Sawshark and elephant fish assessment and bycatch evaluation in the Southern Shark Fishery

Terence I. Walker and Russell J. Hudson

July 2005

Project Number 1999/103

Saw shark and elephant fish assessment and bycatch evaluation in the Southern Shark Fishery

Terence I. Walker and Russell J. Hudson

Published by Primary Industries Research Victoria, Marine and Freshwater Systems, Department of Primary Industries, Queenscliff, Victoria, 3225.

DISCLAIMER

The authors do not warrant that the information in this book is free from errors or omissions. The authors do not accept any form of liability, be it contractual, tortious or otherwise, for the contents of this book or for any consequences arising from its use or any reliance placed upon it. The information, opinions and advice contained in this book may not relate to, or be relevant to, a reader's particular circumstances. Opinions expressed by the authors are the individual opinions of those persons and are not necessarily those of the publisher or research provider.

ISBN 1 74146 416 1

Formatted/designed by Primary Industries Research Victoria Queenscliff

Printed by PIRVic Queenscliff, Victoria

Table of Contents

NON-TECHNICAL SUMMARY

1999/103 Sawshark and elephant fish assessment and bycatch evaluation in the Southern Shark Fishery

Principal Investigator: Terence I. Walker

Address: Primary Industries Research Victoria
PO Box 114 Queenscliff, Victoria 3225, Australia
Tel: (03) 5258 0111 Fax: (03) 5258 0270
Email: Terry.Walker@dpi.vic.gov.au

Objectives:

1. Determine population parameters required for fishery stock assessment of the non-target species common saw shark, southern saw shark and elephant fish.
2. Provide stock assessment of each of these three non-target species in Bass Strait.
3. Provide data for assessment of bycatch, discards and damaged shark in the shark fishery of southern Australia (part of Gillnet Hook and Trap Fishery).

Non Technical Summary:

This project investigated the fisheries population biology of two sawshark and one elephant fish species and evaluated target catch, byproduct catch, bycatch and damaged shark in the shark gillnet and shark hook sectors of the Gillnet Hook and Trap Fishery (GHATF). The species investigated were common sawshark (*Pristiophorus cirratus*), southern sawshark (*P. nudipinnis*), and elephant fish (*Callorhinchus milii*).

In addressing the three project objectives, data were used from field studies undertaken during 1973–76 and 1986–87 as part of earlier projects and from field studies undertaken during 1998–01 as part of the present project. Determination of population parameters (Objective 1) was based on data from all three periods. Stock assessment of sawshark and elephant fish (Objective 2) was based on these parameter values and several other data sets. The other data sets include length-frequency data from all three periods and time series of commercial catch (1950–03), fishing effort (1976–03), and catch per unit effort (1976–03) data for shark longlines and for each mesh-size of shark gillnets. The assessments also included catch for Danish seine (1950–03) and demersal trawl from the South East Trawl Fishery (1985–03) and Great Australian Bight Trawl Fishery (1988–03). The first stock assessment for each of common sawshark, southern sawshark, and elephant fish covering only the Bass Strait region was undertaken and presented to the 20–21 May 2004 SharkRAG meeting. These assessments were subsequently reviewed and extended to include Bass Strait, Tasmania and South Australia and, for elephant fish only, New South Wales, and were then presented to the 9–10 September 2004 SharkRAG meeting. Target catch, byproduct catch, bycatch and damaged shark evaluation in the shark fishery (Objective 3) was based on data collected during 1973–76 and 1998–01. All three objectives of the project were met completely.

Determination of population parameters

The gillnet selectivity parameters, von Bertalanffy growth parameters, and reproductive parameters for each of common sawshark, southern sawshark, and elephant fish were determined and applied for stock assessment undertaken through SharkRAG during 2004. New methods for estimating the values of reproductive parameters were developed and applied to new and available data on school shark

reproduction. Descriptions of the structure and function of some of the reproductive structures of the elephant fish were also made.

The elephant fish belongs to the small taxonomic group known as holocephalans or chimaeras, which form part of class chondrichthyes (sharks, rays and chimaeras) and which has received limited scientific attention in the past. This presented major challenges for the present project when investigating the elephant fish. To address basic biological uncertainties, several small-scale sub-projects were parcelled out to university postgraduate students supervised by the Principal Investigator and by Professor William C. Hamlett, of the University of Indiana, Indiana, USA to investigate the microanatomy, structure, and function of some of the male and female genital ducts. Similar more limited sub-projects were undertaken for common sawshark and southern sawshark. Although this work is not central to the objectives of the present project, it provides a basis for better understanding the reproductive biology of these species and determining maturity, maternity, and timing of mating and egg laying in elephant fish or birth in sawshark.

The at-sea, sampling component of the present project enabled opportunistic collection of data on the reproductive biology of school shark (*Galeorhinus galeus*) and gummy shark (*Mustelus antarcticus*) at no extra cost. The additional data sets for school shark and gummy shark augment earlier data sets and enable addressing certain uncertainties about their reproductive biology evident from the earlier data sets. The results for school shark are incorporated into the special book chapter on reproduction methodology and the gummy shark results are presented in a manuscript currently in preparation (excluded from the present report).

Sawshark and elephant fish stock assessments

The data sets for common sawshark, southern sawshark, and elephant fish off southern Australia are limited and stock assessment would be not be possible, had the present project not been undertaken. The only monitoring data on which an assessment of sawshark (species combined) and elephant fish can be based are catches and catch-rates. The types of long time series of catch length-frequency data and tag release-recapture data available for gummy shark and for school shark are not available for sawshark or elephant fish. Although most of the catch is from Bass Strait and there are generally low catches in waters off Tasmania and South Australia, the assessments undertaken apply to this entire region of waters on the continental shelf and slope for sawshark and to New South Wales for elephant fish. Catch-rate indices for these species were developed on the assumption that the effort estimated to be targeted at gummy shark is also targeted at sawshark and elephant fish. Catches of sawshark are distinguished between common sawshark and southern sawshark on the basis of catches recorded as scientific observations during 1973–76, 1986–87 and 1998–01. The assessments are based on a non-spatial, and age- and sex-based population dynamics model similar to that used for the 1996 stock assessment of school shark (*Galeorhinus galeus*) (Punt and Walker 1998). The results of the base-case assessments indicate that both sawshark and elephant fish are depleted to below 40% of the 1950 pup production level but that the rate of decline in the size of these resources has decreased substantially since the mid-1980s. Sensitivity tests indicate that the uncertainty range of depletion levels is less for elephant fish (14–22%) than for sawshark (17–39%). Pup production is assessed at 32% for common shark, 26% for southern shark, and 20% for elephant fish of the 1950 levels.

Evaluation of target, byproduct, bycatch and damaged catch

Catches were evaluated using data recorded during 1973–76 as part of a population study of gummy shark (*Mustelus antarcticus*) on the continental shelf of south-eastern Australia and data recorded during 1998–01 on commercial vessels as part of the present study. During 1973–76, catches of all species taken in gillnets of eight mesh-sizes 2–9 inches and on longlines were recorded; this provides valuable baseline data of the relative abundance of fish at that time. Comparing catch rates of all species by gillnets of 6-inch mesh-size between 1973–1976 and 1998–01 provides a basis for evaluating potential changes in the abundance of each species impacted by commercial fishing.

During 1973–1976 and 1998–01, a much higher number of animals and a higher number of species were caught by gillnets (mesh-sizes 2–9 inches) than by longlines (hook-sizes Mustad 2/O–11/O). Several important conclusions can be made about the catch rates of gillnets and longlines deployed on the continental shelf in the depth range 9–130 m. Shark gillnets and shark longlines are both much more effective at catching chondrichthyan species than at catching teleost species, and catches of species of cephalopoda, bivalvia, gastropoda, mammalia, aves and reptilia are negligible. The effect of gillnet mesh-size on catch rates is strong, whereas the effects of gillnet hanging ratio, hook size, hook shank length, and hook space are weak. Overall catch rates of chondrichthyan and teleost fishes by mesh-size are very different. For chondrichthyans, the modal catch rate is by 4-inch mesh-size with decreasing catch rates for both increasing and decreasing mesh-size, whereas for teleosts the modal catch rate is by 2-inch mesh-size with decreasing catch rates as mesh-size increases.

For chondrichthyes, the top four species taken by gillnet across 8 mesh-sizes—*Squalus megalops, Mustelus antarcticus, Heterodontus portusjacksoni,* and *Galeorhinus galeus*—are similar to the top four species taken by longline across 8 hook sizes—*Squalus megalops, M. antarcticus, Cephaloscyllium laticeps,* and *G. galeus.* The only difference is that *H. portusjacksoni* is more prevalent than *C. laticeps* in the gillnet catch, whereas the converse occurs for the longline catch. For teleostei, *Platycephalus bassensis* is the most prevalent species caught by both gillnets across 8 mesh-sizes and longlines across 8 hook sizes. *Neosebastes scorpaenoides* is the second most prevalent species caught by longline and the third most prevalent species caught by gillnet. The second most prevalent species taken by gillnet—*Trachurus novaezelandiae*—is not caught by longline.

For chondrichthyes in Bass Strait, there has been about a one-third overall reduction in abundance across all species combined between 1973–76 and 1998–01. About half of this reduction is attributable to an 87% reduction in the catch per unit effort (CPUE) of *Galeorhinus galeus* and a 54% reduction in the CPUE of *Cephaloscyllium laticeps.*

Only small proportions of the commercial catch of chondrichthyan (3%) and teleost (2%) animals taken by demersal gillnets of 6-inch and 6½-inch mesh-size coming on-board dead are discarded. The discarded animals are mostly *Cephaloscyllium laticeps, Heterodontus portusjacksoni, Squalus megalops,* and *Myliobatis australis,* which come on-board live.

Wildlife interactions occur occasionally with Australian fur seals (*Arctocephalus pusillus dorfer*) and common dolphin (*Delphinus delphis*). Of ten chondrichthyan species on the continental shelf and continental slope identified by the IUCN Shark Specialist Group as threatened, two are identified by the present study as caught by shark fishing. White shark (*Carcharodon carcharias*) is taken occasionally and *Galeorhinus galeus,* once the primary target species, is presently taken as significant byproduct catch (195 t during 2002). Both these species are now carefully managed.

Damage to shark carcasses from predation by invertebrates, fish and mammals to sharks landed on-board from gillnets of 6-inch or 6½-inch mesh-size was investigated on-board nine vessels operating under normal commercial fishing conditions. 'Lost carcass mass' from predation for gummy shark and school shark combined is estimated at 4.9% (4.7% for gummy shark and 6.9% for school shark); it is slightly higher in South Australia (5.3%) than in Bass Strait (4.7%). 'Lost carcass mass' for common sawshark and southern sawshark combined is estimated at 2.3% (2.1% for common sawshark and 3.5% for southern sawshark) and for elephant fish is estimated at 3.4%. 'Devalued retained carcass mass' from major damage is estimated at 9.2% for gummy shark and school shark combined (9.0% for gummy shark and 12.8% for school shark), 4.2% for common sawshark and southern sawshark combined (4.0% for common sawshark and 5.5% for southern sawshark), and 6.1% for elephant fish.

OUTCOMES ACHIEVED

All three objectives of the project were met completely and there are several important outcomes.

All basic gear selectivity parameters, von Bertalanffy growth parameters, and reproductive parameters required for appropriate fishery stock assessment are available for ongoing assessments. Available catch (1950–03), effort (1976–03), and catch per unit effort (1976–03) data for sawshark and elephant fish have been assembled in a secure and accessible SAS database and have been appropriately extracted and used for stock assessment.

The first stock assessment for each of common sawshark, southern sawshark, and elephant fish was undertaken and presented to 20–21 May 2004 SharkRAG meeting. These assessments were subsequently reviewed and extended and then presented to the 9–10 September 2004 SharkRAG meeting. These full assessments provided a basis for setting sawshark and elephant fish total allowable catches for the entire Southern and Eastern Scalefish and Shark Fishery. Whereas there are not the rich data sets that underlie the renowned gummy shark and school shark assessments, there are now sufficient data to provide defensible assessments for the sawshark and elephant fish. Gummy shark and school shark also have long-term length-frequency and tag release-recapture data sets, which contribute markedly to reducing the bounds of uncertainty in those assessments.

Evaluating target catch, byproduct catch, and bycatch in the shark fishery of southern Australia has met a first step data requirement for several important processes presently under way and these results form an important part of legislatively prescribed documentation associated with these processes. One of these processes is strategic assessment of fisheries under the Australian Environment Protection and Biodiversity Conservation Act 1999 and AFMA has drawn heavily on the results of the present project in its documentation. As part of an independent process, AFMA has also drawn on these results for preparation of the Bycatch Action Plan for the Gillnet Hook and Trap Fishery required legislatively under the Australian Fisheries Act 1991. Catch evaluation in the shark fishery meets one of the requirements of Australia's National Plan of Action for the Conservation and Management of Sharks launched 26 May 2004. The results were a crucial input for Ecological Risk Assessment for the Effects of Fishing for scoping and a level 1 assessment for each of five components (target species, byproduct and bycatch species, threatened and protected species, habitats, and communities) associated with the use of shark gillnets and shark longlines. The catch evaluation data now available on shark fishing has reduced uncertainty about the impacts of shark fishing (see section Further Development). Most bycatch comes on-board live and can be discarded live and there is negligible bycatch discarded dead. The results dispel myths and beliefs that shark gillnets and shark longlines have high bycatch. Industry, fishery managers, scientists and other beneficiaries find the data and results extremely informative and valuable.

Keywords: Gillnet Hook and Trap Fishery, sawshark, elephant fish, bycatch, and stock assessment

Acknowledgments

Acknowledgment is due to the many people who during 1998–01 participated in collection of sawsharks or elephant sharks at sea, in recording of targeted catch, byproduct catch, and bycatch at sea, in laboratory dissection and ageing of sharks, and in providing statistical advice for analysis of the data. Capture of the animals was undertaken by professional fishers Ron Anthony, Ron Atterton, Stephen Brockwell, Rod Casement, Mick Cook, Harry Ferrier, Jon Gazam, Mark Goulden, Neil Hosking, Peter Riseley, Adrian Rodgers, Arthur Sifford, and Robert White. These fishers made the animals either available for dissection at sea or returned them to port for dissection in the laboratory. Lauren Brown of the Marine and Freshwater Systems, Primary Industries Research Victoria Queenscliff Centre (PIRVic) and Dr Jeremy Prince of Biospherics Pty Ltd participated in monitoring bycatch and dissecting sharks at sea during 1998. Postgraduate students Justin Bell, Jessica Creek, Stephen Dwyer, Jessica Whitlock, Amy Beck, Tracey King, Stephen Leporati, Rachel Smith, Matthew Reardon, Megan Storrie, and Stephanie Van't Hoff assisted with the laboratory dissections. Corey Green and Thérèse Stokie of the Central Ageing Facility at PIRVic undertook laboratory ageing of animals. Anne Gason of PIRVic provided advice on statistical methods for analysis of the data using the statistical package SAS. Professor William C. Hamlett of the Department of Anatomy and Cell Biology, Indiana University School of Medicine, and Dr Rob W. Day of the Department of Zoology, University of Melbourne, served as academic supervisors of students participating in the project.

Acknowledgment is also due to the former technical staff of PIRVic and the many professional fishers who participated in field sampling of sharks during 1973–76 and 1986–87. Collection of data during these two earlier periods was funded from the former Australian Fishing Industry Research Trust Account and the Victorian Treasury. Collection of data during 1998, as part of the Pilot Fixed Site Stations Survey, was funded by the Australian Fisheries Management Authority. Collection of data during 1999–01 and subsequent analysis and reporting of the data were funded by the Australian Fisheries Research and Development Corporation (FRDC) as part of FRDC Project 1999/103. Catch and effort data used for stock assessment of sawshark and elephant fish were sourced from the fisheries agencies of Victoria, Tasmania, and South Australia and from AFMA and were assembled as part of the AFMA funded Southern Shark Fishery Monitoring Project. Species names were based on the Codes for Australian Aquatic Biota (www.marine.csiro.au/caab/caabsearch). Dr André Punt of CSIRO Marine Research and University of Washington is acknowledged for applying available computer software for stock assessment to the sawshark and elephant fish data collated and made available as part of the present project. This software was originally developed for school shark and gummy shark through SharkRAG processes. SharkRAG is acknowledged for its ongoing specification, advice and scrutiny of the sawshark and elephant fish assessments.

FINAL REPORT

1999/103 Saw shark and elephant fish assessment and by-catch evaluation in the Southern Shark Fishery

Background

Acronyms

AFMA	Australian Fisheries Management Authority
CSIRO	CSIRO Marine Research
FIRTA	Fishing Industry Research Trust Account
FRDC	Fisheries Research and Development Corporation
GHATF	Gillnet Hook and Trap Fishery
IPOA-Sharks	International Plan of Action for the Conservation and Management of Sharks
NPOA-Sharks	National Plan of Action for the Conservation and Management of Sharks
PIRVic	Primary Industries Research Victoria
SharkRAG	Southern Shark Resource Assessment Group

Structure of report

The present project investigates the population dynamics of two sawshark and one elephant fish species and evaluates target catch, byproduct catch, and bycatch taken by shark gillnet and shark longline in the Gillnet Hook and Trap Fishery (GHATF). The species investigated were common sawshark (*Pristiophorus cirratus*), southern sawshark (*P. nudipinnis*), and elephant fish (*Callorhinchus milii*). The report has all the prescribed sections of a standard FRDC report, but, because of the diverse range of subjects, much of the detailed information is presented in separate appendices prepared as ten manuscripts and two reports. These follow the standard Appendix 1 (Intellectual Property) and Appendix 2 (Staff). Appendices 3a–3g provide details relating to determination of population parameters (gillnet selectivity, length at-age, and reproduction) for common sawshark, southern sawshark, and elephant fish (Objective 1). Appendix 4 provides details of stock assessment of these three species (Objective 2). Appendices 5a–5b provide details of evaluation of catches of target, byproduct and bycatch species taken by shark gillnets and shark longlines and details of catch evaluation of damaged shark taken by shark gillnets in the Gillnet Hook and Trap Fishery (Objective 3).

Of the ten manuscripts, nine were prepared for internationally reviewed journals and one in a chapter of a book on chondrichthyan reproduction. The book chapter and four of the nine manuscripts are published, five manuscripts are in preparation. The manuscripts and reports are referenced as follows.

Hamlett WC, Reardon M, Clark J, and Walker TI (2002) Ultrastructure of sperm storage and male genital ducts in a male holocephalan, the elephant fish, *Callorhynchus milii*. *Journal of Experimental Zoology* **292**, 111–128.

Hudson RJ, Walker TI, and Day RW (in prep) Reproductive biology of common sawshark, *Pristiophorus cirratus*, in the shark fishery of southern Australia. (Prepared for *Marine and Freshwater Research*).

Punt, A. E., Walker, T. I., and Gason, A. S. (2004) Chapter 10: Initial assessments of sawshark (*Pristiophorus* *cirratus* and *P. nudipinnis*) and elephant fish (*Callorhinchus milii*). Report to SharkFAG Meeting 16–17 September 2004. SharkFAG Document 2004/D18. 35 pp. (CSIRO Marine Research, Hobart, Tasmania, and Primary Industries Research Victoria: Queenscliff, Victoria, Australia).

Reardon MB, Walker TI, and Hamlett WC (2002) Microanatomy of spermatophore formation and male genital ducts in the holocephalan, *Callorhynchus milii*. In 'Proceedings of Sixth Indo-Pacific Fish Conference'. Special Issue of *Marine and Freshwater Research* 53, 591–600.

Smith RM, Walker TI, and Hamlett WC (2004) Microscopic organisation of the oviducal gland of the holocephalan elephant fish *Callorhynchus milii*. *Marine and Freshwater Research* 55, 155–164.

Walker TI (in prep) Gillnet selectivity for four chondrichthyan species harvested off southern Australia. (Prepared for *Marine and Freshwater Research*).

Walker TI (2005) 4 Reproduction in fisheries science. In 'Reproductive Biology and Phylogeny of Chondrichthyes: Sharks, Skates, Stingrays and Chimaeras". (Ed. WC Hamlett) pp. 81–127 (Science Publishers, Inc.: Enfield, New Hampshire 03478, USA)

Walker TI, Hudson, RJ (in prep) Reproductive biology of southern sawshark (*Pristiophorus nudipinnis*) harvested off southern Australia. (Prepared for *Marine and Freshwater Research*).

Walker TI, Hudson, RJ (2005) Predation damage rates to shark in the Gillnet Hook and Trap Fishery. 10 pp. Primary Industries Research Victoria, Queenscliff, Victoria, Australia. (Prepared for SharkRAG.)

Walker TI, Hudson RJ, Bell JD, Reardon MB, Smith RM, Hamlett WC (in prep) Reproductive biology of elephant fish (*Callorhynchus milii*) harvested off southern Australia. (Prepared for *Marine and Freshwater Research*).

Walker TI, Hudson RJ, Gason AS (2005) Catch evaluation of target, byproduct, and bycatch species taken by gillnets and longlines in the shark fishery of south-eastern Australia. In 'Proceedings of North Atlantic Fisheries Organisation Symposium. Elasmobranch Fisheries: Managing for Sustainable Use and Biodiversity Conservation'. 11–13 September 2002. Santiago de Compostela, Spain. *Journal of Northwest Atlantic Fishery Science* 35, 505–530.

Walker TI, Hudson RJ, and Green C (in prep) Age and growth studies of two sawshark and one chimaera species harvested off southern Australia. (Prepared for *Marine and Freshwater Research*).

In addition to preparing these 10 manuscripts for scientific papers and two reports, the following five theses were prepared on two sawshark and one elephant fish species.

Beck ALN (2001) The male reproductive system of the southern sawshark, *Pristiophorus nudipinnis*: structure and function. 43 pp. B.Sc. (Hons) thesis, University of Melbourne, Parkville, Victoria 3052, Australia.

Bell JD (2003) Fisheries and reproductive biology of the elephant fish, *Callorhynchus milii*, in southern Australia. 109 pp. B.Sc. (Hons) thesis, Deakin University, Warrnambool, Victoria 3280, Australia.

