

## Preliminary study on the use of neural arches in the age determination of bluntnose sixgill sharks (*Hexanchus griseus*)

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Conventional structures used for age determinations of teleost fishes (e.g. fin rays, otoliths, scales) cannot be used for elasmobranchs in which these structures are cartilaginous. However, vertebral centra with systematic deposits of calcium phosphate, have been used for age estimation in a number of elasmobranch species such as the tiger shark (*Galeocerdo cuvier*), scalloped hammerhead (*Sphyrna lewini*), and several smoothhound sharks (*Mustelus* spp.) (Cailliet, 1990). Cailliet et al. (1983) provided preparation techniques for enhancing and examining vertebral bands in these elasmobranchs. Unfortunately, the vertebral centra of a number of sharks, including bluntnose sixgill sharks (*Hexanchus griseus*), are too poorly calcified to provide age information (Cailliet, 1990). Previous attempts to age sixgill sharks by using vertebral centra have been unsuccessful (Ebert, 1986). Sharks with poorly calcified vertebral centra tend to be either deep-water species or from relatively primitive families (Cailliet, 1990). However, systematic deposits of calcium phosphate have also been found in chondrocranium, jaws, visceral arches, fin cartilage, claspers, neural and haemal arches (Clement, 1992).

Bluntnose sixgill sharks (hereafter referred to as simply "sixgill sharks") are one of the largest and most primi-

tive species of elasmobranchs. They have a world-wide distribution and in the north-east Pacific Ocean range from the Aleutian Islands to Baja, California (Hart, 1973). Because they are deep-water inhabitants occupying depths up to 2500 m along the outer continental shelf and upper slope waters (Compagno, 1984; Ebert, 1994), little is known about their biology. Sixgill sharks are about 65–70 cm at birth and the maximum length recorded is 482 cm (Castro, 1983). They are ovoviviparous and have reported litter sizes ranging from 22 to 108 (Compagno, 1984; Ebert, 1992). The estimated size-at-maturity for females is 396 cm (Ebert, 1992), although capture of mature sixgill sharks is rare. It is likely that the young inhabit inshore waters (Compagno, 1984).

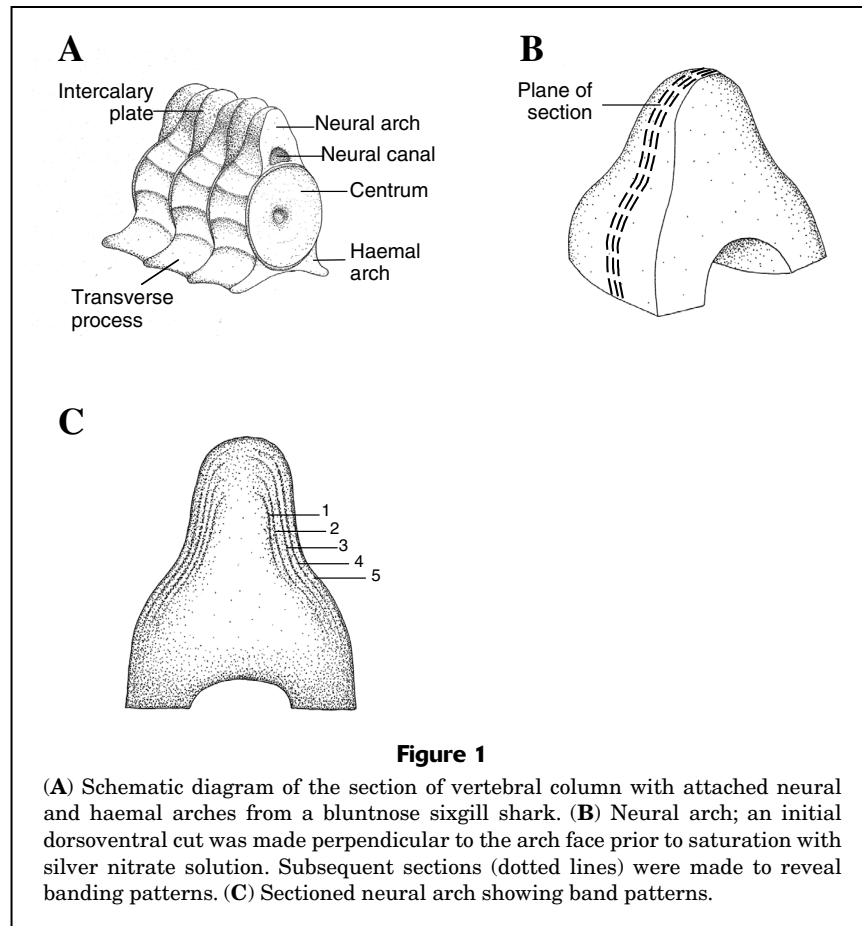
Off the west coast of Canada, an experimental fishery for sixgill sharks was initiated in the early 1990s but was terminated because of conservation concerns. Recently, there has been a renewed interest in a fishery, both commercial and sport, for the species. Incomplete knowledge regarding the biology and life history of sixgill sharks remains a concern. Given the continuing interest in a fishery (commercial and sport) and potential conservation concerns, we investigated the use of neural arches as an alternate body structure to use for the age determination of sixgill sharks. We describe the staining technique used and the bands (annuli) observed. We suggest that our technique represents a possible avenue for developing an aging technique for

