6. Age and growth of elasmobranch fishes

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6.1 INTRODUCTION

The ability to perform age determinations based on the examination of hard anatomical parts is of fundamental importance in fisheries research. Precise and accurate age information enables quality estimates of growth and other vital rates such as natural mortality and longevity, and is essential for successful fisheries management. The effect of inaccurate age determinations on population dynamics studies can lead to serious errors in stock assessment resulting in overexploitation (Hoenig and Gruber, 1990; Hoff and Musick, 1990; Officer *et al.*, 1996; Musick, 1999; Campana, 2001). Fish age and growth are also critical correlates with which to evaluate many other biological (and pathological) processes, such as productivity, yield per recruit, prey availability, habitat suitability and even feeding kinematics (DeVries and Frie, 1996; Campana, 2001; Robinson and Motta, 2002). While age and growth are always used together in phraseology, it is important to remember that each term has its own distinct meaning, which was eloquently stated by DeVries and Frie (1996):

> *"Age refers to some quantitative description of the length of time that an organism has lived, whereas growth is the change in body or body part size between two points in time, and growth rate is a measure of change in some metric of fish size as a function of time."*

Concentric growth bands have been documented in the vertebral centra of most elasmobranchs for over 80 years (Ridewood, 1921). Counts of opaque and translucent banding patterns in vertebrae, dorsal spines, caudal thorns and neural arches have provided the only information on growth rates in these fishes as they lack the hard parts, such as otoliths, scales and bones typically used in age and growth studies of teleost fishes (Cailliet, Radtke and Weldon, 1986; Cailliet, 1990; Gallagher and Nolan, 1999; McFarlane, King and Saunders, 2002). Unfortunately, the vertebral centra of many elasmobranch species (such as numerous deep water species) are too poorly calcified to provide information on age, most species have no dorsal spines and there may be no tangible relationship between observed banding patterns and growth (Caillet *et al.*, 1986; Cailliet, 1990; Natanson and Cailliet, 1990; McFarlane, King and Saunders, 2002). These circumstances continue to cause difficulties in estimating age for many species.

Centrum banding patterns may be related to physiological changes induced by changes in environmental parameters such as temperature and photoperiod (Cailliet, Radtke and Weldon 1986, Branstetter 1987). However, some species such as the little skate (*Leucoraja erinacea*) (Natanson, 1993) and the Pacific angel shark (*Squatina californica*) do not reflect such relationships (Natanson and Cailliet, 1990; Cailliet *et al.*, 1992). Vertebral growth is inevitably linked to food intake and a lack of food for short periods of time can cause subtle bands to appear in vertebral centra of some species

(J. Gelsleichter, Mote Marine Laboratory, USA, pers comm.) Considerable variability exists in the amount and pattern of calicification within and among taxonomic groups of elasmobranch fishes and much of the variation observed in several species has not yet been explained (Branstetter, 1990; Branstetter and Musick, 1994; Wintner and Cliff, 1999). These factors make it inherently risky to assume that the vertebral banding pattern of one species is representative of another species or under all conditions, necessitating a species-specific approach.

The age determination process consists of the following steps: collection of hard part samples, preparation of the hard part for age determination, examination (age reading), assessment of the validity and reliability of the resulting data and interpretation (modeling growth). The purpose of this chapter is to provide a concise overview of basic methodologies and statistical analyses that can be used to quantify age and estimate rates of growth from vertebral centra and dorsal fin spines in elasmobranch fishes. I provide a few web-based references at the end of the chapter and cite additional literature sources throughout that can be obtained to conduct specific staining techniques and age validation methods that are more expensive, complex and technology based. Additional methods of assessing the age-length relationship can also be conducted, but as the purpose of this chapter relates solely to age and growth via hard part analysis, alternative methods such as size mode or length frequency analysis and monitoring captive growth are not covered herein. (See Gulland and Holt, 1959; Francis, 1988; Cailliet *et al.*, 1992; Natanson, Mello and Campana, 2002 and Section 4 this volume for size mode and length frequency analysis; see Van Dykhuizen and Mollet (1992), Mollet, Ezcurra and O'Sullivan (2002) and Mohan, Clark and Schmid (2004) for monitoring captive growth).

6.2 VERTEBRAL OR FIN SPINE COLLECTION AND PREPARATION

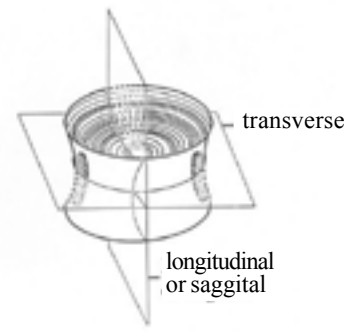
6.2.1 Background

Whole vertebral centra, as well as transverse and sagittally (i.e. longitudinally) sectioned centra have been used for ageing elasmobranchs (Figure 6.1). Transverse sectioning will prevent bands on opposing halves from obscuring each other when illuminated from below. However, determining the age of older animals can still be problematic as bands become more tightly grouped at the outer edge of vertebrae and may be inadvertently grouped and counted together thereby causing underestimates of age (Cailliet *et al.*, 1983a; Branstetter, 1987). As such, sagittally sectioned vertebrae should be used for ageing unless it can be unequivocally demonstrated that identical ages can repeatedly be obtained from a given species using whole centra.

Dorsal fin spines have been another useful hard part for ageing some elasmobranchs, most notably dogfish sharks of the family Squalidae (Ketchen, 1975; Nammack, Musick and Colvocoresses, 1985; McFarlane and Beamish, 1987a). Spines from the second dorsal fin are preferred for ageing as the tips of first dorsal fin spines tend to be more worn down, which leads to an underestimation of age.

Novel approaches to ageing various elasmobranchs continue to arise and researchers may want to begin collecting additional hard parts from specimens in the field to be experimented with in the laboratory. For example, Gallagher and Nolan (1999) used caudal thorns along with vertebral centra to determine age in four bathyrajid species, demonstrating high precision in ages between the two parts. Gallagher, Nolan and Jeal (2005) further elaborated on the

FIGURE 6.1
Diagram of the two sectioning planes that can be used on vertebral centra (courtesy of G.M.Cailliet, Moss Landing Marine Laboratory).



structure and seasonal growth processes in the caudal thorns of the broadnose skate. (*Bathyraja brachyurops)*. Comparing counts in more than one hard part is a common age verification technique used in teleost ageing studies. However, it is not often conducted on elasmobranchs due to the lack of multiple hard parts for comparison. The use of thorns as a reliable hard part for ageing, where appropriate, has the potential to greatly aid in our understanding of the life histories of several species of skate and ray. Further, McFarlane, King and Saunders (2002) have provided preliminary evidence that neural arches stained with silver nitrate may be useful in assessing the ages of sharks with poorly calcified vertebral centra (see Section 6.2.3).

6.2.2 Field sampling and storage

Upon capture, precaudal, fork and total length (PCL, FL and TL, respectively) of sharks should be measured on a straight line, while disc width at its widest point and total length should be measured for skates and stingrays (Section 3.1). While disc width is likely to be the more statistically useful length measurement for skates and rays, total length can be taken for comparison (and used in growth models if it provides better statistical results). Sex should be recorded and clasper length of males should be measured (Section 7). Weights should be obtained from all specimens prior to the removal of any tissues, organs or hard parts.

The location in the vertebral column from which samples are taken for ageing can have a statistically significant effect on increment counts (Officer *et al.*, 1996). This emphasizes the importance of standardizing the vertebral sampling region for all ageing studies, allowing for precise, valid comparisons among individuals within a population and for more accurate comparisons between populations. A section numbering between 10 and 15 of the largest (usually thoracic) vertebrae should be removed from the fish. The largest vertebrae may be located in slightly different areas depending upon the species, but they are typically located directly in front of, or under, the first dorsal fin in sharks and at the thickest body point in skates and rays. The vertebral section should be bagged, labeled and frozen until ready for preparation (Section 6.2.1). If freezing is not an option, vertebrae can be fixed in 10% formalin for 24 hrs and preserved in alcohol.

Second dorsal fin spines should be removed by cutting horizontally just above the notochord to ensure that the spine base and stem are intact. Spines can be bagged, labeled and frozen until returned to the lab or placed immediately in 70-95% ethyl alcohol or 95% isopropyl alcohol.