Reardon M (2001) Seasonality and microanatomy of spermatophore formation in a holocephalan, the elephant fish, *Callorhynchus milii*. 44 pp. B.Sc. (Hons) thesis, University of Melbourne, Parkville, Victoria

3052, Australia.

Smith RM (2001) The reproductive biology of the female elephant fish, *Callorhynchus milii*, with particular reference to the oviducal gland. 60 pp. B.Sc. (Hons) thesis, University of Melbourne, Parkville, Victoria 3052, Australia.

Stevens B (2002) Uterine and oviducal mechanisms for gestation in the common sawshark, *Pristiophorus cirratus*. 41 pp. B.Sc. (Hons) thesis, University of Melbourne, Parkville, Victoria 3052, Australia.

The species

Three endemic species of sawshark whose distributions have not been described precisely occur off southern Australia. Common sawshark (*Pristiophorus cirratus*) is reported to range from Jurien Bay in Western Australia to Eden in New South Wales, including Tasmania, to depths of 310 m. Southern sawshark (*P. nudipinnis*) is reported to range from the western region of the Great Australian Bight to eastern Gippsland in Victoria, including Tasmania, to depths of 70 m. Eastern sawshark (*Pristiophorus* sp. A) is reported to range from about Lakes Entrance in Victoria to Coffs Harbour in NSW at depths of 100–630 m (Last and Stevens 1994). For assessment purposes, all sawsharks south of the Victoria–NSW border are assumed to be common sawshark and southern sawshark, whereas those north of this border are assumed to be eastern sawshark.

The elephant fish is distributed from Esperence in Western Australia to Sydney in New South Wales, including Tasmania, at depths to at least 200 m. Elephant fish also occur in New Zealand, but are assumed to be a separate stock from the population in southern Australia (Last and Stevens 1994).

Although these species have wide distributions off southern Australia, ~90% of the sawshark catch in the Gillnet Hook and Shark Fishery (GHATF) is taken in Bass Strait and ~99% of the elephant fish catch is taken in Bass Strait and off the eastern and south-eastern coasts of Tasmania. Most of the sawshark catch is not distinguished between the three species of sawshark; it is simply reported as 'sawshark' in their catch and effort logbooks. Of the total sawshark catch of 284 tonnes reported for 2002, the GHATF took 59%, the South East Trawl Fishery took 24%, the Great Australia Bight Trawl Fishery took 7%, Tasmanian state fisheries took 5%, and NSW state fisheries took 5%. Of the total elephant fish catch of 71 tonnes reported for 2002, the GHATF took 57%, the South East Trawl Fishery took 21%, Tasmanian state fisheries took 18%, and Victorian state fisheries took 4% (Walker, Taylor *et al.* 2003).

It is uncertain whether each of these species form single stocks or multiple stocks. Limited tag and release data provide evidence of movement within Bass Strait for *P. cirratus*, and of movement between Bass Strait and southern Tasmania for *P. nudipinnis* and *C. milii*. Neither species of sawshark appears to move into distinct pupping grounds, but each year mature elephant fish migrate into large estuaries and inshore bays to lay their eggs (unpublished data).

Previous research

Most previous research of sharks in the GHATF was focused on gummy shark (*Mustelus antarcticus*) and school shark (*Galeorhinus galeus*), but some biological data on reproduction, morphometrics, and gillnet selectivity were collected opportunistically for common sawshark, southern sawshark, and elephant fish during 1973–76 and 1986–87. In addition, small quantities of tag data were collected for these species during 1973–76 and 1990–01. However, it was not until undertaking the present project that there have been sufficient data to determine all the basic fisheries population parameters required for stock assessment of these species.

The present report draws both on data collected as part of the present project (1998–01) and on the earlier data collected opportunistically. The earlier biological data came from four projects: 'Investigations of the Gummy Shark from South-eastern Australian waters' (FIRTA 1973–76), Southern Shark Assessment (FIRTA 1985–88), 'Southern Shark Tagging' (FRDC Project 93/066), and 'Southern Shark Tag Database' (FRDC Project 96/162). In addition to the present project, available byproduct and bycatch data are from the FIRTA

1973–76 Project and the 'Pilot Fixed Site Station Survey (AFMA 1998). Fishers logbook catch and effort data, which are used for the sawshark and elephant fish stock assessments in the present study, were during 1973–88 compiled as part of the four earlier FIRTA and FRDC projects and during 1988–03 compiled as part of an ongoing project. The ongoing project, presently referred to as the GHATF Monitoring Project, was initially funded by the former Australian Fisheries Service and, then subsequently, by AFMA.

The present project is the first study to investigate any species of sawshark worldwide. There have been previous studies of the elephant fish in southern Australia and New Zealand, but this is the first attempt to assemble all the data appropriate for a detailed stock assessment. Taxonomically, the elephant fish is a holocephalan species, for which less than 40 species are described worldwide. Hence, when the present project began, the male and female reproductive systems had not been described for any species of sawshark (family *Pristiophoridae*) (7 species described worldwide) and had not been well described for any species in the holocephalan group. Hence, description of the reproductive systems for *P. cirratus*, *P. nudipinnis*, and *C. milii* was addressed through collaboration with William C. Hamlett, Professor of Anatomy and Cell Biology at the Indiana University School of Medicine. Professor Hamlett is an authority on the structure and function of the reproductive systems of sharks and other chondrichthyans. This collaboration involved several projects undertaken by students for B.Sc. (Hons) degrees at the Zoology Department of the University of Melbourne. Through the involvement of Professor Hamlett and these projects, several journal papers have been published or are presently in the process of publication. These papers provide descriptions of the structure and function of the reproductive tracts of these three species (Beck, Walker *et al.* submitted; Bell 2003; Hamlett, Reardon *et al.* 2002; Reardon, Walker *et al.* 2002; Smith, Walker *et al.* 2004).

Need

The present project addresses three items listed as high priority in the Southern Shark Fishery Five Year Strategic Research Plan 1998; these items are under the key area 'resource status' of FRDC's program 'resources sustainability'. The three items are (1) investigation of non-quota species, (2) analysis of bycatch, and (3) effect of high grading through discarding on the TAC setting process.

The shark component of the GHATF presently targets mainly gummy shark and has almost completely phased out the targeting of school shark, and takes a range of chondrichthyan and teleost species as byproduct and bycatch. The most important by-product species are common saw shark, southern saw shark, and elephant fish. There are occasional interactions with the white shark (*Carcharodon carcharias*), mammals and sea birds, which are protected species. Apart from the need to evaluate bycatch, there is the need to address public perceptions of discards associated with the use of demersal gillnets, which are sometimes confused with surface-set driftnets.

The shark fishery is based on several species of temperate-water sharks inhabiting the continental shelf and slope, with most of the catch is taken in waters less than 75 m deep. The total annual catch ranged 2305–4226 t during 1970–2002 and had an annual value at first sale of $14.2 million ($559,000 for sawshark and $101,000 for elephant fish) during 2002. The catch during the 23-year period 1970–2002 comprised 50% gummy shark, 37% school shark and 13% by-product species. The 13% of byproduct catch comprised 7% sawshark, 2% elephant fish, 1% dogfish, and 3% 'other species'. A total of 159 vessels licensed with Commonwealth Shark Permits and 460 vessels with State-only licences took shark during 2001 using bottom-set gillnets and longlines. Most of the catch is consumed in Victoria (Walker, Taylor *et al.* 2003).

Stock assessments of gummy shark and school shark are periodically updated and refined through SharkRAG, but, prior to the present study, fully quantitative assessments had never been made for any of the non-target species. Current assessments for the GHATF indicate that the stocks of gummy shark are sound, but those of school shark are markedly depleted (Punt, Pribac *et al.* 2000). Catch quota management, adopted for gummy shark and school shark on 1 January 2000 and for sawshark and elephant fish on 1 January 2001, is designed to reduce the catch of school shark and prevent escalation of the catches of the other species. During 2002, gummy shark provided 73% of the catch, with school shark at 10%, sawshark at 8%, elephant fish at 2%, and other shark species at 7%. There is a need to ensure that the byproduct species are harvested sustainably and that the stocks of bycatch species (discarded species) are not depleted. It is therefore essential to provide the basic data needed for assessment of byproduct and bycatch species (Walker, Taylor *et al.* 2003).

Catches of the two species of sawshark combined from the GHATF rose from 52 tonnes during 1970 to reach a peak of 359 tonnes during 1995, before declining to 167 tonnes during 2002. Catches of elephant fish rose from 4 tonnes during 1971, peaked at 118 tonnes during 1985, and then declined to 40 tonnes by 2002. Sawshark and elephant fish catches have not been reliably monitored in the South East Trawl Fishery or in the Great Australia Bight Trawl Fishery prior to 2001. The reported demersal trawl catches for sawshark and elephant fish during 2002 were 88 and 15 tonnes, respectively. This gives total catches of 284 tonnes for sawshark and 71 tonnes for elephant fish across all fisheries during 2002 (Walker, Taylor *et al.* 2003).

Several initiatives taken in recent years have created a requirement to better evaluate catches in Australian fisheries. The requirement applies to both targeted and non-targeted species, and, of the non-targeted species, both the retained species (byproduct) and discarded (bycatch) species.

Australia's Commonwealth *Fisheries Management Act 1991* requires management arrangements to "ensure that the exploitation of fisheries resources and the carrying on of related activities are conducted in a manner consistent with the principles of ecologically sustainable development and the exercise of the precautionary principle, in particular the need to have regard to the impact of fishing activities on non-target species and the long-term sustainability of the marine environment". Hence, in accordance with these legislative obligations and Commonwealth Government policy prescribed under Australia's Ocean Policy regarding the impact of fishing activities on non-target species and the environment, the Australian Fisheries Management Authority developed bycatch action plans for major Australian fisheries.

More recently, the Commonwealth *Environment Protection and Biodiversity Conservation Act (EPBC) 1999* requires fisheries managed under Commonwealth jurisdiction or fisheries producing products for export to be 'strategically assessed'. This process involves assessing each fishery for ecological impacts on (a) target and by-product species, (b) bycatch species, (c) threatened, endangered and protected species, (d) marine habitats, and (e) marine food chains. The process requires collection of appropriate data, risk assessment, and appropriate management responses.

At a world level, concern for the condition of the stocks of chondrichthyan species led to the International Plan of Action for the Conservation and Management of Sharks (IPOA-Sharks), developed recently by the Food and Agriculture Organisation of the United Nations. The IPOA-Sharks recognises that the life-history characteristics of chondrichthyan species can make for low 'biological productivity' and cause these animals to be generally more susceptible to overexploitation from fishing than teleost and invertebrate species. The IPOA-Sharks also recognises that these species require special management, research, and monitoring if they are to be harvested sustainably (Anon. 2000). As a signatory to the IPOA-Sharks, Australia has developed a National Plan of Action for the Conservation and Management of Shark (NPOA-Sharks), which has been ratified by the Commonwealth, Territory and State Governments and was launched 26 May 2004.

The catches of most chondrichthyan species have not been reported and it is likely that many species, particularly those taken as bycatch, are already at high risk without it being recognised (Walker 1998). 'Critical bycatches' are bycatches of species or populations that are in danger of extinction, and 'unsustainable bycatches' are bycatches of species or populations that are not currently at risk but will decline at current levels of bycatch (Hall 1996).

The present study is designed to evaluate the catch composition and catch rates in the gillnet and longline components of the GHATF. The catch of each species was evaluated in terms of whether the animals were landed on-board 'live' or 'dead' and whether they were 'retained' or 'discarded'. The study addresses catches taken both by demersal monofilament gillnets and demersal longlines from data available for the two periods of 1973–76 and 1998–01. The study also addresses the effects of mesh-size and hanging ratio of gillnets and hook-size, hook-shank length, and hook-spacing on catch.

In the GHAT, a carcass in the catch is graded and valued according to its size and to the extent of damage to it. Damage to sharks captured in the gear from several sources can markedly reduce their value. Sharks that die in gillnets are susceptible to being partly or totally eaten by sea lice, seals, teleosts, and other species of shark.

With the introduction of quota management in the GHATF, there is uncertainty on the quantities of shark that are damaged at sea and how much is discarded at sea. Lower valued gummy shark, school shark, common sawshark, southern sawshark, and elephant fish might be discarded at sea for higher graded carcasses. From on-board observations there is a need to provide estimates of (a) quantities of sharks damaged and marketed, (b) quantities of sharks damaged and discarded, and (c) quantities of undamaged sharks discarded because of lower prices.

Objectives

1. Determine population parameters required for fishery stock assessment of the non-target species common saw shark, southern saw shark and elephant fish.
2. Provide stock assessment of each of these three non-target species in Bass Strait.
3. Provide data for evaluation of bycatch, discards and damaged shark in the Southern Shark Fishery (now Gillnet Hook and Trap Fishery).

Methods

The methods are referred to briefly under three separate headings, one to address each of the three project objectives. Full details of the methods are contained in the various manuscripts presented as appendices to the main report.

Determination of population parameters

Determination of population parameters for gillnet selectivity, von Bertalanffy growth, and reproduction (Objective 1) was variously based on data from three separate periods: 1973–76, 1986–87, and 1998–01.

Gillnet selectivity

Full details of the methods are published for gummy shark (Kirkwood and Walker 1986) and given for sawshark, elephant fish and school shark in Appendix 3a. Gillnet selectivity was investigated for sawshark and elephant fish using data collected during two separate experiments when gillnet selectivity parameters for gummy shark were investigated. The first experiment was undertaken during 1973–76 on-board research vessels and the second 1986–87 on-board vessels engaged in commercial fishing. For each of these two periods, experimental gillnets were standardised by constructing them with identical length of net, diameter of headline and footline, number of floats attached to the headline and number of lead weights attached to the footline. Height of net varied slightly to standardise hanging coefficient at 0.60. In all cases, the monofilament polyamide webbing was double-knotted and double-selvedge, but during 1973–76 the thickness and breaking strain of the webbing varied with increasing mesh-size. The nets were set on the seabed at least 100 m apart and not joined to avoid the potential effect of nets of small mesh-size herding fish to nets of larger mesh-size. The fishing times of the different gillnets were controlled to ensure that their fishing times were similar. The species, sex and total length of each shark caught was recorded from each gillnet during field operations.

During 1973–76, eight gillnets had mesh-sizes ranging 2–9 inches (51–229 mm), in steps of 1 inch (25 mm). Each net had a standard length of 250 m and height of ~1.7 m. The eight nets were set at 73 sites on the continental shelf off south-eastern Australia at depths of 9–79 m. The number of meshes deep, the thickness of the filaments of the webbing and the breaking strain of the filaments varied with mesh size. The gear was set before sunrise and hauled after sunrise with a mean fishing time of 5.8 h (Kirkwood and Walker 1986; Walker, Hudson *et al.* in press). During 1986–87, four gillnets had mesh-sizes ranging 5–8 inches (127–203 mm), in steps of 1 inch (25 mm). Each net had a standard length of 500 m and height of ~1.7 m. The number of meshes deep varied with mesh-size, but the thickness and breaking strain of the webbing filaments for all mesh-sizes were standardised at 0.90 mm and 359 N, respectively. The four gillnets were set 144 times on the continental shelf off south-eastern Australia at depths of 17–130 m. The gillnets were set, usually twice a day, at various times throughout the day and night, depending on stage of the tidal cycle. Mean fishing time for the gillnets was 4.8 h.

Relative selectivity, μ_{li}, expressed as a function of length of fish, l, and mesh-size, m_i, is given by

$$\mu_{li} = (l / \alpha_i \beta_i)^{\alpha_i} e^{(\alpha_i - l/\beta_i)},$$

where α_i and β_i are specified in terms of the mesh-size, m_i, and length l, and the length at maximum selectivity for gillnet i is proportional to the mesh-size such that

$$\alpha_i \beta_i = \theta_1 m_i$$

where θ_1 is a constant and the variance θ_2 is constant over different gillnets. These assumptions lead to a quadratic equation for positive β_i such that

$$\beta_i = -0.5\left(\theta_1 m_i - (\theta_1^2 m_i^2 + 4\theta_2)^{0.5}\right).$$

The maximum likelihood estimates of the main parameters of interest, θ_1 and θ_2, were estimated using the Nelder–Mead simplex algorithm (Nelder and Mead 1965). Standard errors for each of θ_1 and θ_2 were estimated according standard methods (Venzon and Moolgavkor 1988).

Length-at-age

Full details of the methods are presented in a manuscript in preparation (Appendix 3b). Stained whole vertebrae of sawsharks and sectioned dorsal spines of elephant fish were used for estimating the age of common sawshark, southern sawshark and elephant fish. Ages were estimated by assuming counted growth-increment bands are deposited annually.

The length-at-age data were fitted to the von Bertalanffy growth (VBG) model reparameterised by the Francis method (Francis 1988a; Francis 1988b) and adapted to correct for sampling bias caused by length-specific gillnet selectivity (Dow 1992). The VBG model has the equation

$$l_a = L_\infty \{1 - e^{-K(a-a_0)}\}$$

where K, L_∞ and a_0 are the VBG parameters and l_a is the length of a shark at age a. For length-at-age data, these parameters are replaced by l_ϕ, l_χ and l_ψ the mean lengths of fish estimated by the model at the arbitrary ages of ϕ, χ and ψ, respectively, where

$$\chi = (\phi + \psi)/2$$

and ϕ and ψ are chosen to represent the range of the data.

Reproductive biology

A full description of the methods adopted for determining the reproductive parameters required for fishery stock assessment are described in detail in each of three quantitative reproductive papers in preparation attached for common sawshark (Appendix 3c), southern sawshark (Appendix 3d), and elephant fish (Appendix 3e). A more general paper in press develops methods for application to chondrichthyans in general, which are applied to school shark (Appendix 3f). Parts of work associated with preliminary investigation of the peculiarities of the structure and function of various reproductive organs and tissues of elephant fish have been published in scientific journals (Appendix 3g).

The total body mass, w, to total length (TL), l, relationship was determined using the power curve

$$w = acl^b,$$

where a and b are parameters determined by linear regression of the natural logarithm of w against the natural logarithm of l, and c is a factor correcting for biases caused by natural logarithmic transformation (Beauchamp and Olson 1973; Walker in press).

The linear relationship between the number of macroscopically visible *in utero* embryos, p, and maternal TL, l is given by

$$p = a + bl,$$

where a and b are parameters estimated by linear regression.

The period of gestation and growth of embryos were determined by plotting mean TL of embryos observed in pregnant females with *in utero* embryos and mean TL values of 0 for *in utero* eggs observed in pregnant females against month and then evaluating the seasonal pattern.

The ovarian cycle was investigated by examining the ovary and measuring the diameters of the largest follicles in animals caught throughout the year. The largest follicle diameter (LFD) varied widely between individual animals and varied depending on uterus condition, so seasonal pattern in LFD for each of the six uterus conditions was examined separately. Pregnant females with macroscopically visible *in utero* embryos provided the least ambiguous basis for determining seasonal growth rates of follicles. Annual growth rate of follicles for pregnant females with macroscopically visible embryos was determined by the linear relationship between LFD, o, and Julian day, t, given by

$$o = a + bt,$$

where a and b are parameters estimated by linear regression. Scattergrams of LFD against Julian day for each uterus condition were compared with the regression line and its and 95% prediction intervals used as a basis for distinguishing between annual, biennial, and longer ovarian cycles.

Size-at-maturity and size-at-maternity were determined as the proportion of the population of animals mature at any TL by classing each animal as in mature condition or immature condition and applying logistic regression for females and males separately. Similarly, for females, the proportion of the population of animals in maternal condition at any TL can be determined by classing each animal as in maternal condition or non-maternal condition and applying logistic regression. A female was classed as in mature condition if the largest ovarian follicle was >3 mm in diameter (size at first yolking); otherwise it was classed as in immature condition. Given uncertainty of the best indicator of maturity of males, the results from methods based on alternative criteria for assuming the mature condition and the immature condition were compared. Males were classed as mature or immature on the basis of testis development, seminal vesicle condition, and clasper calcification. A female was classed in maternal condition at the time of capture, if, had it survived, it would have given birth to young before or soon after the following 1 January; all other females were classed in non-maternal condition.

Logistic regression was adopted to determine the proportion of females in mature condition, the proportion of males in mature condition, and the proportion of females in maternal condition as a function of TL. Females or males in mature condition were assigned a maturity condition value of 1, whereas those in immature condition were assigned a maturity condition value of 0. Similarly, females in maternal condition were assigned a maternal condition value of 1, whereas females in non-maternal condition were assigned a maternal condition value of 0.

The logistic equation adopted to express P as a function of l is given by

$$P = P_{\max}\left(1+e^{-\ln(19)\left(\frac{l-l_{50}}{l_{95}-l_{50}}\right)}\right)^{-1},$$

where $P_{\max}$ is the maximum proportion of animals in mature condition or maternal condition, and l_{50} and l_{95} are the lengths at which 50% and 95% of the maximum proportion of animals in mature condition or maternal condition (Walker in press). The parameters $P_{\max}$, l_{50} and l_{95}, with 95% confidence intervals, were estimated by the method of maximum likelihood using the probit procedure (Proc Probit) of the computer statistical package SAS (SAS Institute, Cary, North Carolina, USA). This applies a modified Newton–Raphson algorithm for estimation. $P_{\max}$ normally has a value of 1.00 except when parturition frequency is biennial ($P_{\max} = 0.500$), triennial, ($P_{\max} = 0.333$) or some other period (Walker in press).

Stock assessment

Full details of the assessments are presented in Appendix 4. Stock assessment (Objective 2) was based on the parameter values and length-frequency data from all three periods and time series of catch (1950–03), fishing effort (1976–03), and catch per unit effort (1976–03) data for shark longlines and for each mesh-size of shark gillnets. The assessments also included catch for Danish seine (1950–03) and demersal trawl from the South East Trawl Fishery (1985–03), and Great Australia Bight Trawl Fishery (1988–03). The first stock assessment for each of common sawshark, southern sawshark, and elephant fish covering only the Bass Strait region was undertaken and presented to the 20–21 May 2004 SharkRAG meeting. These assessments were subsequently reviewed and extended to include Bass Strait, Tasmania and South Australia and, for elephant fish only, New South Wales and then presented to the 9–10 September 2004 SharkRAG meeting.

