Northwest Atlantic Fisheries Organization

SCIENTIFIC COUNCIL MEETING – SEPTEMBER 2002

The Structure and Growth Processes of Caudal Thorns
(Elasmobranch Fisheries – Poster)

by

M. J. Gallagher[*], C. P. Nolan, and F. Jeal.

Zoology Department, Trinity College, Dublin 2, Ireland.
gallagher@bim.ie nolancp@tcd.ie fjeal@tcd.ie

Abstract

Caudal thorns from bathyrajids, for which surface band patterns have previously been correlated with an annual cycle of vertebral band deposition, were examined to ascertain the underlying growth band structure with a view to understanding their growth processes. Caudal thorn samples from *Bathyraja brachyurops*, a commercial ray species from the Falkland Islands were sectioned transversely using both resin embedding and decalcification/wax embedding procedures. Sectioning revealed that internal bands formed as a series of cones underlying each other, and that broad surface broad bands formed annually during periods of rapid somatic growth. Surface ridges representing a near stasis in somatic growth formed at the periphery of each cone. This proposed growth process was further verified by the incorporation of tetracycline at the periphery of caudal thorns from a tag-recaptured bathyrajid. The benefits of using sectioning to enhance band resolution of larger specimens of slower growing species, particularly in the case of worn and tightly spaced surface band patterns are also discussed.

Introduction

In the majority of ray species caudal thorns are found securely embedded in the caudal tissue overlying the spinal column. It is probable that they have a defensive function, and may also assist in the emergence from the egg case. The surface bands of caudal thorns from bathyrajids have previously been correlated with an annual cycle of vertebral band deposition (Gallagher and Nolan, 1999). For a structure to be used as a valid ageing tool it is important to have an understanding of its growth and band formation processes (Cailliet, *et al.*, 1983; Casselman, 1987). To date, however it is unclear how growth bands form in caudal thorns, and how the surface sculpture relates to the underlying morphology. Furthermore bathyrajids are typically slow growing species (Gallagher and Nolan, 1999) and therefore difficulties have been encountered in resolving both worn bands near the thorn tip and tightly spaced bands towards the periphery (Gallagher, 2000). Sectioning of growth structures has previously been recommended for both vertebral centra and dorsal fin spines to help improve band resolution, particularly in slow growing elasmobranchs (Cailliet, 1990; Branstetter and Musick, 1994).

This study investigates the structure and growth processes of caudal thorns from *Bathyraja brachyurops*, a commercially important bathyrajid from the Falkland Islands, which an ageing study using whole caudal thorns has been carried out previously (Gallagher and Nolan, 1999).

---

[*] Current address; Irish Sea Fisheries Board (BIM), Crofton Road, Dun Laoghaire, Co. Dublin, Ireland.

Materials and Methods

Thorn growth *versus* somatic growth

A total of 110 thorn samples of *B. brachyurops* collected for a previous study (Gallagher and Nolan, 1999) were used in this study to assess the relationship between caudal thorn and somatic growth. Whole thorns were measured (mm) by drawing the outline of the thorn viewed under a dissecting microscope using a drawing tube attachment, and derived measurements were plotted against total body length (cm) to assess their relationship.

Structure of caudal thorns

Gross structure of caudal thorns

Whole air-dried caudal thorns and vertebral centra from [*B. brachyurops* collected for a previous study (Gallagher and Nolan, 1999)] were re-examined to assess respective band patterns (Fig. 1). To enhance the surface bands 5 thorns were stained using a silver nitrate staining technique (Gallagher and Nolan, 1999) and were viewed under a dissecting microscope using reflected light. In addition, a total of 5 vertebral centra were sectioned and stained using a crystal violet staining technique (Gallagher and Nolan 1999), and viewed under a dissecting microscope using transmitted light.

Histological structure of caudal thorns

To assess the underlying growth band pattern of caudal thorns, a total of 20 *B. brachyurops* specimens, representing the main size ranges of this species (5 specimens between 20-40 cm, 40-60 cm, 60-80 cm and 80-100 cm (total length)), were collected by scientific observers from commercial fishing vessels within the Falkland Islands' Interim Conservation zone (FICZ) in 1997 (Falkland Islands: 51.45°S, 59.30°W). The first five caudal thorns, posterior to the first haemal arch of the vertebral column, were removed from each specimen. Tissue was removed from the thorn by immersing the segments in hot water (circa. 70-85°C). Thorns were further cleaned in cold running water, and subsequently placed in formalin (10%) solution, buffered in seawater. Samples were packed and labelled, with all relevant details, and transported to the Zoology Department, Trinity College, Dublin, for further analysis.