6.2.3 Cleaning, cutting and mounting

Vertebral samples need to be thawed if frozen, or washed if preserved in alcohol, and cleaned of excess tissue and separated into individual centra. While the removal of all muscle tissue is required, I recommend that the neural arches (Figure 6.2) be removed from only ½ of the vertebral sample, and that the vertebrae with neural arches attached, along with a subsample of the fully cleaned (whole) centra, be kept frozen. Neural arches may be useful for ageing if centra are not (see Section 6.3.3) and additional centra will be needed if staining is necessary. Haemal arches (sometimes referred to as transverse processes) should be removed. If manual cleaning is not sufficient to remove all of the surrounding tissue, or if working with dried vertebrae, several options are available to assist in complete cleaning of vertebral sections. However, soaking them in a 5% sodium hypochlorite solution is a simple and effective method. Soak times can range from 5 minutes to 1 hour depending on the size of the vertebrae and should be followed by soaking centra in distilled water for 30 to 45 minutes (Johnson, 1979; Schwartz, 1983). This method also assists in removal of the vertebral fascia between centra and does not affect the staining process, should any be conducted. Centra are typically permanently stored in 70-95% ethyl alcohol or 95% isopropyl alcohol; however, a sub-sample of

centra should be permanently stored in a freezer as long-term exposure to alcohol may reduce the resolution of the banding pattern (Allen and Wintner, 2002; Wintner *et al.*, 2002). Centra that are to be analysed should remain in one of the above alcohol solutions for at least 24 hrs prior to any further preparation (i.e. being sectioned). Vertebrae should not be permanently stored in formalin as it may damage centra making them unreadable, nor should they be stored dry (in air) as this may result in cracking. Ages can be obtained in most cases from cracked vertebrae, however, accurate centrum measurements may be difficult to obtain from them.

Vertebral sectioning is typically done with a low-speed diamond-bladed saw (e.g. Isomet rotary diamond saw), but can also be done with small handsaws and even scalpels when working with very small centra. Each centrum should be sagittally sectioned immediately adjacent to the center of its focus (Figure 6.2) (so that the center of the focus is at the edge of the cut) and then cut again approximately 1.5 mm off-center. Accuracy and precision in these cuts (i.e. always including the center point of the focus) will reduce centrum measurement error among individuals. A double-bladed saw can be used to eliminate the problem of cutting a small section off of one-half of a vertebral centrum (Figure 6.3). Spacing between blades should be no less than 0.6 mm to allow for some sanding and, or polishing. Large vertebrae can be hand-held for cutting, whereas imbedding small vertebrae in resin (thermoplastic cement) and then cutting may prove easier. If not using a rotary saw, small vertebrae can be sanded in half, mounted, sanded thin and polished. A grinder may be used to section large vertebrae, which can then be mounted, sanded thin and polished.

FIGURE 6.2
Photograph of an individual vertebral centrum showing neural and haemal arches, spinal cord and focus (courtesy of S.E. Campana, Bedford Institute of Oceanography, Canada).

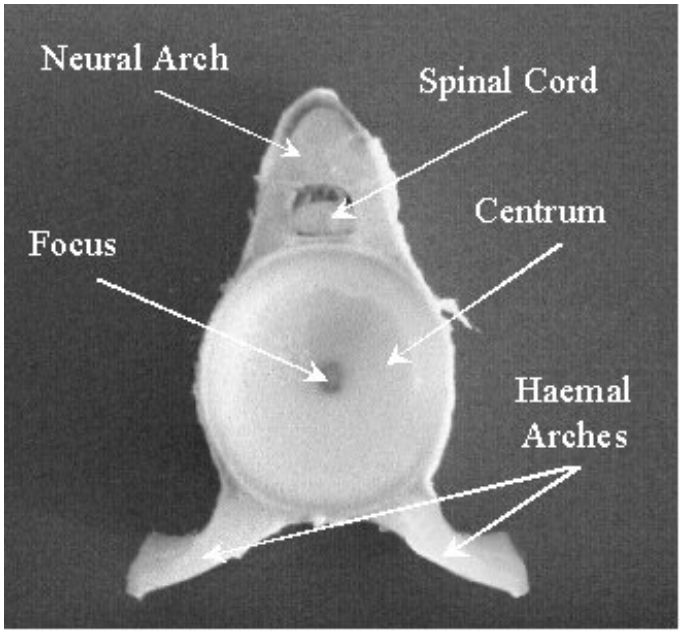
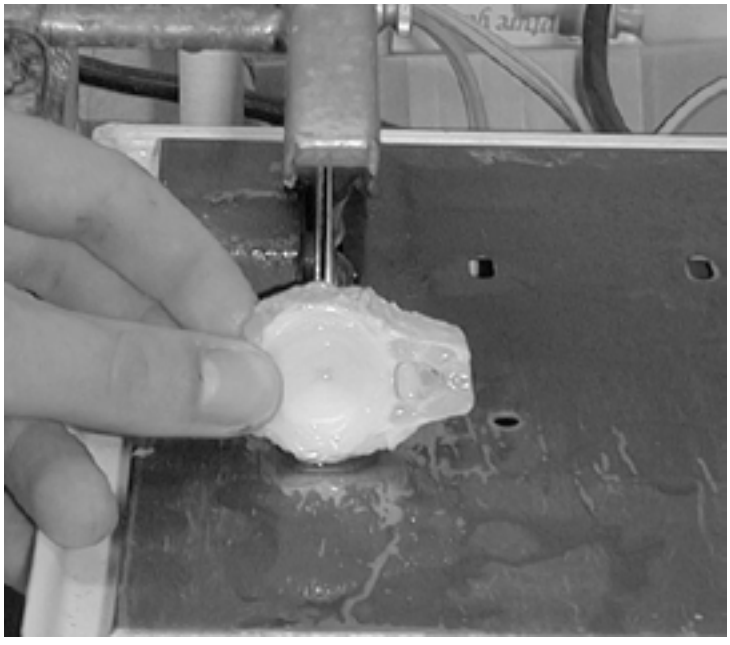


FIGURE 6.3
Photograph showing a vertebral centrum being sectioned (side-to-side) with a double bladed saw (courtesy of S.E. Campana, Bedford Institute of Oceanography, Canada).



If working with lamniform or other vertebrae with small numbers of radials, pressing the sagittally cut (bowtie-shaped) sections between two pieces of plexiglas and placing a weight on the top sheet during drying will prevent warping, which can effect increment and centrum radius measurements. Sectioned vertebrae should be air-dried for 24 hrs (under a ventilation hood if possible) and then mounted onto microscope slides. The focus side of the vertebral section must be consistently placed face down on the slide when mounting to avoid adding to centrum measurement error, which will lead to subsequent analysis error. Any typical slide-mounting medium (e.g. Permount™) will suffice for attaching vertebral sections. After the mounting medium is completely dry (24-36 hrs), sections should be sanded with wet fine grit sand paper in a series (grades 320, 400 and finally 600 for polishing) to approximately 0.3-0.5 mm and air-dried. A binocular dissecting microscope with transmitted light is generally used for identification of growth rings and image analysis (see Section 6.3).

It is important to the age-determination process that at least the majority of vertebral sections include the calcified radials of the intermedialia, but this is not always easy (Figure 6.4). For example, the radials of the intermedialia of carcharhinid sharks are relatively hard, robust and numerous, making their centra nearly solid. In contrast, the radials of the intermedialia in lamnoid sharks are less numerous, softer and quite fragile. Large interstitial spaces between radials can prevent intermedialia from being present in a sectioned centrum. Conducting several preliminary "test cuts" should reveal the best location to make a sagittal cut that will include intermedialia. Once the best location is found, all cuts need to be consistent (i.e. made in the same location on each centrum) in order to minimize error in centrum measurements, which are critically important for centrum edge analyses and back-calculations. In my experience, the best 'cut' to obtain the radials of the intermedialia has most frequently been obtained from a side-to-side cut from the vertebral centrum versus a top-to-bottom one (Figure 6.3).

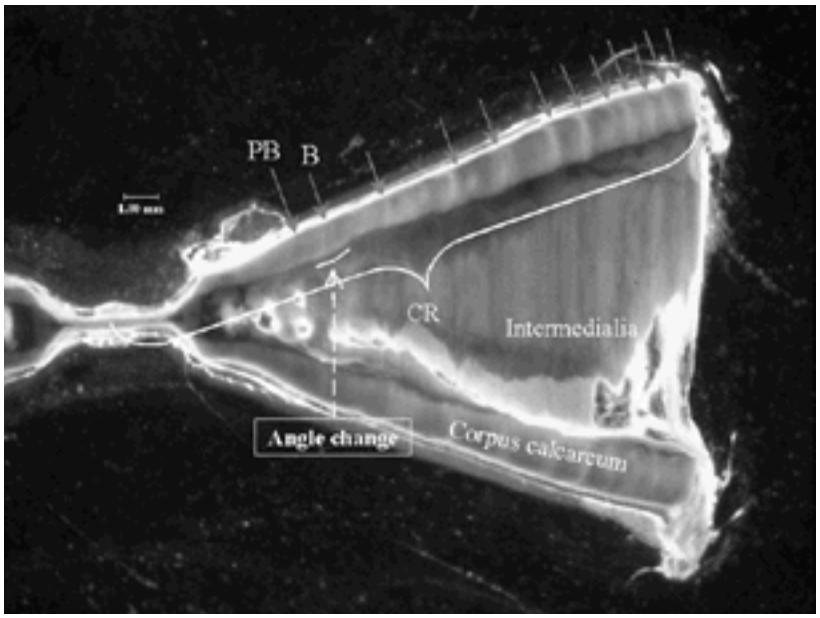
Second dorsal fin spines can be permanently stored dry, in 70-95% ethyl alcohol or in 95% isopropyl alcohol, but should be air-dried for at least 24 hrs before reading. Spines can be read whole (without further preparation), by wet-sanding the enamel and pigment off the surface and polishing the spine or from the exposed surface resulting from a longitudinal cut (Ketchen, 1975; McFarlane and Beamish, 1987a). Spines should also be cross-sectioned as this has provided age assessments for some squaloids and chimaeras (Sullivan, 1977; Freer and Griffiths, 1993; Clark, Connolly and Bracken, 2002a and b; Calis *et al.*, in press).