The data sets for common sawshark, southern sawshark, and elephant fish off southern Australia are limited and stock assessment would be not be possible, had the present project not been undertaken. The only monitoring data on which an assessment of sawshark (species combined) and elephant fish can be based are catches and catch-rates. The types of long time series of catch length-frequency data and tag release-recapture data available for gummy shark and for school shark are not available for sawshark or elephant fish. Although most of the catch is from Bass Strait and there are generally low catches in waters off Tasmania and South Australia, the assessments undertaken apply to this entire area of waters on the continental shelf and slope for sawshark and also includes New South Wales for elephant fish. Catch-rate indices for these species are developed based on the assumption that the effort estimated to be targeted at gummy shark is also targeted at sawshark and elephant fish. Catches of sawshark are distinguished between common sawshark and southern sawshark on the basis of catches recorded as scientific observations during 1973–76, 1986–87 and 1998–01. The assessments are based on a non spatial, and age- and sex-based population dynamics model similar to that used for the 1996 stock assessment of school shark (*Galeorhinus galeus*) (Punt and Walker 1998). The results of the base-case assessments indicate that both sawshark and elephant fish are depleted to below 40% of the 1950 pup production level but that the rate of decline in the size of these resources has decreased substantially since the mid-1980s. Sensitivity tests indicate that the uncertainty range of depletion levels is less for elephant fish (14–22%) than for sawshark (17–39%). Pup production is assessed at 32% for common shark, 26% for southern shark, and 20% for elephant fish of the 1950 levels.

Evaluation of target, byproduct, bycatch and damaged catch

Full details of the methods on evaluation on target catch of shark, byproduct species of shark, and bycatch are presented in Appendix 5a. The present study is designed to evaluate the catch composition and catch rates in the shark fishery of southern Australia. The catch of each species was evaluated in terms of whether the animals were landed on-board 'live' or 'dead' and whether they were 'retained' or 'discarded'. The study addresses catches taken both by demersal monofilament gillnets and demersal longlines from data available for the two periods of 1973–1976 and 1998–2001.

Data used for catch evaluation were collected opportunistically during three separate investigations. Data from the first of these investigations were collected on two research vessels during 1973–1976. Data from the second of these investigations were collected on two commercial fishing vessels during 1998 as part a pilot fixed-station fishery-independent survey designed to determine survey intensity for monitoring abundance of harvested species. Data from the third investigation were collected on eight fishing vessels during 1999–01 when collecting biological samples of common sawshark, southern sawshark, and elephant fish as part of the present study.

During 1973–1976, most of the research sampling was undertaken in Bass Strait, with a small amount of sampling undertaken in waters off the east and south coasts of Tasmania and in waters off South Australia. Five separate experiments were undertaken to test for the effects of gillnet mesh size, gillnet hanging ratio, hook size, hook shank length and hook spacing on catch rate. During 1998–2001, sampling was undertaken during normal commercial fishing operations in Bass Strait and South Australia. For Bass Strait, comparisons of catch rates from gillnet with 6-inch mesh-size were made between 1973–1976 and 1998–2001. Other than recording mesh size of gillnets, it was not possible to control the design of the fishing gear or undertake experiments during the second period. Catch rates for gillnet 7-inch mesh-size and longlines with Mustad 11/O long-shank hooks during 1973–1976 are also presented for Bass Strait, because these gears were used extensively by the fishing industry during that period. For Tasmania, similar data were presented for 1973–1976, but there are no data for 1998–2001. For South Australia, there are insufficient data for 1973–1976, but data for gillnets with 6-inch and 6½-inch mesh-size are presented for 1998–2001. During 1998–2001, most of the fishing gear deployed in South Australia and Tasmania was gillnets with 6½-inch mesh-size and most of the fishing gear deployed in Bass Strait was gillnets with 6-inch mesh-size.

Catch rates were statistically tested for each of the five experiments separately and for each of three regions adopted for comparisons of the fishing gears used most widely in the shark fishery during 1973–1976 and 1998–2001. For each experiment, the data were pooled over all fishing sites, whereas, for inter-period and commercial gear comparisons, the data were separated into the three regions Bass Strait, Tasmania, and South Australia. A one-way analysis of variance was applied to test for the effect of each of several explanatory (independent) variables on catch rate (dependent variable) separately for each species and each major taxonomic group. For each analysis separately, the variance was tested for homogeneity and, where this was true, the following model was applied.

$$\text{Catch rate} = \text{Explanatory variable(s)} + \varepsilon.$$

In the model, ε is the error term and catch rate is the number of animals caught divided by the fishing effort, where fishing effort was applied separately in the model for each of several alternative units. For gillnets, the unit of fishing effort applied was 'metre-lift-hours', and, for longlines, the unit of fishing effort applied was 'hook-lifts' (number of hooks). The explanatory variable in the model varied depending on experiment or on region for the inter-period or gear comparisons. The explanatory variable was mesh size for Experiment 1, hanging ratio for Experiment 2, and hook size for Experiment 3, and the three explanatory variables were hook size, hook shank-length, and hook-space for each of Experiments 4 and 5. For inter-period comparisons, the explanatory variable was sampling period for gillnet with 6-inch mesh-size in Bass Strait and, for commercial gear comparisons, the explanatory variable was mesh size for gillnet with 6-inch and 6½-inch mesh-size in South Australia during 1998–01. No statistical test was applied to the data presented for Tasmania during 1973–76.

Full details of the methods for determining levels of damage to carcasses of gummy shark, school shark, common sawshark, southern sawshark and elephant fish from the effects of predation are presented in

Appendix 5b. 'Shark carcass' refers to a beheaded and eviscerated shark with the tail, all fins, and, for males, the claspers, attached, and a 'damaged carcass' consists of two portions: the 'lost damaged portion' and the 'retained damaged portion'. The two damaged portions contribute to loss of income to the fishing industry in two ways. There is loss of income through the 'lost damaged portion' being unavailable for marketing, and there is loss of income through the 'retained damaged portion' being devalued on the market through a reduced price per kilogram. Hence, for the purpose of the present report, there are an 'undamaged catch' (sum of all the sharks with zero damage to the carcasses), a 'lost catch' (sum of all the 'lost damaged portions' from the 'damaged shark carcasses'), and 'devalued catch' (sum of all the 'retained damaged portions'). Several steps were required to calculate 'lost carcass mass' and 'devalued retained carcass mass'. The 'total mass' and 'carcass mass' (i.e. expected mass assuming no damage) were estimated for each shark from TL and the masses of the 'lost damaged portion' and the 'retained damaged portion' were estimated for each damaged shark from its TL and %-loss value. For each species separately, 'lost carcass mass' of each animal was estimated by calculating the mass of the 'lost damaged portion' as carcass mass x '%-loss/100'. Similarly, 'devalued retained carcass mass' of each animal was estimated by calculating the 'retained damaged portion' as carcass mass x (1 – '%-loss'/100). Then total 'lost carcass mass' was determined by summing 'lost carcass mass' over all individual animals in the catch and total 'devalued retained carcass mass' was determined by summing 'devalued retained carcass mass' over all individual animals in the catch

Results/Discussion

Determination of population parameters

The gillnet selectivity parameters (Appendix 3a), von Bertalanffy growth parameters (Appendix 3b), and reproductive parameters (Appendices 3cde) required for fishery stock assessment of each common sawshark, southern sawshark, and elephant fish have all been determined and applied through SharkRAG for stock assessment undertaken early 2004. The parameter values are presented as a summary in Table 1 of this overview report and applied in the three preliminary fishery stock assessments (Appendix 4).

Stock assessment

The stocks of each of common saw shark, southern sawshark, and elephant fish are depleted to below 40% of the levels in the early 1970s (Appendix 4) and although not severely depleted will need careful monitoring. The species have medium productivity. Although neonate and juvenile stocks appear to be distributed away from the main shark fishing grounds, the selectivity characteristics of gillnets of 6–6½-inch mesh-size are such that they mainly catch mature animals and pregnant females. Modelling of gummy shark and school shark indicate that shark stocks are most secure when the mid-sized sharks are harvest by gillnets through creating a gauntlet effect (Prince in press; Walker 1998), where pre-recruits and breeding fish receive minimum impact.

Through SharkRAG, these assessments will be markedly upgraded over the next few months to include estimates of catch from the South East Trawl Fishery and Great Australian Bight Trawl Fishery from logbook effort and catch per unit effort recorded by observers from the Integrated Scientific Monitoring Program in this fishery.

Evaluation of target, byproduct, bycatch and damaged catch

The extensive results from analyses of catches from the various experiments are presented in Appendix 5a. In summary, for 1973–1976 and 1998–2001 combined, a much higher number of animals and a higher number of species were caught by gillnets (22 918 animals, 124 species) than by longlines (4 006 animals, 54 species). The wider range of gillnet mesh sizes and longline hook sizes deployed caught both a higher number of animals and higher number of species during 1973–1976 (16 657 animals, 112 species) than during 1998–2001 (10 267 animals, 65 species), despite a much lower fishing effort during 1973–1976. Some of the differences in numbers of animals and numbers of species caught between the two periods can be explained by longlines being used only during 1973–1976 (4 006 animals, 54 species). However, most of the differences in the numbers caught is explained by eight mesh sizes (2–9 inch) used during 1973–1976 (12 651 animals, 104 species) and only two mesh sizes (6 and 6½ inch) during 1998–2001 (10 267 animals, 65 species). The catch comprised mostly chondrichthyes (21 633 animals, 33 species) and teleosts (5 118, 87), with small quantities of cephalopoda (26, 4), bivalvia (14, 1), gastropoda (9, 1), crustacea (121, 3), and mammalia (3, 2).

Ten important summary points can be made about the catch rates of gillnets and longlines deployed in the shark fishery of southern Australia on the continental shelf in the depth range 9–130 m.

1. Both gillnets and longlines are much more effective at catching chondrichthyan species than at catching teleost species, and catches of species of cephalopoda, bivalvia, gastropoda, mammalia, aves and reptilia are negligible.

2. The effect of gillnet mesh size on catch rates is strong, whereas the effects of gillnet hanging ratio, hook size, hook shank length, and hook space are weak.

3. Overall catch rates of chondrichthyan and teleost fishes by gillnet mesh size are very different. For chondrichthyans, the modal catch rate is by 4-inch mesh-size with decreasing catch rates for both increasing and decreasing mesh size, whereas for teleosts the modal catch rate is by 2 inch mesh-size with decreasing catch rates as mesh size increases.

4. For gillnets, there is linear increase in the ratio of the number of chondrichthyan fishes divided by the number of teleost fishes with increasing mesh size, whereas for hooks the ratio is approximately constant with increasing hook size.

5. For chondrichthyes, the top four species taken by gillnet across 8 mesh sizes (Experiment 1), *Squalus megalops, Mustelus antarcticus, Heterodontus portusjacksoni,* and *Galeorhinus galeus,* are similar to the top four species taken by longline across 8 hook sizes (Experiment 3), *Squalus megalops, M. antarcticus, Cephaloscyllium laticeps,* and *G. galeus.* The only difference is that *H. portusjacksoni* is more prevalent than *C. laticeps* in the gillnet catch, whereas the converse occurs for the longline catch.

6. For teleostei, *Platycephalus bassensis* is the most prevalent species caught by both gillnets across 8 mesh sizes (Experiment 1) and longlines across 8 hook sizes (Experiment 3). *Neosebastes scorpaenoides* is the second most prevalent species caught by longline and the third most prevalent species caught by gillnet. The second most prevalent species taken by gillnet—*Trachurus novaezelandiae*—is not caught by longline.

7. For chondrichthyes in Bass Strait, there has been about one-third overall reduction in abundance across all species combined between 1973–76 and 1998–01. About half of this reduction is attributable to an 87% reduction in the catch per unit effort (CPUE) of *Galeorhinus galeus* and a 54% reduction in the CPUE of *Cephaloscyllium laticeps.* The decline in CPUE for *Cephaloscyllium laticeps* might partly be attributable to avoidance of high densities of this species by fishermen during 1998–01, but the marked reduction in CPUE for *Galeorhinus galeus* is consistent with trends evident in fishermen's logbook data

8. Only small proportions of the commercial catch of chondrichthyan (3%) and teleost (2%) animals taken by demersal gillnets of 6 in and 6½ in mesh size coming on-board dead are discarded. The discarded animals are mostly *Cephaloscyllium laticeps, Heterodontus portusjacksoni, Squalus megalops,* and *Myliobatis australis,* which come on-board live.

9. Wildlife interactions occur occasionally with the Australian fur seal (*Arctocephalus pusillus dorfer*) and common dolphin (*Delphinus delphis*).

10. Of ten chondrichthyan species on the continental shelf and continental slope identified by the IUCN Shark Specialist Group as threatened, two are identified by the present study as caught by the fishery. White shark (*Carcharodon carcharias*) are taken occasionally and *Galeorhinus galeus,* once the primary target species, is presently taken as significant byproduct (253 t during 2000).

Damage to shark carcasses from predation to sharks landed on-board from gillnets of 6-inch or 6½-inch mesh-size was investigated on-board nine vessels operating under normal commercial fishing conditions (Appendix 5b). The work was undertaken during November 1998–February 2001 at 153 fishing sites (91 sites in Bass Strait and 62 sites off South Australia). Of 3187 gummy sharks (*Mustelus antarcticus*), 145 school sharks (*Galeorhinus galeus*), 1099 common sawshark (*Pristiophorus cirratus*), 315 southern sawshark (*P. nudipinnis*), and 916 elephant fish (*Callorhinchus milii*) examined for carcass damage; 42% of the animals were landed on the deck alive without damage. Part of the catch of animals landed on-board dead had damage to carcasses resulting in 'lost carcass mass' and 'devalued retained carcass mass'. 'Lost carcass mass' from predation for gummy shark and school shark combined is estimated at 4.9% (4.7% for gummy shark and 6.9% for school shark); it is slightly higher in South Australia (5.3%) than in Bass Strait (4.7%). 'Lost carcass mass' for common sawshark and southern sawshark combined is estimated at 2.3% (2.1% for common sawshark and 3.5% for southern sawshark) and for elephant fish is estimated at 3.4%. 'Devalued retained carcass mass' from major damage is estimated at 9.2% for gummy shark and school shark combined (9.0% for gummy shark and 12.8% for school shark), 4.2% for common sawshark and southern sawshark combined (4.0% for common sawshark and 5.5% for southern sawshark), and 6.1% for elephant fish.

Benefits and Adoption

Benefits from the present project are allocated as 98% to the Commercial Sector (90% Commonwealth, 5% Victoria, 2% Tasmania, 1% South Australia) and 2% Recreational Sector (1% Victoria and 1% Tasmania). Benefits from stock assessment of common sawshark, southern sawshark and elephant fish will flow to the GHATF, SETF, GABTF, and coastal fisheries of Victoria, Tasmania, and South Australia, which all take these three species. Evaluation of catches of byproduct and bycatch species will benefit the shark gillnet sub-fishery and longline sub-fishery directly and other fisheries indirectly. Published survey catch rates of the byproduct and bycatch species provide baseline abundance estimates that can be used for future monitoring of abundance of these species. Knowledge of these trends is not only relevant to the shark fishery but to other Commonwealth and state fisheries that impact these species.

Following documentation of the results from the present project, most future work will relate to ongoing stock assessment and harvest strategy evaluation. All data are managed in accessible databases in SAS. As these data sets are updated they will be made available to SharkRAG as required. Additional work has begun to also include data sets on sawshark and elephant fish from the ongoing Integrated Scientific Monitoring Programs for each of the South East Trawl Fishery and Great Australia Bight Trawl Fishery.

The results for catch evaluation were a crucial input for Ecological Risk Assessment for the Effects of Fishing for scoping and a level 1 assessment for each of five components (target species, byproduct and bycatch species, threatened and protected species, habitats, and communities) associated with a sub-fishery. Where a level 1 assessment identifies hazards judged to have moderate or higher consequences, there is a need to progress to a level 2 assessment or adopt a Risk Management Response to avoid or reduce the consequence of each hazard posing risk. Similarly, where a level 2 assessment identifies a medium, high or extreme risk, there is a need to progress to a level 3 assessment or adopt an appropriate Risk Management Response.

Level 1 assessment (Scale Intensity and Consequence Analysis) is complete for each of the Shark Gillnet Sub-fishery and Shark Longline Sub-fishery of the Gillnet Hook and Trap Fishery (GHATF). For the Shark Gillnet Sub-fishery, of 21 impact-causing activities internal to the fishery, only 2 internal activity-impact combinations were identified as having a moderate (score 3) or above impact. For the Shark Demersal Longline Sub-fishery, 31 of 32 activities were identified as leading to some form of impact (Hazard Identification). Of 25 'impact causing' activities internal to the fishery, only 1 internal activity-impact combination was identified as having an impact of moderate or above. The level 1 assessment indicated that the only activity internal to the fishery to have a consequence score above 2 is 'capture fishing'. Of the five components of ERAEF, this occurred for each of three components (target, byproduct and bycatch, and TEP species), but not for the other two components (habitat and community). All other activities internal to the fishery had a consequence score of 1 (negligible) or 2 (minor). Having completed stock assessments for common sawshark, southern sawshark, and elephant fish mean that level 3 assessments were complete for these species.

Further Development

The present project has provided the basic demographic and gear selectivity parameters required for stock assessment of common sawshark, southern sawshark, and elephant fish. The project provided the basis to undertake the first stock assessment for each species and provides a basis for ongoing assessments through SharkRAG. The assessments will be periodically undertaken with updated monitoring data and modelling enhancements. In addition, the project has provided a description of catch rates of byproduct and bycatch species from the shark component of the GHATF, which will be monitored through the AFMA funded Fixed Site Monitoring Program. Further monitoring can be compared with assembled baseline catch rate data provided by the present project for the periods 1973–76 and 1998–01.

To ensure the scientific defensibility of the results produced from the project and defensibility of future stock assessments dependent on the project outputs, the work is being prepared for publication in internationally reviewed scientific journals. To date three manuscripts have been published in scientific journals and five student B.Sc. (Hons) theses have been submitted and assessed. These papers mainly deal with uncertainty of the basic reproductive biology of the elephant fish, which belongs to the poorly understood chondrichthyan group know as the holocephalans. A fourth paper is in press in a scientific journal; this describes the results of evaluation of target catch, byproduct and bycatch from the shark component of the Gillnet Hook and Trap Fishery. A fifth paper in press is a chapter of a book on the reproduction of chondrichthyans; the chapter describes the methods applied to the quantitative approach adopted in the present project, much of which is new. These five papers have all passed through peer review processes. Seven other manuscripts are in preparation and not yet submitted for publication. These include three manuscripts on reproductive biology, one on each of the three species, a fourth on age and growth of the three species, a fifth on gillnet selectivity of the three species, a sixth on sawshark stock assessment, and a seventh on elephant fish stock assessment. During the months ahead these manuscripts will be refined and submitted for scientific review.

Reduction of uncertainty in the assessments will require investment in initiating time-series of additional data sets such as catch length-frequency, catch length-at-age composition, and tag release recapture. An uncertainty recently identified is that of the effects of fishing mortality on female breeding elephant fish entering inshore shallow waters during March–May to lay their eggs in soft-sediment areas.

Over the next year or two it is expected that these assessments will lead to management actions designed to improve the sustainability of byproduct and bycatch species and reduce environmental impacts of shark fishing.

Planned Outcomes

Outcomes from the project include accessibility of basic selectivity parameters, biological parameters and monitoring data sets for ongoing stock assessment and fishery management advice. Both the basic biological data and catch and effort, length-at-age and length-frequency data sets are secure and readily accessible from SAS databases. Steps are being taken to incorporate into the assessments, data on sawshark and elephant fish from the ongoing Integrated Scientific Monitoring Programs for each of the South East Trawl Fishery and Great Australia Bight Trawl Fishery. The data will be made available to scientists for ongoing assessment of sawshark and elephant fish as required.

Data and advice will continue to be provided for refining strategic assessment, bycatch action plans, ecological risk assessment, and initiatives associated with Australia's National Plan of Action for the Conservation and Management of Sharks.

All results from the project will become available to scientists, fishery managers, industry personnel, and other beneficiaries in the form of the present report final to FRDC and ten scientific papers and two reports as they are published.

Conclusion

All gillnet selectivity parameters (Appendix 3a) and basic biological parameters required for age-based stock assessment models associated with Objective 1 are now available for common sawshark, southern sawshark and elephant fish (Table 1). The required biological parameters prescribe the von Bertalanffy length–age (Appendix 3b), logistic maternity–length, logistic maturity–length, linear litter-size–maternal length, and power mass–length (Appendices 3c–e) relationships. The project provided the opportunity to develop new methods for better determining the reproductive parameters required for stock assessment (Appendix 3f). Using data collected opportunistically during field operations, the project also provided the opportunity to provide better estimates of these reproductive parameters for school shark (Appendix 3f) and gummy shark (manuscript in preparation).

In addition to the required biological parameters, all available catch and effort, length-at-age, and length-frequency data required for fishery stock assessment as part of Objective 2 are secure and readily available for each of common saw shark, southern sawshark, and elephant fish from southern Australia. The stocks of each of these species are depleted to below 40% of the levels in the early 1970s and although not severely depleted will need careful monitoring and management. The species have medium productivity. Although neonate and juvenile stocks appear to be distributed away from the main shark fishing grounds, the selectivity characteristics of gillnets of 6–6½-inch mesh-size are such that they mainly catch mature animals and pregnant females (Appendix 4). Modelling of gummy shark and school shark indicate that shark stocks are most secure when the mid-sized sharks are harvest by gillnets through creating a gauntlet effect, where pre-recruits and large breeding fish receive minimum impact.

Apart from impacts on target and byproduct species, shark gillnets and shark longlines on the present fishing grounds of the continental shelf estimated as part of Objective 3 have minimal impact on other chondrichthyan and teleost species. Most bycatch species come on-board in live and vigorous condition; there is negligible discard of species dead and the level interactions with protected fauna is comparatively low (Appendix 5a). There is some damage to shark carcasses from predation by invertebrates, fish and mammals to sharks landed commercial vessels from gillnets of 6-inch or 6½-inch mesh-size. 'Lost carcass mass' from predation is estimated at 4.9% for gummy shark and school shark combined, 2.3% for common sawshark and southern sawshark combined, and 3.4% for elephant fish. 'Devalued retained carcass mass' from major damage is estimated at 9.2% for gummy shark and school shark combined, 4.2% for common sawshark and southern sawshark combined, and 6.1% for elephant fish (Appendix 5b).

