A novel method for the estimation of age and growth in rajids using caudal thorns

M. Gallagher and C.P. Nolan

Abstract: The caudal thorns of four bathyrajid species from the Falkland Islands exhibit well-defined surface ridges. The apparent periodicity of ridge deposition suggests that caudal thorns exhibiting this sculpture have potential for use as ageing devices. Caudal thorns and vertebrae were removed from selected specimens. Thorn surface band counts were compared with counts from longitudinal sections of vertebral centra. Structures were read three times by each of three readers in a double-blind study. Differences in precision were found between readers. Estimates made by the most experienced reader showed least variation. A consistent difference between thorn and centrum band counts was recorded in the males of two species, with centrum counts lower than thorn counts by one growth increment. These differences were attributed to difficulties in interpreting the centrum sections of these species. In all other groups, counts were highly correlated with no significant difference between group means. Caudal thorns represent a novel ageing structure that provides estimates with precision similar to that of more conventional vertebral ageing techniques. Centrum and thorn edge analysis, supported by evidence from a tetracycline-marked individual of a related species, confirms the nature of band formation in both structures. The use of thorns as ageing structures, where appropriate, has the potential to be of significant benefit to the resource assessment and management of skate and ray fisheries.

Résumé : Les épines caudales de quatre espèces de Rajidae des Malouines ont à leur surface des crêtes bien définies. La périodicité apparente du dépôt de ces crêtes laisse penser que les épines caudales portant ces structures pourraient servir à la détermination de l'âge des poissons. On a prélevé les épines caudales et les vertèbres de spécimens choisis. On a comparé les comptes des bandes présentes à la surface des épines aux comptes des bandes de coupes longitudinales des corps vertébraux. Dans le cadre d'une étude à double insu, les structures ont été comptées trois fois par chacune des trois personnes impliquées. On a observé des différences dans la précision des mesures prises par ces personnes. Les estimations réalisées par la personne la plus expérimentée ont montré la plus faible variation. On a obtenu régulièrement une différence entre les comptes des bandes des épines et des corps vertébraux chez les mâles de deux espèces, les comptes des corps vertébraux étant inférieurs d'un incrément de croissance à ceux des épines. Ces différences étaient attribuables aux difficultés inhérentes à l'interprétation des coupes des corps vertébraux de ces espèces. Dans tous les autres groupes, les comptes étaient fortement corrélés et il n'y avait pas de différence significative entre les moyennes des groupes. Les épines caudales sont des structures employées depuis peu pour la détermination de l'âge qui fournissent des estimations aussi précises que les techniques plus classiques utilisant les vertèbres. Les résultats des analyses des corps vertébraux et du bord des épines, combinés à ceux obtenus chez un individu d'une espèce apparentée marqué à la tétracycline, confirment la nature de la formation des bandes sur ces deux structures. L'utilisation des épines comme structures pour la détermination de l'âge peut, quand elle est indiquée, présenter de grands avantages dans l'évaluation des stocks de raies et la gestion de leur pêche.

[Traduit par la Rédaction]

Introduction

The determination of age in commercially important fishes is an important component of fisheries resource assessment (Younger 1975; Casselman 1983). Growth rates, longevity, cohort structure, and ages at other significant periods within the life cycle (maturation, migration, etc.) can be determined and subsequently used in stock assessment and population models.

Due to the commercial importance of rajiformes, age and growth studies have been investigated in this group since the early part of this century (e.g., Steven 1936; Ishiyama 1951). Ageing rajiformes as with most elasmobranch species has historically been accomplished through the analysis of band formation in the vertebral centra (Ishiyama 1951, 1958; Daiber 1960; Taylor and Holden 1964; Du Buit 1972; Cailliet et al. 1983*a*; Waring 1984; Abdel-Aziz 1992; Zeiner and Wolf 1993). To date, few studies have validated the temporal nature of bands observed in vertebral centra (Holden and Vince 1973; Ryland and Ajayi 1984; Natanson 1993), although in many instances, validation often proves unfeasible due to the practical difficulties of recapturing marked animals and the facilities needed for laboratory growth studies.

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Verification is often the only method of assessing the temporal periodicity of band formation. Derived ages have been verified using marginal increment analysis of centra (e.g., Ishiyama 1951; Waring 1984; Abdel-Aziz 1992) and length–frequency analysis (e.g., Brander and Palmer 1985; Smith and Merriner 1987; Martin and Cailliet 1988). The application of length–frequency analysis is, however, generally restricted to discerning the discrete modes of the younger year-classes in a population as subsequent modes become progressively more difficult to resolve and interpret as cohorts increase in age (Fitz and Daiber 1963; Brander and Palmer 1985; Abdel-Aziz 1992).

Other approaches used to determine age in elasmobranchs have included radiometric dating, eye lens weight, tooth replacement rates, and the vertebral accumulation of mercury. The application of these techniques has, however, met with limited success (Strasburg 1963; Applegate 1965; Moss 1977; Ketchen 1975; Welden et al. 1987; Siezen 1989).

The comparison of age estimates between different calcified structures in teleost fish has been routinely employed as a procedure to verify estimates of age (Beamish and McFarlane 1987). In large oceanic pelagic fish (such as tuna and billfishes), otoliths, scales, finrays, vertebrae, sagittae, and operculae have been used successfully for this purpose (Casselman 1983).