various elasmobranchs, particularly those with poorly calcified vertebral centra.

### Methods

From May through September 1994, as part of a co-operative industry–government sixgill shark tagging program, 259 sixgill sharks were captured with hook and line gear off the west coast of Vancouver Island, British Columbia, Canada. Fishing occurred within five main areas: Kyoquot Sound (50°00'N and 127°20'W), Esperanza Inlet (49°45'N 127°00'W), Nootka Sound (49°25'N and 126°40'W), Tofino Inlet (49°05'N and 125°40'W), and Barkley Sound (48°50'N and 125°20'W). A sample of ten sharks was obtained for age determination research. Total length (TL, cm) and sex were recorded for each shark.

A portion of the vertebral column containing 15–20 vertebrae, including the neural and haemal arches, was removed just posterior to the head and immediately frozen. In the laboratory the vertebral column section was thawed. The connective tissue and the outer layer of cartilage were removed from each vertebra and neural arch (Fig. 1A) by soaking them in bleach for 15 minutes and rinsing in distilled water. Some teasing away of tissue was required. For each set of vertebrae, five neural arches were separated from the vertebral centra (Fig. 1B). A single dorsoventral cut was made in each neural arch to expose the inner portions (Fig. 1B). The silver nitrate staining technique for revealing calcium deposits in vertebral centra of other shark species (Cailliet et al., 1983) was modified and applied to the neural arches. Each arch was soaked in 150 mL of 1% silver nitrate while exposed to wide spectrum light (320–400 nm). Soak times in silver nitrate varied according to the size of the arch, but all soak times lasted at least one hour. The degree of staining was assessed on an initial arch for each shark. If required, subsequent arches were restained in the silver nitrate solution and removed at 15–30 minute intervals after removal of the first arch.



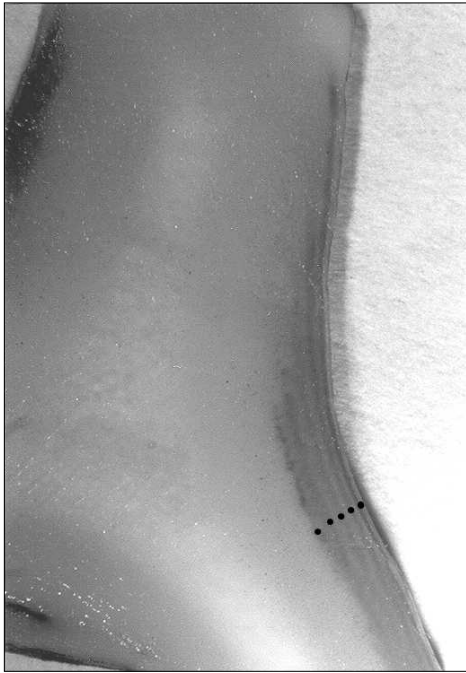
After soaking in silver nitrate, each neural arch was rinsed with distilled water to remove any excess solution. A scalpel was used to cut sections approximately 2–3 mm thick, parallel to the initial cut (Fig. 1B). Sections were placed under the white light (400–700 nm) of a dissecting microscope for a few minutes to allow bands to appear (Fig. 1C). A 2–3 minute soak in 5% sodium thiosulphate (Cailliet et al., 1983) was unsuccessful in halting the development process and fixing the chemical substitution of calcium salts for silver nitrate. However, a 3–5 minute soak in Kodak Stop Bath SB-1a® (1.0 L water; 125 mL 28% acetic acid) did halt the development process. The stained neural arch sections were mounted on a microscope slide with an acrylic toluene mounting medium.