References

Anon. (2000) Fisheries management 1. Conservation and management of sharks. In 'FAO Technical Guidelines for Responsible Fisheries' pp. 37. (Food and Agriculture Organisation of the United Nations: Rome)

Beauchamp JJ, Olson JS (1973) Corrections for bias in regression estimates after logarithmic transformation. Ecology 54, 1403–1407.

Beck ALN, Walker TI, Hamlett WC (submitted) Histology of the testis, male genital ducts and spermatozeugmata formation in the Southern sawshark, Pristiophorus nudipinnis. Marine and Freshwater

Research 00, 00-00.

Bell JD (2003) Fisheries and reproductive biology of the elephant fish, Callorhinchus milii, in south-eastern Australia. B.Sc. (Hons) thesis, Deakin University.

Dow NG (1992) Growth parameter estimation from tagging and ageing data. In 'The Measurement of Age and Growth in Fish and Shellfish, Australian Society for Fish Biology Workshop'. (Ed. DA Hancock) pp. 185–192. (Bureau of Rural Resources, Canberra: Lorne)

Francis RICC (1988a) Are growth parameters estimated from tagging and age-length data comparable? Canadian Journal of Fisheries and Aquatic Sciences 45, 936–942.

Francis RICC (1988b) Maximum likelihood estimation of growth and growth variability from tagging data. New Zealand Journal of Marine and Freshwater Research 22, 42–51.

Hall MA (1996) On bycatches. Reviews in Fish Biology and Fisheries 6, 319–352.

Hamlett WC, Reardon M, Clark J, Walker TI (2002) Ultrastructure of sperm storage and male genital ducts in a male holocephalan, the elephant fish, Callorhynchus milii. Journal of Experimental Zoology 292, 111-128.

Kirkwood GP, Walker TI (1986) Gill net mesh selectivities for gummy shark, Mustelus antarcticus Günther, taken in south-eastern Australian waters. Australian Journal of Marine and Freshwater Research 37, 689–697.

Last PR, Stevens JD (1994) 'Sharks and rays of Australia.' (CSIRO Australia: Melbourne)

Nelder JA, Mead R (1965) A simplex method for function minimization. The Computer Journal 7, 308–313.

Prince JD (in press) Gauntlet fisheries for elasmobranchs – the secret of sustainable shark fisheries. Journal of Northwest Atlantic Fishery Science 00, 00–00.

Punt AE, Pribac F, Walker TI, Taylor BL, Prince JD (2000) Stock assessment of school shark Galeorhinus galeus based on a spatially-explicit population dynamics model. Marine and Freshwater Research 51, 205–220.

Punt AE, Walker TI (1998) Stock assessment and risk analysis for the school shark (Galeorhinus galeus) off southern Australia. Marine and Freshwater Research 49, 719–731.

Reardon MB, Walker TI, Hamlett WC (2002) Microanatomy of spermatophore formation and male genital ducts in the holocephalan, Callorhynchus milii. In 'Proceedings of Sixth Indo-Pacific Fish Conference'. Special Issue of. Marine and Freshwater Research 53, 591-600.

Smith RM, Walker TI, Hamlett WC (2004) Microscopic organisation of the oviducal gland of the elephant fish, Callorhynchus milii. Marine and Freshwater Research 55, 155–164.

Venzon DJ, Moolgavkor SH (1988) A method for computing profile-likelihood-based confidence intervals. Applied Statistics 37, 87–94.

Walker TI (1998) Can shark resources be harvested sustainably? A question revisited with a review of shark fisheries. Marine and Freshwater Research 49, 553–572.

Walker TI (2005) 4 Reproduction in fisheries science. In 'Reproductive Biology and Phylogeny of Chondrichthyes: Sharks, Skates, Stingrays and Chimaeras''. (Ed. WC Hamlett) pp. 81–127 (Science Publishers, Inc.: Enfield, New Hampshire 03478, USA)

Walker TI, Hudson RJ, Gason AS (in press) Catch evaluation of target, byproduct, and bycatch species in the shark fishery of south-eastern Australia. In 'Proceedings of North Atlantic Fisheries Organisation Symposium. Elasmobranch Fisheries: Managing for Sustainable Use and Biodiversity Conservation'. 11-13 September 2002. Santiago de Compostela, Spain. Journal of Northwest Atlantic Fishery Science 35, 505-530.

Walker TI, Taylor BL, Gason AS (2003) 'Southern shark catch and effort 1970–2002 report to Australian Fisheries Management Authority.' Marine and Freshwater Resources Institute, Queenscliff, Victoria, Australia.

Table 1. Biological and gear selectivity parameter values for common sawshark, southern sawshark and elephant fish required for stock assessment

Selectivity parameters were determiined using the 8 mesh sizes 2–9 inches during 1973–76; common sawshark and southern sawshark data pooled.

Parameter	Common sawshark		Southern sawshark		Elephant fish	
	Male	Female	Male	Female	Male	Female
von Bertalanffy growth						
$L\infty$(mm)	1165	1502	971	1047	770	1049
K ($year^{-1}$)	0.309	0.149	0.575	0.488	0.400	0.238
t_0 (year)	-1.00	-1.76	-1.00	-0.49	-0.04	-0.05
Mass (kg) – TL (mm)						
Non pregnant						
a (se range) ($x10^{-9}$)	1.520 (0.991–2.330)	0.990 (0.824–1.190)	0.078 (0.035–0.172)	0.060 (0.034–0.107)	0.063 (0.044–0.089)	0.591 (0.438–0.798)
b (se)	3.015 (0.062)	3.292 (0.062)	3.450 (0.115)	3.498 (0.086)	3.688 (0.054)	3.337 (0.046)
Pregnant						
a (se range) ($x10^{-9}$)		0.423 (0.163–1.100)		354.0 (80.80–1550.0)		
b (se)		3.231 (0.134)		2.252 (0.214)		
Litter size – TL (mm)						
a (se)		–14.52 (2.79)		–8.36 (4.63)		–2.37 (11.70)
b (se)		0.0205 (0.0022)		0.0184 (0.0045)		0.0279 (0.0144)
Maturity – TL (mm)						
P_{max}		1.00		1.00		1.00
l_{50} (mm)		1128 (1116, 1138)		866 (827, 892)		607 (585, 624)
l_{95} (mm)		1383 (1362, 1407)		1056 (1037, 1083)		731 (717, 746)
Maternity – TL (mm)						
P_{max}		0.50		0.50		1.00
l_{50} (mm) (95% Cl)		1156 (1155, 1157)		944		659 (636, 678)
l_{95} (mm) (95% Cl)		1239 (1238, 1241)		954		888 (871, 910)
Gillnet selectivity						
$\theta 1$	237.91		237.91		154.23	
$\theta 2$	185075		185075		185097	

Equations as in Punt and Walker (1998), except fecundity is different: Fecundity = a + b TL

Appendix 1: Intellectual Property

No intellectual property has arisen from the research that is likely to lead to significant commercial benefits, patents or licences. Intellectual property associated with information produced from the project will be shared equally by the Fisheries Research and Development Corporation and by the Victorian Department of Primary Industries.

Appendix 2: Staff

Organisation, position, period on the project and percentage of time each year on the project are listed for each staff member at the Marine and Freshwater Resources Institute.

Terry Walker	Principal Investigator	1 Jul 99–30 Jun 00	10%
		1 Jul 00–30 Jun 01	10%
Russell Hudson	Fisheries Scientist	1 Jul 99–30 Jun 00	60%
		1 Jul 00–30 Jun 01	60%
Lauren Brown	Fisheries Scientist	1 Jul 99–30 Jun 00	10%
Corey Green	Technical Officer	1 Jul 99–30 Jun 00	10%
		1 Jul 00–30 Jun 01	15%
Thérèse Stokie	Technical Officer	1 Jul 00–30 Jun 01	5%

Appendix 3a: Gillnet selectivity

This appendix contains a manuscript in preparation which includes the results of gillnet selectivity trials for common sawshark, southern sawshark and elephant fish.

Gillnet selectivity for four chondrichthyan species harvested off southern Australia

Contents

List of Tables and Figure

Draft manuscript in preparation for submission to a scientific journal for publication (17 July 2005)

Gillnet selectivity for four major non-target chondrichthyan species harvested off southern Australia

Terence I. Walker[A]

Primary Industries Research Victoria, PO Box 114, Queenscliff, Victoria 3225, Australia.
[A]Corresponding author. Email Terry.Walker@dpi.vic.gov.au

Abstract

Gillnet selectivity parameters are estimated from catches of four species of shark by two experiments undertaken in southern Australia during 1973–76 and 1986–87 using a selectivity function derived from the gamma probability distribution function. The data are fitted to the data by maximum likelihood. The school shark (*Galeorhinus galeus*) parameter estimates are based on four mesh-sizes (5–8 inch) used during 1986–87. The elephant fish (*Callorhinchus milii*) parameter estimates based on six mesh-sizes (4–9 inch) during 1973–76. Data from the two species common sawshark (*Prisiophorus cirratus*) and southern sawshark (*P. nudipinnis*) were pooled to provide combined selectivity parameters from eight mesh-sizes (2–9 inch) during 1973–76. The presence of the rostrum with rostral spines on the sawshark and large variation in body shape between these species produce marked differences in the length at maximum selectivity and width of the selectivity curves for any mesh-size.

Key words: Fishing gear selectivity; Fisheries; Gillnet; Australia

Introduction

Chondrichthyan fish are mostly captured in fisheries by gillnet, longline and demersal trawl (Walker 1998), yet little attempt has been made to investigate the effects of gear selectivity on the size of these animals caught. The few investigations undertaken are mainly for sharks caught in gillnets (Kirkwood and Walker 1986; McLoughlin and Stevens 1994; Simpfendorfer and Unsworth 1998; Carlson and Cortés 2003). All are based on the same method for determining selectivity parameters (Kirkwood and Walker 1986). The investigations involved the use of experimental gillnets constructed with range of mesh-sizes and designed to resemble the standard gears used in the respective fisheries. Another investigation applying the same method determined the gillnet selectivity parameters for a ray using fishery-dependent data selected to minimise departure from the assumptions of the method (Márquez-Farias 2005).

The present study also adopts this method using experimental gillnets to estimate gillnet selectivity parameters for three species of shark and one species of holocephalan. The species of shark investigated are school shark (*Galeorhinus galeus*), common sawshark (*Pristiophorus cirratus*), and southern sawshark (*Pristiophorus nudipinnis*) and the holocephalan species is the elephant fish (*Callorhinchus milii*).

Galeorhinus galeus, a triakid, occurs as six genetically distinct populations (Ward and Gardner 1997) off western North America, eastern South America, southern Africa, southern Australia and New Zealand, and in the eastern North Atlantic Ocean (Compagno 1984). There is some mixing by a small proportion of the large animals undertaking migrations between New Zealand and Australia (Hurst *et al.* 1999), but genetic studies indicate that there is no interbreeding between these populations (Ward and Gardner 1997). As a long-lived, low-productivity species, the Australian population has been severely depleted by fishing over an 80-year period (Punt and Walker 1998; Punt *et al.* 2000).

Pristiophorus cirratus and *P. nudipinnis* are two of four species of the family *Pristiophoridae* endemic to Australia. *Pristiophorus cirratus* is distributed from Jurien Bay in Western Australia to Eden in southern New South Wales, around Tasmania, at depths to 310 m. *Pristiophorus nudipinnis* is distributed from the western regions of the Great Australian Bight in South Australia to Eastern Victoria, around Tasmania, at depths to 70 m (Last and Stevens 1994).

Callorhinchus milii occurs in southern Australian (Last and Stevens 1994) and New Zealand (Francis 1998) waters and is one of only three species in the chimaeroid family (*Callorhynchidae*). The other species are *C. capensis* off South Africa (Freer and Griffiths 1993) and *C. callorhynchus* off eastern South America (Di Giácomo and Perier 1994). *Callorhinchus milii* is distributed at depths to at least 200 m from Esperence in Western Australia to Sydney in New South Wales and around Tasmania (Last and Stevens 1994).

All four species are caught mostly as byproduct gummy shark (*Mustelus antarcticus*) are when targeted in the Gillnet Hook and Trap Fishery (GHATF) of southern Australia. None of the four species is commonly targeted. However, school shark was the primary target species from the mid-1920s when shark fishing began until the early 1970s, when demersal gillnets replaced longlines as the main fishing method in the fishery, and gummy sharks became the principal target species. Present management arrangements in the fishery discourage targeting of school sharks.

Present legislation prescribes a minimum mesh-size of 150 mm (~6 inches) and a maximum mesh-size of 165 mm (~6½ inches) for gillnets. This mesh-size range has proved to be very effective for gummy shark in that it provides for the highest sustainable catches. Smaller mesh-sizes catch the animals when they are small and reduce the yield from the fishery; larger mesh-sizes catch the large most fecund animals, raising the risk of depletion through impacting the recruitment processes (Walker 1998). A fishery harvest strategy designed to capture mid-sized animals with a narrow mesh-size range, a gauntlet-style fishery, has been shown to provide for stable and secure shark fisheries (Prince 2005). The 6–6½-inch mesh-size range is also effective for minimising bycatch from the shark fishery of southern Australia (Walker *et al.* 2005).

Materials and Methods

Field experiment

Gillnet selectivity was investigated during two separate periods. The first period was from 8 June 1973 to 29 November 1976 aboard research vessels and the second period was from 28 February 1986 to 9 December 1987 aboard vessels engaged in commercial fishing. For each of these two periods, experimental gillnets were standardised by constructing them with identical length of net, diameter of headline and footline, number of floats attached to the headline and number of lead weights attached to the footline. Height of net varied slightly to standardise hanging coefficient at 0.60. In all cases, the monofilament polyamide webbing was double-knotted and double-selveged, but during 1973–76 the thickness and breaking strain of the webbing varied with increasing mesh-size. The nets were set on the seabed at least 100 m apart and not joined to avoid the potential effect of nets of small mesh-size herding fish to nets of larger mesh-size. The fishing times of the different gillnets were controlled to ensure that their fishing times were similar. The species, sex and total length of each shark caught was recorded from each gillnet during field operations.

During 1973–76, eight gillnets had mesh-sizes ranging 2–9 inches (51–229 mm), in steps of 1 inch (25 mm). Each net had a standard length of 250 m and height of ~1.7 m. The eight nets were set at 73 sites on the continental shelf between Streaky Bay in South Australia and Gabo Island in Victoria, and off eastern and southern Tasmania at depths of 9–79 m. The number of meshes deep, the thickness of the filaments of the webbing and the breaking strain of the filaments varied with mesh size (Table 1). The gear was set before sunrise and hauled after sunrise with a mean fishing time of 5.8 h (Kirkwood and Walker 1986; Walker *et al.* 2005).

During 1986–87, four gillnets had mesh-sizes ranging 5–8 inches (127–203 mm), in steps of 1 inch (25 mm). Each net had a standard length of 500 m and height of ~1.7 m. The number of meshes deep varied with mesh-size, but the thickness and breaking strain of the webbing filaments for all mesh-sizes were standardised at 0.90 mm and 359 N, respectively. The four gillnets were set 144 times on the continental shelf between Western Australia-South Australia border and Lakes Entrance in eastern Victoria at depths of 9–201 m. The gillnets were set, usually twice a day, at various times throughout the day and night, depending on stage of the tidal cycle. Mean fishing time for the gillnets was 4.8 h.

Data analysis

The method adopted for analysis of the data was initially developed to analyse experimental data for *Mustelus antarcticus* (Kirkwood and Walker 1986). A review of techniques for determining gillnet selectivity advocated this approach because it simultaneously fits to catches from a range of mesh-sizes (Millar 2000). For this method, absolute selectivity of a gillnet for each size of fish is defined as the probability that, if a fish of that size encounters the gillnet, it is captured and retained in the gillnet. These probabilities cannot be readily estimated, so it is necessary to work with relative selectivities, rather than absolute selectivities. Relative selectivity for a particular size of fish is proportional to the absolute selectivity, with the constant of proportionality such that the maximum relative selectivity over all sizes is one.

The method is a convenient, flexible, two-parameter model that can display varying amounts of right skew to account for large fish entangling rather than enmeshing in a net. The model is derived from the probability density function of a gamma distribution with parameters α and β and argument x and has the probability density

$$\left(x^{\alpha}e^{(-x/\beta)}/\Gamma(\alpha+1)\right)/\beta^{(\alpha+1)}$$

with a single mode at $x=\alpha\beta$ and variance $(\alpha+1)\beta^2$. In this expression, $\Gamma(.)$ is the standard gamma function.

For this method, m_i is mesh-size of gillnet i, where $i=1,2,...,I$ for I separate gillnets; l_j is mid-length of length-class j, where $j=1,2,...,J$ for J separate length-classes; n_{ij} is number of fish from length-class j caught by gillnet i; μ_j is relative proportion in the population from length-class j; S_{ij} is mean relative selectivity for a fish in gillnet mesh-size i from length-class j; and η_j is fishing power at the size of maximum selectivity, relative to the maximum power over all I gillnets. Then for suitably scaled μ_j,

$$n_{ij}=\eta_i\mu_j S_{ij}.$$

To model relative selectivities S_{ij} with a maximum of one, this expression is rescaled so that the modal value is one. The model has a number of simplifying assumptions.

1. The gillnets have equal fishing power.

2. The selectivities S_{ij} and the mode and variance of the probability density function can be modelled by simple functions of m_i and l_j.

3. For each gillnet i and length-class j, the catches n_{ij} are independent observations from a Poisson distribution with mean $\mu_j S_{ij}$.

4. The full set of experimental nets encounter the same population.

5. The catch by a net is not influenced by the presence of any other net.

The log-likelihood of the whole data set is given by

$$L = \sum_{i=1}^{I} \sum_{j=1}^{J} \left(n_{ij} \ln(\mu_j S_{ij}) - \mu_j S_{jo} \right)$$

where

$$\mu_j = \frac{\sum_{i=1}^{I} n_{ij}}{\sum S_{ij}}.$$

Relative selectivity, μ_{li}, expressed as a function of length of fish, l, and mesh-size, m_i, is given by

$$\mu_{li} = (l / \alpha_i \beta_i)^{\alpha_i} e^{(\alpha_i - l / \beta_i)},$$

where α_i and β_i are specified in terms of the mesh-size, m_i, and length l, and the length at maximum selectivity for gillnet i is proportional to the mesh-size such that

$$\alpha_i \beta_i = \theta_1 m_i$$

where θ_1 is a constant and the variance θ_2 is constant over different gillnets. These assumptions lead to a quadratic equation for positive β_i such that

$$\beta_i = -0.5\left(\theta_1 m_i - (\theta_1^2 m_i^2 + 4\theta_2)^{0.5} \right).$$

The maximum likelihood estimates of the main parameters of interest, θ_1 and θ_2, were estimated using the Nelder–Mead simplex algorithm (Nelder and Mead 1965). Standard errors for each of θ_1 and θ_2 were estimated according standard methods (Venzon and Moolgavkor 1988).

Results and Discussion

Catches of the four species *Galeorhinus galeus*, *Pristiophorus cirratus*, *P. nudipinnis*, and *Callorhinchus milii* were pooled for each species separately over the 73 fishing sites where all eight nets of 2–9-inch mesh-sizes were set during 1973–76. Similarly the catches were pooled over the 144 fishing sites where all four gillnets were set during 1986–87. The number of sharks caught in each 100-mm length-class and the number of km-hours fished are presented for each mesh-size in Table 2.

Field observations indicated that some of the caught sharks were entangled rather than enmeshed by the nose or around the gills. This effect was more prevalent for large sharks than for small ones; i.e., for a given net the selectivity falls off more slowly for larger (right tail) than smaller sharks (left tail). Accordingly, a right-skewed model such as the gamma distribution is more appropriate for the data than a symmetric model.

The validity of the assumptions of equal fishing powers at the size of maximum selectivity and constant variance of selectivities across nets cannot be assessed. The assumption that mess, the second assumption appears reasonable, Shark length is proportional to girth (T. 1. Walker, unpublished data), which is consistent with the assumption that length at maximum selectivity is proportonal to mesh-size.

The 1973–76 data have the advantage of eight mesh-sizes over the four mesh-sizes for the 1986–87 data, but there was better coverage school shark during 1986–87 than the 1973–76. Fishing was mainly directed variously as both school shark and gummy shark during the 1986–87 whereas it was directed mainly at gummy shark during 1973–76. The 1973–76 data have adequate coverage of common sawshark, southern sawshark and elephant fish, which tend to be taken as byproduct when gummy shark are targeted. Hence, gillnet selectivity parameters for school shark were estimated from the 1986–87 with four mesh-sizes, whereas the parameters for sawshark and elephant fish were estimated from the 1973–76 data. To improve data coverage, data for common sawshark and southern sawshark were pooled and the model fitted the data adequately with all eight mesh-sizes (2–9 inches). For elephant fish, the model fitted the available data adequately for the six mesh-sizes 4–9 inches but failed to fit the data adequately for all eight mesh-sizes 2–9 inches. Mesh-sizes 2 and 3 inches were excluded from the analysis.

Applying the maximum likelihood estimation procedure to catches gave the following estimates of θ_1 and θ_2 and the selectivity curves are presented in Figure 1.

Species	θ_1 (s.e.)	θ_2 (s.e.)	Data	Mesh-size (inches)
Galeorhinus galeus	188.4 (3.5)	55920 (7)	1986–87	5–8
Pristiophorus spp	237.9 (3.1)	185075 (5)	1973–76	2–9
Callorhinchus milii	154.3 (5.9)	185097 (6)	1973–76	4–9

For any mesh-size, the presence of rostral spines on the rostrum of sawshark and large variation in body shape between these species produce marked differences in the length at maximum selectivity and width of the selectivity curves.

Given the highly selective nature of gillnets, as a fishing gear, proper interpretation of catch data taken with this gear cannot be made without taking account of selectivity. This applies to commercial catch and effort data, tag release-recapture data, and biological data such as length-at-age.