To date, the comparison of age estimates derived from different hard parts has not been applied successfully to the assessment of age in elasmobranchs. This paper addresses this deficit and investigates the comparative use of vertebral centra and caudal thorns in the determination of age estimates for four commercially important skate species. A commercial fishery for skates and rays began around the Falkland Islands in 1991. The fishery is multispecies in composition and has realised between 3500 and 8500 t (unprocessed weight) per annum between 1991 and 1997 (Falkland Islands Government 1998). Little biological information is available on the species targeted and the management approach to this fishery has been conservative as a result.

Materials and methods

Collection methodology

A total of 104 *Bathyraja brachyurops*, 126 *Bathyraja griseocauda*, 47 *Bathyraja scaphiops*, and 51 *Bathyraja albomaculata* were obtained by scientific fisheries observers from commercial fishing vessels and scientific research cruises within the Falkland Islands Interim Conservation and Management Zone between 1994 and 1996 (Falkland Islands: 51.45°S, 59.30°W). Individuals were selected from the size range of each species. An optimum of five specimens from each 5-cm size-class by sex formed the sampling objective (Fig. 1).

A vertebral segment containing the first 10 vertebrae immediately posterior to the scapular origin of the vertebral column was extracted and frozen. The first five caudal thorns, posterior to the first haemal arch of the vertebral column (Hubbs and Ishiyama 1968), were also removed from each specimen. A slice was made from posterior to anterior that bisected the caudal vertebral column horizontally and in so doing removed a segment of the tail containing the required thorns.

Tissue was removed from both the vertebral and thorn segments by immersing the segments in hot water. Thorns were removed from surrounding tissue using forceps and cleaned gently in running water prior to drying. Vertebral segments and extracted thorns were subsequently air dried for storage prior to further processing.

Preparation and processing techniques

Vertebral centra

Vertebral sections were immersed in a 5% solution of trypsin for 24 h to remove the neural and haemal arches and the intervertebral connective tissue. The two largest centra from the thoracic region of each vertebral column were selected for preparation, as the internal pattern of vertebrae is generally more difficult to read with decreasing size (Taylor and Holden 1964; Cailliet et al. 1983*b*; Officer et al. 1996). The centra were sectioned longitudinally with a bench grinder and polished by hand with progressively finer grades of carborundum paper (P620–P1200) using tap water as a lubricant. The polished faces of the centra were embedded in epoxy resin and were further ground and polished to a section thickness of 60–90 μm. Very small, fragile centra (<8 mm centrum diameter) were embedded whole in epoxy resin before grinding.

Previously successful enhancement techniques for elasmobranch centra were assessed for each of the species examined (e.g., alizarin red (LaMarca 1966), silver nitrate (Stevens 1975), cobalt nitrate (Hoenig and Brown 1988)). The most effective methodology proved to be a modification of a grinding and polishing technique described by Smith (1984) followed by staining.

Centrum segments were etched in a dilute solution of acid alcohol (5%) to enhance the band pattern and subsequently immersed for 1–4 h, depending on the species, in a crystal violet solution (0.005%) (Schwartz 1983). The 0.005% solution was used instead of the standard 0.01% solution (Schwartz 1983), as it was found that a longer immersion time in a more dilute solution increased band resolution. Staining times were not dependent on centrum size for *B. albomaculata* and *B. scaphiops*. Centra from these species were stained for 140 min. For *B. brachyurops* and *B. griseocauda*, staining times were increased with increasing centrum size. The optimum staining times were 120 and 70 min, respectively, for centra from these species.

Caudal thorns

Caudal thorns were cleaned by immersion in a 5% solution of trypsin for 12 h. After cleaning, the thorns appeared slightly translucent, with an external band pattern apparent on the thorn surface of all species examined.

