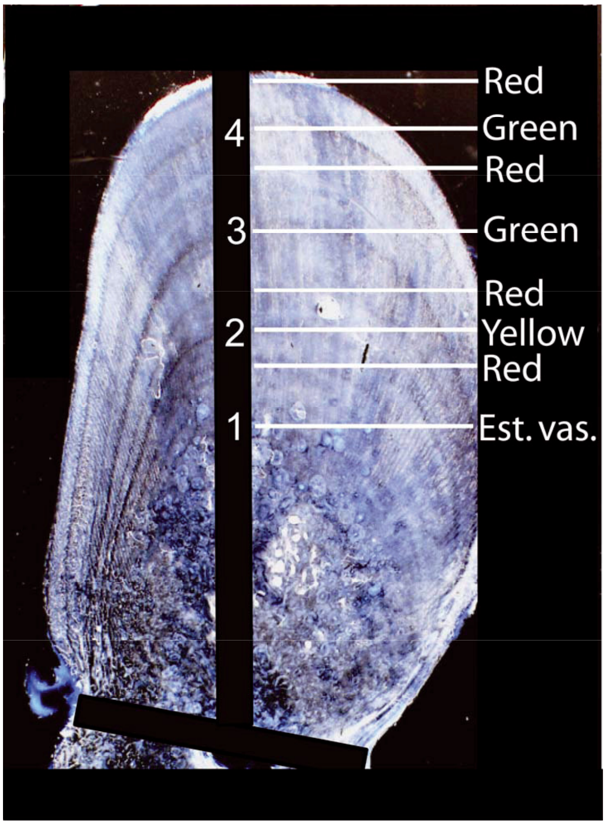

Fig. 4. Diagram of a dorsal fin spine section from a striped marlin, *Kajikia audax* showing annulus classification scheme including green, yellow, and red. The final age estimate for this fish was four, which included two green annuli, one yellow annulus, and one annulus lost due to vascularization (Est. vas.).

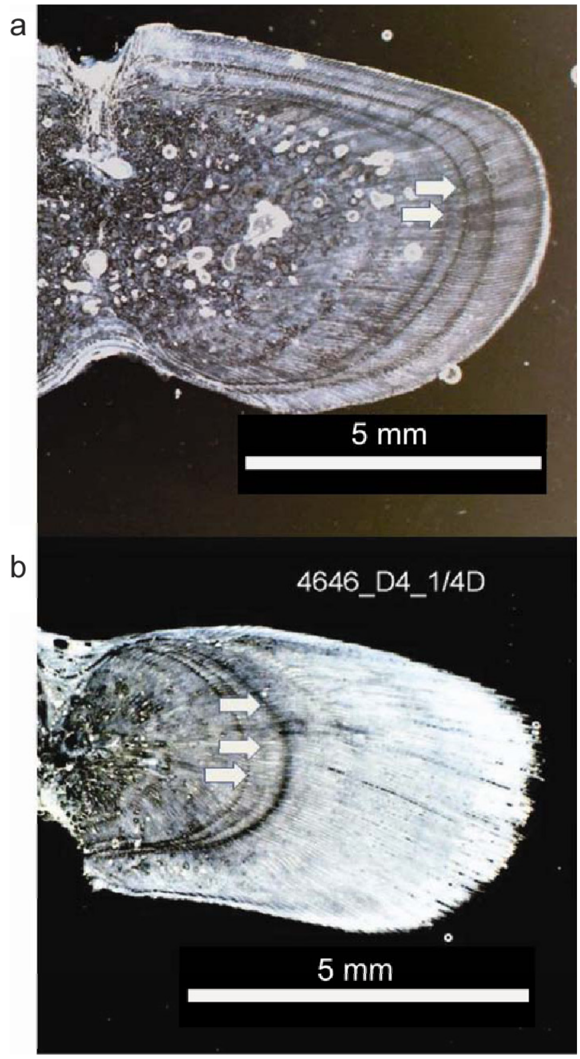

Fig. 5. Diagram of a striped marlin, *Kajikia audax* fin spine section with arrows pointing to a false double annulus (a) and a false triplet annulus in a juvenile (b).

