A REVIEW OF SEVERAL METHODS FOR AGING ELASMOBRANCHS

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ABSTRACT.

Fisheries for elasmobranch fishes, especially for use as human food, are increasing at a tremendous rate in California waters. Unfortunately, critical aspects of their life histories, such as their age structure, growth rates and reproductive habits, are unknown for a majority of the species presently being fished. The evidence that does exist suggests that elasmobranchs have relatively slow growth rates, a late age of first reproductive maturity, low fecundities, and long gestation periods. This combination makes them susceptible to over-fishing due to the close relationship between stock and recruitment and the paucity of catch statistics relating to size, age and state of reproductive maturity. Since elasmobranchs lack hard, bony parts used to age other fishes, little is known about their age or growth rates. This has led Holden (1977) to plead for "establishing acceptable techniques in age determination of elasmobranchs". Alternating hyaline and opaque zones (circuli) deposited in their vertebral centra appear to be promising tools for aging, much like those in teleost scales, otoliths and bones. However, the amount of calcification varies considerably among elasmobranch vertebrae, and it is necessary to determine which methods are best at discerning these circuli. Since 1979, we have collected more than 700 specimens of at least 25 species, taken several body measurements on them, examined their reproductive tracts, and removed a section of their vertebral columns. From more than 300 individuals of 8 species, we have attempted to discern circuli using several techniques from the literature, modified versions of these techniques, and independently developed methods. Here, we report on three methods which worked well for select species: silver nitrate impregnation, x-radiography and cedarwood oil clearing. We also discuss those methods which have either proven ineffective or which we have not yet been able to evaluate. Finally, we discuss ways of approaching preparation and counting procedures objectively, and the problems associated with interpreting the annular nature of such counts.

INTRODUCTION

Utilization of elasmobranch fishes is increasing at a tremendous rate in California waters. Historically, sharks were used for their oils and for reduction purposes (Byers, 1940), the vitamins in their livers (Frey, 1971) and as human food (Frey, 1971; Hart, 1973). Their use as human food is rapidly gaining considerable favor. According to the National Marine Fisheries Service (Fishery Market News), at least six species of elasmobranchs are commonly landed and catches often reach several hundred thousand pounds in a two-week period in San Pedro, California alone. A major problem that arises with this increased utilization of elasmobranchs is that very little is known of the life history characteristics that would be essential to effectively manage any on of these species of fishes. For example, age determination has not been evaluated sufficiently for the majority of elasmobranchs in California, and therefore, age at first reproduction is not known. Since the usual means of aging fishes, by examining scales, otoliths or bones, are not applicable to elasmobranchs, it has been thought that they cannot be easily aged (Stevens, 1975; Holden, 1977). However,

the evidence that does exist indicates that growth rates may be slow and time to sexual maturity long, estimated as approximately 0.6 to 0.9 of the asymptotic length (Holden, 1977). For example, sexual maturity occurs at about nine years for Galeorhinus zyopterus (Holden, 1977) and for Raja clavata (Steven, 1936), and fourteen to twenty-three years or longer for Squalus acanthias (Ketchen, 1972, 1975; Holden, 1973; Jones and Green, 1977a, b). Also, their fecundity is relatively low (Holden, 1973, 1974, 1977). Even though the number of eggs and embryos of species such as Squalus acanthias increases with age and length of the parent (Bonham, et al., 1949), ultimately, the available uterine space in females is limiting and therefore, the maximum number of embryos is limited. Since elasmobranchs have varied reproductive strategies (viviparous, ovoiviparous and oviparous: Breder and Rosen, 1966), it has been difficult to determine the number of young produced annually by most shark, skate and ray species. Also, gestation periods fluctuate widely among species and are relatively long. Ford (1921), for example, determined in Squalus acanthias that pregnancy for Galeorhinus zyopterus and Babel (1967) estimated only a three-month gestation period for Urolophus halleri. This variability in reproductive habits, coupled with the problem of aging, makes elasmobranch populations difficult to manage intelligently. Since fishing the premature early life stages could deleteriously affect the total population size of elasmobranchs very quickly, due to the close relation between stock and recruitment, the information on age and size at which reproduction first occurs is essential to effective management.

