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Feasibility of ageing pale ghost sharks (*Hydrolagus bemisi*)

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National Institute of Water and Atmospheric Research

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Final Research Report

Report Title: Feasibility of ageing pale ghost sharks (*Hydrolagus bemisi*)

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2. **Contractor:** National Institute of Water and Atmospheric Research Limited

3. **Project Title:** Age and growth of pale and dark ghost sharks

4. **Project Code:** GSH2002/01

5. **Project Leader:** Malcolm Francis

6. **Duration of Project:**

   Start date: 1 March 2003
   Completion date: 31 December 2003

7. **Executive Summary:**

Eye lenses and dorsal fin spines of pale ghost sharks were examined in an attempt to develop a suitable ageing technique for this species. Lenses were measured and weighed, and frequency distributions of these variables examined for modal structure that might correspond with age classes. Spines were sectioned and examined under a variety of lighting and after staining to visualise growth bands.

The utility of eye lens diameters and weights as indicators of the age of pale ghost sharks could not be determined. Samples contained too few juveniles to assess the presence or absence of modes that might correspond with age classes. Juvenile pale ghost sharks are uncommon in research trawl samples and commercial catches, and this is a considerable obstacle to determining the feasibility of this technique.

Sections of dorsal fin spines show much greater promise for ageing pale ghost sharks. Growth bands are visible, and their readability is greatly enhanced by DIC lighting, and to a lesser extent, staining with silver nitrate or Meyer's haematoxylin, and viewing under ultraviolet light. Two readers had moderately good agreement in band counts for smaller fish. Length-at-age estimates by both readers corresponded moderately well with growth curves generated by MULTIFAN analysis of length-frequency data in a previous study, although spine counts suggested a faster initial

growth rate than did length-frequency data. We conclude that band counts on sections of dorsal fin spines offer a feasible method for ageing pale ghost sharks. Recommendations are made for further development of the technique. Spine bands and length-frequency analyses both suggest that pale ghost sharks are moderately fast growing and have medium longevity.

8. Objectives:

2. To investigate the feasibility of ageing pale ghost sharks in GSP 1.

9. Introduction

In 1999–2000, NIWA was contracted by the Ministry of Fisheries to carry out project GSH1999/01, Age and growth of ghost sharks. The specific objective of that project was to determine the feasibility of ageing dark and pale ghost sharks. In that project, we used length-frequency data to develop growth curves for both dark ghost shark (*Hydrolagus novaezealandiae*) and pale ghost shark (*H. bemisi*, formerly known as *Hydrolagus* sp. B2; (Didier 2002)) from a number of regions around southern New Zealand. We also investigated the utility of vertebrae, eye lenses, and thick sections of dorsal fin spines in ageing the two species, but focused mainly on dark ghost shark (Francis & Ó Maolagáin 2000).

In 2000–01, we carried out a more detailed investigation of the feasibility of ageing dark ghost shark in project MOF2000/03C (Francis & Ó Maolagáin 2001). The present report, prepared under project GSH2002/01, is the third in the series. It presents a more detailed study of eye lenses and fin spines of pale ghost shark in an attempt to develop an ageing technique for this species.

10. Methods:

Pale ghost shark samples

Pale ghost sharks were collected from the Chatham Rise, the Stewart Island–Snares Shelf, and Campbell Plateau during research trawl surveys (Table 1). Most samples came from a December–January middle depths trawl survey of Chatham Rise.

Table 1: Samples of pale ghost shark collected for age determination on *Tangaroa* voyages.

			Number of ghost sharks		
Date	Voyage	Region	Female	Male	Total
Jun–Jul 2002	TAN0208	NW Chatham Rise	7	1	8
Sep–Oct 2002	TAN0213	SW Chatham Rise	18	4	22
Nov–Dec 2002	TAN0219	Stewart-Snares-Campbell	27	31	58
Dec 2002–Jan 2003	TAN0301	Chatham Rise	141	144	286
Total			193	180	374

Ghost sharks were sexed and measured fresh to the centimetre below caudal length (CL; distance between the tip of the snout and the posterior end of the caudal fin, excluding the tail filament).

Eye lenses

The analysis of eye lenses was restricted to data collected from the same region at the same time, to avoid potential confounding of results. The Chatham Rise sample collected during voyage TAN0301 was used for this purpose, as it was the largest sample available.