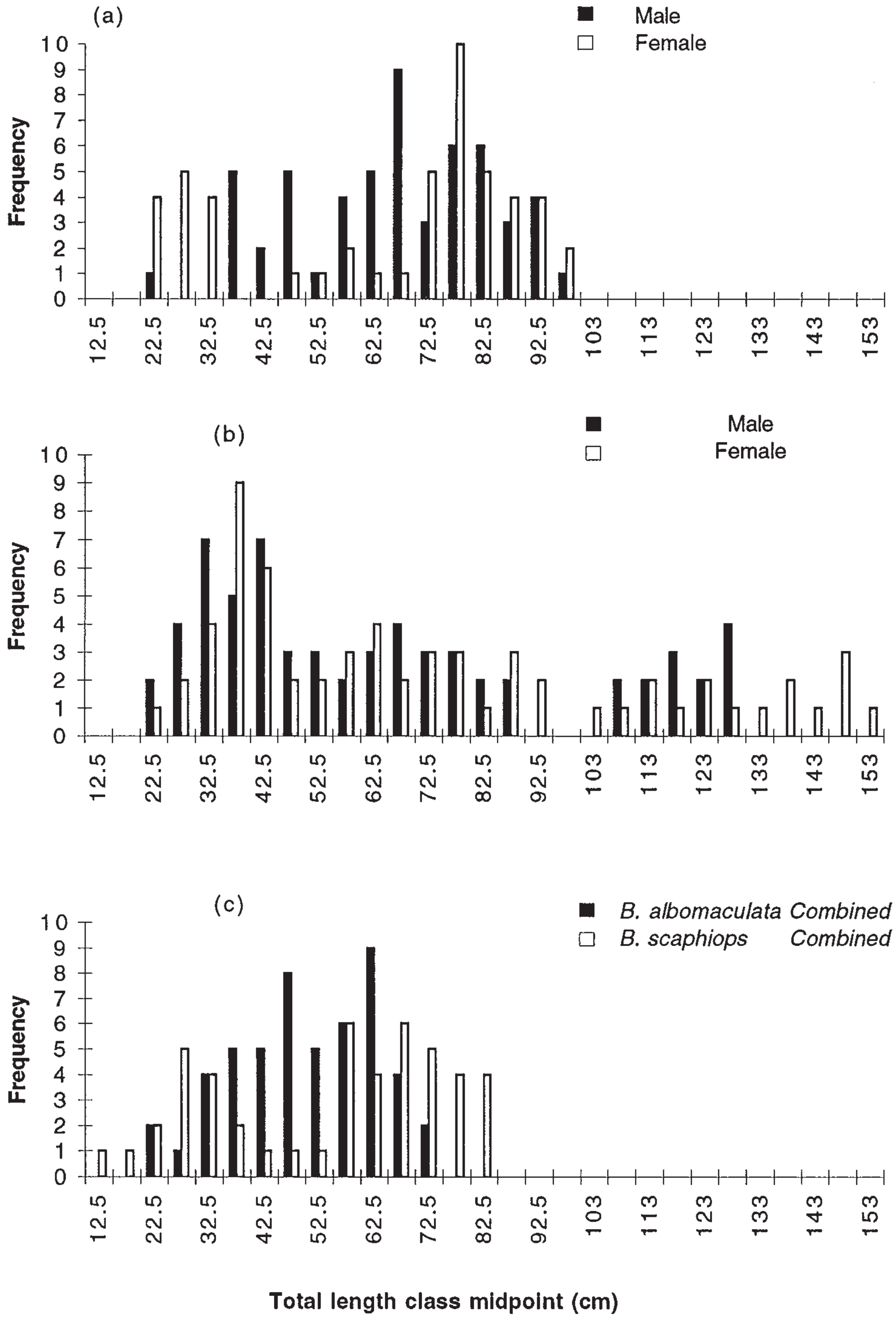
The clarity of the banding pattern was improved after etching the thorns in a 5% solution of ethylenediaminetetraacetic acid for periods of between 5 and 10 min. Further enhancement of the band pattern was accomplished by staining the etched thorns. Several staining techniques were assessed (e.g., alizarin red, cobalt nitrate). A modification of a staining technique using silver nitrate (Stevens 1975) proved to be most effective. Thorns were immersed in a 1% solution of silver nitrate for a period of 40–70 min, which was followed by exposure to ultraviolet light.

This technique involves the substitution of calcium in calcium salts in the matrix of the thorn by silver, which is subsequently reduced to black metallic silver. The reaction was terminated by washing in distilled water for 30 s followed by immersion for 10 min in a 5% solution of sodium thiosulphate. The process enhanced the resolution of calcified bands on the thorns, with these areas appearing as dark ridges on the external surface of an opaque thorn matrix.

Reading methodology

Banding patterns on whole thorns and centrum sections were read using a stereomicroscope at a magnification between 6.7 and 40×. A fibreoptic light source was used to illuminate the vertebrae and thorns dorsally and laterally, which enabled resolution of bands using both transmitted and reflected light.

Fig. 1. Length–frequency distribution by sex of specimens of (*a*) *B. brachyurops*, (*b*) *B. griseocauda*, and (*c*) *B. albomaculata* and *B. scaphiops* from which vertebrae and thorns were extracted and processed in this study.