Elasmobranchs lack the calcareous otoliths and bones of teleosts and their placoid scales are too small to be aged. This has led to alternate methods of age determination utilizing tagging, size-frequency analysis, tooth-replacement rates, stage of development of sexually dimorphic characters, and numbers of rings in spines and vertebral centra. Life history characteristics of elasmobranch fishes vary considerably, depending of species and the methods used to age them. From tagging and recapture studies, Steven (1936), Kauffman (1955) and Babel (1967) suggested that both male and female elasmobranchs (Raja clavata, Squalus acanthias and Urolophus halleri, respectively) grow at similarly slow rates. Analysis of length-frequency data has been used by Olsen (1954) for Galeorhinus australis, by Templeman (1944) for Squalus acanthias, by Aasen (1963) for Lamna nasus, by Parker and Scott (1965) for Cetorhinus maximus and by Johnson and Horton (1972) for the holocephalan Hydroagus colliei. This method has not proved to be very successful, because the growth rates of elasmobranchs are very slow, there are sampling difficulties, and the method is very time-consuming and costly. Moss (1972) conducted a tooth-replacement study to determine growth rates of Mustelus canis. He found that maturity is attained at one year of life and maximum size is not reached until six to seven years, a pattern that differs from the possibly more common one exhibited by the long-lived, slow-growing Squalus acanthias or Galeorhinus australis. Johnson and Horton (1972) could only categorize ratfish into "young, immature and adult age groups", using the developmental state of secondary sex characters. Kaganovskaia (1933), Templeman (1944), Bonham, et al. (1949), Aasen (1961), Holden and Meadows (1962) and Ketchen (1975) examined dorsal spines and all suggested, but fail to substantiate, annular deposition of rings in the spines of Squalus acanthias. Even if spines served as useful aging structures, they would not apply to most elasmobranchs which lack them.

Rings deposited in vertebral centra are more promising as tools for aging elasmobranchs and appear as hyaline and opaque zones. Ishiyama (1951), working with Raja fusca, tentatively concluded that the zones were laid down in winter and were therfore annual. Diaber (1960) and Richards, et al. (1963), working with Raja eglanteria and R. erinacea, respectively, also were unable to prove that the zones were annual, but an analysis of their data using the von Bertalanffy growth equation (von Bertalanffy, 1938), indicates that they were probably annular. Stevens (1975) impregnated vertebral centra of Prionace glauca with silver nitrate and counted the resulting rings. He then evaluated the annular nature of these rings by correlating them with Aasen's (1966) length-frequency data, thus enabling him to construct a growth curve. He also fitted these curves to the observed data using the von Bertalanffy growth equation, indicating slow rates and late age at maturity. More recently, Jones and Geen (1977a), using x-ray spectrometry, were able to eliminate many of the problems in counting rings of vertebral centra. Peaks of the elements calcium and phosphorus, which were presumably deposited annually in the vertebral centra of Squalus acanthias, were detected using an energy-dispersive x-ray spectrometric system. Results of their analysis indicate that this technique is very promising for verifying age determinations in elasmobranchs. Several investigators have used tetracycline to mark bony

structures such as scales and bones in fishes. This approach may also prove valuable in verifying age determinations in elsamobranchs (Weber and Ridgway, 1962; Holden and Vince, 1973; Simkiss, 1974). Indeed, it may provide an answer to Holden's (1977) plea for "establishing acceptable techniques in age determination of elasmobranchs".