6.3 AGE DETERMINATION

6.3.1 Annulus formation and identification

The most commonly distinguishable banding pattern in sectioned centra when viewed microscopically is one of wide bands separated by distinct narrow bands (Figure 6.4). The terms opaque and translucent are commonly used to describe these bands and they tend to occur in summer and winter, respectively. However, the opacity and translucency of these bands varies considerably with species, light source and methodology (Cailliet, Radtke and Weldon, 1986; Cailliet, 1990; Wintner *et al.*, 2002). It should not be assumed that the opaque and translucent nature of vertebral bands in different species will be similar; however, the pattern of wide and narrow banding tends to be consistent (Figure 6.4). In temperate waters, the wide bands represent faster fish growth during the summer months when water temperatures are warmest and the narrow bands represent slower growth during the colder winter months. An annulus is usually defined as the winter band. The difference in appearance between summer (wide) and winter (narrow) growth bands provides the basis for age determinations. In many species, this so-called winter band actually forms in the spring (Sminkey and Musick 1995). While tropical teleosts have sometimes proven difficult to age (due to

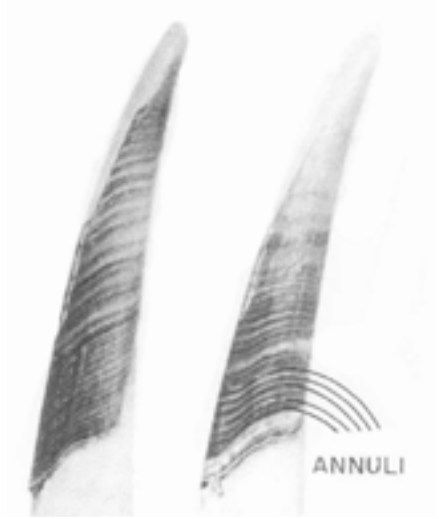
FIGURE 6.4
Sagittal section of a vertebral centrum from a 10 year old salmon shark (*Lamna ditropis*), showing the typical banding pattern in this species. CR=centrum radius. PB = pre-birth ring, B = birth ring, and arrows indicate rings or age (photograph, K.J. Goldman).



the lack of seasonality and relatively consistent photoperiod), this does not appear to be the case with tropical elasmobranchs, such as the lemon shark (*Negaprion brevirostris*) (Brown and Gruber 1988).

In elasmobranch vertebral sections, each pair of wide/narrow bands that extends across one arm of the corpus calcareum, across the intermedialia and across the opposing corpus calcareum arm is considered to represent an annual growth cycle; the narrow bands, hereafter referred to as "rings" or "annuli," are what are counted (Figure 6.4). It must be noted that counting these rings, at this point in the process carries with it the assumption that each one represents a year's growth; however, the validity of this assumption must be tested (Section 6.4). (The term annulus is defined as a ring-like figure, part, structure or marking, but annuli must be shown to be annual in their deposition). The age determination process (i.e. enumeration of rings, measurements and back-calculations) for spines is virtually identical to that for vertebrae (Figure 6.5); however, Ketchen's (1975), (see also Nammack, Musick and Colvocoresses, 1985) method for calculating age from worn spines should be used instead of discarding them. This method uses an age to spine-base-diameter regression for unworn spines to allow an estimation of age for individuals with worn spines. The best-fit regression line is used to obtain the number of years that are to be added to the age of an individual based on the diameter of a

FIGURE 6.5
Photograph of spiny dogfish (*Squalus acanthias*), second dorsal fin spines showing annuli. First spine was aged at 42 years; second spine aged at 46 years (courtesy of G.A. McFarlane, Pacific Biological Station, Canada).



spine at its "no wear point" (see Ketchen, 1975 for details on worn spine criterion and specific examples).

While transmitted light is the most commonly used method of illuminating sectioned centra, I strongly recommend comparing transmitted light with reflected light, translucent and other filtered light, as well as ultraviolet (UV) illumination even if staining or tetracycline injection has not been conducted (see Sections 6.2.3 and 6.3.3). Altering the intensity of each type of light and making finite adjustments to the optical focus of the microscope can often provide visual enhancement of the banding pattern.

6.3.2 Ageing protocols

Age and growth studies require interpretation of banding patterns in the hard parts of fishes. As such, they incorporate several sources of variability and error. While the individuals used in an ageing study provide a source of natural variability, variability between sexes and among geographic locations may also exist (Parsons, 1993; Carlson and Parsons, 1997; Yamaguchi, Taniuchi and Shimizu, 1998). Other potential sources of variability and error include the method used to count growth increments, effects of within and between reader variability and bias, effects of staining, variation in increment counts from different hard parts and variation in increment counts from within the same region of the vertebral column and from different regions of the vertebral column (Officer *et al.*, 1996; Campana, 2001). Developing an ageing protocol brings consistency to the ageing process leading to better precision thus minimizing error. The most important aspect of any ageing protocol is that it produces repeatable ages within and between readers (i.e. precision). Ageing protocols have two key components: (a) determination of which marks on vertebral centra or spines will be counted and (b) checking for reader agreement and precision and testing for bias within and between readers after age determinations are completed (Section 6.3.4). Every ageing protocol, whenever possible, should have two readers independently age all centra two times in blind, randomized trials without knowledge of each specimen's length or disc width (Section 6.3.4).

One of the more common problems in age determination occurs due to deviations in typical growth patterns observed in vertebral centra, which can lead to inaccurate counts. These deviations can result from false checks or split bands occurring within the corpus calcareum, the intermedialia or both, and the vertebral intermedialia of many species possess a great deal of "background noise". As such, it is important that these accessory bands be recognized as anomalies when assigning an age to a specimen. Checks tend to be discontinuous, weak or diffuse, and inconsistent with the general growth pattern of true annuli. Developing some familiarity with the typical "look" of the banding pattern in a given species' centra to aid in distinguishing checks from annuli is recommended. If the ageing study is an ongoing one, regular review of reference collections and comparing summaries of age-length data from one season to the next also helps maintain accuracy, precision and reduce bias in age determinations (Officer *et al.*, 1996; Campana, 2001). In addition, because the intermedialia of the centrum in many species is not robust, it may warp in a concave manner during the drying process. When this occurs, the rings near the outer edge of the intermedialia become "bunched up" and indistinguishable. The rings on the corpus calcareum also become more tightly grouped at the outer edge, particularly in larger and older animals; however, they have a tendency to remain distinguishable due to the stronger (more robust) nature of the structure (see Figure 6.4). For these reasons, the corpus calcareum should always be used as the primary counting and measuring surface, with the distinct rings in the intermedialia and any additional features (see below) used for "confirmation" of a ring or annulus.

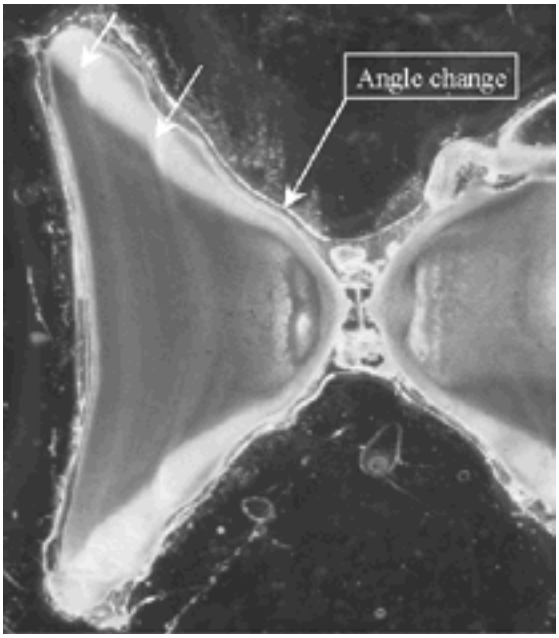
Additional difficulties in ageing elasmobranch fishes can include determining the birthmark and first growth ring. Birthmarks are usually represented by an angle change

along the centrum face of whole vertebrae or along intermedialia-corpus calcareum interface with an associated ring on the corpus calcareum in sectioned centra (Figures 6.4 and 6.6), but this feature may not be distinct in either. While the birthmark usually can be found on the whole centrum surface (i.e. the outside wall of the corpus calcareum), the variability in this mark is such that it may be distinct only within the sagittally cut section (Figure 6.4). Further, "pre-birth rings" have been reported in some species (Branstetter and Musick, 1994; Nagasawa, 1998; Goldman, 2002) (Figure 6.4). Once the angle change is located, pre-birth rings can easily be distinguished from the first growth ring. The first growth ring may consist of minimal growth around the focus of a vertebra and can appear faint relative to other annuli (Campana, 2001), and can also differ in its opacity or translucency (Wintner and Dudley, 2000; Allen and Wintner 2002). Being able to consistently locate a birthmark and (particularly) the first annulus are obviously of critical importance to accurate age assessment. Knowledge of the pupping (or hatching) time of a given species can help in determining if the first annulus is expected to be very small (first winter is soon after birth) or large (first winter is a considerable time after birth).