The left and right eye lenses were removed, sealed in zip-lock plastic bags, and frozen. The lenses consist of a solid crystalline core surrounded by a sticky gelatinous fluid, and encapsulated in a tough membrane. After thawing, lens cores were dissected out and the greatest core diameter was measured to the nearest 0.001 mm with a digital micrometer. The core was also weighed wet to the nearest 0.0001 g. Diameters were grouped into 0.2 mm class intervals, and weights were grouped into 0.05 g intervals for analysis. Only data for the left lenses are presented here.

Eye lens measurements from samples collected in 1999–2000 from the Chatham Rise at the same time of year (i.e. December–January), and presented in our earlier study (GSH1999/01), were compared with the 2003 data.

Fin spines

The dorsal fin spine was removed from sub-samples of pale ghost sharks for sectioning. Ghost shark spines grow by deposition of new cones of dentine inside the older cones. Sections taken too close to the spine tip do not contain the most recently deposited cones, and sections taken too close to the base do not contain the earliest cones. The best sectioning location (ca. 5–7 mm from the spine tip) was determined by taking serial transverse sections at different distances from the tip, and identifying the location where the greatest number of bands was present (Francis & Ó Maolagáin 2000). However, as we cannot be sure that all growth bands were present and recognisable in these sections, a further section was taken approximately 10 mm from the tip.

In preparation for sectioning, spines were cleaned in household-strength bleach for 30–45 minutes, washed in water, air dried for about one week, and embedded in a block of epoxy resin (Araldite K142). Thick sections were cut with a dual-bladed, precision diamond saw (Struers Accutom 2). One side of each section was polished using a graduated series of carborundum paper, then glued to a glass microscope slide using thermoplastic cement. The other side of the section was similarly polished until rings became discernible under the microscope. Final section thickness ranged from 0.15 to 0.30 mm.

Some sections (4–6 depending on the stain) were stained with silver nitrate, Meyer's haematoxylin, or alizarin in an attempt to improve the clarity of the bands.

Growth bands in the spine sections were counted by two readers at 40–100x magnification using transmitted light, with and without differential interference contrast (DIC), and reflected ultraviolet light. DIC "converts specimen optical path gradients into amplitude differences that can be visualized as improved contrast in the resulting image" (http://micro.magnet.fsu.edu/primer/techniques/dic/dichome.html).

Reader 1 counted the sections with knowledge of the length of the fish, whereas Reader 2 counted them without knowledge of the length. The outermost band was omitted from the counts because it was assumed to be laid down in the egg case (ghost sharks hatch with a well developed spine). Band counts were assessed for between-reader ageing bias using an age-bias plot (Campana et al. 1995).

11. Results:

Eye lenses

Most of the 2002–03 Chatham Rise pale ghost sharks available for eye lens analyses were large mature individuals exceeding 60 cm CL (Figures 1 & 2). Only 26% of females and 27% of males were less than 60 cm CL. Individuals less than 40 cm long are rarely caught on the Chatham Rise or elsewhere in New Zealand (Horn 1997; Francis & Ó Maolagáin 2000). The small sample size of juveniles means that any modal structure present in lens diameter or weight frequencies is difficult to detect. There are possible modes at about 13 mm and 14 mm lens diameter for both sexes, and at 14.5 mm for males (middle panels of Figures 1 & 2), but these may be artifacts of the length distributions of pale ghost sharks, which have corresponding modes (top panels).

The lens weight distributions (bottom panels of Figures 1 & 2) are similarly difficult to interpret: there are one or two modes at small lens weights (less than 0.8 g) but because there are no obvious length-frequency modes for fish shorter than 60 cm, it is not clear whether these weight modes correspond with individual year classes or an aggregation of two or more year classes, or whether they are artifacts of the length distributions.

Data collected from the Chatham Rise in 1999–2000 are also shown in Figures 1 & 2 for comparison. There was a marked absence of small fish in this sample, and virtually no modal structure. These data therefore offer little help in interpreting the 2002–03 data.

Fin spines

In spine sections viewed with white light and ultraviolet light, growth bands were poorly defined and very difficult to count. Ultraviolet light enhanced the bands in some sections, but not others. DIC with transmitted white light gave much better results, though counts were still difficult. Thick sections viewed with DIC light for a range of ghost shark sizes (17–83 cm CL) are shown in Figure 3.

Of the three stains tested, Meyer's haematoxylin and silver nitrate both slightly improved the contrast and the readability of bands, while alizarin appeared to make no difference.