Since 1979, we have been approaching age determination of Californian elasmobranchs; our approach has been twofold. First, we have been testing several aging techniques, some of which we found cited in the literature and others which we either modified or developed independently. We have been applying the more successful of these techniques to as many of the commercial and potentially commercial elasmobranch species as possible from California waters. In this paper, we report upon those techniques we have tried and present preliminary results from some of the elasmobranchs we have sampled. Ultimately, we hope that our information on the age, size, growth and age-specific reproductive characteristics of common California elasmobranch fishes will allow a more informed approach to managing this emerging resource.

We wish to thank Dr. Michael Lagios for suggesting we use radiography, and Kevin Lohman for helping prepare many vertebrae in the early stages of this project. This research was sponsored in part by NOAA, National Sea Grant College Program, Department of Commerce, under Project No. R/F-57, through the California Sea Grant College Program.

COLLECTING, PROCESSING AND PREPARATION PROCEDURES

To obtain specimens for testing the various aging techniques, we have utilized several sources. Locally, we have sampled the catches at the two shark derbies conducted in Elkhorn Slough (see Herald, et al. 1969). This has provided us with numerous individuals of a range of sizes of the leopard shark (Triakis semifasciata), brown smoothhound (Mustelus henlei) and bat ray (Myliobatis californica). We have also undertaken trawl and gill net collections to obtain other local coastal species. To complement these collections and to broaden our coverage of commercially important elasmobranchs, we have concentrated most of our efforts in central and southern California on subsampling the commercial gill net, trammel net and trawl fishing fleet catches. This has been most effective for obtaining specimens of such commercial species as the pelagic thresher (Alopias vulpinus), bonito shark (Isurus oxyrinchus), spiny dogfish (Squalus acanthias), blue shark (Prionace glauca), soupfin shark (Galeorhinus zyopterus), Pacific angel shark (Squatina californica), longnose skate (Raja rhina), and big skate (R. bionculata). To date, we have collected more than 700 specimens representing at least 25 species. We are presently developing a cooperative sampling program with the observer program of the California Department of Fish and Game to increase our sample sizes of the commonly caught commercial elasmobranchs in southern California.

For each individual specimen collected, measurements are taken, the reproductive tract is examined, and several vertebrae are removed, usually from below the origin of the dorsal fin. Measurements include length, girth, weight and several other potentially important features. To assess reproductive condition in males, several approaches are taken. The configuration of the vas deferens duct and the condition, size and development of the claspers are noted. For some species, sperm smears are made and microscopically examined (see Pratt, 1979). For females, the number and size of eggs in the ovaries are recorded, the embryos, if present, are measured and sexed, and several other features, such as shell gland and oviduct dimensions, and presence or absence of uterine scars, are noted. This information will later be applied to our age and growth models to determine the size and age at which the different species reach sexual maturity.

The vertebrae collected are superficially cleaned and are frozen in plastic bags for later analysis in the laboratory. Once defrosted, a vertebral centrum must be cleaned before it can be prepared for the various manipulations to expose rings. First, the connective tissues must be removed. This is accomplished using one of several techniques, depending upon the species. For the Pacific angel shark, bonito shark, thresher shark, blue shark and white shark, a five-minute soak in distilled water, followed by air drying, effectively

allows the connective tissue to be peeled away from the body of the centrum. For the leopard shark, the gray and brown smoothhounds, the spiny dogfish, the bat ray, the big skate and the longnose skate, soaking in bleach is more effective for removing this connective tissue. The larger the centrum, the longer the soaking time needed, with immersion intervals ranging from five to thirty minutes. Finally, the centrum is rinsed well in tap water. Preliminary work indicates that soaking in enzyme detergent solutions and subjecting the centrum to ultrasonic cleaning procedures did not significantly enhance the cleaning that had already resulted from bleach immersion.

TECHNIQUES FOR DISCERNING RINGS IN VERTEBRAL CENTRA

For the several species we have studied, three techniques have proven useful at elucidating the rings in the vertebral centra, while several other techniques have either proven ineffective or have not yet been evaluated. In this section, we will describe the three useful techniques, list those which have either not proven effective or which we have not utilized, and further discuss some of the considerations which need to be made when interpreting the rings found in elasmobranch vertebral centra.