8 Validation

Most age estimates conducted on billfishes have not been validated in any form (Skillman and Yong 1976; Hedgepeth and Jolley 1983; Davie and Hall 1990; Melo-Barrera et al. 2003; Kopf et al. 2005; Hoolihan 2006). In the absence of an absolute form of age validation, the periodicity of annulus formation and location of the first annulus should be identified (Campana 2001). Penha et al. (2004) present a statistical method for determining whether the first annulus is missing and this method appears suitable for billfishes. DeMartini et al. (2007) presented a novel method for identifying the location of the first annulus that compares daily otolith micro-increment counts with fin spine radius at 365 days. This method may be useful to identify the location of the first annulus in other billfishes but requires matching samples of otoliths and fin spines from young of the year fish. Additionally, the time of year when annuli form needs to be confirmed since it is improbable that the first annulus forms exactly 365 days after hatching. Processing the small and fragile otoliths of billfishes for daily age estimation is also challenging and requires specialized techniques (Prince et al. 1991).

The most common and feasible method used to validate fin spine age estimates of billfishes has been marginal increment analysis. Unfortunately, marginal increment analysis is also the most commonly abused method of age validation (see Campana 2001). Yearly periodicity of annulus formation has been validated in swordfish (Sun et al. 2002; DeMartini et al. 2007) and sailfish (Chiang et al. 2004; Alvardo-Castillo and Felix-Uraga 1996) using marginal increment analysis. Marginal increment analysis compares monthly or seasonal changes in the most recently formed translucent or opaque zone. The margin or edge of sections extends from the perimeter of the section to the border of the previous translucent zone. Previous studies on billfishes have found that the timing of annulus formation coincides with the end of the growing season or during the spawning season (Sun et al. 2002; Chiang et al. 2004; DeMartini et al. 2007).

We recommend use of the Marginal Increment Ratio described by the equation $MIR = (S - r_n)/(r_n - r_{n-1})$, where $S =$ spine radius, r_n = radius of the ultimate translucent zone, and r_{n-1} = radius of the penultimate translucent zone. The MIR calculates the percentage of annulus completed at the edge by comparing the width of the margin to the width of the previous annulus. When the mean and standard error of the MIR is