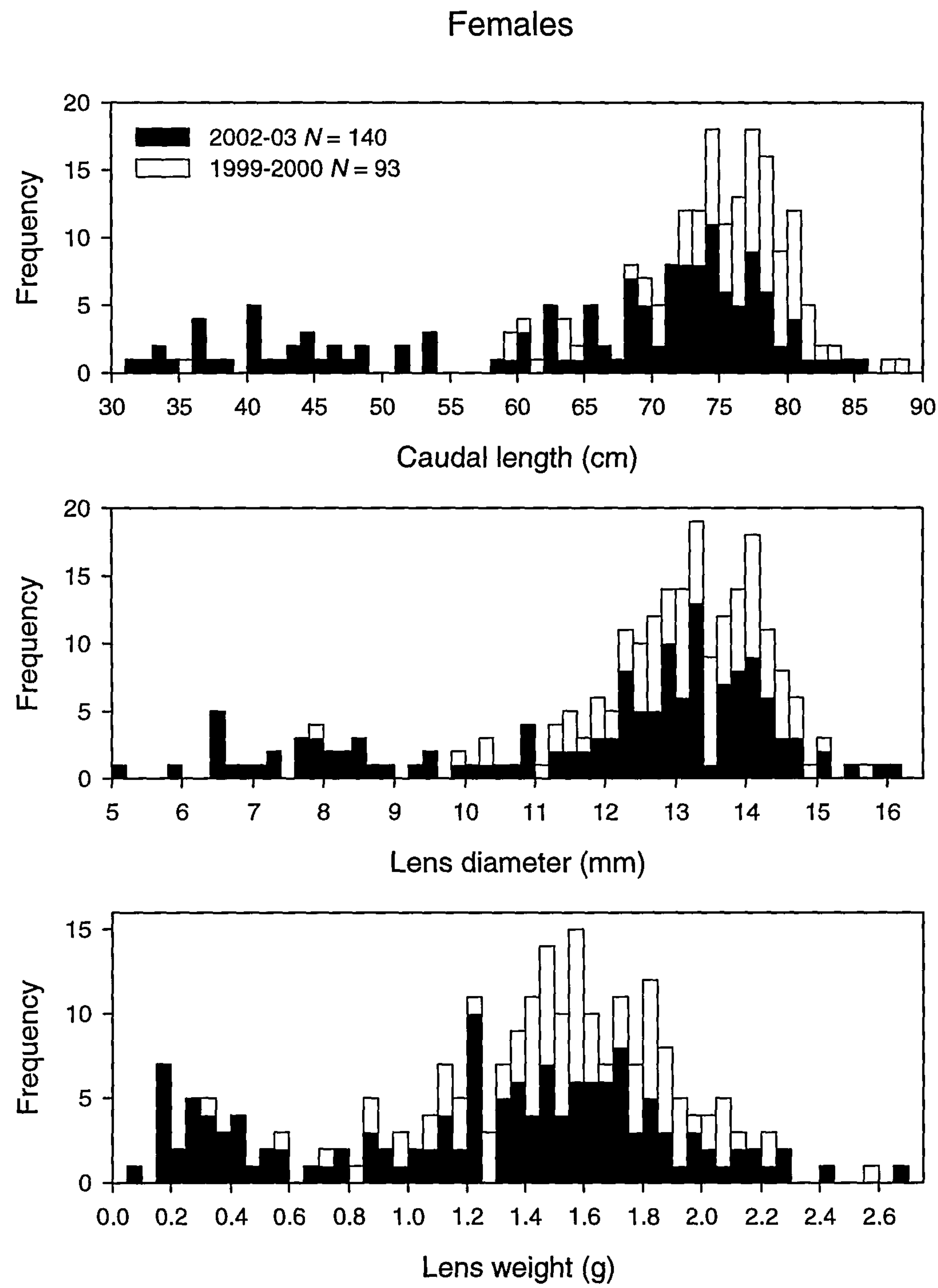


Figure 1. **Frequency distributions of caudal length, eye lens diameter, and eye lens weight for female pale ghost shark collected from the Chatham Rise in December 2002–January 2003 and December 1999–January 2000.**