The first technique is one which was adopted by Stevens (1975) to elucidate rings in blue shark vertebrae. It basically involved replacing the calcium salts in the centrum with silver, providing distinct silver-impregnated rings which become quite dark after illumination under ultraviolet light. An advantage of this technique is that vertebrae which have been preserved in 70% alcohol may be used as well as fresh specimens. For our purposes, it was necessary to further modify Stevens' procedures. To assure the chemical substitution of silver for calcium, all connective tissue must be removed from the centrum body. This is accomplished by one of the previous cleaning methods. The centrum is then soaked in distilled water for approximately fifteen minutes and placed in a 1% silver nitrate solution and immediately placed in a chamber, where it is illuminated by an ultraviolet light source. The length of light exposure ranges from three to fifteen minutes, depending upon the species tested and the size of the centrum. The centrum is then rinsed in distilled water to remove excess silver nitrate.

Usually, a disceting microscope with transmitted illumination focused laterally on the centrum is used to count circuli and to measure the widths of these circuli along a predetermined axis. Since staining clarity can be inconsistent, several centra from each specimen are stained and counted for replicate analysis. After these counts and measurements are made on the newly stained centra, they are soaked in a 5% sodium thiosulfate solution for two to three minutes. This procedure removes excess silver and fixes the chemical substitution. Fixation also eradicates the very narrow rings; therefore, counts should occur before and after fixation. The final step is storage in 70% isopropyl alcohol.

Of the species so far tested with our version of this technique, rings were made more discernible on blue, white, leopard and bonito shark centra, several of which are shown in Figure 1. Using Stevens' (1975) procedure, no rings were observable on leopard shark centra, but Stevens reported success with Lamna and Galeocerto. Our preliminary results indicate that the silver nitrate technique, as modified, will elucidate rings on the longnose skate, but not on the bat ray centra. It may, therefore, have variable but relatively wide applicability to elasmobranch aging studies.

In preparing vertebral centra for the two other successful techniques we have used, they are clenaed using either bleach or sodium hydroxide and cross-sectioned so that the resultant halves present opposite faces of the same centrum. This sectioning may be especially needed for centra which are relatively long, as opposed to those which are longitudinally flat or disk-like (see Figure 2). Large vertebrae are cut in half with a small circular saw attachment for a jeweler's drill, secured in a vice. For smaller specimens, half of the centra are ground away using both aluminum oxide wheel points and fine sandpaper attachments for the same tool. This procedure prevents rings on these opposing halves from obscuring each other when observed after further preparation.