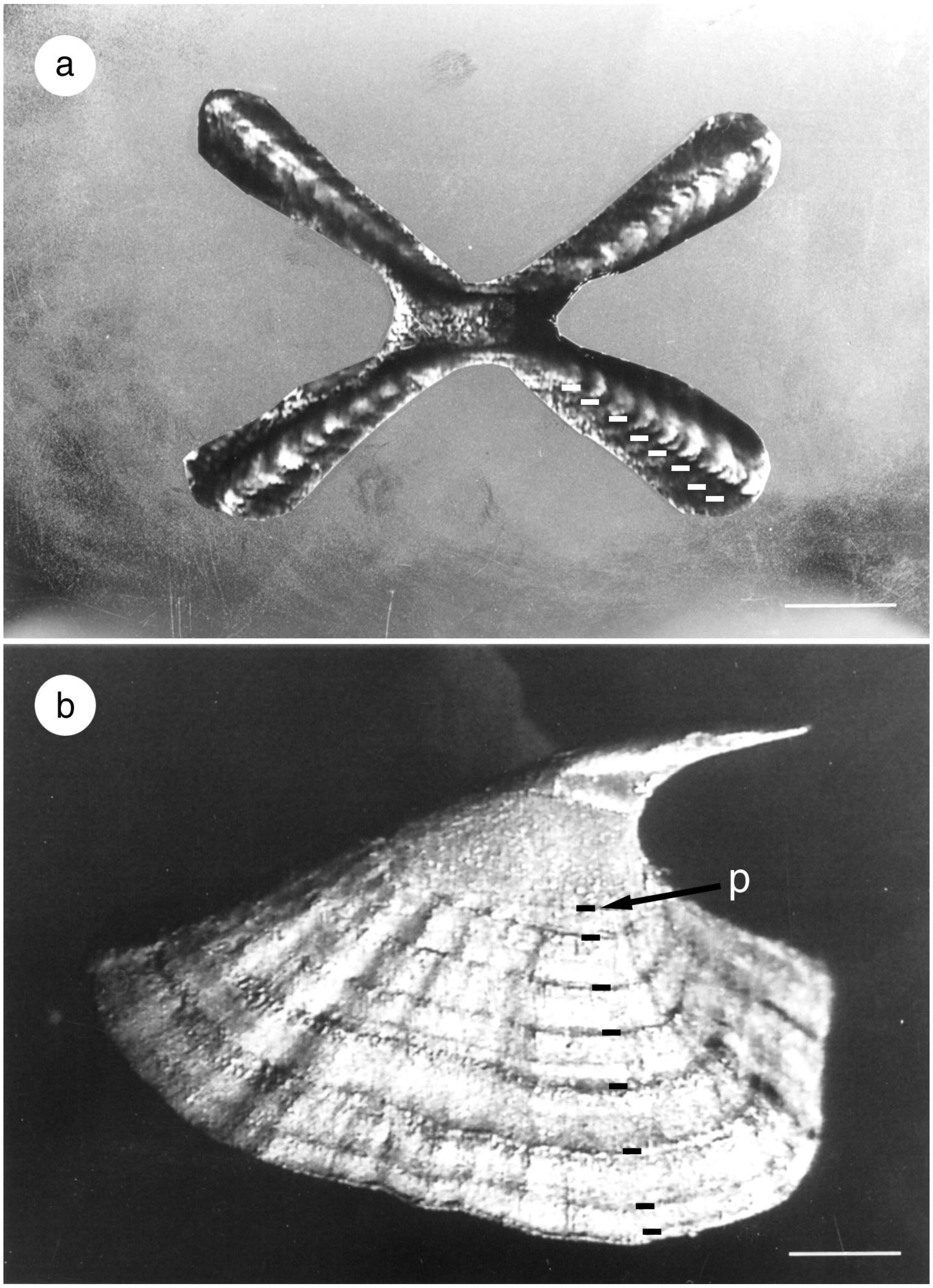


In centrum sections, the axis of the centra showing a complete and distinct series of stained bands was chosen as the main reading axis. Bands were counted from the centre of the section to the distal margin along this axis. The three remaining axes on each section were used during the reading process to verify band occurrences on the main axis and provide corroboration for band counts in areas of poor resolution on the primary axis.

Banding patterns on caudal thorns were read with reference to a protothorn or embryonic thorn that was evident on the apex of all thorns examined (Fig. 2). The base of the protothorn was used as the datum from which all other subsequent bands on the anterior surface were counted. Each of the prepared centra and thorns were read on three independent occasions by one reader in a double-blind study. A subsample of thorns (32 from *B. brachyurops*, 35 from *B. griseocauda*, 24 from *B. scaphiops*, and 26 from *B. albomaculata*) and centra (37 from *B. brachyurops*, 33 from *B. griseocauda*, 26 from *B. scaphiops*, and 26 from *B. albomaculata*) was also read by a second and third reader on three separate occasions, independently, for a within- and between-reader comparison.

Thorn and centrum band count precision was assessed on this subsample of centra and thorns from each of the species examined using Chang's (1982) coefficient of variation (*V*) and index of precision (*D*) described as follows:

Fig. 2. (*a*) Photomicrograph of a vertebral centrum in transverse section stained with 0.005% crystal violet. Scale bar = 1 mm. (*b*) Photomicrograph of a caudal thorn in lateral view stained with silver nitrate. Scale bar = 1.1 mm; p = protothorn. Both structures were extracted from an immature male *B. griseocauda* of 654 mm total length, estimated to be 7 years old. Annual increments are indicated by small bars on each structure (white bars on Fig. 2*a* and black on Fig. 2*b*).



$$V = \frac{s}{\bar{Y}} \times 100$$

$$D = \frac{\left(\frac{s}{\bar{Y}} \times 100\right)}{\sqrt{R}}$$

where s is the standard deviation of repeated counts, R is the number of counts per individual, and $\bar{Y}$ is the mean of repeated counts. The index of precision represents the percent error contributed by each reading to the average of multiple readings of the same structure.

The similarity of thorn and centrum band counts for each of the specimens processed by the primary reader was examined using a paired t test, while the correlation between counts was assessed using Pearson's product-moment correlation. Male and female *B. brachyurops* and *B. griseocauda* were treated separately, while the sexes were combined for *B. scaphiops* and *B. albomaculata* due to their small sample sizes.

Centrum and thorn edge analysis

Periodicity of band formation on the surface of thorns and within the structure of the vertebral centrum was assessed by correlating the date of capture with the position of the peripheral band

Table 1. Chang's (1982) coefficient of variation ($\bar{V}$) and mean reader precision ($\bar{D}$) for band counts made by multiple readers on samples of vertebral centra and caudal thorns from *B. brachyurops*, *B. griseocauda, B. albomaculata*, and *B. scaphiops*.