Excess tissue was further trimmed off the formalin preserved caudal thorns. Thorns were decalcified in De-Cal (Raymond Alam) for between 12 and 20 hours depending on thorn size, and subsequently stored in 70% ethanol. Decalcified thorns were embedded in Paramat wax (melting point 56-58°C). Transverse sections (Fig. 1) of between 10µm and 15µm were produced using a rotary microtome (Leitz), and were placed onto glass slides. Sections were then stained for between 20 min. and 1 hr in Harris's heamatoxylin. Stained sections were subsequently examined under a compound microscope (x40) using transmitted light.

A total of 20 thorns were selected from the air-dried samples of *B. brachyurops* (Gallagher and Nolan, 1999) and were embedded in resin and transverse sections (90-120µm) were produced. Sections were examined under a compound microscope (x20-x40) using transmitted light.

Tetracycline incorporation

Thorns from a single tetracycline marked recaptured specimen from a Falkland Island ray-tagging programme were examined. The specimen, which is currently being described (*Bathyraja* sp.), is a co-habitor and morphologically similar to *B. brachyurops*. The recaptured specimen was an immature male, which was at liberty for 10 months (tagged 8 July 1994, recaptured 6 May 1995). Thorns were viewed under a dissecting microscope using ultra violet light.

Results

Thorn growth versus somatic growth

A logarithmic relationship between thorn size and total length was apparent for caudal thorns for each species (Fig. 2). For males this is described as $y = 3.778\ln(x)-7.230$ ($r^2 = 0.810$) and for females $y = 3.733\ln(x)-7.599$ ($r^2 = 0.723$)

Whole thorns

Regularly spaced, concentric surface bands were readily evident on *B. brachyurops* thorns (Fig. 3). In larger specimens thorn wear was evident on occasion near the thorn apex and surface bands were more tightly spaced nearer the distal margins. These however were not sufficient to reduce resolution.

Histological structure of caudal thorns

Histological and resin embedded (Fig. 4.) transverse sections displayed an internal 'inverted V shaped' band morphology under the proto-thorn, with the base of each of these bands being externally expressed as a ridge on the thorn surface Each new band was formed under the previously formed band, and therefore the innermost internal band represents the most recently formed.

Tetracycline incorporation

Tetracycline incorporation was readily apparent as a distinct intensely fluorescent band on the distal margins of caudal thorns and was concurrent with ridge formation of surface bands in caudal thorns (Fig. 5).

Discussion

Caudal thorns are placoid scale in origin, and are formed from minerals deposited by epidermal and dermal cells. Placoid scales are typically composed of a vascular (supplied with blood) inner core of pulp, a middle layer of dentine and a hard enamel-like outer layer of vitrodentine (Helfman *et al.,* 1997). In longitudinal section the tips of successively formed cones are apparent under the proto-thorn as 'inverted V' shaped bands, with the inner most band under the tip representing the most recently formed, and the surface ridges on the caudal thorns are external expressions of each cone base. This is further corroborated in this study by the fact that tetracycline from a specimen at liberty for under a year was incorporated into the most recently formed ridge near the distal margin of thorn. This growth and band formation process is somewhat analogous to that of dorsal fin spines on the spurdog, *Squalus acanthias*, which are also of placoid scale origin (Holden and Meadows, 1962; McFarlane and Beamish, 1987; Beamish and McFarlane, 1987).

It is probable that the surface ridges on the caudal thorns represent a near stasis in somatic growth, whereas the broader bands represent periods of more rapid growth. This is suggested by the fact that tetracycline was incorporated into a surface ridge (Gallagher and Nolan, 1999). The specimen was injected with tetracycline during the winter period (austral), when somatic growth would have been presumably slowest. An important component of age and growth studies is the assurance that the structure being used to derive age presents a continuous record of growth (Casselman, 1983; Beamish and McFarlane, 1987). Analysis of the relationship between caudal thorn and somatic growth reveals a logarithmic relationship, which suggests that thorn growth slows relative to increased body length. Casselman (1987, 1990) suggested that in very large specimens of slow growing species, structures that are involved in supporting mass, such as vertebral centra, may give a better record of body growth than those that cover the body, such as scales. This can largely be attributed to the fact that although a species may approach its asymptotic length, its mass continues to increase, therefore centra will continue to grow to support this extra mass, whereas scales growth will virtually cease (Casselman, 1987; 1990). This near cessation in growth of scales in older fish has lead to gross inaccuracies in age assessments, where scales have been used exclusively as the primary ageing structure of slow growing teleost species (Beamish and McFarlane, 1987). There were initial concerns that caudal thorns, as they form on the body surface, and grow more slowly with increasing body size, may not accurately reflect the age of larger specimens. Results, however, revealed that even in larger specimens, band resolution was higher on thorns than on vertebral centra for the majority species examined (Gallagher and Nolan, 1999). An exception was evident for larger specimens of female *B. griseocauda,* where resolution of bands on