The vertebral centra of some species may also possess features that can assist in ageing specimens. For example, sagittally cut vertebral sections of some species reveal distinct notches along either the inside or outside edge of the corpus calcareum at each ring providing an additional ageing feature (Figure 6.6). This can be particularly useful in ageing vertebral sections where the cut has excluded the radials of the intermedialia and in distinguishing growth checks from annuli.

If examination of vertebral centra reveal no discernable banding patterns or reveal rings that are difficult to interpret, centra (either whole or sectioned) can be stained to enhance growth-bands for enumeration.

FIGURE 6.6
Sagittal section of a vertebral centrum from a two year old smooth dogfish (*Mustelus canis*), showing the distinct notching pattern (white arrows) that accompanied the distinct banding pattern (courtesy of C. Conrath, Virginia Institute of Marine Science, USA).



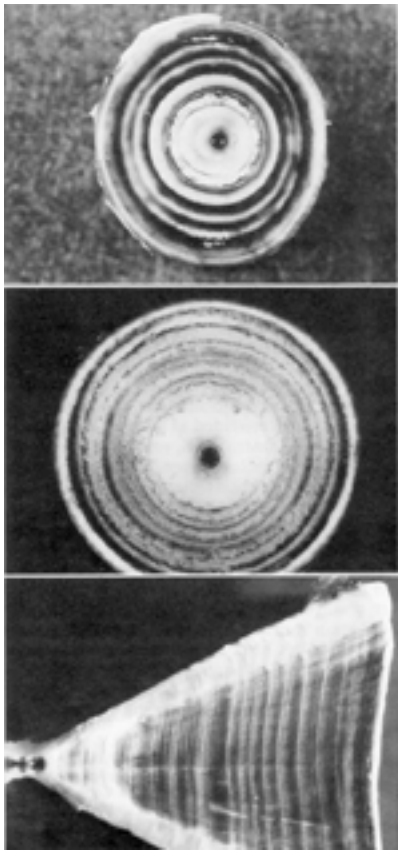
6.3.3 Staining methods

Numerous techniques have been used in attempts to enhance the visibility of growth bands in elasmobranch vertebral centra. The list includes alcohol immersion (Richards, Merriman and Calhoun, 1963), xylene impregnation (Daiber, 1960), histology (Ishiyama, 1951; Casey, Pratt and Stillwell, 1985; Natanson and Cailliet, 1990), X-radiography (Aasen, 1963; Cailliet *et al.*, 1983a, b; Natanson and Cailliet, 1990), X-ray spectrometry (Jones and Green, 1977), cedarwood oil (Cailliet *et al.*, 1983a; Neer and Cailliet, 2001), alizarin red (LaMarca, 1966; Gruber and Stout, 1983; Cailliet *et al.*, 1983a), silver nitrate (Stevens, 1975; Schwartz, 1983; Cailliet *et al.*, 1983a, b), crystal violet (Johnshon, 1979; Schwartz, 1983; Carlson, Cortés and Bethea, 2003), graphite microtopography (Parsons, 1983; Parsons, 1985; Neer and Cailliet, 2001), a combination of cobalt nitrate and ammonium sulfide (Hoenig and Brown, 1988) and the use of copper, lead and iron based salts (Gelsleichter, Piercy and Musick, 1998). Many of these studies used multiple techniques on a number of species for comparison, particularly Schwartz (1983) and

Cailliet *et al.* (1983a). These studies show that the success of each technique is often species specific and that slight modifications in technique may enhance the results.

In addition to their effectiveness, the various techniques mentioned vary in their simplicity, cost and technological requirements. Histological processes have proven useful, but require specialized equipment, a number of chemicals and are relatively time consuming. However, the resulting staining process resulted in no color change in vertebral sections after 15 years (Casey, Pratt and Stillwell, 1985). X-radiography has proven useful in many studies, but has the obvious necessity of an appropriate X-ray machine and film processing capabilities, and while X-ray spectrometry may hold promise (Jones and Geen, 1977; Casselman, 1983), it is time consuming and expensive. Simpler, less expensive and time-efficient staining techniques, such as crystal violet, silver nitrate, cedarwood oil, graphite microtopography and alizarin red should be used first prior to considering other, more elaborate methods. While these techniques have been tried, many have not yet been thoroughly evaluated. For example, the cobalt nitrate and ammonium sulfide stain suggested by Hoenig and Brown (1988) is easy to use, time efficient and provided quality results for two species (Figure 6.7), but has not been extensively applied. A microradiographic method using injected fluorochrome dyes to aid in resolving individual hypermineralized increments was applied to captive gummy sharks (*Mustelus antarcticus*), with success (Officer *et al.*, 1997), but this method has also not been extensively applied. This method may also have application as a validation technique, but this needs to be investigated.

FIGURE 6.7
Vertebrae stained using the cobalt nitrate and ammonium sulfide method of Hoenig and Brown (1988). The top image is a smooth dogfish, centrum, the middle and bottom images are of lemon shark (*Negaprion brevirostris*), centra (courtesy of J.M. Hoenig, Virginia Institute of Marine Science).



Two of the simplest staining techniques are crystal violet and silver nitrate, which are described below. The appropriate literature (cited here) should be acquired for detailed directions on other staining or enhancement techniques as well as modifications of the techniques. The wide-ranging subtle differences between studies using the same staining technique and the use of whole versus sectioned vertebrae make presenting a single formula difficult. As such, a general timeline range for the methods is presented and may require some adjustment for the best results. Mini-modifications are made by many researchers in attempts to accentuate the vertebral rings in the centra of their study species.

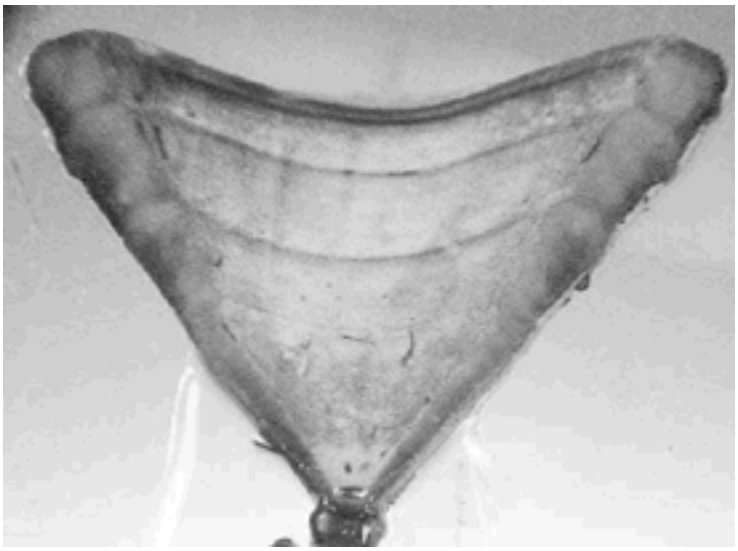
6.3.4 Standard protocols

Crystal violet protocol

Perhaps the simplest staining technique involves the use of crystal violet (Figure 6.8). An advantage of this technique is that it can be performed on fresh vertebrae as well as those stored in alcohol. After each vertebra has been cleaned of excess tissue, it is soaked in a 0.01% solution of crystal violet. Johnson (1979) suggested soak times ranging from 10 minutes to 4 hours depending on the size of vertebrae, but this was for teleost fishes. Schwartz (1983) used soak time ranging from 10–15 minutes for 12 different

elasmobranch species (10 minutes for sharks < 70 cm FL, 15 minutes for sharks > 100 cm FL). Carlson, Cortés and Bethea (2003) used similar soak times as Schwartz (1983) for sectioned finetooth shark (*Carcharhinus isodon*) vertebrae (Figure 6.8), and for whole centra from blacknose shark (*Carcharhinus acronotus*) (J.K. Carlson, NOAA/NMFS/SEFSC Panama City Laboratory, USA, pers. comm.). The best ring definition may occur if vertebrae are initially overstained and then destained for no more than 1 minute in 50% isopropyl alcohol (Schwartz, 1983).

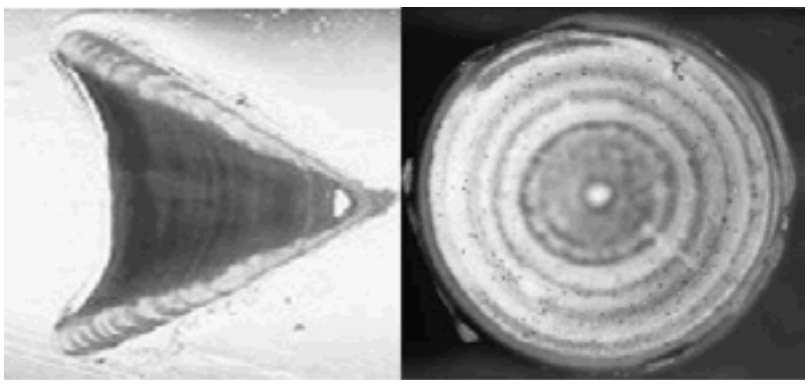
FIGURE 6.8
Sagittal section of a vertebral centrum from a 3 year old fine-tooth shark stained with crystal violet (courtesy of J.K. Carlson, NOAA/NMFS/SEFSC Panama City Laboratory, USA).