		Primary reader			Reader 2			Reader 3		
Species	Structure	N	$\bar{V}$	$\bar{D}$	N	$\bar{V}$	$\bar{D}$	N	$\bar{V}$	$\bar{D}$
B. brachyurops	Centra	37	0.055	0.032	37	0.087	0.050	37	0.107	0.062
	Thorns	32	0.041	0.024	32	0.084	0.049	32	0.088	0.051
B. griseocauda	Centra	33	0.049	0.029	33	0.119	0.069	33	0.155	0.090
	Thorns	35	0.067	0.039	35	0.099	0.057	35	0.121	0.070
B. albomaculata	Centra	26	0.069	0.040	26	0.110	0.064	26	0.113	0.065
	Thorns	24	0.056	0.032	24	0.093	0.054	24	0.086	0.050
B. scaphiops	Centra	26	0.088	0.051	26	0.097	0.056	26	0.088	0.051
	Thorns	26	0.092	0.053	26	0.086	0.049	26	0.106	0.061

forming subsequent to the last fully formed band on a selection of samples from each species.

The band structure evident in the distal edge zone of each of the structures was categorised using a modification of the grading system described by Yudin and Cailliet (1990). The grades were as follows. (1) Centrum: opaque band forming at the marginal edge; thorn: narrow ridge forming at the marginal edge; (2) centrum: translucent band beginning to form at the marginal edge; thorn: broad band beginning to form at the marginal edge; (3) centrum: translucent band well formed at the marginal edge; thorn: broad band well formed at the marginal edge.

Mark–recapture

In support of this study, a programme of mark–recapture involving intraperitoneal injection of oxytetracycline (25 mg·kg body mass^{-1}) has accounted for 550 bathyrajids being tagged to date. This approach has proven to be successful in the validation of several species of elasmobranchs (e.g., Gruber and Stout 1983; Ryland and Ajayi 1984; Smith 1984; Casey et al. 1985; Tucker 1985; Branstetter 1987).

Results

Of the conventional staining techniques previously used on elasmobranch vertebral centra, staining with silver nitrate was the only technique to significantly enhance the banding pattern on thorns from the species examined. The process proved to be very effective and consistent on the thorns of all species and was particularly quick and efficient in its application.

The precision, consistency, and repeatability of thorn and centra counts revealed no significant differences within readers when tested using Chang's (1982) coefficient of variation and index of precision (Table 1).

Differences in precision among readers were apparent, however, when the counts from samples of equal size were compared. With the exception of *B. scaphiops*, there was greater variation and less precision in the centra and thorn count estimates made by the second and third readers than those made by the more experienced primary reader. Thorn band count precision was generally of the same magnitude as that for centra within species. In general, only small differences in precision were recorded between structures by each reader. In the majority of cases, however, the precision of thorn band counts was greater than that of counts from vertebral centra.

Comparison of individual thorn and centra counts by sex revealed similar differences related to structure. All groups tested exhibited a high product-moment correlation (>0.85), implying the existence of a relatively consistent relationship between thorn and centra counts (Table 2). Differences between the sexes were seen between counts in *B. brachyurops* and *B. griseocauda*. In both species, there was greater agreement between counts in females, although this was not significant in either case.

Figure 3 presents the distribution of count differences for the primary reader. Centra and thorn counts agreed exactly in 129 (39%) of 328 measurements made by the primary reader over the entire range of samples examined. Centra counts were greater than thorn counts in 18% (58) of cases and less than thorn counts in 26% (85) of cases. In all species except *B. brachyurops*, the distribution of count differences was skewed, with centra counts being lower than thorn counts. Male *B. brachyurops* and female *B. griseocauda* showed the greatest difference in this regard.

Edge analysis of thorns and centra in all species showed similar patterns of calcification in temporally similar samples. Although variability is apparent in the timing of band formation between species and structures, a trend in band formation can be established. At the end of the autumn and entering winter (June), the grade 3 phase dominated the edge characteristics of the majority of samples (Fig. 4). Opaque band formation (grade 1) was also apparent in this month, implying that June and July are most likely to be the changeover period from translucent to opaque band formation in vertebrae and from broad band to narrow ridge deposition in thorns. The grade 1 phase was prevalent in samples of vertebrae from *B. brachyurops* collected during the spring (September). For samples collected entering the summer months (October), the grade 1 phase was also apparent. November is the probable period of changeover from grade 1 to grade 2. This would appear to occur at a slightly earlier stage in thorns than in vertebrae, as grade 1 appeared to dominate in vertebrae and grade 2 dominated in thorns collected during this month. An annual cycle of translucent and opaque bands in centra and a ridge-defined growth cycle in caudal thorns are therefore supported by these observations.

A single tagged and tetracycline-marked specimen of a related bathyrajid species was recovered. The animal was an immature male and was at liberty for 10 months (tagged 8 July 1994, recaptured 6 May 1995). This species is morphologically similar to and a cohabitor with *B. brachyurops* and consequently is often misidentified as *B. brachyurops*. A de-

Table 2. Comparison of the caudal thorn and vertebral centrum band counts between the sexes in *B.brachyurops* and *B. griseocauda*, and combined by sex in *B. albomaculata* and *B. scaphiops*.