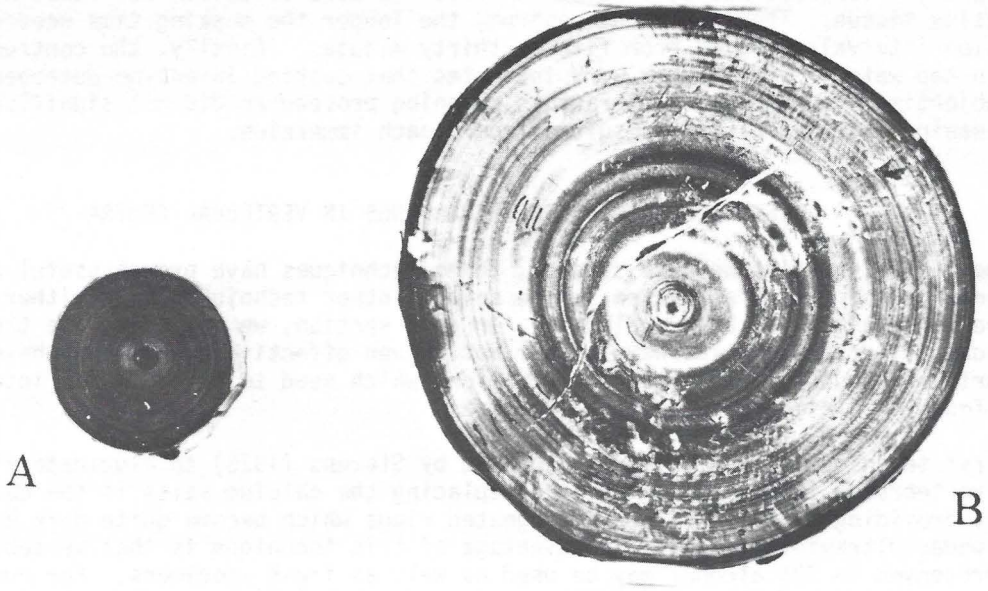

Figure 1. Centra from two elasmobranch species stained using silver nitrate technique: (A) Bonito shark (Isurus oxyrinchus) 211.0 cm total length, centrum diameter 26 mm; (B) Great white shark (Carcharodon carcharias) 487.7 cm total length, centrum diameter 74 mm. (*Photograph by Lynn McMasters*).

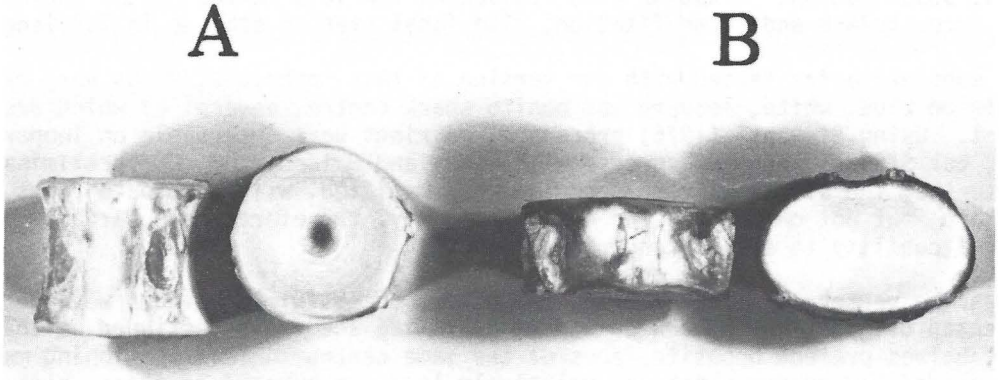

Figure 2. Typical vertebral centra shapes: (A) Leopard shark (Triakis semifasciata) 134.2 cm total length, centrum diameter 19 mm; (B) Pacific angel shark (Squatina californica) 109.4 cm total length, widest centrum diameter 19 mm. (*Photograph by Lynn McMasters*).

The second technique involves taking x-radiographs of half-centra as prepared above. We have used a Hewlett-Packard Faxitron Series X-Ray System (Model No. 43805N) with Kodak Industrex M film (Readypack M-2), as suggested by Miller and Tucker (1979). X-radiographs of the bat ray centra are viewed through a dissecting microscope with a combination of reflected and transmitted light. X-radiographs of centra from the Pacific angel shark, thresher shark, bonito shark and both species of smoothhounds are viewed through a compound microscope using transmitted light. In all of the above species, distinct rings were discernible from x-radiographs. Use of this x-ray technique on centra of the leopard shark and the longnose and big skates has been unsuccessful, apparently because the structural components of these vertebrae interfere with the clarity of the rings. Prints made from x-radiographs of several species are shown in Figure 3. We feel x-radiography will prove to be a very useful method for delineating circuli in many elasmobranch vertebrae.

The third technique involves applying cedarwood oil to the face of each centrum to increase the clarity of circuli by eliminating superficial irregularities. Frequently, prior scraping of the centrum face with a scalpel enhances the clarity of the finer circuli. The circuli of the centrum thus prepared are viewed under a dissecting microscope using a fiber optics light transmitted both vertically and horizontally. This technique has been used successfully on bat rays and longnose and big skates.