thorns relative to centra was generally lower. It is probable that growth in this large species slows dramatically, particularly in larger female specimens. Therefore the distances between the surface bands and ridges become less apparent on the distal margins of caudal thorns, leading to reduced resolution. Wear on the thorn tip was also readily evident for these large specimens, and this probably occluded the resolution of bands within this region. Similar wear on the dorsal fin spine of large specimens of the spurdog, *Squalus acanthias* has regularly been encountered, and has also led to reduced band counts (Ketchen, 1975; Soldat, 1982). This problem was overcome somewhat in ageing studies of this species, by measuring the diameter of the spine below the worn area, and estimating the number of bands in the worn area from a smaller specimen where spine wear was not evident (Soldat, 1982). This procedure could also be developed for caudal thorns from larger specimens of slow growing bathyrajid species (e.g. *B. griseocauda*). Longitudinal sectioning, which is commonly used on the dorsal fin spines of the spurdog to increase resolution of bands for ageing purposes (Beamish and McFarlane, 1987), could also be carried out on caudal thorns for these larger specimens.

Caudal thorns proved unsuccessful as an ageing tool for four Irish species investigated (Gallagher, 2000). Although a faint band pattern was discernible on distal margins of thorns from *Raja clavata*, their resolution tended to be variable, and it could not be established if they were formed in a temporally predictable manner. For the other three species no band pattern could be resolved. Although it is unlikely that the thorn growth processes for the Irish species examined are any different to those of the Falkland Islands species, the lack of surface bands, may be due to the fact that the cessation in somatic growth, which most likely causes the ridge-defined band pattern on caudal thorns from the Falkland Islands species, is not sufficiently abrupt to cause surface ridge formation in the Irish species. Further research into the cellular structure of caudal thorns is required to ascertain how the thorn ultra structure changes with different stages of somatic growth.

References

BEAMISH, R. J. and G. A. McFARLANE. 1987. Current trends in age determination methodology. *In*: R. C. Summerfelt and G. E. Hall (eds.) The Age and Growth of Fish, 15-42. The Iowa State University Press, Ames, Iowa.

BRANSTETTER, S. and J. A. MUSICK. 1994. Age and Growth Estimates for the Sand Tiger in the Northwestern Atlantic Ocean. *Trans. Am. Fish. Soc.*, **123**; 242-254.

CAILLIET, G. M. 1990. Elasmobranch age determination and verification: An updated review. *In*: H. L. Pratt, S. H. Gruber and T. Taniuchi (eds.) Elasmobranchs as living resources: Advances in the biology, ecology, systematics and the status of the fisheries., **90**:157-165. US Department of Commerce, Washington.

CASSELMAN, J. M. 1983. Age and growth assessment of fish from their calcified tissue-techniques and tools. *In*: E. D. Prince and P. L. M (eds.) Proceedings of the International Workshop on Age determination of Oceanic Pelagic Fishes: Tunas, Billfishes and Sharks. *NOAA Technical Reports NMFS*, **8**: 157-166. US Department of Commerce, Washington.

CASSELMAN, J. M. 1987. Determination of age and growth. *In*: S. Gill (ed). The biology of fish growth. Academic Press, London, London.

CASSELMAN, J. M. 1990. Growth and Relative Size of Calcified Structures of Fish. *Trans. Am. Fish. Soc.*, **119**: 673-688.

GALLAGHER, M. and C. P. Nolan. 1999. A novel method for the estimation of age and growth in rajids using caudal thorns. *Can. J. Fish. Aquat. Sci.*, **56**: 1590-1599.

GALLAGHER, M. J., 2000. The fisheries biology of commercial ray species from two geographically distinct regions. Ph.D. Thesis, Department of Zoology, University of Dublin, Trinity College, Dublin 2, Ireland.

HOLDEN, M. J. and P. S. MEADOWS. 1962. The structure of the spine of the Spur Dogfish (*Squalus acanthias* L.) and its use for age determination. *J. Mar. Biol. Assoc. UK*, **42**: 179-197.

KETCHEN, K. S. 1975. Age and growth of dogfish *Squalus acanthias* in British Colombia Waters. *J. Fish. Res. Board Can.*, **32**: 43-59.