Species	Count type	df	*N*	Mean	Variance	*t*	Mean of paired difference	*P*	R^2
B. brachyurops									
Male	Thorn	54	55	8.946	8.867	0.866	0.127	0.390	0.886
	Vertebral	54	55	9.073	10.402				
Female	Thorn	48	49	8.449	18.919	–0.125	–0.020	0.901	0.934
	Vertebral	48	49	8.429	19.833				
B. griseocauda									
Male	Thorn	62	63	6.142	13.641	–4.073	–0.397	<0.0001	0.965
	Vertebral	62	63	5.746	15.773				
Female	Thorn	62	63	6.683	15.833	–1.206	–0.206	0.232	0.909
	Vertebral	62	63	6.476	19.737				
B. albomaculata (combined sexes)	Thorn	50	51	8.64	12.953	–0.922	–0.177	0.361	0.862
	Vertebral	50	51	8.471	13.134				
B. scaphiops (combined sexes)	Thorn	46	47	6.617	12.894	–0.375	–0.064	0.710	0.899
	Vertebral	46	47	6.553	13.427				

Note: Student's paired *t* statistic with associated probablity values (*P*) are given; Pearson's product-moment correlation (R^2) values, indicating the correlation between thorn and vertebral bands, are also presented.

scription of this species is currently being prepared by the second author.

Examination of unstained centrum sections and caudal thorns from this winter-tagged animal under ultraviolet light revealed a distinct fluorescent mark on both structures. On centrum sections and in thorns, growth was apparent distal to the position of the tetracycline mark. A variety of staining techniques were used on centrum sections (crystal violet, alizarin red, and silver nitrate) that demonstrated that tetracycline was incorporated into a calcium-dense opaque band at this time of year. The formation of a translucent band distal to the heavily stained band was evident. The centrum edge was thus characterised as approaching grade 3, which corroborates the evidence from the centrum edge study conducted on each of the species.

Tetracycline was incorporated in a narrow ridge in the thorns, suggesting that ridge formation occurs during the winter. A broad band was apparent distal to the fluorescent mark and the edge categorised as approaching grade 3. Although this recaptured specimen was not one of the species investigated in this study, analysis of the stained vertebral sections and caudal thorns revealed that the animal had eight bands at tagging (the assumed point of tetracycline incorporation). This agrees with the size at age for *B. brachyurops*, and therefore, it is assumed that band formation occurs at a similar rate in this recaptured species as in the other species investigated in this study.