Silver nitrate protocol

The silver nitrate technique replaces calcium salts in the centrum with silver, providing bands that darken when illuminated with ultraviolet light (Figure 6.9). As with crystal violet, this technique can be performed on fresh vertebrae as well as those stored in alcohol. All connective tissue must be removed from the centrum to ensure chemical substitution. While Cailliet *et al.* (1983a) soaked vertebral centra in 88% formic acid for 2–4 minutes to remove any traces of bleach they had used in the cleaning process and etch the centrum surface for staining, this may not be required, as neither Stevens (1975) or Schwartz (1983) did this. Regardless of whether this step is taken, all centra should be repeatedly washed for 5–15 minutes in distilled water prior to applying the stain. Centra can then be placed in 1% silver nitrate solution for 1–3 minutes and simultaneously illuminated with an ultraviolet light source for anywhere between 2 and 4 minutes and depending on the species and size of the centrum (Stevens, 1975; Schwartz, 1983), although times used by Cailliet *et al.* (1983a) ranged from 3 to 15 minutes. Submerging whole centra in solution is recommended for ensuring the extreme edges of the vertebra are stained. Checking the centrum every 30 seconds or so will allow determination of the proper immersion time and prevent over-staining, which can easily occur (Schwartz, 1983). Centra should then be rinsed

FIGURE 6.9
Images of two vertebrae stained with silver nitrate. Left-hand image is a sagittal sectioned centrum of an 11 year old leopard shark, *Triakis semifasciata* (courtesy of G.M. Cailliet, Moss Landing Marine Laboratories, USA) and the right-hand image is of a 4 year old spot-tail shark, *Carcharhinus sorrah* (courtesy of J.D. Stevens, CSIRO, Australia).



with distilled water to remove excess silver nitrate and may then be read or sagittally sectioned and read.

Cailliet *et al.* (1983a) used a dissecting scope with reflected illumination focused laterally on the centra to make counts; however, either reflected or transmitted light can be used for sectioned vertebrae. After counts are completed, centra should be soaked in a 5% sodium thiosulfate solution for 2–3 minutes, rinsed with distilled water and stored in 70% isopropyl alcohol (Stevens, 1975; Schwartz, 1983; Cailliet *et al.*, 1983a; 1983b). This process fixes the chemical substitution, but may also eradicate very narrow rings. Counts should be made before and after fixation to estimate the bias caused by the process (Cailliet *et al.*, 1983b).

Calcium deposits have been documented in the neural arches of elasmobranch fishes (Peignoux-Deville, Lallier and Vidal, 1982; Cailliet, 1990), but they had not been used for ageing. McFarlane, King and Saunders (2002) recently introduced the first attempt using this structure for ageing elasmobranchs by silver nitrate staining the neural arches of sixgill sharks (*Hexanchus griseus*). The results from this preliminary study indicate that neural arches may provide another ageing structure for elasmobranch species where their vertebral centra are poorly calcified, but the method has not been validated. Attempts are currently underway to refine this method by determining the most appropriate sectioning methods and thickness, staining times and solution concentration (McFarlane, King and Saunders, 2002). The technique is also being applied to several other elasmobranchs with poorly calcified vertebrae (G.A. McFarlane, Pacific Biological Station, Canada, pers. comm.).

6.3.5 Reader agreement, precision and bias

Precise and accurate age estimation is a critical component of any ageing study. It is important to keep in mind that the consistent reproducibility of age estimates from vertebral centra will achieve high precision, but that these age estimates may not be accurate (i.e. reflect the true or absolute age), and that precision should never be used as a substitute for accuracy. Accurate age determination requires validation of absolute age not just the frequency of increment formation in vertebral centra or spines (Beamish and McFarlane, 1983; Cailliet, 1990; Campana, 2001) (Section 6.4).

Using two readers to independently age all centra two times in blind, randomized trials without knowledge of each specimen's length or disc width allows two calculations of between-reader agreement and precision and helps prevent reader bias that can be caused by "predetermination" of age based on knowledge of length (i.e. prevent subjectivity). When there is a disagreement between readers, a final age determination should be made by the two readers viewing the centrum together, as a single age is needed from each specimen for input into growth models. If no consensus can be reached, the sample should be eliminated from the study.

The most commonly used methods for evaluating precision in age determination have been the average percent error (APE) technique of Beamish and Fournier (1981) and the modification of their method by Chang (1982). However, Hoenig, Morgan and Brown (1995) and Evans and Hoenig (1998) have demonstrated that there may be differences in precision that these methods obscure because the APE assumes that the variability among observations of individual fish can be averaged over all age groups and that this variability can be expressed in relative terms. Also, APE does not result in values that are independent of the age estimates. APE indices do not test for systematic differences, do not distinguish all sources of variability (such as differences in precision with age) and do not take experimental design among studies into account (i.e. number of times each sample was read in each study) (Hoenig, Morgan and Brown, 1995). Within a given ageing study, however, APE indices may serve as good relative indicators of precision within, and between, readers provided that each reader ages each vertebra the same number of times. However, even this appears only to inform which

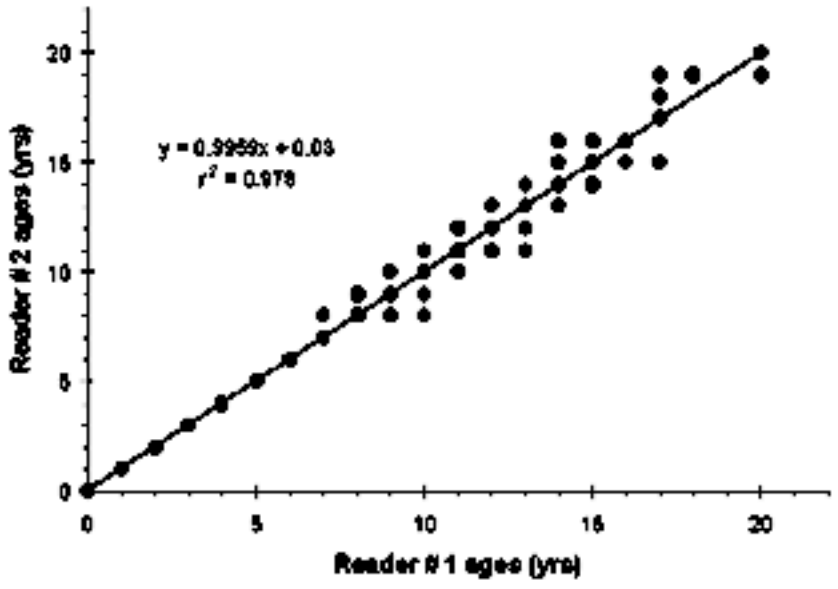
reader was less variable, not which is better or if either were biased. The comparison of precision between ageing studies would appear to have limited value, and I can find no references that compare precision estimates for a given species (APE or otherwise) to other studies, although a conversion factor relating the two precision estimators has been derived based on 14 papers that used both APE methods (Campana, 2001). Comparing precision between studies would seem to be important only if the study species is the same, but caution should be used if samples are from different areas.

A simple and accurate approach to estimating precision is to: (a) calculate the percent reader agreement (PA=[No. agreed/No. read] x 100) within and between readers for all samples; (b) calculate the percent agreement plus or minus one year (PA +/- 1 year) within and between readers for all samples; (c) calculate the percent agreement within and between readers, with individuals divided into appropriate length or disc width groups (e.g. 5–10 cm increments) as an estimate of precision (this should be done with sexes separate and together) and (d) test for bias using one or more of the methods below. The criticism of percent agreement as a measure of precision has been that it varies widely among species and ages within a species (Beamish and Fournier, 1981; Campana, 2001). However, there is validity in using percent agreement with individuals grouped by length as a test of precision because it does not rely on ages (which have been estimated), but rather on lengths, which are empirical values. Age could be used if, and only if, validation of absolute age for all available age classes had been achieved (Section 6.4).

Several methods can be used to compare counts (ages) by multiple readers such as regression analysis of the first reader counts versus the second reader counts, a paired t-test of the two readers' counts and a Wilcoxon matched pairs signed-ranks test (DeVries and Frie, 1996). Campana, Annand and Mcmillan (1995) stated the importance of a separate measure for bias and that bias should even be tested for prior to running any tests for precision. They suggest an age-bias plot, graphing one reader versus the other, which is interpreted by referencing the results to the equivalence line of the two readers (45° line through the origin) (Figure 6.10). Similarly, Hoenig,

FIGURE 6.10

An age-bias plot, graphing one reader versus the other, showing good agreement and no bias. The x^2 tests of symmetry were also conducted on these data and gave no indication that differences between and within readers were systematic rather than due to random error (from Goldman 2002).