McFARLANE, G. A. and R. J. BEAMISH. 1987. Validation of the dorsal spine method of age determination for spiny dogfish. *In*: R. C. Summerfelt and G. E. Hall (eds.) The Age and Growth of Fish. 287-300. The Iowa State University Press, Ames, Iowa.

SOLDAT, V. T. 1982. Age and size of spiny dogfish, *Squalus acanthias*, in the Northwest Atlantic. *N. Atl. Fish. Org. Sci. Council Stud.*, **3**: 47-52.
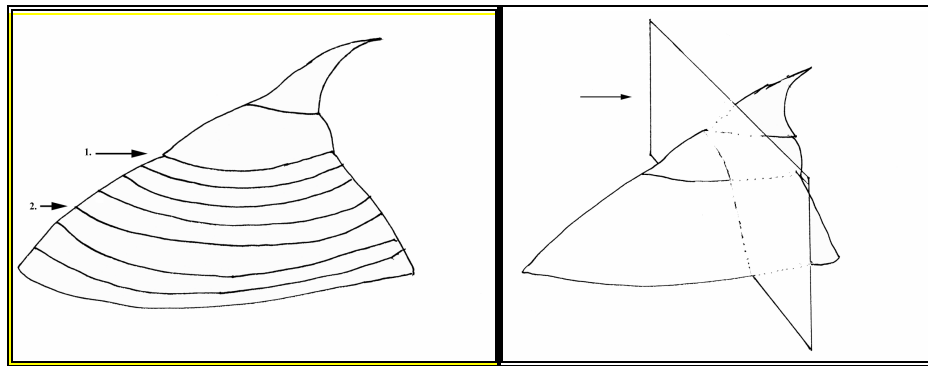
Fig. 1.   Diagrammatic representation of; Left; a whole caudal thorn from *Bathyraja brachyurops.* Arrow 1., Base of proto-thorn; Arrow 2; Surface band and Right; the plane of a transverse section (indicated by arrow) of a resin embedded caudal thorn from a Falkland Islands' ray specimen.
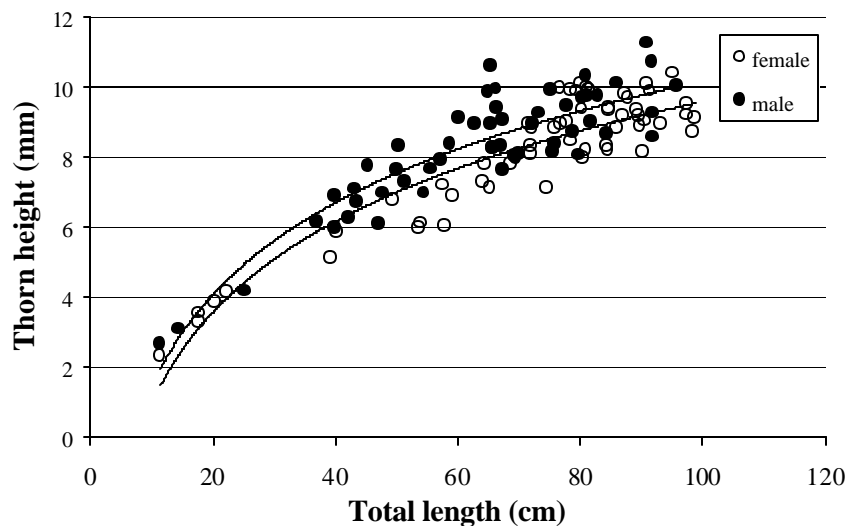