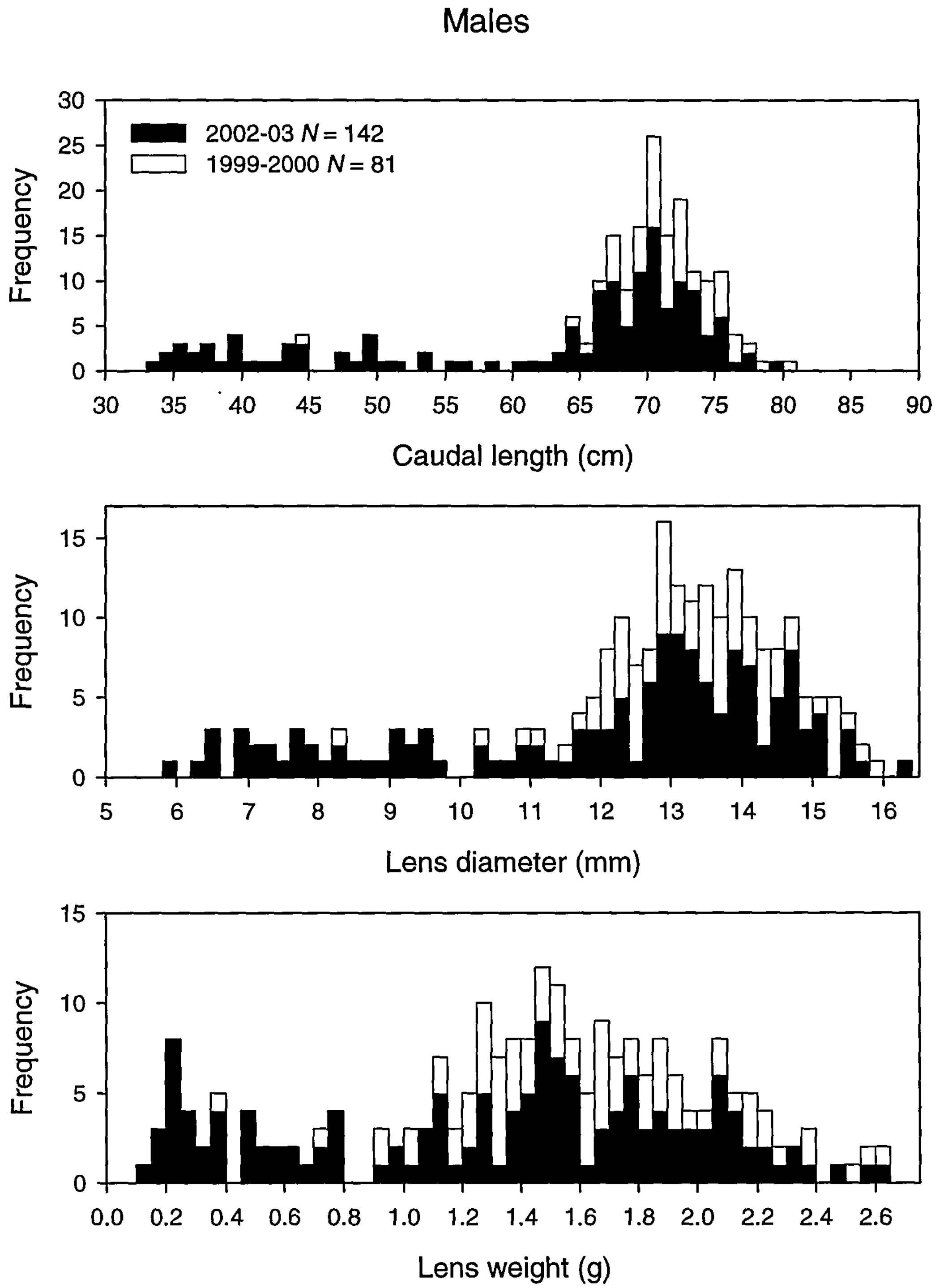
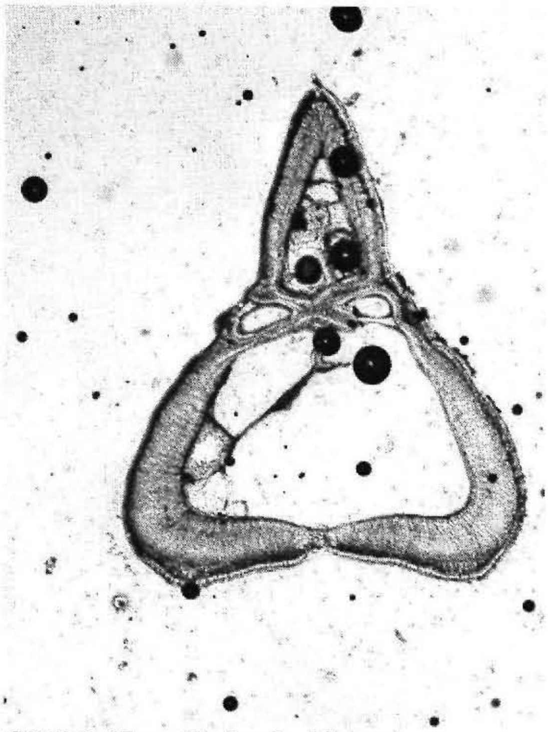
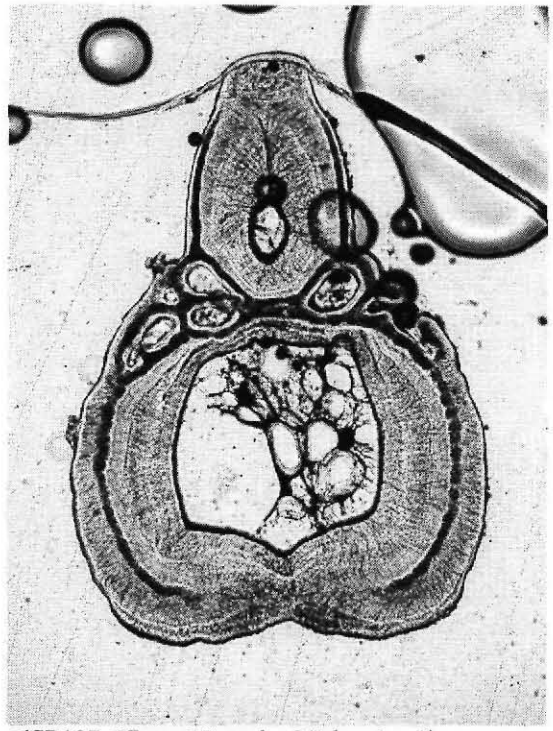