Acknowledgments

Acknowledgment is due to a large number of former and present staff of the Primary Industries Reseach Victoria Queenscliff Centre who participated in field sampling of sharks and laboratory processing. The shark fishing skippers and crews of various fishing vessels are thanked providing accommodation, assistance, and hospitality on-board their fishing vessels. Analysis of the data was funded by the Fisheries Research and Development Corporation (FRDC) of Australia as part of FRDC Project 1999/103. Collection of the data was funded by FRDC through earlier projects.

References

Carlson JK, Cortés E (2003) Gillnet selectivity of small coastal sharks off the southeastern United States. *Fisheries Research* **60**, 405–414.

Compagno LJV (1984) FAO species catalogue. Vol. 4. Sharks of the world. An annotated and illustrated catalogue of shark species known to date. Part 2. Carcharhiniformes. *FAO Fisheries Synopsis* **125**, 251–655.

Di Giácomo EE, Perier MR (1994) Reproductive biology of the cockfish, *Callorhynchus callorhynchus* (Holocephali: Callorhychidae), in Patagonian waters (Argentina). *Fishery Bulletin* **92**, 531–539.

Francis MP (1998) New Zealand shark fisheries: development, size and management. *Marine and Freshwater Research* **49**, 579–591.

Freer DWL, Griffiths CL (1993) The fishery for, and general biology of, the St Joseph *Callorhinchus capensis* (Dumeril) of the south-western cape, South Africa. *South African Journal of Marine Science* **13**, 63–74.

Hurst RJ, Bagley NW, McGregor GA, Francis MP (1999) Movement of the New Zealand school shark, *Galeorhinus galeus*, from tag returns. *New Zealand Journal of Marine and Freshwater Research* **33**, 29–48.

Kirkwood GP, Walker TI (1986) Gill net mesh selectivities for gummy shark, *Mustelus antarcticus* Günther, taken in south-eastern Australian waters. *Australian Journal of Marine and Freshwater Research* **37**, 689–697.

Last PR, Stevens JD (1994) 'Sharks and rays of Australia.' (CSIRO Australia: Melbourne)

Márquez-Farias JF (2005) Gillnet mesh selectivity for the shovelnose guitarfish (*Rhinobatos productus*) from fishery-dependent data in the artisanal ray fishery of the Gulf of California, Mexico. *Journal of Northwest Atlantic Fishery Science* **35**, 443–452.

McLoughlin KL, Stevens JD (1994) Gill-net mesh selectivities for two species of commercial carcharhinid shark taken in northern Australia. *Australian Journal of Marine and Freshwater Research* **45**, 521–534.

Millar RB (2000) Untangling the confusion surrounding gillnet selectivity. *Canadian Journal of Fisheries and Aquatic Science* **57**, 507–511.

Nelder JA, Mead R (1965) A simplex method for function minimization. *The Computer Journal* **7**, 308–313.

Prince JD (2005) Gauntlet fisheries for elasmobranchs – the secret of sustainable shark fisheries. *Journal of Northwest Atlantic Fishery Science* **35**, 407–416.

Punt AE, Pribac F, Walker TI, Taylor BL, Prince JD (2000) Stock assessment of school shark *Galeorhinus galeus* based on a spatially-explicit population dynamics model. *Marine and Freshwater Research* **51**, 205–220.

Punt AE, Walker TI (1998) Stock assessment and risk analysis for the school shark (*Galeorhinus galeus*) off southern Australia. *Marine and Freshwater Research* **49**, 719–731.

Simpfendorfer CA, Unsworth P (1998) Gill-net mesh selectivity of dusky sharks (*Carcharhinus obscurus*) and whiskery sharks (*Furgaleus macki*) from south-western Australia. *Marine and Freshwater Research* **49**, 713–718.

Venzon DJ, Moolgavkor SH (1988) A method for computing profile-likelihood-based confidence intervals. *Applied Statistics* **37**, 87–94.

Walker TI (1998) Can shark resources be harvested sustainably? A question revisited with a review of shark fisheries. *Marine and Freshwater Research* **49**, 553–572.

Walker TI, Hudson RJ, Gason AS (2005) Catch evaluation of target, byproduct, and bycatch species taken by gillnets and longlines in the shark fishery of south-eastern Australia. *Journal of Northwest Atlantic Fishery Science* **35**, 505–530.

Ward RD, Gardner MG (1997) Stock structure and species identification of school and gummy sharks in Australasian waters. February 1997. FRRF Project 93/11 and FRDC Project 93/64. 92 pp. CSIRO Marine Research, Hobart, Tasmania, Australia.

Table 1. Variable characteristics of the eight 1973–76 experimental gillnets

Mesh size (inch)	No. meshes deep	Filament thickness (mm)	Breaking strain (N)
2	42	0.47	101
3	28	0.57	146
4	21	0.66	193
5	17	0.74	240
6	14	0.81	285
7	12	0.87	326
8	10	0.90	348
9	9	1.05	467

Table 1. Observed catches for two gillnet selectivity experiments

Variable	Number of sharks caught during each period in gillnets of each mesh-size (inch)																		
	1973–76									1986–87					973–76 & 1986–87 combine				
	2	3	4	5	6	7	8	9	Total	5	6	7	8	Total	5	6	7	8	Total
Fishing effort (km)																			
Fishing effort (km-h)	108	107	103	105	109	105	105	105	847	335	335	335	335	1340	440	444	440	440	1764
Length-class (mm)																			
Galeorhinus galeus																			
300–399	21	2	3				1		27	2	1			3	2	1		1	4
400–499	1	14							15	1				1	1				1
500–599		10	5	1					16	1			1	2	2			1	3
600–699	1	10	32	8			1		52	74				74	82			1	83
700–799	1	4	15	11		2	1		34	96	22	1	2	121	107	22	3	3	135
800–899	3	2	9	20	1	2			37	99	35	1		135	119	36	3		158
900–999	4		3	6	1	2		1	17	52	40	12	5	109	58	41	14	5	118
1000–1099	1	3	4	5	2	4			19	31	40	11	3	85	36	42	15	3	96
1100–1199	3	1	1	1	5	3	3	1	18	16	22	25	7	70	17	27	28	10	82
1200–1299			1		3	7	4		15	13	15	31	17	76	13	18	38	21	90
1300–1399			1	2	3	4	2	1	13	6	17	31	21	75	8	20	35	23	86
1400–1499	1	2	2		6	9	6	6	32	14	16	28	30	88	14	22	37	36	109
1500–1599	1	2	3	1	1	13	13	17	51	8	13	19	25	65	9	14	32	38	93
1600–1699	2						2	2	6	1		3	6	10	1		3	8	12
1700–1799											1	1		2	0	1	1		2
Total	39	50	79	55	22	46	33	28	352	414	222	163	117	916	469	244	209	150	1072
Pristiophorus cirratus																			
300–399			1	1					2	0	1	2		3	1	1	2		4
400–499	0	1							1										
500–599	2	4							6	1				1	1				
600–699	1		2						3										
700–799	1	1	4						6	2			1	3	2			1	3
800–899	4	2	5						11	10	1	2	4	17	10	1	2	4	17
900–999	8	16	10	23	3	2	2		64	61	14	6	1	82	84	17	8	3	112
1000–1099	17	17	21	50	23	5	2		135	100	63	25	17	205	150	86	30	19	285
1100–1199	3	11	7	10	13	3	1		48	41	39	14	9	103	51	52	17	10	130
1200–1299	1	3	2	5	1		3		15	20	24	18	16	78	25	25	18	19	87
1300–1399	1					2			3	13	11	8	9	41	13	11	10	9	43
1400–1499				1					1	0			1	1	1			1	2
Total	38	55	52	90	40	12	8	0	295	248	153	75	58	534	338	193	87	66	683
Pristiophorus nudipinnis																			
300–399		1							1	1				1	1				1
400–499	1								1										
500–599	1	5							6				1	1				1	1
600–699	2	1							3		1	1		2		1	1		2
700–799	1	4	1	1					7	2		1		3	3		1		4
800–899	0	3	1	1		2			7	3	1	3	1	8	4	1	5	1	11
900–999	20	26	38	20	6	1	3		114	75	21	15	10	121	95	27	16	13	151
1000–1099	4	11	7	14	6		1	1	44	33	16	8	6	63	47	22	8	7	84
1100–1199		1			1				2		4	1		5		5	1		6
1200–1299												1	1	2			1	1	2
Total	29	52	47	36	13	3	4	1	185	114	43	30	19	206	150	56	33	23	262
Callorhinchus milii																			
300–399		1			2			1	4	1				1	1	2			3
400–499		4		1					5						1				1
500–599		2	3	2	1	1			9	1			1	2	3	1	1	1	6
600–699	1	9	23	41	10	13	2	8	107	27	19	15	10	71	68	29	28	12	137
700–799	2	4	16	33	16	11	3	4	89	54	27	13	7	101	87	43	24	10	164
800–899	5	12	11	15	22	13	12	5	95	2	2	6	3	13	17	24	19	15	75
900–999		4	4	7	7	13	2	4	41		1	2	1	4	7	8	15	3	33
1000–1099				1					1						1				1
Total	8	36	57	100	58	51	19	22	351	85	49	36	22	192	185	107	87	41	420

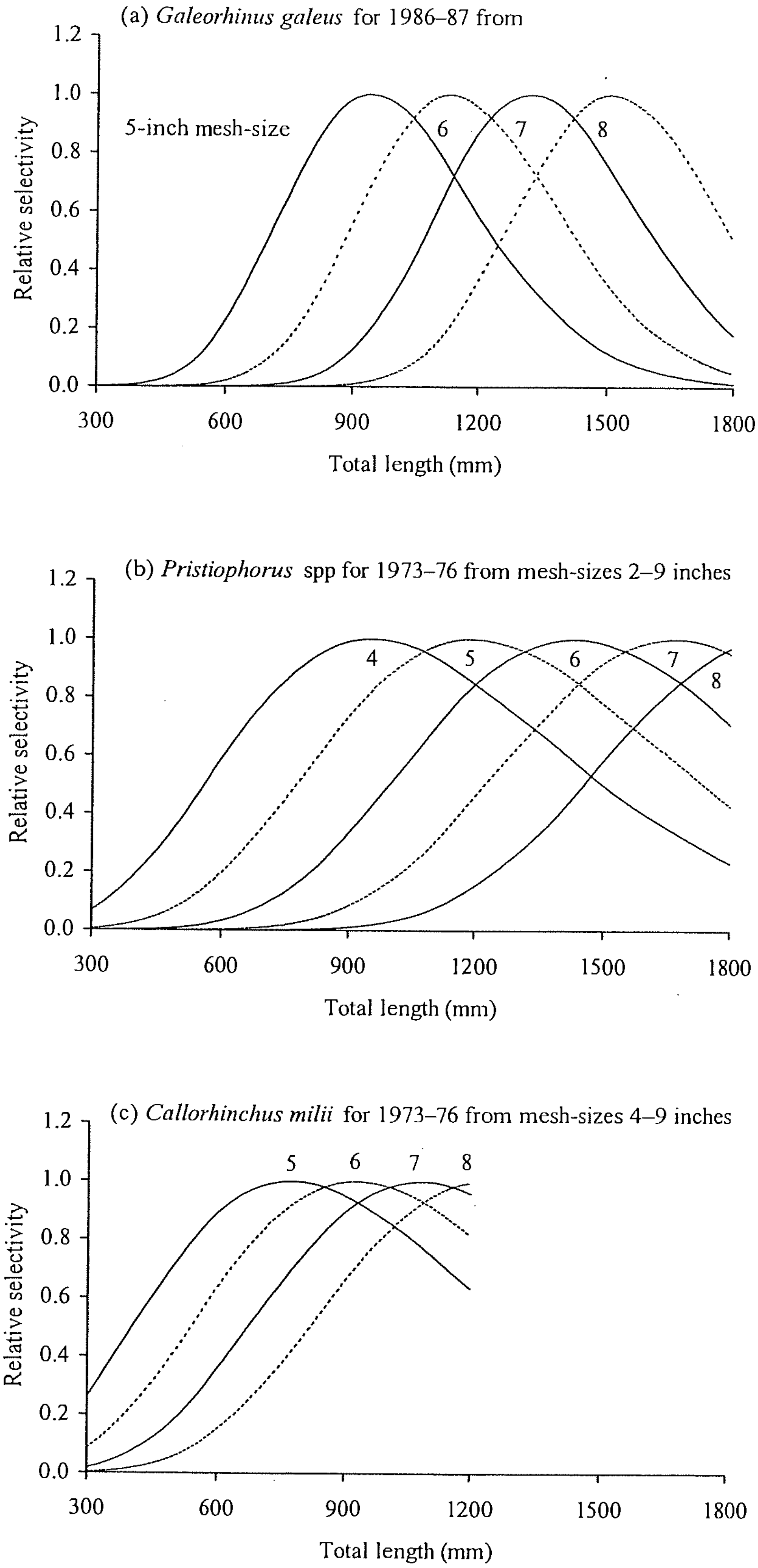


Fig. 1. Estimated gillnet selectivity curves for three species

Appendix 3b: Age and growth

This appendix contains a manuscript in preparation of the results of age and growth studies for common sawshark, southern sawshark and elephant fish.

Age and growth of common sawshark, southern sawshark, and elephant fish harvested off southern Australia

Contents

List of Tables and Figures

Draft manuscript in preparation for submission to a scientific journal for publication (17 July 2005)

Age and growth of common sawshark, southern sawshark, and elephant fish harvested off southern Australia

Terence I. Walker[A,C], Russell J. Hudson[A,B] and Corey Green

[A]Primary Industries Research Victoria, PO Box 114, Queenscliff, Victoria 3225, Australia
[B]Ocean Grove, Victoria 322X, Australia.
[C]Corresponding author. Email Terry.Walker@dpi.vic.gov.au

Abstract

Length-at-age data were obtained by counting stained growth-increment bands on whole vertebral centra of common sawshark (*Pristiophorus cirratus*) and southern sawshark (*P. nudipinnis*) and by counting growth-increment bands in transverse sections of the dorsal spine of elephant fish (*Callorhinchus milii*). The data were fitted to the von Bertalanffy growth model reparameterised by the Francis method and adapted to correct for the effects of sampling bias caused by length-specific gillnet selectivity.

Key words: Growth; Length-at-age; Gillnet selectivity; Fisheries; Australia

Introduction

Age and growth studies of harvested species are required for fishery assessment to apply age-structured models, age-based production models, yield per recruit models, or, usually, simple demographic models. Such studies also contribute for ecological risk assessment (Walker 2004) or species assessment for risk of extinction against criteria established by the IUCN (Hilton-Taylor 2000).

Pristiophorus cirratus and *P. nudipinnis* are two of four species of the family *Pristiophoridae* endemic to Australia. *Pristiophorus cirratus* is distributed from Jurien Bay in Western Australia, around Tasmania, to Eden in southern New South Wales at depths to 310 m. *Pristiophorus nudipinnis* is distributed from the western regions of the Great Australian Bight in South Australia, around Tasmania, to Eastern Victoria at depths to 70 m (Last and Stevens 1994).

Callorhinchus milii occurs in southern Australian (Last and Stevens 1994) and New Zealand (Francis 1998) waters and is one of only three species in the chimaeroid family (*Callorhynchidae*). The other species are *C. capensis* off South Africa (Freer and Griffiths 1993) and *C. callorhynchus* off eastern South America (Di Giácomo and Perier 1994). *Callorhinchus milii* is distributed at depths to at least 200 m from Esperence in Western Australia, around Tasmania, to Sydney in New South Wales (Last and Stevens 1994).

In the present study, *P. cirratus* and *P. nudipinnis* were aged from growth-increment bands in the postcranial vertebrae and C. milii were aged from estimating growth-increment bands in the dorsal spine. The relationship between length and age and heterogeneity in length-at-age was determined using the Francis model (Francis 1988b; Francis 1988a) which involves reparameterisation of the von Bertalanffy model (Von Bertalanffy 1938). The Francis model was enhanced to correct for sampling bias (Dow 1992) caused by the effects of length-selectivity when collecting the animals for ageing using gillnets (Kirkwood and Walker 1986).

Materials and Methods

Field sampling

Animals for ageing were collected during 1998–01 at 153 fishing sites (91 sites in BS and 62 sites in SA) by gillnets of 6-inch (152 mm) or 6½-inch (165 mm) mesh-size on board commercial fishing vessels. The animals were measured to the nearest millimetre as total length; the tail of each shark was allowed to take a natural position and the caudal lobe placed parallel with the body axis. Several postcranial vertebrae of *Pristiophorus cirratus* and *P. nudipinnis* and the dorsal spine of *Callorhinchus milii* were excised, placed in a labelled vial, and stored in a refrigerator at about –4°C.

Laboratory preparation

Sawshark vertebrae

In the laboratory, the sawshark vertebrae and elephant fish spines were stored at –22°C. In preparation for age determinations, the vertebrae were thawed, separated, trimmed of connective tissue, including the neural and haemal arches, and soaked in a 2.5% sodium hypochlorite solution until the fascia material could be removed effectively. Small vertebrae (3–5 mm diameter) were cleaned effectively in 30–40 min; larger vertebrae (>15 mm diameter) often required soaking for 1–2 h. Over-exposure to sodium hypochlorite produced chalky or partly decalcified vertebrae, which adversely affected the subsequent uptake of the stain. As a safeguard against mistreatment of samples, one or two vertebrae were set aside and not chemically treated. When clean, the treated vertebrae were placed under a flow of tap-water for at least 2 h to remove traces of bleach and then left to air dry for ~1 week.

Initial evaluation of ageing methods for sawshark

Sectioning vertebrae allows the internal structures of vertebrae to be viewed. Using a diamond impregnated rotating blade, vertebrae cut in half along the dorsal-ventral axis. Vertebra halves were immersed in water and illuminated from the side. The intermedialia and the corpus calcareum were assessed for increment clarity.

One method trialed was to grind the vertebrae to a thickness where light could be transmitted through the preparation. Vertebrae were attached to heated glass slides using thermoplastic glue (Crystal Bond™) in a way to achieve a dorsal-ventral plane when ground. Using coarse silica-carbide paper (200 grit size) attached to a rotating disk, vertebrae were ground down until the core was located. The sample was removed from the slide and glued to a secondary slide (ground face down). The remaining vertebra was ground until a thin section was obtained (approximately 400µm). Preparations were viewed under a dissecting microscope with transmitted light. Vertebral staining revealed clear growth increments during the early stages of growth; these stages included the birth band and the next two or three growth-increment bands. Subsequent bands were diffuse and indistinct at the outer margin. Sectioned vertebrae displayed clear growth increments in the corpus calcareum and intermedialia; however, as described for other species (Davenport and Stevens 1988), the birth band and following two or three increments were not as clear. Ground vertebrae displayed poor growth-increment clarity.

As with *Mustelus antarcticus* where the ageing method was validated from recapture of tagged sharks injected with oxytetracycline, it was considered that reading whole vertebrae stained with alizarin red was more accurate and more cost-effective than sectioning the vertebrae. Hence, whole vertebrae were used for subsequent age estimation.

Laboratory preparation of elephant fish spines

In preparation to embed the spines, a clear polyester casting resin was poured onto the base of a silicon mould and allowed to partially cure. Spines were then embedded in resin in rows of five and oven cured at 55° C for 24 h. Each block of five spines was cut into about 20 sections using a Gemmasta™

lapidary saw fitted with a diamond-impregnated blade. Each section of five spines was cleaned in alcohol and attached with an appropriate label to a 50-mm x 75-mm glass-slide and protected with a cover slip by thin coatings of resin. The mounted sections were then oven cure at 55° C for 24 h.

Reading vertebra sections

Before attempting to assign an age estimate to a sample, the "reader" first became familiar with the structure of the prepared vertebrae. A subsample of approximately 20 vertebrae from animals of a wide size range sizes was used to "calibrate" the interpretation. This training process was essential to ensure consistent interpretation.

Age estimates were determined by interpreting changes between hypermineralised and hypomineralised zones. Hypermineralised zones appear dark and relatively narrow compared with the optically lighter and wider hypomineralised zones. The birth band was defined as the first visible growth-increment, highlighted by the alizarin red stain, visible on the articular face. The formation of the first increment after the birthmark was allocated as the first growth increment band. Increments present on the articular face were counted out to the first two increments and used to reinforce the interpretation of increments visible and counted on the corpous calareum. The corpous calcareum was used to locate and count increments close to the edge of the preparation. Ages were assigned to the sub-sample and all samples were then re-aged. This process was repeated until there was minimal error between the repeated band counts. All samples were then aged.

A readability score of 1–5 (Officer *et al.* 1996) was given for each specimen based on clarity of the increments. A sample with unambiguous growth increments was given a score of one. A sample that was uninterpretable was allocated a score of five.

Measuring increment width allows analysis of the vertebral growth rate. To determine the distance between each increment and image analysis system was incorporated into the ageing process. A microscope with a CCD video camera linked to a computer with an image processing card. The image analysis Optimate™ was used to firstly mark a transect from the vertebrae core, along the central part of the corpus calcareum to the vertebra edge. The position of each increment was marked "on screen" and the distance between each increment automatically exported to Microsoft™ Excel (Morison et al. 1998a). The position of the birthmark was also marked. The total age was calculated as the total number of increments marked 'on screen' minus one to adjust for the birth mark.

Reading whole vertebrae

Vertebra staining enhances the contrast of increments by differentially staining the hypermineralized and hypomineralized zones on the articular face. Each whole vertebra was immersed in a freshly prepared dilute alkaline solution of alizarin red prepared from a concentrated solution of alizarin red and 0.1% potassium hydroxide solution in the ratio of 1:9 (Gruber and Stout 1983). The duration of immersion (1–5 min) depended on size of the vertebra. When stained, the vertebra was washed in tap water for 1 min and, while the vertebra was wet, the vertebral bands were counted immediately by viewing the surface of one centrum on each vertebra under a stereomicroscope at appropriate magnification using incident light (x7–x14).

For embryos and newborn sharks a dark stained embryonic growth zone was always present at the centre, but a clear band was visible outside the centrum embryonic growth zone. A band, usually faintly stained, was usually present in the centra of sharks judged to be several months old. It was assumed, therefore, that the first band is laid down shortly before birth. This first band is referred to as the 'birth band'.