Morgan and Brown (1995) and Evans and Hoenig (1998) state that comparisons of precision are only of interest if there is no evidence of systematic disagreement among readers or methods, and suggest testing for systematic differences between readers using Chi-square tests of symmetry such as described by Bowker (1948), McNemar (1947) and their Evans-Hoenig test to determine whether differences between and within readers were systematic (biased) or random. This is of particular importance if initial percent agreement and precision estimates are low. I recommend these tests of symmetry for testing for bias regardless of precision as they place all age values in contingency tables and test the hypothesis that values in a given table are symmetrical about the main diagonal, and because they can be set up to test among all individual age classes or groups of age classes. The test statistic (the x^2 variable) will tend to be large if a systematic difference exists between the two readers.

6.3.6 Back-calculation methods

Back-calculation is a method for describing the growth history of each individual sampled and numerous variations in methodology exist (see Francis (1990) for a thorough review). Back-calculations estimate lengths-at-previous-ages for each individual and should be used if sample sizes are small and if samples have not been obtained from each month. Regression methods are ill advised because they discard information and frequently produce back-calculated lengths that overestimate fish length at capture (Francis 1990) and are not presented here. Back-calculation formulas that follow a hard part or body proportion hypothesis are recommended (Francis, 1990; Campana, 1990; Ricker, 1992). The proportional relationship between animal length or disc width and the radius of the vertebral centrum among different length animals within a population is used as a basis for empirical relationships regarding population and individual growth as is the distance from the focus to each annulus within a given centrum (see below and Section 6.3.2). Centrum radius (CR) and distance to each ring should be measured as a straight line from the central focus to the outer margin of the corpus calcareum (Figure 6.4) to the finest scale possible. When using a compound video microscope with the image analysis system (e.g. UTHSCSA Image Tools 1997 or Optimus - Media Cybernetics 1999), distances can be measured to the nearest 0.001 mm. If no image analysis system is available, measurements should be made with an ocular micrometer (which is easily inserted into an eyepiece of the microscope). Lengths or disc widths should then be plotted against CR to determine the proportional relationship between somatic and vertebral growth (Figure 6.11), which will assist in determining the most appropriate back-calculation method.

Four different proportion-based back-calculation methods are presented here that can be used to compare sample length-at-age data depending on the relationship between CR and length. (Length and disc width are interchangeable in the following equations, but length will be the term used). The results of the method best representing sample data should be used in subsequent growth models (see Section 6.5).

(i) The Dahl-Lea direct proportions method (Carlander, 1969).

This is the most commonly applied proportions method. While it should only be conducted when the linear fit to the relationship between CR and length passes through the origin, it may still provide the most accurate results when compared to sample length-at-age data. Hence, at least one of the other three methods below should also be conducted for comparison. The Dahl-Lea direct proportions equation is:

$$L_i = (L_c/CR_c)\, CR_i \qquad (6.1)$$

where L_i = length at ring i, L_c = length at capture, CR_c = centrum radius at capture, and CR_i =centrum radius at ring i.

(ii) Linear-modified Dahl-Lea method (Francis, 1990).

This method should be applied if the relationship between CR and length is best described by a linear equation and the CR-length relationship does not pass through the origin (Figure 6.11). Parameter estimates from the specific linear fit are incorporated into the back-calculation estimates. The linear-modified Dahl-Lea equation is:

$$L_i = L_c\,[(a+bCR_i)/(a+bCR_c)] \tag{6.2}$$

where *a* and *b* are the linear fit coefficient estimates (Figure 6.11).

(iii) Quadratic-modified Dahl-Lea method (Francis, 1990).

This method should be applied if the relationship between CR and length is best described by a quadratic fit as parameter estimates from the specific quadratic fit are incorporated into the back-calculation estimates. The quadratic-modified Dahl-Lea equation is:

$$L_i = L_c\,[(a+bCR_i+cCR_i^2)/(a+bCR_c+cCR_c^2)] \tag{6.3}$$

where *a*, *b*, and *c* are the quadratic fit parameter estimates (Figure 6.11).

(iv) Size-at-birthmodified Fraser-Lee method (Campana, 1990):

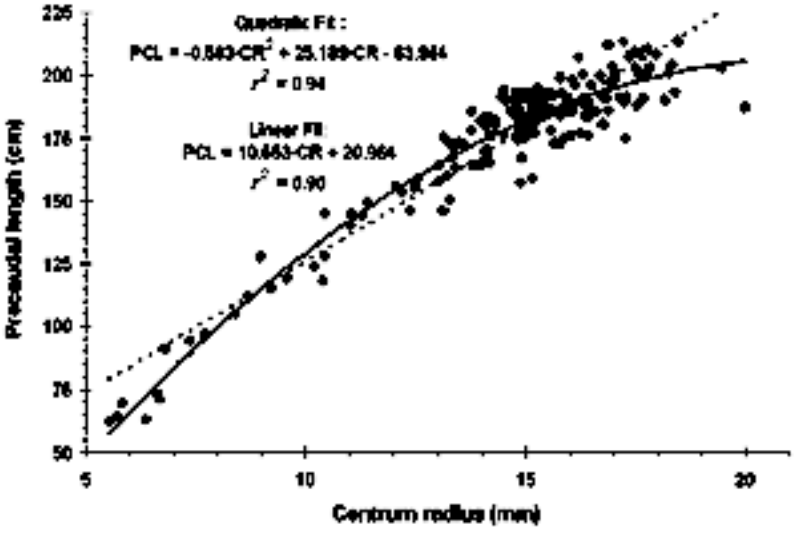
Both Ricker (1992) and Campana (1990) suggested that the point of origin of proportional backcalculations should be related to a biologically derived intercept (i.e. length at birth). This equation is recommended for use anytime the linear fit to the relationship between CR and length does not pass through the origin. The "size-at-birth-modified" Fraser-Lee equation is:

$$L_i = L_c+[(CR_i–CR_c)\,(L_c–L_{Birth})/(CR_c–CR_{Birth})] \tag{6.4}$$

where L_{Birth} = length at birth and CR_{Birth} = centrum radius at birth.

FIGURE 6.11

Relationship between centrum radius and precaudal length for eastern North Pacific salmon sharks (*Lamna ditropis*), showing significant fits given by linear and quadratic equations (sexes combined, n=182). PCL = precaudal length, CR = centrum radius (from Goldman 2002).



Providing biological and statistical reasoning behind the choice of a back-calculation method is extremely important for obtaining accurate life history parameter estimates from a growth function (e.g. the von Bertalanffy model) when using back-calculated data. While one method may show itself to be more statistically appropriate for back-calculation, researchers should conduct several methods for comparison of available sample length-at-age data to verify that statistical significance equates to biological accuracy. Biological accuracy can be determined by plotting the sample mean length-at-age data against the difference between mean back-calculated length-at-age estimates and the sample mean length-at-age data to see which method provides the best results (Goldman, 2002). This plot will show which mean back-calculation length-at-previous-age estimates (from each method) most accurately reflect mean lengths-at-age of sampled individuals.

6.4 VALIDATION

6.4.1 Requirements

Estimates of age, growth rate and longevity in sharks assume that the vertebral rings are an accurate indicator of age. While this is probably true for most species, few studies on elasmobranch growth have validated the temporal periodicity of band deposition in vertebral centra and even fewer have validated the absolute age (Cailliet, Radtke and Weldon, 1986; Cailliet, 1990; Campana, Natanson and Myklevoll, 2002; Natanson, Mello and Campana, 2002). Cailliet (1990) stated that the process of evaluating growth zone deposition in fishes can be categorized into the terms "verification" and "validation," where verification is defined as "confirming an age estimate by comparison with other indeterminate methods" and validation as "proving the accuracy of age estimates by comparison with a determinate method" and these definitions are followed here.

Obtaining the absolute age of individual fish (complete validation) is the ultimate goal of every ageing study, yet it is the frequency of growth ring formation for which validation is typically attempted. The distinction between validating absolute age and validating the periodicity of growth ring formation is important (Beamish and McFarlane, 1983; Cailliet, 1990; Campana, 2001). Validation of the frequency of growth ring formation must prove that the mark being considered an annulus forms once a year (Beamish and McFarlane, 1983). However, it is the consistency of the marks in "number per year" that really matters, be it one or more than one. Two or more marks (rings) may make up an "annulus" if, and only if, consistent multiple marks per year can be proven. Strictly speaking, validation of absolute age is only complete when it has been done for all age classes available, with validation of the first growth increment being the critical component for obtaining absolute ages (Beamish and McFarlane, 1983; Cailliet, 1990; Campana, 2001).

Validation can be achieved via several methods such as chemically tagging wild fish, mark-recapture studies of known-age individuals and bomb carbon dating (see Section 6.3.3). The latter two can also be used to validate absolute age. A combination of using known-aged individuals, tag and recapture, and chemical marking is probably the most robust method for achieving complete validation (Beamish and McFarlane, 1983; Cailliet, 1990; Campana, 2001; Natanson, Mello and Campana, 2002). While this is a rather daunting task to accomplish with most elasmobranch species, the current necessity to obtain age-growth data for fisheries management purposes dictates that it be attempted. The most frequently applied method used with elasmobranchs has been chemical marking of wild fish (see Section 6.4.3) even though recaptures can be difficult to obtain for many species. As validation has proven difficult in elasmobranchs, verification methods such as centrum edge analysis and relative marginal increment analysis are frequently employed.