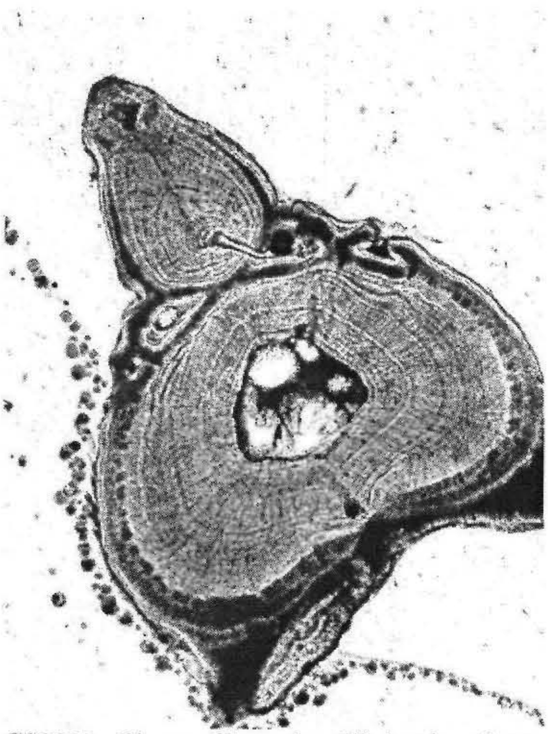
Figure 2. **Frequency distributions of caudal length, eye lens diameter, and eye lens weight for male pale ghost shark collected from the Chatham Rise in December 2002–January 2003 and December 1999–January 2000.**