## Results

Of the ten sharks sampled, eight of the sharks were female (130–349 cm TL), and two were male (140 and 242 cm TL). All sharks were immature. The soak time required for the silver nitrate to penetrate the whole neural arch varied between arches of similarly sized sharks and even between arches from the same shark. Generally arches from sharks less than 200 cm TL, 200–250 cm TL, 250–300 cm TL, and greater than 300 cm TL were removed after 1, 2, 3,

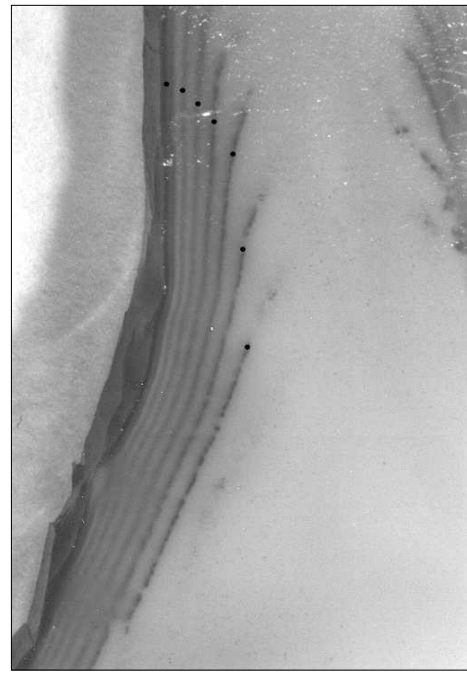
and 4 hours respectively. It was necessary to use several neural arches per shark in order to differentiate a clear set of bands (annuli).

The staining occurred only near the outer portion of each arch, and this left the central portion unstained (Figs. 2 and 3). Distinct, thin, dark bands were discernible in nine of the 10 sets of neural arches examined (e.g. Fig. 2). Bands in the neural arches from the 349 cm female were not all clearly defined even after soaking in the silver nitrate solution for eight hours. In general, bands appeared at regular intervals (Fig. 2). The widths between bands were asymmetrical along the whole edge of the neural arch. These widths were greater along the top edge compared to widths at the side edges (Fig. 3). In some neural arches, the bands were not continuous (Figs. 3 and 4), which made examination of different portions of the arch necessary in order to count the number of bands formed. In some arches, bands were identifiable but fainter towards the inner portions (Fig. 4). These bands appeared granular, but patterns were still detectable (Fig. 4). The number of bands increased linearly with the total length of the shark (Fig. 5). By using 67.5 cm as the median length at birth (Castro, 1983), the mean estimated growth rate was 25 cm/band (SD=4,  $n=9$ ). Because all of the specimens in our study were immature, a change in the growth rate was not observed, as would be expected when size at maturity was reached.



**Figure 2**

A stained neural arch from a 207-cm (total length) female bluntnose sixgill shark. Note that the staining occurs along the outer third portion of the arch. Five bands are indicated by dots.



**Figure 3**

A stained neural arch from a 242-cm (total length) male bluntnose sixgill shark. Seven bands are indicated by dots. Note that the widths between bands are asymmetrical and the bands are discontinuous along the whole perimeter.

## Discussion

The nature of the staining observed on the neural arches suggests that these structures have a potential use in the age determination of elasmobranchs. In sixgill sharks, the bands were distinct and appeared at regular intervals. The number of bands per neural arch increased with total length of the shark, suggesting their potential for age determination. The banding occurred only on the outer portions, indicating that the calcium was deposited after the proximal portion of the arches developed, probably after birth. The methodology used in our study for the staining of calcium deposits in neural arches offers some promise for age determination of sixgill sharks. Future research is required to refine the method by determining sectioning methods and thickness, optimum staining times, or perhaps alternative solution concentrations. For example, it might be more effective to cut neural arches into thin sections prior to soaking in silver nitrate rather than to soak larger portions and then section them.