The stained vertebrae from each sample were examined and the bands on the most evenly and clearly stained centra were counted. A small proportion of bands either were incompletely stained, or appeared as closely paired rings. With increasing shark size, bands near the outer perimeter become compacted and often lacked definition. This was further compounded by the characteristic curvature of the outer perimeter and the difficulty of delineating the point where new growth material was deposited. Therefore, any staining beyond the outermost discernible vertebral band was recorded as present or absent.

Bands on the vertebrae of each sample were counted by a single reader who classed each vertebra as 'readable' or 'unreadable', based on either or both the degree of differentiation effected by the stain and the difficulty in interpreting the arrangement of the vertebral bands. By assuming that vertebral bands were formed annually, and by selecting 1 January as an arbitrary birth date based on growth of embryos *in utero*, ages from band counts were calculated from the formula:

age = number of vertebral bands
− 1 for birth band
+ 1 only if outer perimeter was stained
+ proportion of year from 1 January to capture date.

Data analysis

A random sample of 25% of each species was re-aged to acquire estimates of intra reader variability. Since the interpretation of the vertebrae were the same, the samples were analysed on combined basis. The variation of age was assessed using an index of average percent error (IAPE) (Beamish and Fournier 1981). Bootstrap techniques (Efron and Tibshirani 1993) were applied to the IAPE to determine a bias corrected mean IAPE and 95% confidence intervals (Morison *et al.* 1998).

The length-at-age data were fitted to the von Bertalanffy growth (VBG) model reparameterised by the Francis method (Francis 1988b; Francis 1988a) and adapted to correct for sampling bias caused by length-specific gillnet selectivity (Dow 1992). The VBG model has the equation

$$l_a = L_\infty \{1 - e^{-K(a-a_0)}\}$$

where K, L_∞ and a_0 are the VBG parameters and l_a is the length of a shark at age a. For length-at-age data, these parameters are replaced by l_ϕ, l_χ and l_ψ the mean lengths of fish estimated by the model at the arbitrary ages of ϕ, χ and ψ, respectively, where

$$\chi = (\phi + \psi)/2$$

and ϕ and ψ are chosen to represent the range of the data.

Selectivity of the gillnets used for sampling the shark population for length-at-age data is assumed to be described by the gamma function, where maximum selectivity is set equal to 1. Selectivity is expressed as a function of length of shark and the selectivity parameters α and β, where α and β are in turn expressed as functions of mesh size and the parameters θ_1 and θ_2 (Kirkwood and Walker 1986). The length-frequency distribution of sharks in the population of each age class is described by the gamma probability density function (pdf) (i.e. 'prior distributions') (Dow 1992) rather than by the normal Gaussian pdf usually assumed. The length-frequency distribution for each age class of sharks in the sample captured by gillnets is also described by the gamma pdf ('posterior distribution') (Dow 1992). This is because the product of a gamma pdf (describing the 'prior distribution' of the shark population) and a gamma function (describing the selectivity of gillnets) gives a gamma pdf (describing the 'posterior distribution' of the sample). The likelihood for the 'posterior distribution' is derived (Dow 1992) from the product of the selectivity function of gillnets (Kirkwood and Walker 1986) and the likelihood for the 'prior distribution' (Dow 1992) derived from the likelihood (Francis 1988b; Francis 1988a).

The Francis growth parameters (with standard errors) for each model were estimated by maximising log-likelihood functions ('posterior distributions') using the Nelder–Mead simplex algorithm (Nelder and Mead 1965). Selectivity parameters θ_1 and θ_2 used in these statistical analyses were estimated for from experiments undertaken during each of 1973–76 and 1986–87 from the length-frequencies of sharks caught in the experimental gillnets (Appendix 3b).

Not all data available were used in these analyses. Only length-at-age data from stained vertebrae designated as 'readable' were included in these statistical analyses.

χ^2-tests on likelihood-ratios (Rao 1973; Silvey 1975) were used to determine whether growth varied with sex of the animals. This approach, advocated for comparison of VBG curves has been shown to be more reliable than other methods (Kimura 1980).

By using the Francis transformation equations (Francis 1988a), values for the three VBG parameters K, L_∞ and a_0 were derived from l_ϕ, l_χ and l_ψ. Any age could be used but $a = \chi$ years was selected because it is an age near the centre of the data range where the determined VBG curve is well defined.

Results and Discussion

A summary of the results from analysis of the data is presented in Table 1 for males and females separately for each of common saw shark, southern sawshark, and elephant fish. The table includes estimates of the Francis parameters with standard deviations and standard errors and estimates of the standard VBG parameters derived from the Francis parameters. Variation in length-at-age for two alternative assumptions. One assumes variance in fish length at any age is constant with age and the other assumes variance in fish length at any age is proportional to mean length at any age. The parameter estimates are corrected for the effects of sampling biased caused by the use of gillnets for catching the sharks for ageing purposes. The VBG curves are presented in Figure 1.

Acknowledgments

Acknowledgment is due to a large number of former and present staff of the Primary Industries Research Victoria Queenscliff Centre (PIRVic). Anne Gason of PIRVic is acknowledged for advice on statistical analysis of the data and application of the statistical package SAS and Thérèse Stokie of PIRVic is acknowledged for laboratory preparation of shark vertebrae and elephant fish spines. Many shark fishers who kindly provided accommodation, assistance, and hospitality aboard their fishing vessels are also thanked. The work was funded by the Fisheries Research and Development Corporation of Australia (FRDC Project 1999/103).

References

Beamish R, Fournier DA (1981) A method for comparing the precision of a set of age determinations. *Journal of the Fisheries Research Board of Canada* **36**, 1395–1400.

Davenport S, Stevens JD (1988) Age and growth of two commercially important sharks (*Carcharhinus tilstoni* and *C. sorrah*) from Northern Australia. *Australian Journal of Marine and Freshwater Research* **39**, 417–433.

Di Giácomo EE, Perier MR (1994) Reproductive biology of the cockfish, *Callorhynchus callorhynchus* (Holocephali: Callorhychidae), in Patagonian waters (Argentina). *Fishery Bulletin* **92**, 531–539.

Dow NG (1992) Growth parameter estimation from tagging and ageing data. In 'The Measurement of Age and Growth in Fish and Shellfish, Australian Society for Fish Biology Workshop'. (Ed. DA Hancock) pp. 185–192. (Bureau of Rural Resources, Canberra: Lorne.)

Efron B, Tibshirani RJ (1993) 'An introduction to the bootstrap.' (Chapman and Hall: New York, USA.)

Francis MP (1998) New Zealand shark fisheries: development, size and management. *Marine and Freshwater Research* **49**, 579–591.

Francis RICC (1988a) Are growth parameters estimated from tagging and age-length data comparable? *Canadian Journal of Fisheries and Aquatic Sciences* **45**, 936–942.

Francis RICC (1988b) Maximum likelihood estimation of growth and growth variability from tagging data. *New Zealand Journal of Marine and Freshwater Research* **22**, 42–51.

Freer DWL, Griffiths CL (1993) The fishery for, and general biology of, the St Joseph *Callorhinchus capensis* (Dumeril) of the south-western cape, South Africa. *South African Journal of Marine Science* **13**, 63–74.

Gruber SH, Stout RG (1983) Biological materials for the study of age and growth in a tropical marine elasmobranch, the lemon shark, *Negaprion Brevirostris* (Poey). pp. 193–205. In 'Proceedings of the international workshop on age determination of oceanic pelagic fishes: Tunas, billfishes, and sharks'. (Eds ED Prince and LM Pulos).

Hilton-Taylor C (2000) '2000 IUCN Red List of Threatened Species.' (IUCN The World Conservation Union: Gland, Switzerland.)

Kimura DK (1980) Likelihood methods for the von Bertalanffy growth curve. *Fishery Bulletin* **77**, 765–776.

Kirkwood GP, Walker TI (1986) Gill net mesh selectivities for gummy shark, *Mustelus antarcticus* Günther, taken in south-eastern Australian waters. *Australian Journal of Marine and Freshwater Research* **37**, 689–697.

Last PR, Stevens JD (1994) 'Sharks and rays of Australia.' (CSIRO Australia: Melbourne.)

Morison AK, Robertson SG, Smith DC (1998) An integrated system for production fish ageing:image analysis and quality assurance. *North American Journal of Fisheries Management* **18**, 549–554.

Nelder JA, Mead R (1965) A simplex method for function minimization. *The Computer Journal* **7**, 308–313.

Officer RA, Gason AS, Walker TI, Clement JG (1996) Sources of variation in counts of growth increment lines in vertebrae from gummy shark, *Mustelus antarcticus*, and school shark, *Galeorhinus galeus*: implications for growth determination. *Canadian Journal of Fisheries and Aquatic Sciences* **53**, 1765–1777.

Rao CR (1973) 'Linear Statistical Inference and Its Applications.' (John Wiley and Sons: New York.)

Silvey SD (1975) 'Statistical inference.' (Chapman and Hall: London.)

Von Bertalanffy L (1938) A quantitative theory of organic growth (inquiries on growth laws. II). *Human Biology* **10**, 181–213.

Walker TI (2004) Chapter 13. Management measures. In 'Elasmobranch Fisheries Management Techniques'.pp 285–321 (Eds JA Musick and R Bonfil). 370 pp. (APEC Secretariat: Singapore.)

Table 1. Sawshark and elephant fish growth parameter estimates for during 1998–01

n, sample size; SE, standard error; SD, standard deviation; ML, maximum log-likelihood; l_a, mean length at age; ρ_1 and ρ_2 are constants.

Sex	n	Variance[A]	Gillnet selectivity corrected	Model fit converged	Francis parameters l_3 (SE, SD) (mm)	l_7 (SE, SD) (mm)	l_{11} (SE, SD) (mm)	ρ_1 or ρ_2[B] (SE)	ML	von Bertalanffy parameters K (yr^{-1})	$L\infty$ (mm)	a_0 (yr)
Common sawshark												
Male	273	Constant	No	Yes	849 (7, 59)	1077 (4, 59)	1148 (11, 59)	ρ1 = 3540 (304)	−1502.60	0.290	1180	−1.38
			Yes	Yes	826 (8, 60)	1067 (4, 60)	1137 (12, 60)	ρ1 = 3598 (314)	−1501.57	0.309	1165	−1.00
		Proportional	No	Yes	849 (7, 56)	1077 (5, 63)	1153 (12, 65)	ρ2 = 3.710 (0.318)	−1509.73	0.274	1191	−1.55
			Yes	Yes	830 (7, 56)	1065 (5, 64)	1183 (12, 66)	ρ2 = 3.829 (0.338)	−1509.73	0.274	1183	−1.41
Female	324	Constant	No	Yes	837 (8, 74)	1143 (5, 74)	1311 (7, 74)	ρ1 = 5403 (426)	−1851.57	0.149	1518	−2.38
			Yes	Yes	800 (9, 74)	1132 (5, 74)	1307 (7, 74)	ρ1 = 5545 (449)	−1851.31	0.149	1502	−1.76
		Proportional	No	Yes	837 (7, 64)	1142 (5, 75)	1313 (8, 80)	ρ2 = 4.878 (0.383)	−1850.94	0.147	1526	−2.42
			Yes	Yes	812 (7, 64)	1130 (5, 76)	1308 (8, 82)	ρ2 = 5.085 (0.417)	−1850.94	0.147	1530	−2.16
Southern sawshark												
Male	192	Constant	No	Yes	862 (7, 40)	965 (5, 40)	976 (8, 40)	ρ1 = 1608 (164)	−981.08	0.571	977	−0.75
			Yes	Yes	853 (8, 40)	959 (5, 40)	969 (8, 40)	ρ1 = 1619 (167)	−981.06	0.575	971	−0.67
		Proportional	No	Yes	863 (7, 39)	965 (5, 41)	975 (8, 41)	ρ2 = 1.733 (0.177)	−982.09	0.576	976	−0.74
			Yes	No								
Female	203	Constant	No	Yes	869 (12, 48)	1027 (4, 48)	1050 (10, 48)	ρ1 = 2315 (230)	−1074.21	0.484	1054	−0.60
			Yes	Yes	856 (12, 48)	1020 (5, 48)	1042 (11, 48)	ρ1 = 2340 (235)	−1074.15	0.488	1047	−0.49
		Proportional	No	Yes	871 (11, 45)	1027 (5, 49)	1051 (10, 50)	ρ2 = 2.354 (0.234)	−1075.69	0.473	1055	−0.69
			Yes	Yes	860 (11, 45)	1019 (5, 49)	1043 (11, 50)	ρ2 = 2.401 (0.244)	−1075.69	0.474	1047	−0.63
Elephant fish					l_3 (SE, SD)	l_5 (SE, SD)	l_9 (SE, SD)					
Male	102	Constant	No	Yes	548 (10, 54)	703 (11, 54)	748 (24, 54)	ρ1 = 2898 (409)	−550.90	0.415	766	−0.02
			Yes	Yes	536 (10, 54)	717 (16, 54)	747 (28, 54)	ρ1 = 2903 (410)	−550.86	0.415	766	+0.22
		Proportional	No	Yes	546 (10, 52)	706 (12, 59)	754 (26, 61)	ρ2 = 4.938 (0.692)	−549.65	0.400	775	−0.05
			Yes	Yes	535 (10, 52)	699 (12, 60)	749 (26, 62)	ρ2 = 5.088 (0.734)	−549.66	0.400	770	−0.04
Female	126	Constant	No	Yes	564 (10, 74)	853 (9, 74)	948 (23, 74)	ρ1 = 5478 (699)	−720.92	0.278	995	−0.01
			Yes	No								
		Proportional	No	Yes	560 (10, 69)	806 (8, 83)	927 (19, 89)	ρ2 = 8.480 (1.070)	−725.13	0.238	1042	−0.24
			Yes	Yes	541 (10, 69)	800 (9, 84)	927 (20, 91)	ρ2 = 8.927 (1.186)	−725.13	0.238	1049	−0.05

[A]Variance in shark length at any age constant with mean lengh at age or proportional to mean length at age;
[B]constant variance = ρ_1 and proportional variance = $l_a \ \rho_2$.

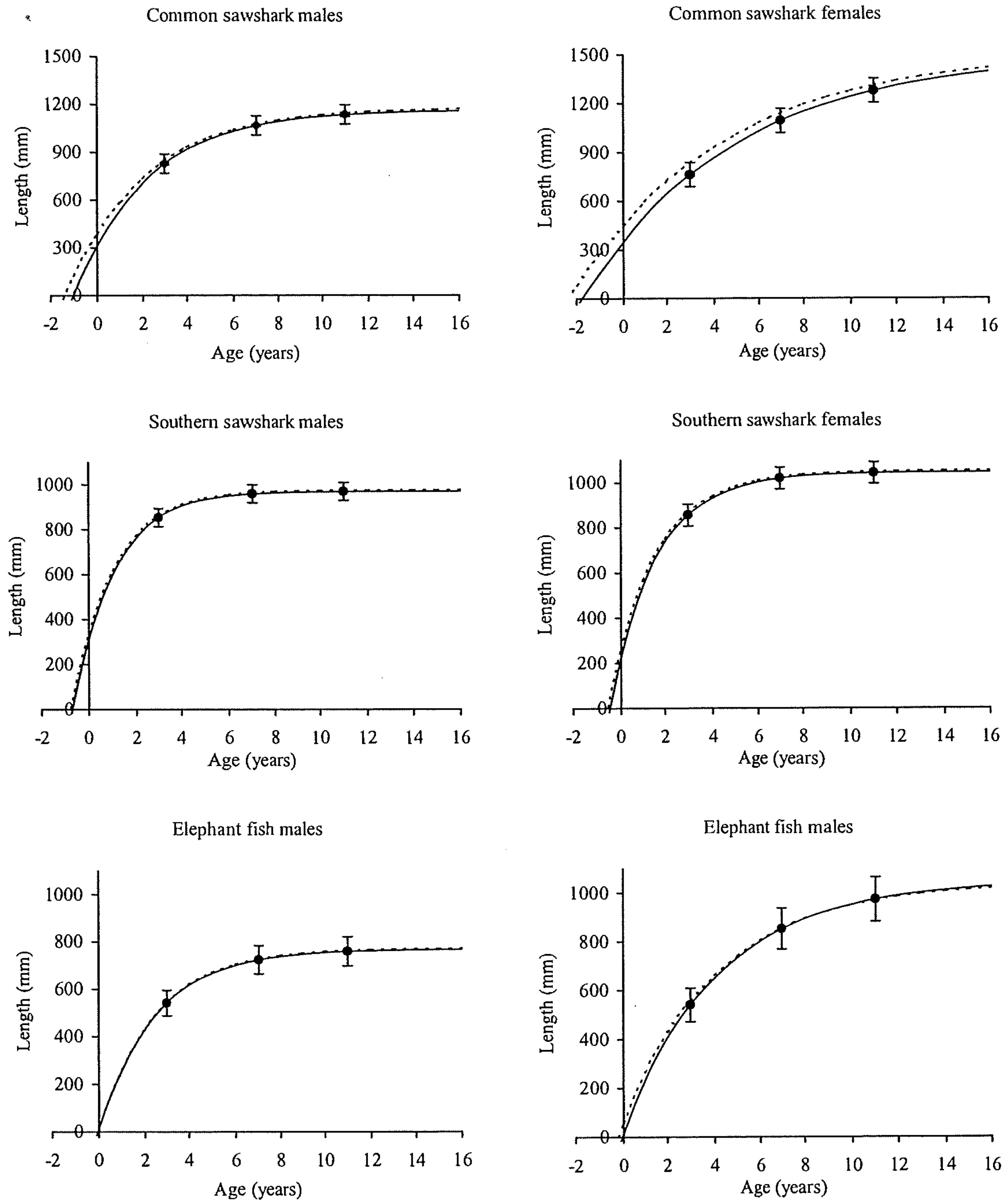


Fig. 1. Von Bertalanffy growth curves for common sawshark, southern sawshark, and elephant fish

Curves are for males and females separately corrected (———) and uncorrected (- - - - -) for length-selective sampling bias based on the assumption of constant variance in length with age. Error bars denote one standard deviation about mean.

Appendix 3c: Common sawshark reproduction

This appendix contains a manuscript in preparation, which presents the results of a study of the reproduction of common sawshark (*Pristiophorus cirratus*) required for fishery stock assessment.

Reproductive biology of common sawshark (*Pristiophorus cirratus*) harvested off southern Australia

Contents

Draft manuscript in preparation for submission to a scientific journal for publication (18 July 2005)

Reproductive biology of common sawshark (*Pristiophorus cirratus*) harvested off southern Australia

Russell J. Hudson[AB], Terence I. Walker[AD] and Robert W. Day[C]

[A]Primary Industries Research Victoria, PO Box 114, Queenscliff, Victoria 3225, Australia.
[B]Ocean Grove, Victoria 322X, Australia.
[C]Zoology Department, The University of Melbourne, Parkville, Victoria 3052, Australia.
[D]Corresponding author. Email Terry.Walker@dpi.vic.gov.au

Abstract

Breeding female *Pristiophorus cirratus* mostly have synchronous 2-year ovarian and parturition cycles. Ovulation occur mostly during mid-July–mid-November; the gestation period is more than 12 months, with *in utero* embryos observed *in utero* over the 16-month period October–December. Ovarian follicle diameter ranges 30–57 mm at ovulation, with a maximum recorded follicle diameter of 63 mm. Mean wet mass gain is about double from *in utero* egg (~37 g) to full-term embryo (~86 g mean maxium for one litter) at ~350 mm total length. The sex ratio of *in utero* embryos is 1:1. Litter size (3–22 embryos per pregnancy) rises linearly with maternal length. The number of eggs *in utero* gives a better indication of litter size than the number of embryos *in utero* because pregnant females carrying embryos close to full term are prone to spontaneous abortion on capture. Female length-at-maturity and length-at-maternity appear not to vary temporally or spatially. Maximum body mass of females is double that of males and, at any length, mean body-mass for pregnant females exceeds that of non-pregnant females, which in turn exceeds that of males.

Key words: Reproduction; Sawshark; Fishery; Australia

Introduction

One of four species of the family *Pristiophoridae* endemic to Australia, the common sawshark (*Pristiophorus cirratus*) is distributed at depths to 310 m from Jurien Bay in Western Australia to Eden southern New South Wales (Last and Stevens 1994). Common sawshark exhibits aplacental viviparity, and, because mothers supplement the yolk nutrients to their embryos with uterine secretions (histotrophy) (Stevens 2002), is classed as matrotrophic (Hamlett *et al.* 2005). It is uncertain whether the species forms a single stock or multiple stocks. Tag release-recapture data indicate movement within Bass Strait, but not of large-scale movements across southern Australia, and there is no evidence of distinct pupping grounds. The species feeds mainly on small demersal teleost species (PIRVic, unpublished data).

The common sawshark, together with the sympatric southern sawshark (*Pristiophorus nudipinnis*), is taken mainly as byproduct when gummy shark (*Mustelus anarcticus*) are targeted in the Gillnet Hook and Trap Fishery (GHATF) of southern Australia. These species are only occasionally targeted. More than 90% of the sawshark catch from the GHATF is taken by fishers operating in Bass Strait with gillnets of 6-inch mesh-size in depths less than 90 m. Since 1974, the annual sawshark catch has exceeded 200 tonnes, peaking during 1995 at 359 tonnes (Walker *et al.* 2003). The catches reported by fishers are not separated by species but catches recorded by scientific observers from gillnets of 6-inch mesh-size indicate most of the catch is common sawshark. These data indicate the ratio of the number of common sawshark to the number southern sawshark caught is 4:1 (Walker *et al.* 2005). Common and southern sawshark are also taken by demersal otter trawl and Danish seine nets off eastern Victoria and by demersal otter trawl off eastern South Australia. The quantities caught are less but have not been as well monitored in the trawl fisheries as in the GHATF.

In this paper we closely follow the quantitative approach to the study of reproduction adopted for school shark (*Galeorhinus galeus*) off southern Australia (Walker 2005). This approach provides the basic reproductive parameters required for stock assessment of a chondrichthyan species. The same parameters are required for ecological risk assessment (Walker 2004) or species assessment against IUCN criteria established under each of several risk of extinction categories (Hilton-Taylor 2000).