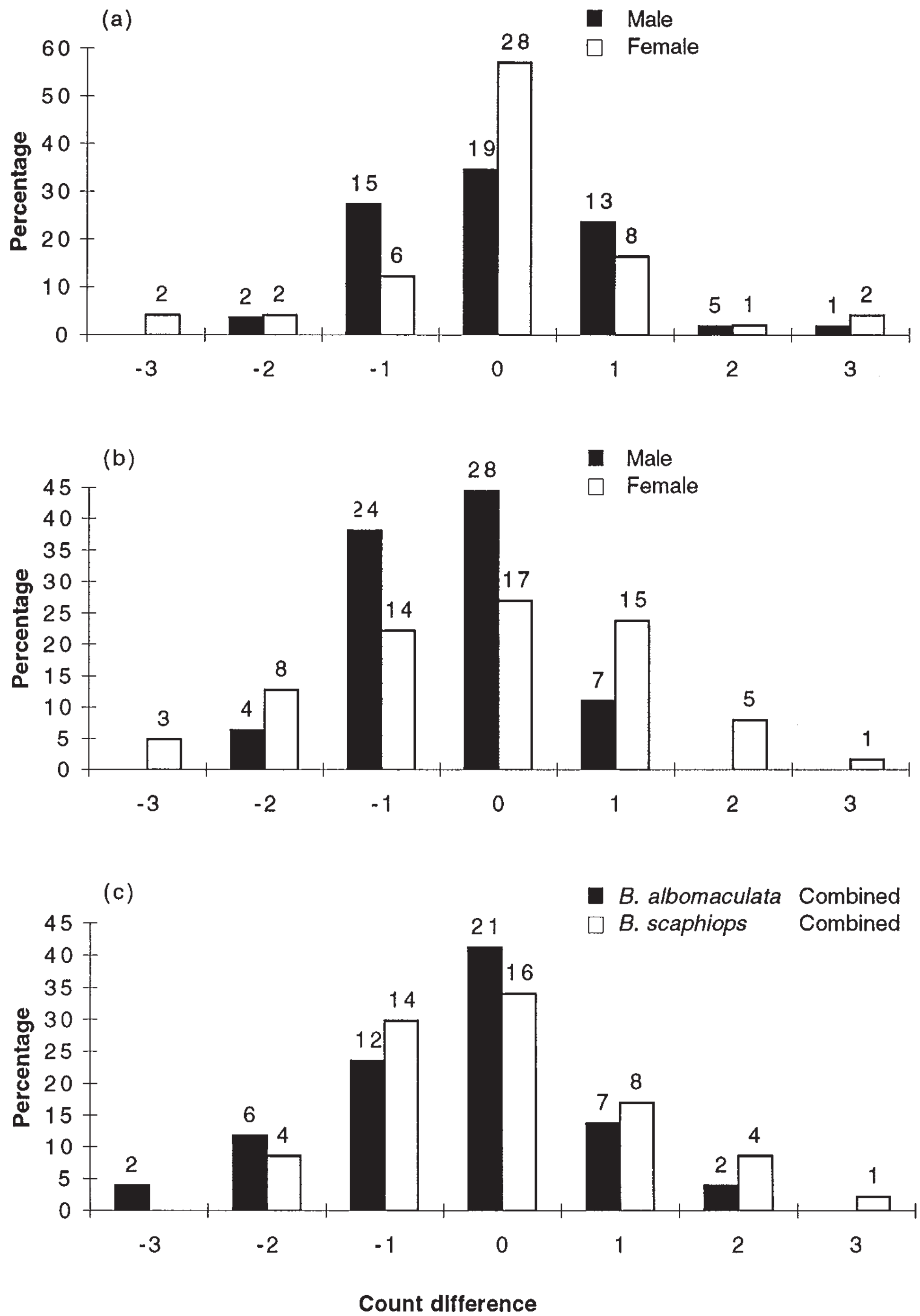
Discussion

To date, the hard parts used to estimate age in elasmobranchs have been restricted to vertebral centra and dorsal fin spines (Correia and Figueiredo 1997). Several studies have effectively used spines in age determination studies. However, comparison of age estimates from spines (e.g., Holden and Meadows 1962; Ketchen 1975) with readings from centra has generally proved inconclusive, due to the incomplete and irregular banding pattern observed in the vertebrae (Kaganovskaia 1933; Soldat 1982; Cailliet et al. 1983*b*; Polat and Gumus 1995). Lack of such agreement is generally accepted and has been highlighted in a number of previous ageing studies using hard parts in bony fish (Casselman 1983; Beamish and McFarlane 1987).

Although of similar defensive purpose and placoid scale origin, the structural differences between elasmobranch dorsal fin spines and the caudal thorns of the rajids are significant. In contrast with the enamel-like structure of fin spines, the caudal thorns of the bathyrajids examined in this study are convexly placoid and securely embedded and anchored within the caudal tissue. The thorns exhibit a definite external sculpture with distinctive circular ridges that vary in definition and resolution among species. The apparent periodicity and definition of the surface sculpture in the species lead to the investigation of these structures as tools for the determination of age. Initial observations reveal that thorn growth and band formation occurs in a manner similar to that of dorsal spines in *Squalus acanthias* (Holden and Meadows 1962). This process is currently being investigated through histological analysis by the senior author.

Thorns have a number of distinct advantages over centra in their use as potential ageing tools. Thorns can be removed and cleaned with comparative ease and, importantly, may be removed unobtrusively and with minimal damage to fish of commercial value. A major advantage with thorns is that they can also be removed without sacrifice of or detrimental effect to the animal, a feature that is likely to prove invaluable in tagging studies where species suiting the technique are the focus of research. Difficulties have arisen in previous studies in the determination of the counting reference point or nucleus and the first growth band (e.g., Brander and Palmer 1985) in centra used as ageing tools. The caudal thorns of the species examined in this study showed that location of the first growth band is perhaps the most distinctive of all the external sculpture and is recognised with relative ease, even prior to staining. Given that previous studies have met with varied levels of success comparing readings

Fig. 3. Percent frequency, by sex, of agreement between vertebral centrum and thorn band counts in (*a*) *B. brachyurops*, (*b*) *B. griseocauda*, and (*c*) *B. scaphiops* and *B. albomaculata* made by the primary reader. Column totals indicate number of individuals. The difference between counts is expressed as the vertebral centrum count less the thorn band count.