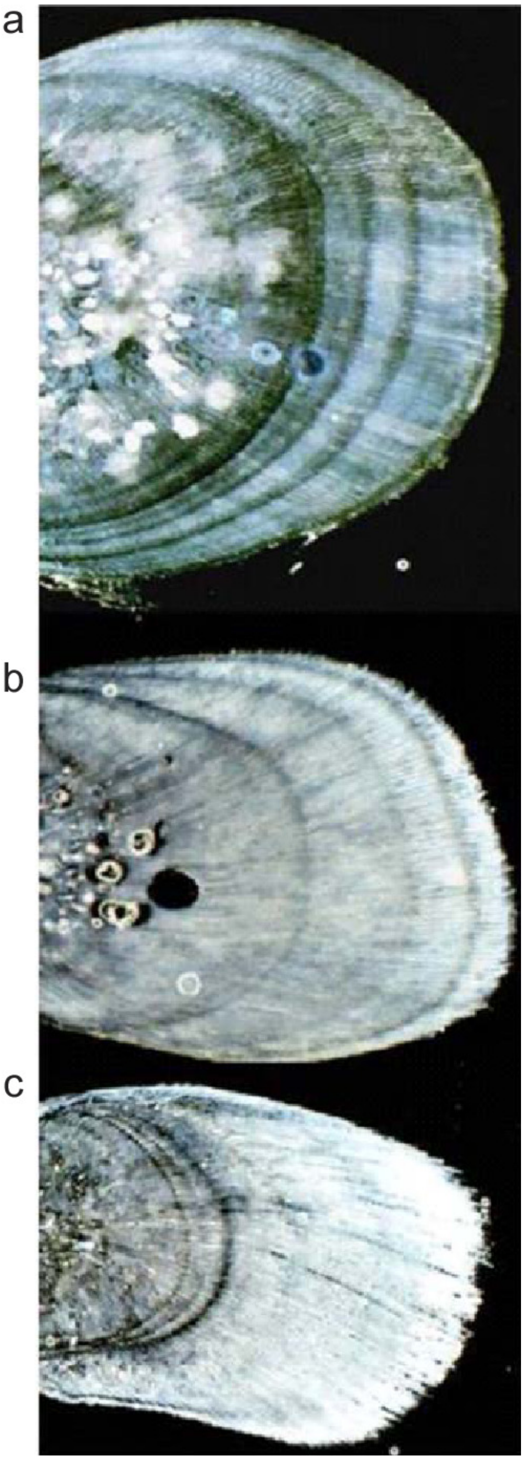

Fig. 6. Fin spine section from a striped marlin, *Kajikia audax* showing different edge types including translucent (a), opaque narrow (b), and opaque wide (c).

plotted each month over 12 months, the trend line should form one sinusoidal cycle if annulus formation is yearly. Conversely, if two annuli are formed per year, two cycles may be observed. If no cycle is observed then annulus formation may not occur each year or may not occur at the same time of year for all individuals. It is recommended that an appropriate statistical measure be used to test for differences in the mean MIR by month,

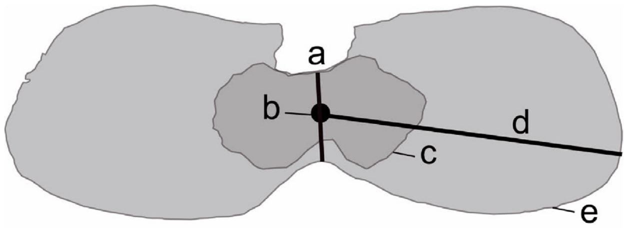

Fig. 7. Measurements and notable features of a fin spine section displaying the vertical axis (a), focus (b.), perimeter of the vascularized area (c), counting path and horizontal axis (d), and the perimeter that delineates the total surface area of the section (e).

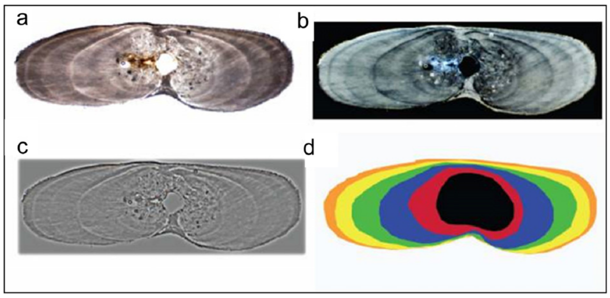

Fig. 8. Examples of digital image modifications used to improve annulus clarity or facilitate measurements. Unmodified image viewed under transmitted light (a), inverted (b), embossed (c), and digitized (d).

season, or year. Due to the difficulties of sampling billfishes during all months of the year and relatively low sample sizes, a non-parametric approach may provide a more robust statistical comparison. We recommend use of the Kruskal-Wallis one-way ANOVA on ranks (see Cailliet et al. 2006).

Absolute age validation of billfishes has not been achieved and there remains considerable uncertainty in longevity estimates and size-at-age, particularly for older age classes (see DeMartini et al. 2007). Prince et al. (1986) examined fin spine sections of a sailfish at large for nearly 11 years and found that sections from all fin spines D1-6 significantly underestimated the actual age. However, Speare (2003) examined fin spine sections from an oxytetracyline injected and recaptured black marlin and found that growth was consistent with a yearly annulus formation. Absolute age validation of billfishes would involve captive maintenance, or release-and-recovery of known-age fish. However, neither of these methods has been achieved because billfishes have not been maintained in captivity for extended periods (see Holland 2003).

Radiometric or bomb radiocarbon ageing (see Andrews et al. 2001; Campana 2001) has not been considered for billfishes due to the small size of their otoliths that would require pooling several hundred samples together. However, it may be worth applying radiometric techniques to fin spines or other bones of billfishes. Bomb radiocarbon dating of fin spines has been used to validate ages of spiny dogfish, *Squalus acanthias* (Campana et al. 2006) but radiometric ageing of fin spines was not useful to validate ages of Atlantic sturgeon, *Acipenser oxyrinchus* (Burton et al. 1999). Large-scale chemical tagging