Several other published techniques were unsuccessful at enhancing circuli in centra from species we studied. Following Daiber's (1960) technique designed for Raja eglanteria, vertebral centra from both the longnose and big skates were soaked in formalin, cleared of connective tissue and placed in 95% and absolute isopropyl alcohol. After immersion in xylene, the centra were heated in paraffin at 60°C and returned to xylene. Most of the centra were only partially cleared, and circuli were indistinguishable. In testing the technique of Richards, et al. (1963), centra from the same two species were either cleaned in sodium hydroxide or scraped, placed first in 70% alcohol and then transferred to 100% alcohol. Circuli, especially on the outer portions of the centra, were unclear, irrespective of cleaning technique. Using a third method (LaMarca, 1966, utilized on Carcharias taurus), a small number of longnose skates were cleared in sodium hydroxide, stained in a saturated solution of alizarin red S in sodium hydroxide, and differentiated in 3% hydrogen peroxide. Although success among vertebral centra was variable and ring contrast was moderate, further attempts with this technique are warranted and may yield better results. Smith (1980) used a technique on the cownose ray (Rhinoptera bonasus), in which vertebrae were stored in alcohol, cleaned, air dried and sectioned longitudinally. After the hourglass-shaped face was polished, the vertebrae were heated at 200°C for two to three minutes. Attempts to apply this technique to our Pacific bat ray were unsuccessful. Finally, our attempt to use Stirling's (1969) method designed for delineating rings in pinniped teeth, involving etching for twenty-four hours with formic acid and formalin, produced poor results with leopard shark vertebrae.

Undoubtedly, there are many other procedures which may prove useful in aiding researchers to discern circuli in elasmobranch vertebrae. One, which we have not yet had the facilities to pursue, is the use of x-ray spectrometry to measure the concentration of such elements as calcium and phosphorus, which are deposited more during fast growth seasons than in slower periods (Jones and Geen, 1977a). This method is somewhat expensive and time-consuming, but should provide valuable assistance, especially for comparing results with other, more practical techniques.

Regardless of the technique used to help clarify circuli in vertebral centra, decisions need to be made regarding procedures for counting these concentric rings. These decisions require definitions of circulus, ring and band, and will vary depending upon the characteristics displayed by the centra of the species under consideration. In order for our counts to be useful to future researchers, we are attempting to make our approach as objective as possible. We define a "circulus" as any concentric line found in an elasmobranch centrum. We have found, from our experience with bat ray and skate vertebrae, that there are two kinds of circuli. The term "band" refers to the wider type of circulus, while the term"ring" denotes the much narrower type, of which the bands are composed (see Figure 3-C). Each broad band in the bat ray, for example, is composed of three to seven rings, and as with the rings within the band, the bands appear as alternating regions of broad light and narrow, dark circuli when viewed by transmitted light. In x-rays, the broad circuli are dark and the narrow circuli are light.