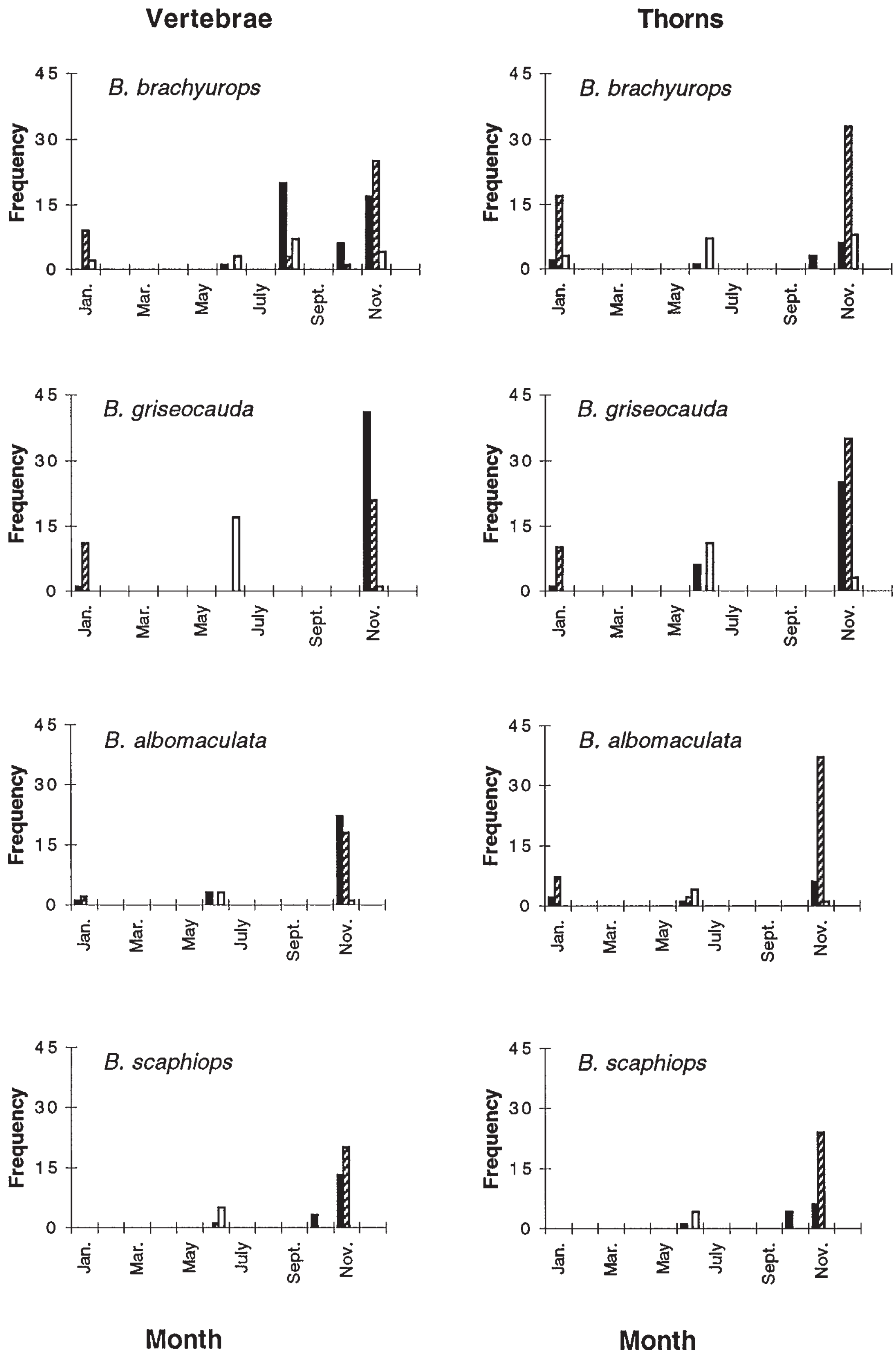
from hard parts and vertebrae, the high level of agreement existing between thorn and centrum band counts in the species examined in this study is encouraging.

Although reader precision appears to be related to thorn morphology and size, the ease with which thorns can be read by readers with little experience, to a high degree of precision, advocates the use of thorns, where possible, in preference to vertebral centra as ageing structures.

In this study, reader precision was highest for thorn band counts from species with large thorns carrying a distinct banding pattern (e.g., *B. albomaculata*). Similarly, the lowest level of precision was associated with small thorns and difficulties experienced resolving thorn banding pattern due to poor and indistinct surface sculpture (e.g., *B. scaphiops*).

The consistent disagreement between counts in male *B. griseocauda* and to some extent in male *B. brachyurops* over the size range examined suggests a possible physiological difference effecting the calcification of the vertebral centra, which may be associated with dietary or temperature changes throughout life (Jones and Geen 1977; Ferreira and

Fig. 4. Temporal characteristics in the structure of the growing edge of vertebral centra and thorns from *B. brachyurops*, *B. griseocauda*, *B. albomaculata*, and *B. scaphiops* described using a modification of the grading system defined by Yudin and Cailliet (1990). Solid bars, grade 1; hatched bars, grade 2; open bars, grade 3.



Vooren 1991). The banding pattern in vertebral sections in both sexes of *B. griseocauda* appears to alter in density and variability with increasing body size. This leads to difficulties in interpreting and resolving bands, a situation that has previously been encountered in other elasmobranch species (e.g., Stevens 1975; Casey et al. 1985; Ferreira and Vooren 1991; Officer et al. 1997).

The propensity for underreading of the banding pattern in centra, relative to thorn band counts, particularly in male individuals, suggests poor resolution of periodic structures within centrum sections that is likely to be most prevalent in the centra of older, slower growing individuals, particularly at the centre and distal margins.

Thorn and centrum edge analysis describes a structural cycle of growth band formation that supports the supposition of annual periodicity. Analysis of the vertebral centra and thorns from a tetracycline-marked and recovered specimen lends support to the conclusions of the centrum edge analysis that a single band pair is laid down annually in the vertebrae of the species investigated in this study. Similarly, the position of the fluorescent mark relative to the date of tagging indicates that tetracycline was incorporated into an opaque, calcium-rich band in vertebral sections. This agrees with previous findings in other elasmobranch species where hypermineralisation of vertebrae occurs when growth is slowest (Ishiyama 1951; Yudin and Cailliet 1990; Officer et al. 1997). Both thorn edge analysis and the position of the fluorescent mark reveal that narrow ridge formation occurs during the slower growth phase in caudal thorns. It is hoped that with further recaptures of tetracycline-marked specimens that validation at the species level will be achieved.

The novel approach to estimating age in skates and rays using caudal thorns is a quick, easy, and reliable method. Given the accepted difficulties dedicating resources to the conventional vertebral ageing of these species, the application of this technique, where appropriate, has the potential to be of significant benefit to the resource assessment and management of skate and ray stocks.

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