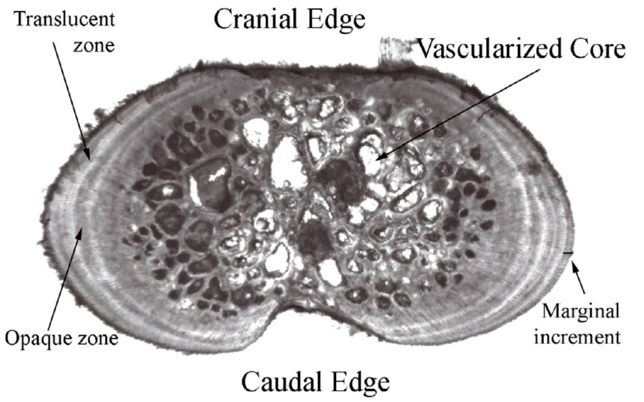

Fig. 9. White marlin, *Kajikia albida* fin spine section with black arrows indicating notable features including the vascularized core, translucent zone, opaque zone, and marginal increment.

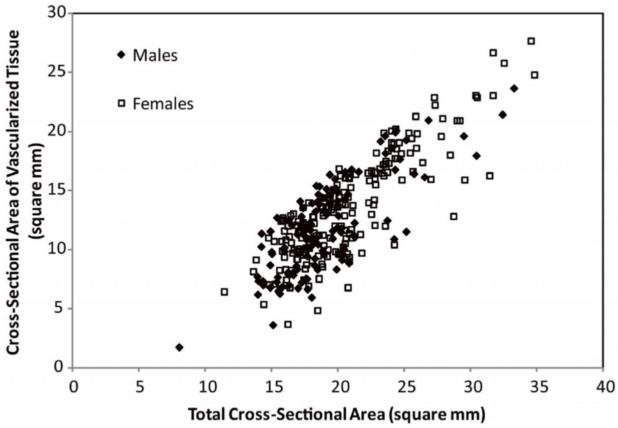

Fig. 10. Area of vascularized tissue compared to the total cross-sectional area of white marlin, *Kajikia albida* fin spine sections.

might be feasible in some recreational or commercial fisheries and this method has resulted in the only direct form of age validation for billfishes (Speare 2003). Unfortunately, tag recapture rates for most billfishes is less than 1% (Ortiz et al. 2003) which makes this a difficult method to use for age validation.

9 Vascularization

Vascularization of the fin spine core, otherwise known as bone remodeling, is a process that has been reported in fin spines of other fishes (Panfili et al. 2001) but appears to be most prominent in fast growing pelagic species. If unaccounted for, vascularization may result in significant age underestimation and overestimation of growth. Vascularization of fin spines was the purported cause of significant age underestimation of a tagged and recaptured sailfish that was at large for nearly 11 years (Prince et al. 1986). Previous research on billfishes suggests that anywhere between one and seven annuli may be reabsorbed due to the process of vascularization (Berkley and Houde 1983; Prince et al. 1986; Speare 2003).

Vascularization is present to some degree in all fin spine sections of white and striped marlin but the proportion vascularized increases directly with body length (Yatomi 1990; Drew et al. 2006) (Figs. 9 and 10). Since the proportion of vascularization increases as fish grow (Fig. 10), older individuals usually have a greater number of annuli obscured than younger fish. Ageing studies on large pelagic fishes have overcome the effects of vascularization by using a statistical replacement method developed by Hill et al. (1989). The radius of each visible annulus is compared to the mean and 95% confidence intervals of annulus radius statistics from smaller, younger fish that have not been affected by vascularization. The number of missing annuli in older fish is then estimated based on the annulus radius statistics from smaller individuals. Hill et al.'s (1989) technique for ageing blue marlin has subsequently been applied to swordfish (Tserpes and Tsimenides 1995), sailfish (Chiang et al. 2004), and striped marlin (Davie and Hall 1990). However, the methods presented by Hill et al. (1989) may not be appropriate for spines that exhibit extensive vascularization or if young individuals are not available.