6.4.2 Indeterminate methods (verification)

Centrum edge analysis and relative marginal increment analysis are simple, indeterminate methods that can be used to verify the temporal periodicity of ring formation in vertebral centra. Each uses the centrum edge in a different manner to assess the timing of band deposition. While relative marginal increment analysis may be slightly more robust, as the technique makes all age classes comparable on a relative scale, it is advantageous to conduct both methods, particularly if electron microprobe spectrometry can be applied (Cailliet, 1990; Wintner and Dudley, 2000; Wintner *et al.*, 2002).

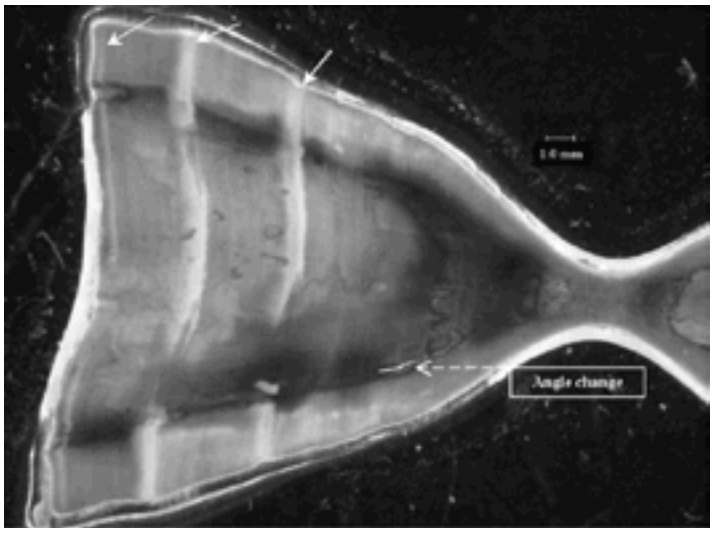
Centrum edge analysis compares the opacity and translucency (width and/or density) of the centrum edge over time in many different individuals to discern seasonal changes in growth. The centrum edge is categorized as opaque or translucent and the band width is measured or graded, then compared to season or time of year (Kusher, Smith and Cailliet, 1992; Wintner and Dudley, 2000; Wintner *et al.*, 2002). A more detailed centrum edge analysis can be conducted by analyzing the levels of calcium and phosphorous at the centrum edge using X-ray or electron microprobe spectrometry (Cailliet, Radtke and Weldon, 1986; Cailliet and Radtke, 1987), which according to Cailliet (1990) has only been conducted in a single study on recaptured nurse sharks that had been injected with tetracycline (Carrier and Radtke, 1988 in Cailliet, 1990).

Relative marginal increment analysis (RMI) – sometimes Marginal Increment Ratio (MIR) – is a useful, direct technique with which to assess seasonal band and ring deposition. The margin, or growth area of a centrum from the last (ultimate) growth ring to the centrum edge, is divided by the width of the last (previously) fully formed (penultimate) annulus (Branstetter and Musick, 1994; Natanson, Casey and Kohler, 1995; Wintner *et al.*, 2002). Resulting MIR values are then plotted against the month of capture to determine the temporal periodicity of band formation. Age-zero animals cannot be used in this analysis since they have no fully formed increments.

6.4.3 Determinate methods (validation)

Validation of absolute age is extremely difficult to achieve with elasmobranch fishes, hence the few studies that have attempted validation in these fishes have focused on validating the temporal periodicity of ring (growth increment) formation. The tetracycline validation method is a standard among fisheries biologists for marking free-swimming individuals (Cailliet, 1990; DeVries and Frie, 1996; Campana, 2001) to test the assumption of annual periodicity of growth rings. Oxytetracycline (OTC), a general antibiotic that can be purchased through veterinary catalogs, binds to calcium and is subsequently deposited at sites of active calcification. It is typically injected intramuscularly at a dose of 25 mg/kg body weight (Tanaka, 1990; Gelsleichter *et al.*, 1998) and an external identification tag is simultaneously attached to each injected animal. OTC produces highly visible marks in vertebral centra and dorsal fin spines of recaptured sharks when viewed under ultraviolet light (Holden and Vince, 1973; McFarlane and Beamish, 1987a and b; Brown and Gruber, 1988; Tanaka, 1990; Kusher, Smith and Cailliet, 1992; Smith, 1984; Gelsleichter *et al.*, 1998; Natanson, Mello and Campana, 2002; Simpfendorfer *et al.*, 2002; Smith, Mitchell and Fuller, 2003) (Figure 6.12). The combination of body growth information and a discrete mark in the calcified structure permit direct comparison of time at liberty with growth band deposition, such that the number of rings deposited in the vertebra or spine since the OTC injection can be counted and related to the time at liberty. Although there may be problems associated with using captive growth as a surrogate to growth in the wild and with recapturing animals that have been at large for long enough periods of time, this method has been used on a number of species in the field and in laboratories (Cailliet, Radtke and Weldon, 1986; Branstetter, 1987; Cailliet, 1990; Cailliet and Goldman, in prep.). While growth in captive animals may be influenced by constant environmental variables (e.g. water temperature and photoperiod) and food availability,

FIGURE 6.12
Sagittally cut vertebral section of OTC injected captive sand tiger shark (*Carcharias taurus*). White arrows indicate three clearly visible OTC marks at the sight of ring formation (photograph K.J. Goldman).



laboratory studies can provide valuable information on growth rates (Tanaka, 1990; Mohan, Clark and Schmid, 2004), assist in verifying or validating the timing of growth ring deposition (Branstetter, 1987; Goldman, 2002), and the results may resemble growth rates observed in field experiments (Branstetter, 1987).

Several other chemical markers such as fluorescein and calcein have been used to validate growth ring periodicity in teleost otoliths, but few studies have evaluated these in elasmobranchs (Gelsleichter *et al.*, 1997; Officer *et al.*, 1997). Gelsleichter *et al.* (1997) found that while doses of 25 mg/kg body weight (typical dose for teleosts) induced physiological stress and mortality in elasmobranchs, doses of 5– 10 mg/kg body weight produced suitable marks without causing physiological trauma or death. Based on this evaluation, any alternative chemical markers tested should consider that doses for teleosts might be too high for elasmobranchs.

Bomb carbon dating is a technique that has recently been applied to age validation in elasmobranchs. A rapid increase in radiocarbon (^{14}C) occurred in the world's oceans due to atmospheric atom bomb testing in the 1950s and 1960s (Druffel and Linick, 1978). Its uptake was virtually synchronous in marine carbonates including corals and fish otoliths, which allowed the period of increase to serve as a dated marker in structures exhibiting growth bands (Druffel and Linick, 1978; Weidman and Jones, 1993; Kalish, 1995; Campana, 1997; Campana, 1999). Hence, all fish born prior to 1958 contain relatively little ^{14}C, all those born after 1968 possess elevated levels of ^{14}C and individuals born in the interim period have intermediate levels of ^{14}C. Matching the ^{14}C chronology in the fish hard part with the published ^{14}C chronologies for the region allows interpretation of age and validation. While this method has been used for ageing several teleost fishes, Campana, Natanson and Myklevoll (2002) reported the first application of bomb radiocarbon to validate ages in long-lived sharks, specifically the porbeagle shark (*Lamna nasus*), and preliminary results for the mako shark (*Isurus oxyrinchus*). This method may provide one of the best approaches to age validation of long-lived fishes; however, it is not viable for short-lived species or younger individuals, and appropriate reference chronologies are not available for some environments (Campana, 2001). Bomb carbon dating requires at least some of the fish in the sample to have been born (or hatched) prior to 1965. It is expensive and requires the use of relatively high technology equipment such as a mass spectrometer, which may make this method unavailable for many researchers. It may, however, resolve certain ageing discrepancies such as questions regarding single versus double ring formation annually in some species, if vertebrae from the appropriate time period can be obtained.

6.5 GROWTH MODELS

6.5.1 Sampling and fitting requirements

A number of models and variations of models exist for estimating growth parameters in fishes, of which the von Bertalanffy (1938) and Gompertz (1825) growth models are the most commonly applied (see Ricker, 1979; Summerfelt and Hall, 1987; and Haddon, 2001 for reviews). The von Bertalanffy growth function has mostly been used to describe fish growth, while the Gompertz curve is often used to describe larval and early life growth of fishes and growth in many invertebrates (Zweifel and Lasker, 1976; Ricker, 1979). These two models are presented in standard form below; however weight can be used in place of length in the von Bertalanffy model, and length may be substituted for weight in the Gompertz model. Many statistical packages include functions that can be used to calculate the best fitting growth parameters for the available length-at-age or weight-at-age data pairs from the equations given in Sections 6.4.2 and 6.4.3. For example, a nonlinear least squares regression algorithm (e.g. 'nls' in S-Plus, Mathsoft Inc., 2000), a maximum likelihood function or the PROC NLIN function in SAS can be used to fit the von Bertalanffy and Gompertz curves to the data (SAS Institute Inc., 1999), and programs such as PC-YIELD (Punt and Hughes, 1989) can calculate a wide range of growth models for comparison (Wintner *et al.*, 2002). Further, FISHPARM (Prager, Saila and Recksick, 1987), a fishery-based statistics program, is simple to use and provides quality statistical results for the two models presented herein. Both models can also be fit to data on a spreadsheet via a non-linear regression using the "solver" function in Microsoft Excel.