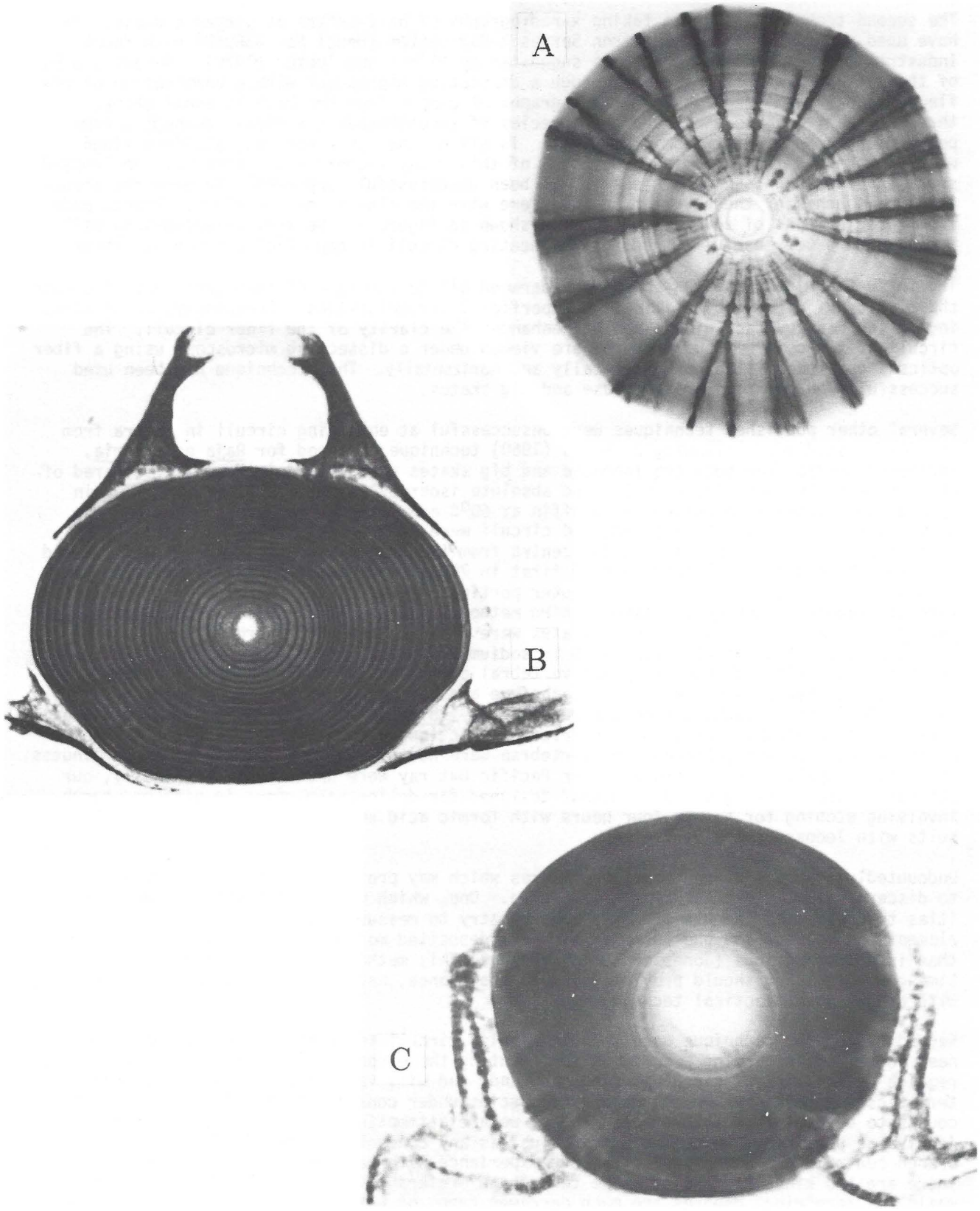

Figure 3. Radiographs from three species of elasmobranchs: (A) Bonito shark (Isurus oxyrinchus) 173 cm total length, centrum diameter 22 mm; (B) Pacific angel shark (Squatina californica) 65 cm total length, widest centrum diameter 11 mm; (C) Bat ray (Myliobatis californica) 102.4 cm disc width, narrowest centrum diameter 9.9 mm. (*Photography by Lynn McMasters*).

Insuring the accuracy of our counts of bands and rings also requires certain decisions about procedures. First, for all species and all techniques we are using, we prepare and analyze several vertebrae from each specimen to alleviate possible differences in preparation or problems of using certain vertebrae. Also, we make replicate, blind counts so as to assure that the readings more accurately reflect the number of bands or rings on an individual centrum.

Finally, we are concerned about the ultimate interpretation of the temporal periodicity that these circuli represent. Are the bands or rings we observe and count on centra daily, weekly, monthly, yearly or even of a larger time frame? It is virtually impossible at this time to verify the time frame for circuli of all of the species we are presently studying. We will have to await results from on-going research projects using size-frequency analysis and growth studies of both captive and wild elasmobranchs involving tetracycline ring-marking techniques and tag return size information.

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