One technique being explored for fin spine sections of white marlin is cluster analysis. An age estimate is assigned to each annulus based on its radius measurement through a partitioning technique called *k*-means cluster (Macqueen 1967). Annuli are assigned to a cluster representing an age using an algorithm that attempts to minimize the distance from the center of the cluster to each observation. The tightest possible cluster is identified by changing both the cluster center and the observations. The center of the cluster is in effect the average annulus radius for that age. Since this technique is sensitive to starting values, and particularly location of the first annulus, k-means clustering should be seeded with realistic values of annulus radii. Annulus radii statistics can be calculated using the statistical replacement technique 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 annulus of a particular age class forms at approximately the same radius for each individual in the population. Ensuring that sections are cut from the same spine and relative level is an important consideration for replacing annuli lost due to vascularization (see *Fin spine preparation and sectioning*). However, the algorithm in the *k*-means cluster technique will smooth out some of the variation observed between fin spine sections.

10 Assigning final age estimates

Final age estimates of individual fish should consider the most appropriate annulus count, the number of annuli lost due to vascularization, and the edge type classification. The precision of age readings should improve with successive readings so that the final reading of the primary reader is the most precise age estimate. The reproducibility of fin spine 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 readings of individual fish and average the error across the sample population. The CV provides a more conservative estimate of precision than APE but both are widely accepted methods.

Intra-laboratory and intra-reader comparisons often generate less precise age estimates and CV's of 10–15% are common for large pelagic fish (DeMartini et al. 2007). Sections with an average percent error of greater than 15% should be re-examined and discarded if a consensus age estimate cannot be reached. As the fin spines of billfishes display a high degree of variability in annulus clarity, it is important to consider acceptability and rejection thresholds. Early fin spine ageing studies rejected between 13 and 47 percent of all sections collected (Berkley and Houde 1983; Hedgepeth and Jolley 1983). However, more recent studies seem to have less conservative rejection thresholds that result in less than 10% of all sections being considered unreadable (Speare 2003; Hoolihan 2006). 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.

Recent fin spine ageing studies have used digital images to conduct age readings (Sun et al. 2002; Chiang et al. 2004; Hoolihan 2006) while other studies (Davie and Hall 1990; Berkley and Houde 1983) have derived age estimates directly using a microscope. Results from a comparison of swordfish fin spine sections suggest that there may not be a significant difference between age estimates made on digital images versus those made directly using a microscope (DeMartini et al. 2007). Digital images are easy to archive and share with researchers in different regions and allow the optical properties of each section to be fixed. Digital imageing programs such as Adobe Photoshop or Image J (Rasband 1997), which is free on-line, have graphic enhancement and measuring tools that can facilitate examination of sections (Fig. 8).

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 may be marked on digital images using Image J or other imageing software (see *Measurements*). Blind readings, which do not show uniquely identifiable characteristics or a scale bar should be conducted. The number of annuli lost due to vascularization should be estimated using the methods described in the *Vascularization* section. Particular care should be taken to ensure that annuli inside the vascularized perimeter are not counted twice. Accounting for the edge type allows individual fish to be placed into appropriate year classes. In some cases fish showing an opaque wide edge type may be moved up to a higher age class. For example, even though a fish may only show one complete annulus (translucent and opaque zone), the individual may have passed its second birthday. If the timing of annulus formation and the spawning season is known, fish can be assigned to an appropriate age class.

11 Conclusion

Fin spine ageing remains the most feasible method to acquire growth information needed for population assessment of many highly migratory fishes. Highly migratory species pass through multiple international jurisdictions and are therefore studied by multiple international scientists that apply a wide array of methods to obtain age and growth information. Since few studies have been able to validate age and growth estimates of billfishes, it is uncertain whether discrepancies in size-at-age and growth can be attributed to authentic biological differences or to methodological differences between investigators. The present review highlighted some of the methodological inconsistencies that can influence fin spine age estimates of billfishes and emphasized the importance of standardizing ageing methods for highly migratory fishes.

The methods reviewed here certainly do not encompass all of the intricacies that can influence fin spine age estimation of white and striped marlins or other species of billfish. In fact, standardizing all aspects of ageing is not achievable and would potentially be counterproductive. We recommend the development of a general framework or code of practice for estimating the ages of closely allied species, so that regional comparisons can be made. An international workshop on age determination of oceanic pelagic fishes (see Prince and Pulos 1983) may provide a platform to discuss new ideas and facilitate the development of codes of practice for estimating the ages of highly migratory fishes.

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