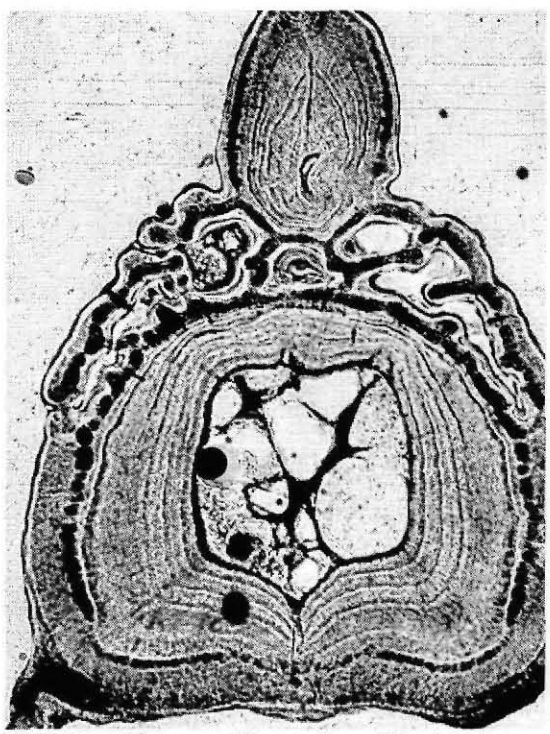
GSP078, 17 cm CL female, 1/1 bands



GSP107, 27 cm CL male, 2/1 bands, silver nitrate

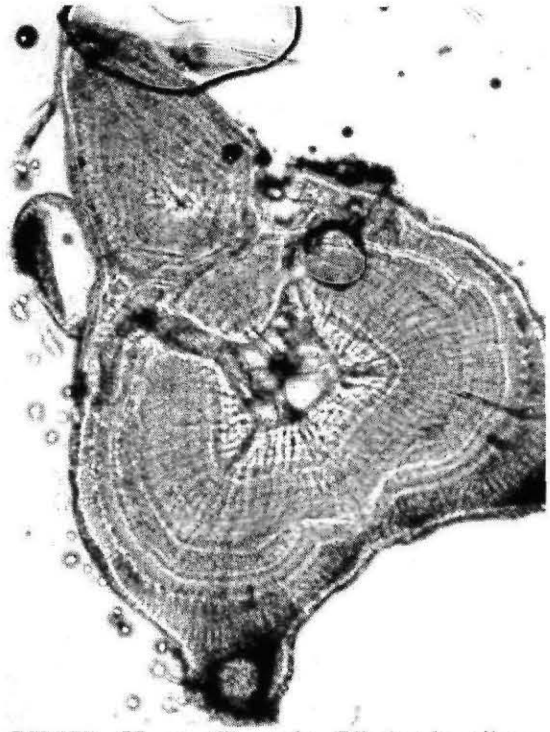


GSP222, 64 cm CL male, 5/6 bands, silver nitrate

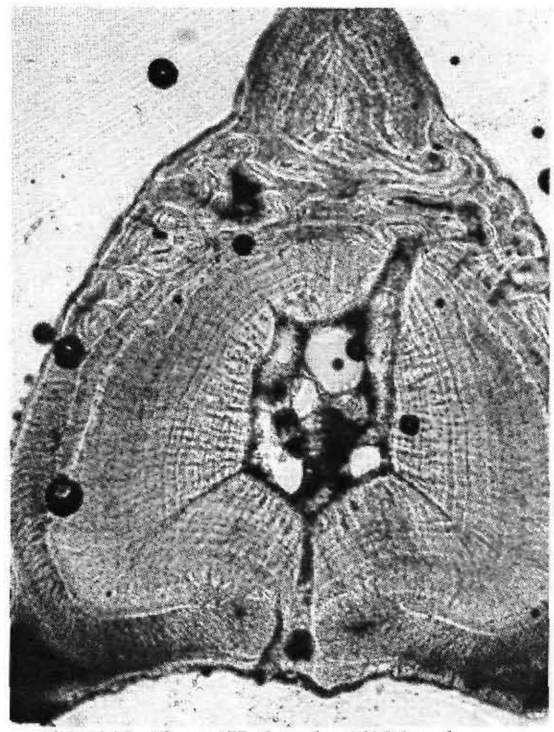


GSP19, 66 cm CL male, 5/5 bands, haematoxylin

Figure 3. Thick sections through the distal part of the dorsal fin spines of pale ghost sharks. Captions provide the specimen number, caudal length, sex, band counts by Reader 1 and Reader 2, and stain (if used). All sections were photographed with transmitted white light and differential interference contrast at 40x magnification. All sections to same scale. (Continued.)



GSP177, 75 cm CL male, 7/8 bands, silver nitrate



GSP118, 83 cm CL female, 9/10 bands

Figure 3 (cont). Thick sections through the distal part of the dorsal fin spines of pale ghost sharks. Captions provide the specimen number, caudal length, sex, band counts by Reader 1 and Reader 2, and stain (if used). All sections were photographed with transmitted white light and differential interference contrast at 40x magnification. All sections to same scale.