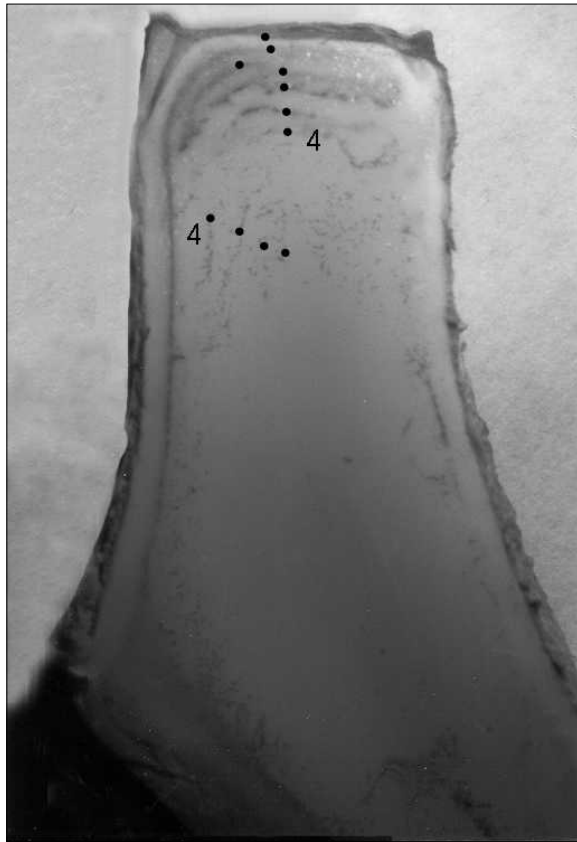
Calcium deposits have been observed in the neural arches of several elasmobranchs (Cailliet, 1990). For example, Peignoux-Deville et al. (1982) provided an extensive description of these deposits for dogfish (*Scyliorhinus canicula*). However, age determination research for elasmobranchs has focused on the calcium deposits in the vertebral centra. Use of the neural arches as aging structures

may provide an alternative age determination method for species in which the vertebral centra are poorly calcified.

Valid age and growth information is fundamental to stock assessment and management. However, Cailliet (1990) lists only 39 species of elasmobranchs for which there are published or ongoing age verification studies. As interest and concern in commercial and recreational fishing of elasmobranchs increases, the need for age determination techniques becomes more pressing. Researchers need to investigate alternative methods for aging elasmobranchs, and we suggest that neural arches may prove to be useful. It is important to note that our observations on calcium banding in sixgill shark neural arches are preliminary and are for immature sixgill sharks only. We stress that fundamental to all aging determination studies is the validation of the method (Beamish and McFarlane, 1983), and future work is required to determine if the bands observed in our study are formed on annual, or regular, intervals.

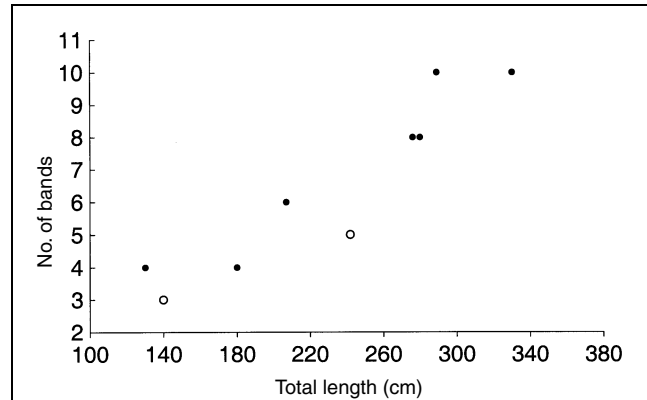
## Acknowledgments

Bill Andrews and Grady O'Neill provided technical assistance in producing the photographs. Maria Surry produced the schematic diagrams of the vertebral columns and neural arches.



**Figure 4**

A stained neural arch from a 330 cm (total length) female bluntnose sixgill shark. The arch was soaked in 1% silver nitrate for 7.25 hours, but the inner portions of the arch were barely stained. Ten bands are indicated by dots, but the first four are difficult to determine. The fourth band is labelled with the number four.



**Figure 5**

Number of bands observed in neural arches by total length (cm) of sixgill shark. Dots represent females; open circles represent males. As the total length of the shark increases, the number of annuli observed increases.

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