Materials and Methods

Collection of specimens

Sampling for *P. cirratus* was undertaken during the three separate periods 1973–76, 1986–87, and 1992–01, mainly in Bass Strait (BS) and waters off South Australia (SA) (Figure 1). During 1973–76, the animals were caught using experimental gillnets of mesh-size ranging 2–9 inches (51–229 mm), in steps of 1 inch (25 mm), and hooks attached to sinking longlines. The sawsharks were captured as a result of fishing at 162 fishing sites mainly in BS (126 sites), but also off eastern Tasmania south of latitude 41° South (20 sites) (grouped with BS samples) and SA (16 sites). During 1986–1987, the animals were caught in experimental gillnets of mesh-size ranging 5–8 inches (127–203 mm), in steps of 1 inch. The sawsharks were captured at 144 fishing sites (60 sites in BS and 84 sites in SA). During 1998–01, the animals were caught at 153 fishing sites (91 sites in BS and 62 sites in SA). All animals were caught by gillnets of 6-inch (152 mm) or 6½-inch (165 mm) mesh-size on board commercial fishing vessels.

Biological sampling

Specimens of *P. cirratus* were dissected to investigate their reproductive biology. They were measured to the nearest millimetre as total length (TL); the tail of each animal was first allowed to take a natural position and the upper caudal lobe placed parallel to the body axis. Sex, TL, fullness of the stomach, and several reproductive indices were recorded for each animal. Also recorded, when the sea conditions permitted (mostly on a research vessel during 1973–76), were the mass of total body, liver, left ovary of females, and left testis of males.

For females, at-sea macroscopic inspection of the condition of the paired uteri, oviducal glands, and ovaries was undertaken to investigate breeding condition, litter size, period of gestation, and growth of embryos. Records were made of the diameters of the three largest oocytes and the presence of *corpora atrecia* or *corpora lutea* in the ovary and, for pregnant animals, the number of *in utero* eggs and embryos in each uterus. In addition, the TL, sex, uterus (left or right), and mass (with and without yolk sac) of each embryo and uterus and mass of each *in utero* egg were recorded for many of the pregnant sharks. Indices were adopted for recording the condition of the ovary, oviducal gland, and uteri from rapid visual inspection. Ovary index (O) was based on size and colour of the follicles (O=1–4). Oviducal gland index (G) was based on shape and size of the gland (G=1–3). Uterus index (U) was based on appearance, size and contents of the uteri (U=1–6) (Table 1).

For males, at-sea macroscopic inspection of condition of the testes, seminal vesicles, and claspers was undertaken to investigate maturity by adopting three indices of breeding condition. Testis index (T) was based on shape, size, and relative predominance of testis tissue to epigonal gland tissue (T=1–3). Seminal vesicle index (V) was based on appearance, thickness of the wall, and presence or absence of seminal fluid (V=1–3). The length of left clasper was measured from the basipterygium to the distal end and clasper index (C) was based on appearance and rigidity (C=1–3) (Table 1).

Terminology

The present study closely follows the methods and terminology of a previous quantitative study designed to provide estimates of the reproductive parameters essential for fisheries assessment or demographic analysis (Walker 2005). That study broadly describes the anatomical structures and functions of the various reproductive organs of chondrichthyan species, and, more importantly, provides explicit definitions of several terms related to population biology. These are briefly repeated here.

Most indicators of female maturity are difficult to determine and to attain consistency between repeated measures and different observers. Animals were assumed to be mature with the onset of vitellinogenesis, which approximates to the ovarian follicle exceeds >3 mm diameter. Animals were assumed immature where the largest follicle was 1–3 mm in diameter. For the present study, the period of the ovarian cycle is defined as the period from completion of one ovulation to completion of the next ovulation. The period of gestation is defined as the period from fertilisation (assumed to shortly after ovulation) to parturition. It is necessary to determine the proportion of the female population contributing to recruitment each year for population modelling. For this purpose, an observed female is defined as being in maternal condition if it is in pregnant condition (in utero eggs or embryos present) and expected to give birth soon after or before 1 January. It is also defined as being in maternal condition if it observed in post-partum condition before 1 January (Walker 2005).

Statistical analysis

Total body mass at TL

The relationship between total body mass, w, and TL, l, was determined using the power curve

$$w = acl^b,$$

adopted commonly for sharks (Olsen 1954) and bony fishes (Ricker 1958) without the constant c, where a and b are parameters determined by linear regression of $\ln(w)$ against $\ln(l)$, and c is a factor correcting for biases caused by natural logarithmic transformation (Beauchamp and Olson 1973).

Period of gestation and growth of embryos

The period of gestation and growth of embryos can be determined by plotting mean TL of embryos observed in pregnant females (U=5 animals) and mean TL values of 0 for *in utero* eggs observed in pregnant females (U=4 animals) against month and then evaluating the seasonal pattern. Mass gain or loss from egg to full-term embryo during gestation was investigated for a sample of pregnant females (U=5 animals). This was undertaken by separately plotting each of four variables against mean embryo TL for U=5 animals. These variables were the mean wet mass of embryos, the mean wet mass of external yolks, the sum of these two quantities, and the mean external yolk wet mass expressed as a proportion of the sum of the two quantities.

Ovarian cycle

The ovarian cycle was investigated by examining the ovary and measuring the diameters of the largest follicles in animals caught throughout the year. The largest follicle diameter (LFD) varied widely between individual animals and varied depending on uterus condition, so seasonal pattern in LFD for each of the six uterus conditions defined in Table 1 was examined separately.

Pregnant females with macroscopically visible *in utero* embryos (U=5 animals) provided the least ambiguous basis for determining seasonal growth rates of follicles and providing a basis for distinguishing between annual, biennial, and longer ovarian cycles. Examination of *in utero* growth of embryos indicate this period covers more than one full year indicating patterns in LFD against Julian day can be adopted for measuring annual rate of follicle growth (Walker 2005).

None of the other five uterus conditions provided such clear information on annual rate of follicle growth. The data indicate that females with uterus condition U=1 exhibit little or no change in LFD over the 12-month period from January to December. Females with uterus conditions U=4 occurred for only several months towards the end of the year or very early in the year, and therefore on their own provide no information on annual growth of follicles. These animals, however, do provide information on the timing of ovulation and on follicle diameter at the time of ovulation.

Animals with uterus conditions U=2, 3, or 6 displayed wide variation in LFD and similar patterns. Some of these animals provide information on follicle diameter immediately prior to ovulation and some with uterus condition U=6 information of follicle diameter during the period immediately following parturition. The uterus condition U=6 was not commonly observed suggests that after parturition the distended uterus contracts to resemble uterus condition U=3. It is generally difficult to distinguish between uterus conditions U=3 and U=6. This implies that animals recorded with uterus condition U=3 might be a mixture of animals approaching first pregnancy (all U=3) and animals between pregnancies (U=6 changing to resemble U=3). Unlike animals with uterus conditions U=5, U=4, and U=6, which can be related to the timing of ovulation and parturition, U=2 and U=3 animals cannot be so reliably related to either of these events.

Annual growth rate for animals of uterus condition U=5 was determined by the linear relationship between LFD, o, and Julian day, t, given by

$$o = a + bt,$$

where a and b are parameters estimated by linear regression. A scattergram of LFD against Julian day for U=5 animals was compare with the U=5 regression and 95% prediction intervals. Similarly, a scattergram LFD against Julian day for each of the U=2, U=3, U=4, and U=6 animals separately was compared with the regression line and 95% prediction intervals for the U=5 animals. Based on clustering of the data points, where it appeared animals data points lay outside the 95% prediction intervals 365 days were added to Julian day. Then a similar regression was undertaken for the U=3, U=4 (ovulating only), and U=6 animals pooled and this regression line was compared with that for the U=5 animals. Differences in these regression lines were tested by comparing slopes and elevations (Kleinbaum *et al.* 1988). These comparisons provided a basis for considering whether the ovarian cycle is annual, biennial, triennial or longer.

Size-at-maturity and size-at-maternity

The proportion of the population of animals mature at any TL can be determined by classing each animal as in mature condition or immature condition and applying logistic regression for females (Mollet *et al.* 2000; Conrath and Musick 2002) and males (Walker 2005) separately. Similarly, for females, the proportion of the population of animals in maternal condition at any TL can be determined by classing each animal as in maternal condition or non-maternal condition and applying logistic regression.

For *Pristiophorus cirratus*, a female was classed as in mature condition if the largest ovarian follicle was >3 mm in diameter (size at first yolking); otherwise it was classed as in immature condition. Given uncertainty of the best indicator of maturity of males, the results from methods based on alternative criteria for assuming the mature condition and the immature condition are compared. Males were classed by testis condition as mature if T=3 and immature if T=1 or T=2. Similarly, they were classed by seminal vesicle condition as mature if V=2 or V=3 and immature if V=1 and by clasper condition as mature if C=2 or C=3 and immature if C=1 (Table 1).

A female was classed in maternal condition at the time of dissection, if it would have given birth to young before or soon after the following 1 January. To implement this criterion, females were classed as in maternal condition if they met any one of three criteria. The first criterion was pregnant with visible embryos (U=5) during January–December except for pregnant females with early-term embryos during October–December or with full-term embryos during January were classed as in non-maternal condition. The second criterion was pregnant with *in utero* eggs (U=4) during January–February, and the third criterion was non-pregnant in post-partum condition with distended uteri (U=6) during October–December. In addition to pregnant females with early-term embryos during October–December or with full-term embryos during January, females were classed as non-maternal if U=1, U=2, U=3, U=4 during July–December, or U=6 during January–September.

Logistic regression was adopted to determine the proportion of females in mature condition, the proportion of males in mature condition, and the proportion of females in maternal condition as a function of TL. Females or males in mature condition were assigned a maturity condition value of 1, whereas those in immature condition were assigned a maturity condition value of 0. Similarly, females in maternal condition were assigned a maternal condition value of 1, whereas females in non-maternal condition were assigned a maternal condition value of 0.

The logistic equation adopted to express P as a function of l is given by

$$P = \frac{c}{(1+e^{-(a+bl)})}$$

where a, b, and c are parameters but to provide parameters that are more biologically meaningful, the equation is reformulated to express P as a function of l by

$$P = P_{\max}\left(1+e^{-\ln(19)\left(\frac{l-l_{50}}{l_{95}-l_{50}}\right)}\right)^{-1},$$

where $P_{\max}$ is the maximum proportion of animals in mature condition or maternal condition, and l_{50} and l_{95} are the lengths at which 50% and 95% of the maximum proportion of animals in mature condition or maternal condition (Walker 2005).

The parameters $P_{\max}$, l_{50} and l_{95}, with 95% confidence intervals, were estimated by the method of maximum likelihood using the probit procedure (Proc Probit) of the computer statistical package SAS (SAS Institute, Cary, North Carolina, USA). This applies a modified Newton–Raphson algorithm for estimation.
The standard error for any length, l, is given by

$$se_l = P_l(1-P_l)/N.$$

The SAS probit procedure sets $1-P_{\max} = 0.000$. This is appropriate for the maturity ogive where all large-sized animals in the population are in mature condition, and hence the proportion of large-sized animals in the population mature is 1.000. Similarly, this is appropriate for the maternity ogive where all of the large-sized animals in the population are in maternal condition, and hence the proportion of large-sized animals in the population in maternal condition is 1.000; parturition frequency is annual. However, this is not appropriate where parturition frequency is biennial, triennial, or some other period.

ovulating U=4 animals (LFD ranged 30–57 mm). These results are consistent with the hypothesis that the ovarian cycle is biennial, and inconsistent with the hypothesis that the ovarian cycle is annual.

Similar regression analysis of LFD against Julian day was undertaken pooling animals with uterus conditions U=2, 3, 4 (ovulating only), and 6. Animals where the follicles were either possibly not growing or undergoing atresia were excluded from the analysis. All U=1 animals (Figure 5b), U=4 animals that had completed ovulation, and U=2, U=3 and U=6 animals where LFD <9 mm were excluded. In addition, from visual inspection of the pattern of clustering of the data points, 365 days were added for U=2, 3 or 6 animals where LFD >20 mm and Julian day <300. The regression indicated that mean annual growth in LFD for these animals was 26 mm y^{-1}. This predicted mean LFD increased from 2 to 28 mm during a 365-day period and from 2 to 54 mm during a 730-day period (Figure 6). Comparison of the two separate linear regression fits for LFD against Julian day between the U=5 animals and the U=2, 3, 4 (ovulating) and 6 animals indicated that these two straight lines had statistically significantly different slopes (t-test, t=5.438, d.f.=513, and P<0.001), but not significantly different elevations (t-test, t=1.027, d.f.=513, and P<0.001). These results indicate that the ovarian cycle is biennial, but the growth of the follicles, and hence rate of vitellogenesis, is a slower for pregnant females with developing embryos than it is for other vitellinogenic females. These results imply that for females with *in utero* embryos during the first year of the biennial ovarian cycle, the growth rate of follicles is higher during the second year than during the first year.

The animals for each uterus condition U=2, U=3, U=4, and U=6 were then examined to assess whether they were consistent with the hypothesis of a 2-year ovarian cycle. Scattergrams of LFD plotted against Julian day for animals for each of these four uterus conditions were compared with the predicted mean LFD trajectory determined for the non-pregnant animals. The LFD trajectory was extrapolated through a second year and displayed for a 1-year period by presenting the trajectories as parallel lines. On each of the four scattergrams, the mean trajectory and trajectories of the lower and upper 95% prediction intervals were presented for the first year and second year on axes displaying 365 Julian days (Figures 5cdef).

Among the animals with U=2 uterus condition, those where LFD <9 mm are interpreted as recently matured from the U=1 uterus condition. The pattern of LFD against Julian day for the rest of these animals is consistent with a 2-year ovarian cycle (Figure 5c). The pattern for animals with uterus condition U=3 is similar to that for animals with the U=2 uterus condition, except there were far fewer animals with small follicles (only three animals had LFD <9 mm). The latest U=3 animal capture with enlarged follicles was 6 October (Julian day 279), suggesting these animals begin ovulating at about that date (Figure 6d).

The scattergram for the U=4 animals is consistent with the 2-year ovarian cycle hypothesis (Figure 5e). These data provided reliable information on the timing of ovulation and on magnitude of LFD at the time of ovulation. The animals were classed as 'ovulating' or 'ovulated' based on LFD, which had two size clusters (ranging 1–9 and 30–57 mm LFD). Animals were classed as ovulating (in the process of ovulation) if they contained eggs *in utero* and ≥30-mm LFD; animals were classed as ovulated (ovulation complete) if they contained eggs *in utero* and <30-mm LFD. Animals found ovulating were captured during the period from 14 July (Julian day 199) to 15 November (Julian day 319). Animals found ovulated were captured during the period from 18 July (Julian day 199) to 7 February (Julian day 38).

For U=6 animals, the individual LFD values have a similar distribution to U=3 animals. They are consistent with the 2-year ovarian cycle by being clustered near both the lower and upper trajectories (Figure 5f). There is uncertainty distinguishing between the U=6 and U=3 uterus conditions and of the possibility of the pregnant females aborting when captured. Many of those clustered near the upper mean trajectory are likely to be a mix of maturing animals approaching first ovulation and animals having given birth the previous year.

Both the stage of maturation as indicated by uterus condition and the stage of the ovarian cycle affected the mass of the ovary. Ovary mass was available only for animals collected during 1973–76, 135 animals (U=1–6) from BS and 6 animals (U=5) from SA. There was little change in ovary mass between U=1 animals (n=59, mean 3.9 g, s.d. 4.1 g, range 1–22 g) and U=2 animals (n=14, mean 9.2 g, s.d. 7.7 g, range 1–22 g). One large U=3 animal (1729 mm TL) had an exceptionally high value of 282 g ovary mass. The ovary mass for the other U=3 animals (n=17, mean 43.8 g, s.d. 28.8 g, range 10–98 g) and U=4 animals (n=13, mean 32.3 g, s.d. 23.6 g, range 4–94 g) had wider variation. With the exception of one individual, U=5 animals (n=36, mean 19.6 g, s.d. 17.8 g, range 4–69 g) had a range less than U=3 and U=4 animals. The exceptional U=5 animal, which was one of only two animals observed in BS carrying full-term embryos and close to ovulation, had a 195 g ovary mass. As might be expected from the LFD values, the few U=5 animals from SA (n=6, mean 38.6 g, s.d. 8.3 g, range 25–47 g) tended to have higher ovary mass than the U=5 animals from BS. Ovary mass was weighed for only one U=6 animal (18 g).

Percentage GSI for U=1 animals (n=58, mean 0.14, s.d. 0.13, range 0.02–0.55%) was similar to that for U=2 animals (n=14, mean 0.16, s.d. 0.13, range 0.02–0.37%). However, percentage GSI was higher for U=3, 4, 5, and 6 animals without any obvious seasonal trends, except for U=5 animals. GSI for U=3 animals (n=16, mean 0.34, s.d. 0.14, range 0.13–0.67%) and U=4 animals (n=14, mean 0.35, s.d. 0.22, range 0.12–0.91%) exceeded GSI for U=5 animals (n=31, mean 0.19, s.d. 0.21, range 0.02–0.47%). The one exception was one of the two pregnant animals in BS close to ovulation, which had a percentage GIS of 1.17%. Only one measurement was taken for U=6 animals (0.20%).

Percentage HSI was similar for U=1 animals (n=102, mean 4.26, s.d. 1.29, range 1.95–8.63%), U=2 animals (n=18, mean 4.85, s.d. 1.24, range 2.02–7.65%), U=3 animals (n=17, mean 4.50, s.d. 1.75, range 2.44–9.10%), U=5 animals (n=57, mean 4.03, s.d. 1.67, range 1.77–11.55%), and U=6 (n=6, mean 4.43, s.d. 1.78, range 2.68–7.28%) animals. Only U=4 animals (n=21, mean 7.08, s.d. 2.16, range 2.17–10.91%) were different. A weak but significant correlation between HSI and LFD (Spearman 0.321, $P<0.01$) for U=3–6 animals provides some evidence for an increase in liver mass with vitellogenesis. There was no significant correlation between HSI and LFD (Spearman –0.213, $P>0.05$) for U=1–2 animals (Figure 7).

Size-at-maturity and size-at-maternity

Testing for the effects of region, period, and region x period interaction on logistic regression models by backward stepwise elimination of statistically non-significant terms by log-likelihood ratio tests indiacted that none of these terms were significant. Hence, available data were pooled over the three regions EBS, WBS, and SA and three periods 1973–76, 1986–87, and 1998–01.

Total length at which 50% and 95% of the animals matured, with 95% confidence limits (CI), derived from the ogives presented in Figure 8 are tabulated as follows.

Condition	L_{50} (95% CI)	L_{95} (95% CI)	P_{max}	n	N
Maturity	1128 (1116, 1138)	1383 (1362, 1407)	1.00	656	1053
Maternity	1156 (1155, 1157)	1239 (1238, 1241)	0.50	321	1049

Litter size and sex ratio of embryos

A total of 118 pregnant females 1095–1434 mm TL were observed to carry 6–18 eggs *in utero* and 208 pregnant females 1015–1492 mm TL were observed to carry 3–22 embryos *in utero*. The male:female ratio was 0.479:0.521, which is not statistically different from 1:1. The linear relationship between the number of macroscopically visible embryos *in utero* and maternal length (Figure 9a) gives a slightly lower litter size at TL and variance in litter size at TL than the relationship between the number of eggs *in utero* and maternal length (Figure 9b).

In utero	a (s.e.)	b (s.e.)	n	r^2	rmse	P
Embryos	−14.52 (±2.79)	0.0205 (±0.0022)	208	0.294	2.434	***
Eggs	−17.87 (±2.57)	0.0235 (±0.0020)	118	0.535	1.790	***

The difference in the relationships is explained by the occasional occurrence of spontaneous abortion of embryos in pregnant females carrying embryos approaching full-term. Infertile eggs, common in *Mustelus antarcticus* and *Galeorhinus galeus*, were not observed in *P. cirratus*; so it can be assumed that the number of eggs *in utero* provides the more reliable indicator of litter size than the number of embryos *in utero*. Hence the second set of parameters is the more appropriate to use for fishery stock assessment and demographic analysis than the first set of parameters.

Synchrony and periodicity of reproductive cycle

Synchrony of the breeding condition and the two-year periodicity of the ovarian cycle and gestation are illustrated in Figures 10 and 11.

Acknowledgments

Acknowledgment is due to a large number of former and present staff of the Primary Industries Reseach Victoria Queenscliff Centre who participated in field sampling of sharks and laboratory processing. Special acknowledgement is due to Anne Gason for advice on statistical analysis of the data and application of the statistical package SAS. The skippers and crews of various shark fishing vessels who kindly provided accommodation, assistance, and hospitality aboard their fishing vessels are also thanked. The work was funded by the Fisheries Research and Development Corporation (FRDC) of Australia as part of FRDC Project 1999/103. Some of the field data were collected opportunitically as part of earlier projects.

References

Beauchamp JJ, Olson JS (1973) Corrections for bias in regression estimates after logarithmic transformation. *Ecology* **54**, 1403–1407.

Conrath CL, Musick JA (2002) Reproductive biology of the smooth dogfish, *Mustelus canis*, in the northwest Atlantic Ocean. *Environment Biology of Fishes* **64**, 367–377.

Hamlett WC, Kormanik G, Storrie MT, Stevens B, Walker TI (2005) Chapter 13. Chondrichthyan parity, lecithotrophy and matrotrophy. pp. 395–434. In 'Reproductive Biology and Phylogeny of Chondrichthyes: Sharks, Rays and Chimaeras'. Ed. WC Hamlett. (Science Publishers, Inc.: Enfield, New Hampshire 03478, USA.

Hilton-Taylor C (2000) '2000 IUCN Red List of Threatened Species.' (IUCN The World Conservation Union: Gland, Switzerland.)

Jones TS, Ugland KI (2001) Reproduction of female spiny dogfish, *Squalus acanthias*, in the Oslofjord. *Fishery Bulletin* **99**, 685–690.