Sample size can have considerable influence on growth model results. Pooling the sexes into one sample can mask differences in sex-specific growth, so growth parameters should be estimated for the sexes separately and combined and tested for significant differences. If smaller and-or medium size age classes are not well represented of one or both sexes, lengths at previous ages should be back-calculated from centra measurements for all animals. Sample (observed) length-at-age data, back-calculated length-at-age data, mean back-calculated length-at-age data, and a combination of back-calculated lengths-at-age and sample data should then each be separately fitted with the appropriate growth function and the resulting parameter estimates compared. As long as large animals are well represented in the sample size, close parameter estimates from these four growth curves would indicate that to provide relatively reliable results sufficient sample size (n) has been obtained. The best parameter estimates in these cases will be those with the smallest standard error; however, significant differences between curves can be tested with a likelihood ratio test. A likelihood ratio test should always be conducted if the resulting four curves have not produced similar parameter estimates and if standard errors are high.

Several methods exist for evaluating differences in growth curves (Gallucci and Quinn, 1979; Kimura, 1980; Bernard, 1981; Kappenman, 1981; Francis, 1996; Wang and Milton, 2000). While several techniques can provide reasonable results, a likelihood ratio test will always equal or surpass other methods in accuracy and reliability and should be used to determine whether significant differences exist between growth curves, such as to see if female and male growth parameter estimates are significantly different or if a single set of growth parameters better describe the data (Kimura, 1980; Cerrato, 1990; Quinn and Deriso, 1999; Haddon, 2001). Likelihood ratio tests can be conducted using a multitude of programs, such as Microsoft Excel following the Kimura (1980) method. Haddon (2001) provides an excellent step-by-step instruction guide to the Kimura (1980) method or one can use the PROC NLIN function in SAS (SAS Institute Inc. 1999).

6.5.2 von Bertalanffy growth function

The von Bertalanffy (1938) growth function has been widely used since its introduction into fisheries by Beverton and Holt (1957) and although it has received much criticism over the years, it is the most widely used growth function in fisheries biology today (Roff, 1982; Haddon, 2001). It maintains its attractiveness, in part, because its approach to modeling growth is based on the biological premise that the size of an organism at any moment depends upon the resultant of two opposing forces: anabolism and catabolism. Further, it is convenient to use and allows for much easier comparison between populations and several alternate forms of the model can be fitted to the age-length data. Haddon (2001) presents a variety of growth models including generalized models as possible alternatives).

The von Bertalanffy (1938) growth function is:

$$L_t = L_\infty (1\text{-}e^{-k\,(t\text{-}to)}) \tag{6.5}$$

where L_t = predicted length at age 't', L_∞= asymptotic or maximum length, k = the growth coefficient, and t_o = age or time when length theoretically equals zero. The growth rate, k, is often misrepresented in its description; it should be remembered that when fitted, this curve represents the average growth rate of population members, i.e. the 'k' coefficient is best described as the average rate at which an organism in the population achieves its maximum length [or size] from its length at birth. The exponent t_o is an extrapolation from available data and is not biologically interpretable.

Small sample size, particularly of small and, or large individuals can cause poor parameter estimates using the von Bertalanffy model (Cailliet and Tanaka, 1990; Francis and Francis, 1992). In lieu of using t_o (due to its lack of biological meaning) some researchers suggest using an estimate of length at birth (L_o) rather than t_o as a more robust method (Goosen and Smale, 1997; Carlson, Cortés and Bethea, 2003; H. Mollet, Monterey Bay Aquarium, Monterey, USA, pers. comm.). This method was first introduced by Fabens (1965) as an alternate equation to the von Bertalanffy growth model. While only a few studies have used Faben's (1965) equation to estimate growth parameters in elasmobranchs, it has provided more realistic parameter estimates for some species when the sample size was small (Goosen and Smale, 1997) and extremely similar results to the von Bertalanffy model when sample size was adequate (Carlson, Cortés and Bethea, 2003). This appears to be an excellent alternative to the von Bertalanffy model and should be applied where appropriate for comparison to other models.

The Fabens (1965) equation is:

$$L_t = L_\infty(1\text{-}be^{-kt}) = L_\infty - (L_\infty - L_o)e^{-kt} \tag{6.6}$$

$$b = (L_\infty - L_o)/L_\infty = e^{kto}$$

where L_o is the length at birth.

6.5.3 Gompertz growth function

The Gompertz (1825) growth function is an S-shaped function (similar to the logistic function—for use of the logistic function and several alternatives to the Gompertz function, see Ricker 1975 and Ricker 1979). The estimated instantaneous growth rate in the Gompertz function is proportional to the difference between the logarithms of the asymptotic disc width or length and the actual disc width or length (Ricker, 1975; 1979). This growth function has been used most often for skates and stingrays (Mollet, Ezcurra and O'Sullivan, 2002) and may be better suited to elasmobranchs that hatch from eggs, but it may also be the most appropriate model for some shark species

(Wintner *et al.*, 2002). This model may be better when the volume of an organism greatly expands with age, such as myliobatiform rays where considerable thickness is added to the animal over time, but not so much disc width or length (W. Smith, *Institute, Country,* pers. comm.). The body mass may be distributed differently than would be readily detectable by length measurement and by the von Bertalanffy model. Further, captive growth rates (particularly when starting with young, small animals) may be better estimated by this function as newly captured specimens may not grow in their typical fashion due to physiological stress or a reduction in feeding that often accompanies that stress, which may cause growth rates to slow (Mollet, Ezcurra and O'Sullivan, 2002).

Three commonly used integral forms of the Gompertz growth function (Gompertz, 1825 as presented by Ricker, 1979) are:

$$w_t = W_o\, e[k(1-e^{-gt})] \tag{6.7}$$

$$w_t = W_\infty\, e[k(1-e^{-gt})] \tag{6.8}$$

$$w_t = W_\infty\, e[-e(-g^{t-to})] \tag{6.9}$$

where: w_t = biomass at any time t, W_0 = hypothetical size (weight or length) at t = 0 (not t_o), W_∞= Maximum estimated weight, k = dimensionless parameter such that "kg" is the size-specific instantaneous rate of growth at t = 0, g = instantaneous growth rate when $t = t_o$, where t_o = the time at which the absolute growth rate starts to decrease (i.e. the inflection point in the curve), and t = age. Equations 6.8 and 6.9 are alternative expressions for Equation 6.7, but the same three parameters (w, k, and g) are solved.

6.6 SAMPLING COVERAGE

The goal of every age and growth study is to accurately and thoroughly describe (through validation) the age-length relationship of a species. In order to achieve that goal, a solid experimental design beginning with field sampling and ending with the calculation and comparison of growth rate estimates is necessary. Thorough sampling coverage is imperative to the successful outcome of ageing studies. The dramatic effect that sample size can have on growth parameter estimates makes it imperative that a representative sample of the population be obtained for ageing studies. It is obvious that a larger number of specimens (i.e. larger sample size) is beneficial to gaining a thorough understanding of the age and growth process of any species. The desired content of this sample is to have specimens from both sexes for each month of the year for the entire size and geographic range of the species. This can be difficult to obtain for many elasmobranchs, but the goal should be to come as close to it as possible. Back-calculations can help to "fill in" the gaps for a low sample size of young and middle age classes, but it must be remembered that while there is considerable value in using back-calculated data, the result is a false increase in sample size, as back-calculation data are not independent values.

A wide range of techniques is available for conducting ageing studies on elasmobranch fishes (Casselman, 1983; Cailliet, Radtke and Weldon, 1986; Cailliet, 1990; Cailliet and Goldman, in prep), and studies should use as many of the available techniques as possible. In this paper I have tried to encompass the majority of options available, but resources cited here should be consulted for more detailed information on specific topics. Researchers more frequently follow the advice given by Beamish and McFarlane (1983) and reiterated in Cailliet, Radtke and Weldon, (1986) and Cailliet (1990) regarding the need to combine techniques, such as the use of OTC marking and tag-recapture data from the field and-or laboratory to validate relative (timing of increment formation) and absolute age. However, much work still needs to be done on

this group of fishes. These practices, along with the development of new techniques, will be needed to further elucidate the nature of growth increment deposition in the vertebral centra, dorsal fin spines, neural arches and caudal thorns and to validate age. Research on physiological aspects related to age and growth, such as the function of the endocrine system in calcium regulation and the deposition of growth increments, should also be undertaken, as we know little about these mechanisms as they relate to growth in elasmobranchs (Cailliet 1990, Gelsleichter and Manire 1997).

6.7 WEB-BASED RESOURCES

- Canadian Shark Research Laboratory, Bedford Institute of Oceanography, Nova Scotia Canada:
  http://www.mar.dfo-mpo.gc.ca/science/shark/english/index.htm
- NMFS/NOAA/SEFSC Shark Population Assessment Program, Panama City, FL, U.S.A.:
  http://www.sefscpanamalab.noaa.gov/shark/shark_final_1.htm
- NOAA/NMFS/NEFSC Apex Predator Program, Narragansett, RI, U.S.A.:
  http://na.nefsc.noaa.gov/sharks/

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