Fig. 2.   The relationship between total length (cm) and thorn height (mm) for male (N= 54) and female (N= 56) *Bathyraja brachyurops.*
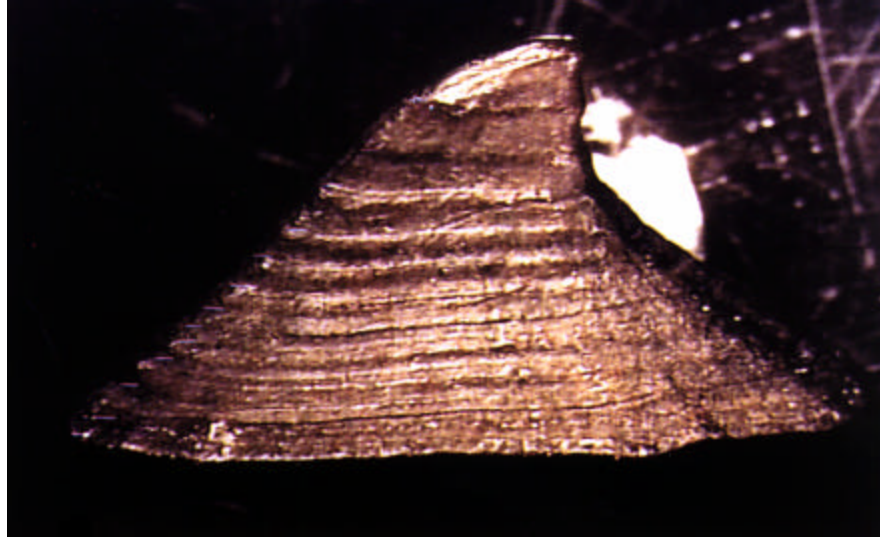
Fig. 3. *Bathyraja sp*.; photomicrograph of a whole unstained caudal thorn from a tetracycline tag-recaptured specimen (Scale bar = 0.94 mm). White arrow denotes area of tetracycline incorporation.
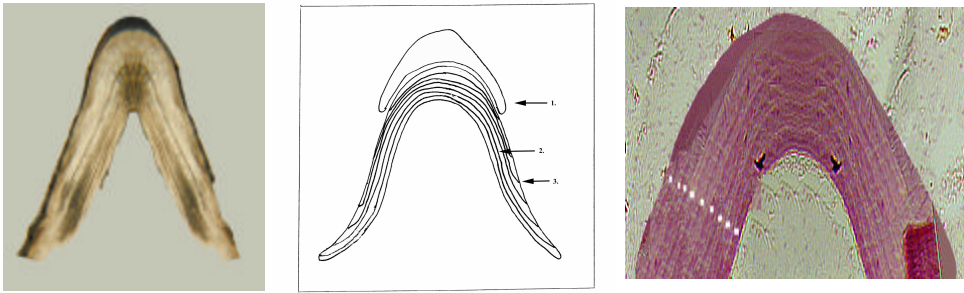

Fig. 4. *Bathyraja brachyurops*; Left; photomicrograph of the apex of a resin embedded caudal thorn in longitudinal section. White bars denote internal band pattern. Scale bar = 0.42 mm; Centre; Diagrammatic representation of a transverse section of a caudal thorn from *B. brachyurops*, showing the relationship between the internal and surface band pattern. 1. Proto-thorn; 2. Internal band; 3. Surface band; Right; photomicrograph of the apex of a decalcified caudal thorn in longitudinal section, stained in haematoxylin and eosin. White bars denote internal band pattern. Scale bar = 0.35 mm.
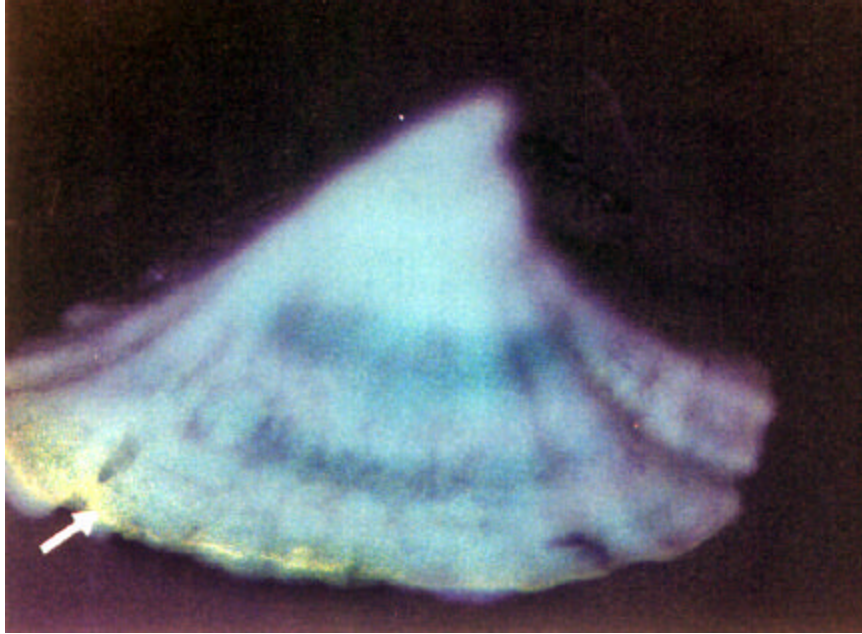

Fig. 5. *Bathyraja sp*.; photomicrograph of a whole unstained caudal thorn from a tetracycline tag-recaptured specimen (Scale bar = 0.94 mm). White arrow denotes area of tetracycline incorporation.