Counts of fin spine bands by Reader 1, who knew the caudal length of the specimens while counting, were closely correlated with length (Figure 4). Counts by Reader 2, who did not know the lengths, were more variable, and when they were plotted against caudal length, three outliers were apparent. These three spine sections were re-examined: two were judged unreadable and the third was re-counted. This last section was aged by Reader 2 after staining with silver nitrate. The staining process had increased the visibility of fine lines near the spine lumen, and these were initially included in the count by Reader 2, but later discounted.

Final band counts by Reader 2 were more variable in relation to caudal length than counts by Reader 1 (Figure 4). Both readers counted similar numbers of bands in the smaller ghost sharks, but Reader 2 tended to count more bands than Reader 1 in the larger specimens (Figures 4 & 5). An age-bias plot indicated a change from under-reading to over-reading by Reader 2 relative to Reader 1, though the differences were not significant, probably because of the small sample sizes. However this comparison is not strictly valid because of the different reading protocols used by the two readers (i.e. caudal length was known by one reader but not the other).

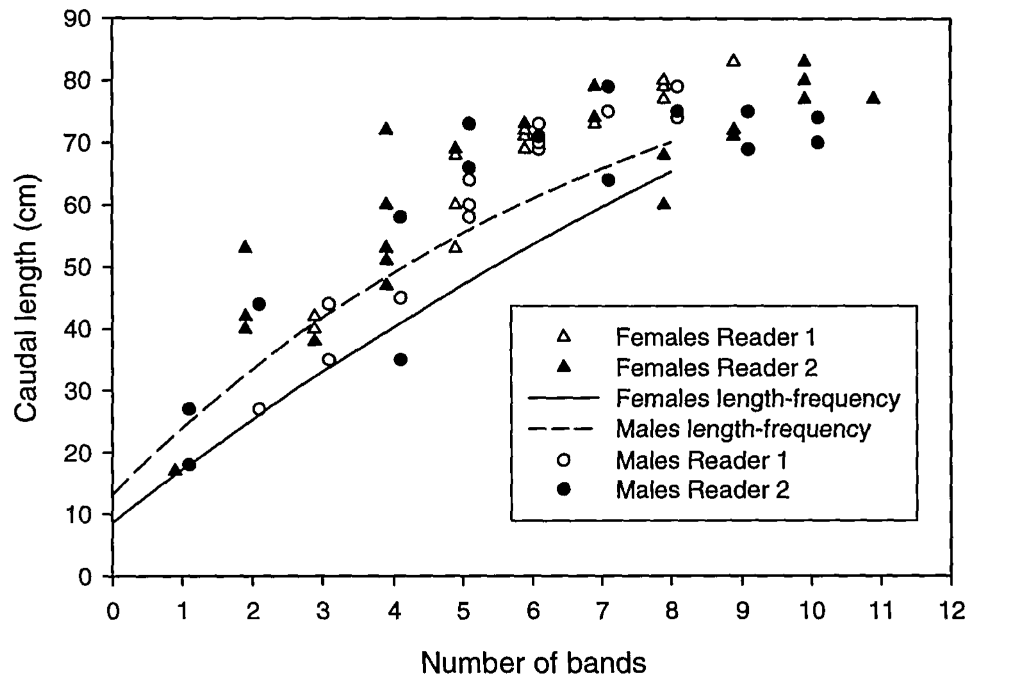


Figure 4. **Relationship between caudal length and number of bands for two readers. The growth curves were derived by MULTIFAN analysis of length-frequency data in a previous study by Francis & Ó Maolagáin (2000). Males and females have been slightly offset horizontally for clarity.**

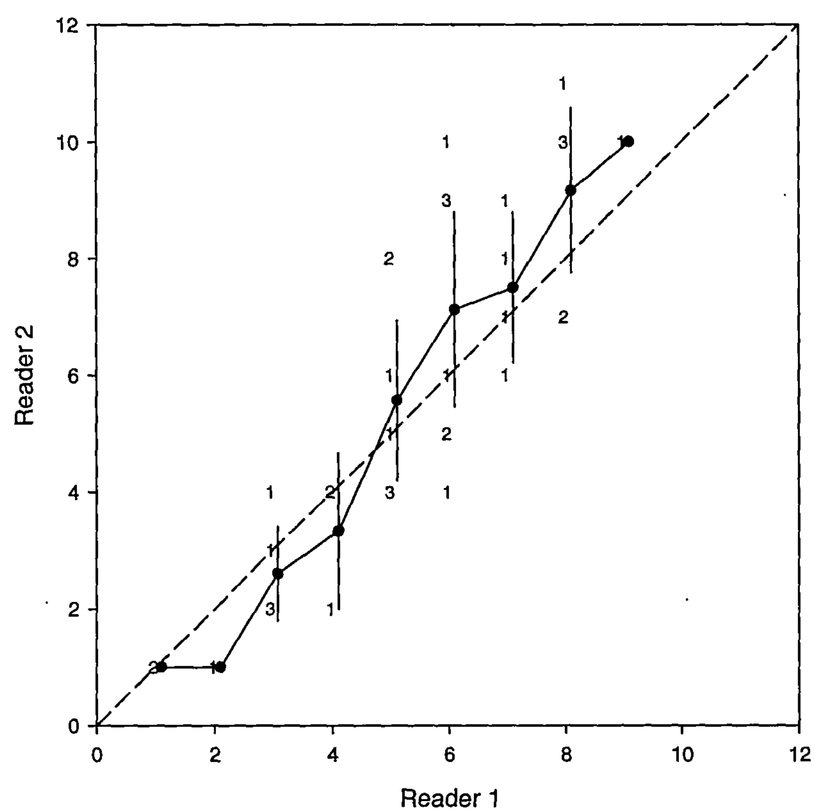


Figure 5. **Comparison of spine section band counts between Reader 1 and Reader 2 (*N* = 37). The dashed line is the 1:1 line, and the solid line with error bars (twice the standard error) is the age-bias plot (slightly offset). The numerals are sample sizes.**

12. Discussion:

The utility of eye lens diameters and weights as indicators of the age of pale ghost sharks could not be determined. Samples contained too few juveniles to assess the presence or absence of modes that might correspond with age classes. Juvenile pale ghost sharks are uncommon in research trawl samples (see Appendix 1 in Francis & Ó Maolagáin 2000) and the commercial fishery, and this is a considerable obstacle to determining the feasibility of this technique. Our previous work with dark ghost sharks indicated that lens diameters are a possible indicator of age in that species (Francis & Ó Maolagáin 2001).

Sections of dorsal fin spines show much greater promise for ageing pale ghost sharks. Growth bands are visible, and their readability is greatly enhanced by DIC lighting, and to a lesser extent, staining with silver nitrate or Meyer's haematoxylin. Surprisingly, ultraviolet light did not enhance the bands as well as it did in dark ghost sharks (Francis & Ó Maolagáin 2001).

For pale ghost sharks, the two readers had moderately good agreement in band counts for smaller fish, but they differed in their interpretation of the number of bands present in some of the larger fish. Length-at-age estimates by both readers corresponded moderately well with growth curves generated by MULTIFAN analysis of length-frequency data in a previous study (see Figure 4), although spine counts suggested a faster initial growth rate than did length-frequency data.

We conclude that band counts on sections of dorsal fin spines offer a feasible method for ageing pale ghost sharks. However, further development of the technique is required, particularly in the following areas:

- Examination of a larger sample size so that the interpretation of bands improves as readers become more experienced
- Determination of the size of pale ghost sharks at hatching from the egg case, and the composition of the fin spine at hatching, in order to confirm the starting point for band counts
- Closer study of the process of band formation in the spine lumen of large animals, in an attempt to determine whether deposition ceases, or bands become too narrow to be resolved, in large animals, thus leading to under-estimation of age
- Validation of annual deposition of the bands

Subject to these caveats, spine bands and length-frequency analyses both suggest that pale ghost sharks are moderately fast growing and have medium longevity.

13. References

Campana, S.E.; Annand, M.C.; McMillan, J.I. (1995). Graphical and statistical methods for determining the consistency of age determinations. *Transactions of the American Fisheries Society 124*: 131-138.

Didier, D.A. (2002). Two new species of chimaeroid fishes from the southwestern Pacific Ocean (Holocephali, Chimaeridae). *Ichthyological Research 49*: 299-306.

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Horn, P.L. (1997). Biology and commercial landings and a stock assessment of ghost sharks (*Hydrolagus* spp.) in New Zealand waters. *New Zealand Fisheries Assessment Research Document* 97/3. 36 p.

14. Publications:

Nil.

15. Data Storage:

The data have been stored on the MFish *age* database.