Kleinbaum DG, Kupper LL, Muller KE (1988) 'Applied regression analysis and other multivariable methods.' (PWS–Kent Publishing Company: Boston, MA.)

Last PR, Stevens JD (1994) 'Sharks and rays of Australia.' (CSIRO Australia: Melbourne.)

Mollet HF, Cliff G, Pratt Jnr HL, Stevens JD (2000) Reproductive biology of the female shortfin mako, *Isurus oxyrinchus* Rafinesque, 1810, with comments on the embryonic development of lamnoids. *Fishery Bulletin* **98**, 299–318.

Olsen AM (1954) The biology, migration, and growth rate of the school shark, *Galeorhinus australis* (Macleay) (Carcharhinidae) in south-eastern Australian waters. *Australian Journal of Marine and Freshwater Research* **5**, 353–410.

Ricker WE (1958) Handbook of computations for biological statistics of fish populations. *Bulletin of Fisheries Research Board of Canada* **119**, 1–191.

Stevens B (2002) Uterine and oviducal mechanisms for gestation in the common sawshark, *Pristiophorus cirratus*. B.Sc. (Hons) thesis, University of Melbourne, Parkville, Victoria 3052, Australia.

Walker TI (2004) Chapter 13. Management measures. pp 285–321. In 'Technical manual for the management of elasmobranchs'. Eds JA Musick and R Bonfil. (Asia Pacific Economic Cooperation, Secretariat: Singapore.)

Walker TI (2005) 4 Reproduction in fisheries science. pp. 81–127. In 'Reproductive Biology and Phylogeny of Chondrichthyes: Sharks, Rays and Chimaeras'. Ed. WC Hamlett. (Science Publishers, Inc.: Enfield, New Hampshire 03478, USA.)

Walker TI, Hudson RJ, Gason AS (2005) Catch evaluation of target, byproduct, and bycatch species taken by gillnets and longlines in the shark fishery of south-eastern Australia. In 'Proceedings of North Atlantic Fisheries Organisation Symposium. Elasmobranch Fisheries: Managing for Sustainable Use and Biodiversity Conservation'. 11–13 September 2002. Santiago de Compostela, Spain. *Journal of Northwest Atlantic Fishery Science* **35**, 505–530.

Walker TI, Taylor BL, Gason AS (2003) 'Southern shark catch and effort 1970–2002 report to Australian Fisheries Management Authority.' Marine and Freshwater Resources Institute, Queenscliff, Victoria, Australia.

Table 1. Indices adopted for staging reproductive condition

Various assumption made on maturity when analysing the data are also listed.

Organ or tissue	Index	Description	Maturity assumption
Female			
Ovary	O=1	Largest follicles white and of diameter <2 mm	Immature
	O=2	Largest oocytes yolking and of diameter 2–3 mm	Immature
	O=3	Largest oocytes with yellowish yolk and of diameter >3 mm	Mature
	O=4	Yolked oocytes of diameter >3 mm and extensive corpora atretica present	Mature
Oviducal gland	G=1	Indistinct from anterior oviduct	Immature
	G=2	Distinct but only partly formed (Hamlett et al 1998)	Immature
	G=3	Enlarged with ram horn-shaped lobes	Mature
Uterus	U=1	Uniformly thin tubular structure	Immature
	U=2	Thin tubular structure partly enlarged posteriorly	
	U=3	Uniformly enlarged tubular structure	
	U=4	In utero eggs present without macroscopically visible embryos present	Mature
	U=5	In utero embryos macroscopically visible	Mature
	U=6	Enlarged tubular structure distended	Mature
Male			
Testis	T=1	Thin tissue strip with epigonal gland predominant	Immature
	T=2	Thickened strip with epigonal gland tissue extensive	Immature
	T=3	Enlarged and predominant with epigonal gland tissue negligible	Mature
Seminal vescicle	V=1	Thin translucent walls and seminal fluids absent	Immature
	V=2	Thickened opaque walls and seminal fluids present	Mature
	V=3	Thickened opaque walls and seminal fluids absent	Mature
Clasper[A]	C=1	Pliable with no calcification	Immature
	C=2	Partly calcified	Immature
	C=3	Rigid and fully calcified	Mature

[A]Adopted for periods 1986–87 and 1992–01, but not for period 1973–76.

Table 2. Comparison of diameters of three largest ovarian follicles by uterus condition

n, sample size; s.e., standard error

Uterus condition[A]	n	Mean diameter (±s.e.) of ovarian follicle (mm)		
		Oocyte 1	Oocyte 2	Oocyte 3
U=1	112	1.26±0.06	1.22±0.06	1.22±.06
U=2	220	11.33±0.89	11.14±0.89	10.81±0.89
U=3	174	30.93±0.55	30.02±0.52	29.37±0.51
U=4	134	6.92±0.91	5.30±0.65	4.97±0.60
U=5	234	10.21±0.48	9.76±0.46	9.44±0.46
U=6	22	25.36±2.96	25.00±2.81	24.73±2.92

[A]Defined in Table 1.

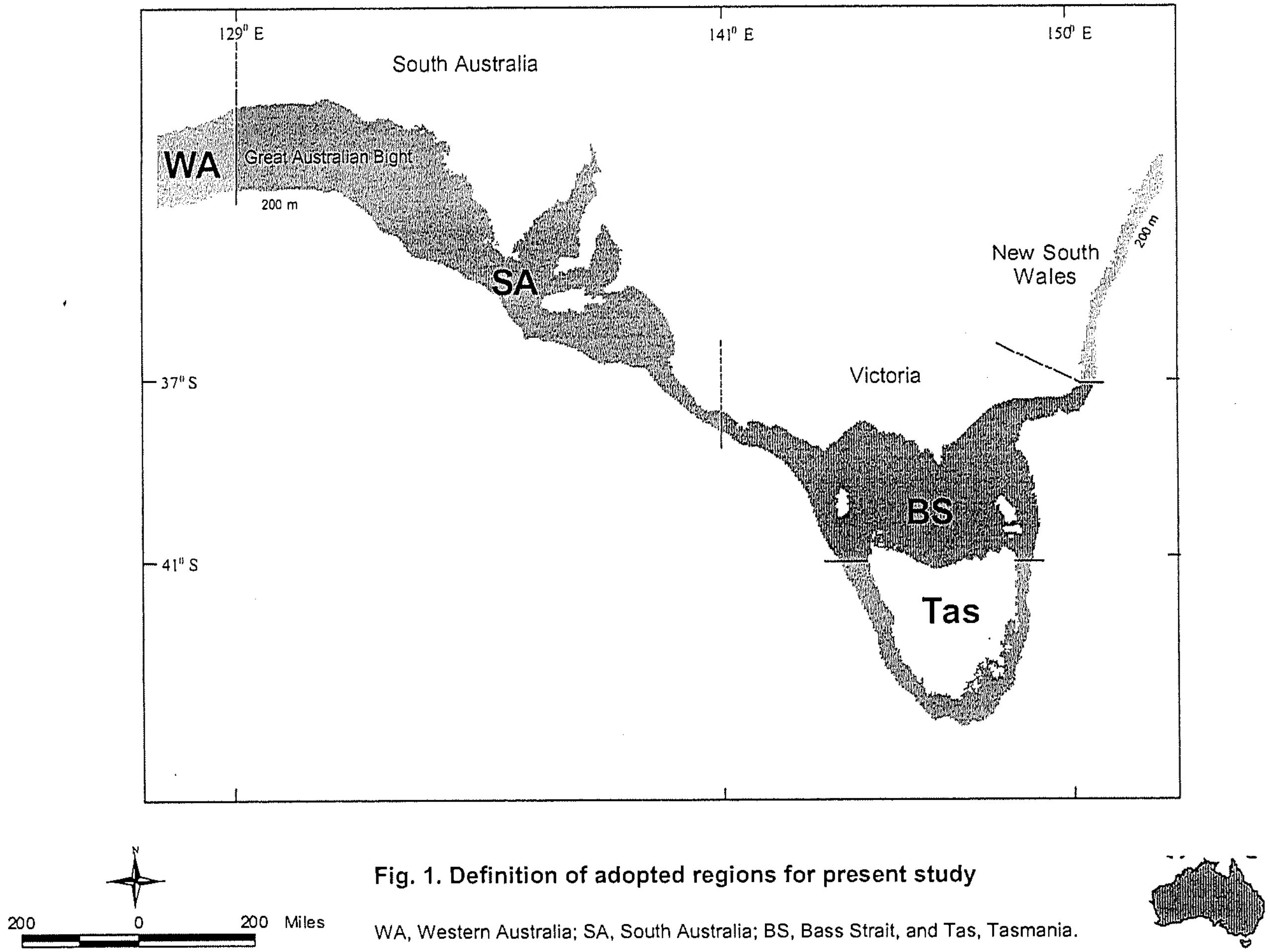


Fig. 1. Definition of adopted regions for present study

WA, Western Australia; SA, South Australia; BS, Bass Strait, and Tas, Tasmania.

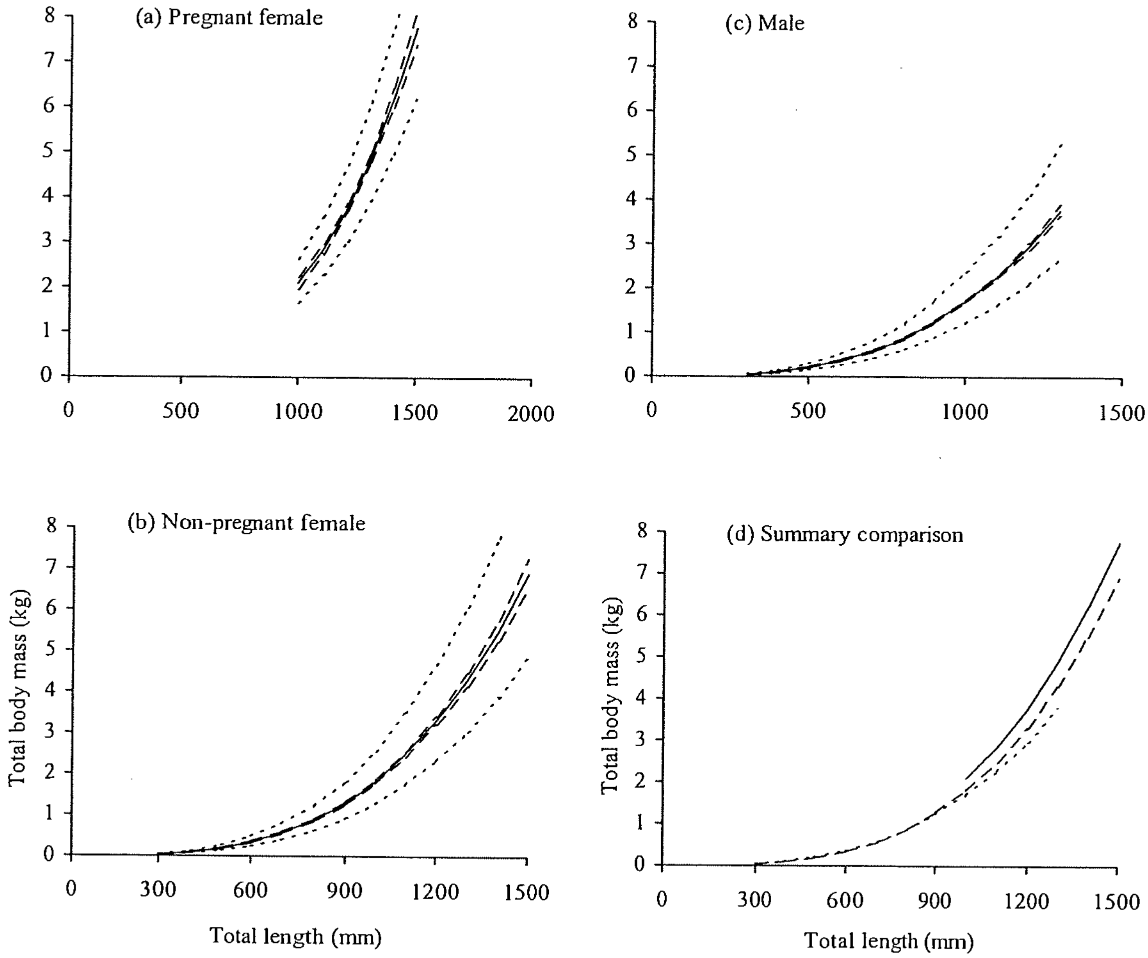


Fig. 2. Relationships between total body mass and total length.

Plots of mean total mass against TL (——), with 95% confidence limits (– – – –) and 95% prediction intervals (- - - - -), for pregnant female (a), non-pregnant females (b), and males (c), and comparison of the mean curves for pregnant females (——), non-pregnant females (– – – –), and males (- - - - -) (d) in southern Australia during the periods 1973–76, 1986–87, and 1998–01 combined. Values for parameters and statistical quantities from linear regression analysis to derive the equation $w=acl^b$ are given in the following tablulation:

Shark category	a (s.e. range) x 10^{-9}	b(se)	c	n	r^2	rmse	P
Pregnant female	0.423 (0.163–1.100)	3.231 (0.134)	1.006	136	0.804	0.111	***
Non-pregnant female	0.238 (0.154–0.366)	3.292 (0.062)	1.016	206	0.932	0.175	***
Males	1.520 (0.991–2.330)	3.015 (0.062)	1.014	463	0.834	0.167	***
Pregnant female (U=4)	69.00 (6.660–715.0)	2.513 (0.327)	1.008	30	0.678	0.128	***
Pregnant female (U=5)	0.097 (0.036–0.259)	3.439 (0.138)	1.005	106	0.846	0.101	***

where w is total body mass, l is total length, a and b are parameters, c is the Beauchamp and Olson (1973) correction factor, n is sample size, r^2 is square of correlation coefficient, and rmse is root mean square error for this regression (*P<0.1; **P<0.01; ***P<0.001) for the regression equation ln(w)=a+bln(l).

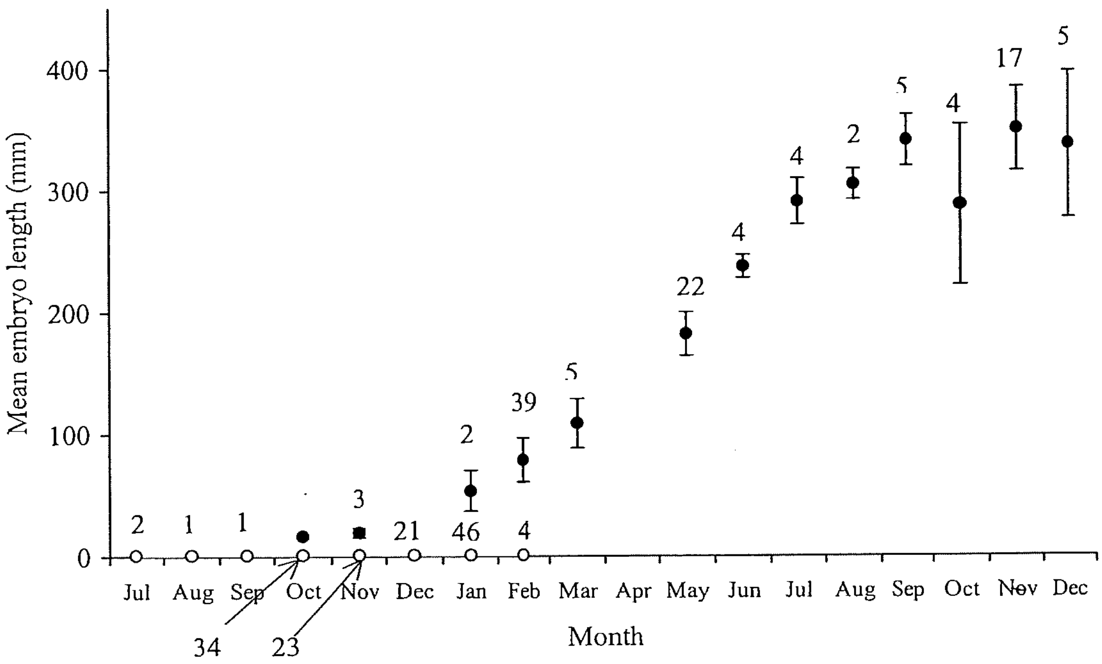


Fig. 3. Mean embryo length against month

Derived from the mean embryo length of the litter from each of 132 pregnant animals with macroscopically visible embryos and of 149 pregnant animals with only *in utero* eggs; •, overall mean; bars, standard deviation .

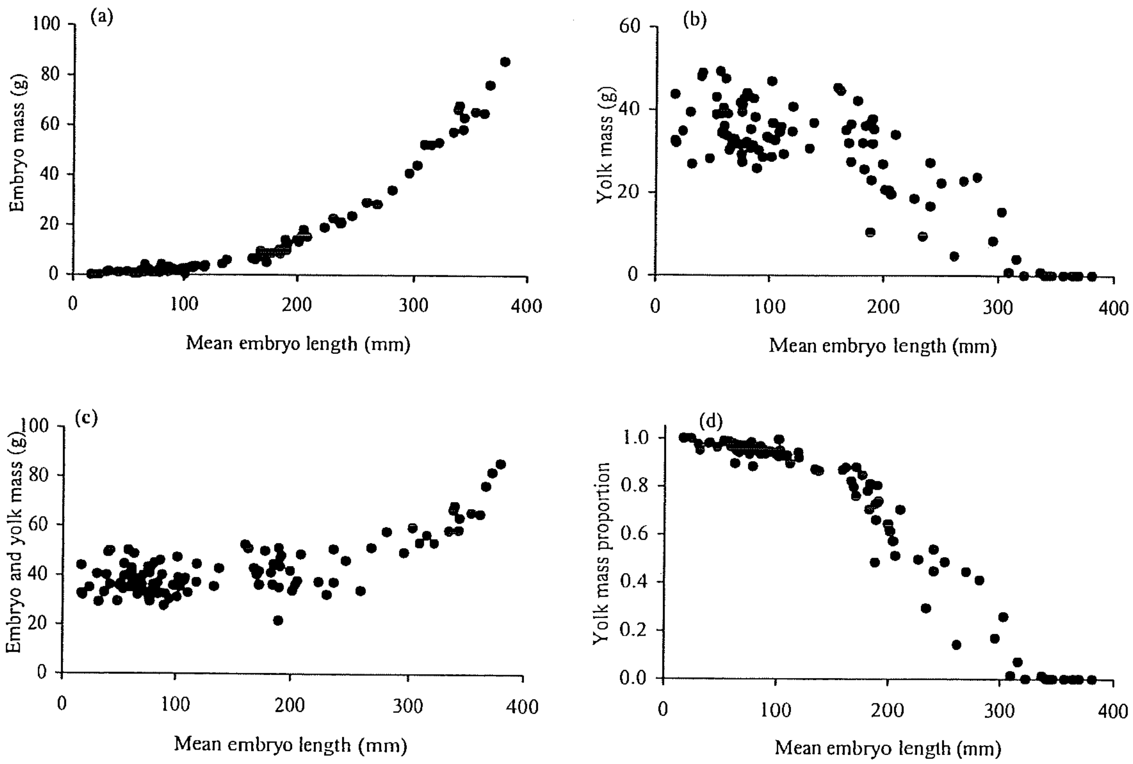


Fig. 4. Mass gain of embryos and mass loss from yolk sac during gestation

Mean mass of embryos (a) mean mass of yolk sacs (b), and yolk sac as a proportion of sum yolk sac and embryo mass (c) against mean embryo length. Each data point is derived from the mean embryo mass, mean yolk mass, and mean embryo length determined for the litter of each of 113 pregnant animals with macroscopically visible embryos. Yolk mass proportion is yolk sac mass/(embryo mass + yolk sac mass).

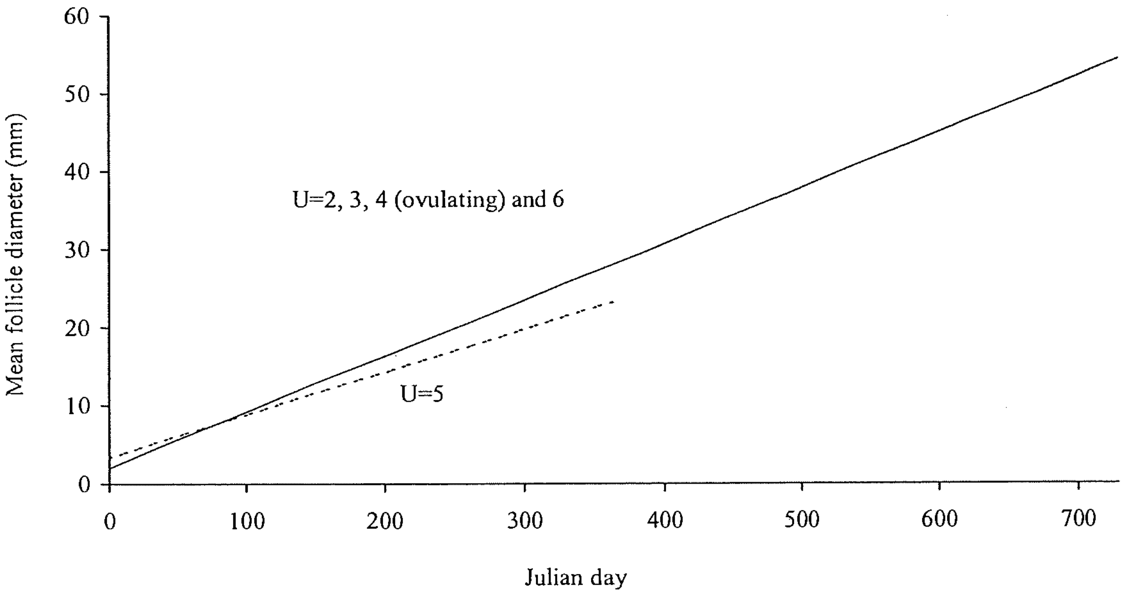


Fig. 6. Comparison of relationships of ovarian follicle diameter against Julan day

Mean ovarian follicle diameter against Julian day for females with uterus condition U=5 (- - - -) with uterus conditions U=2, 3, 4 (ovulating), and 6 (——) collected from Bass Strait and South Australia during 1973–76, 1986–87, and 1998–01 combined (from Fig. 5).

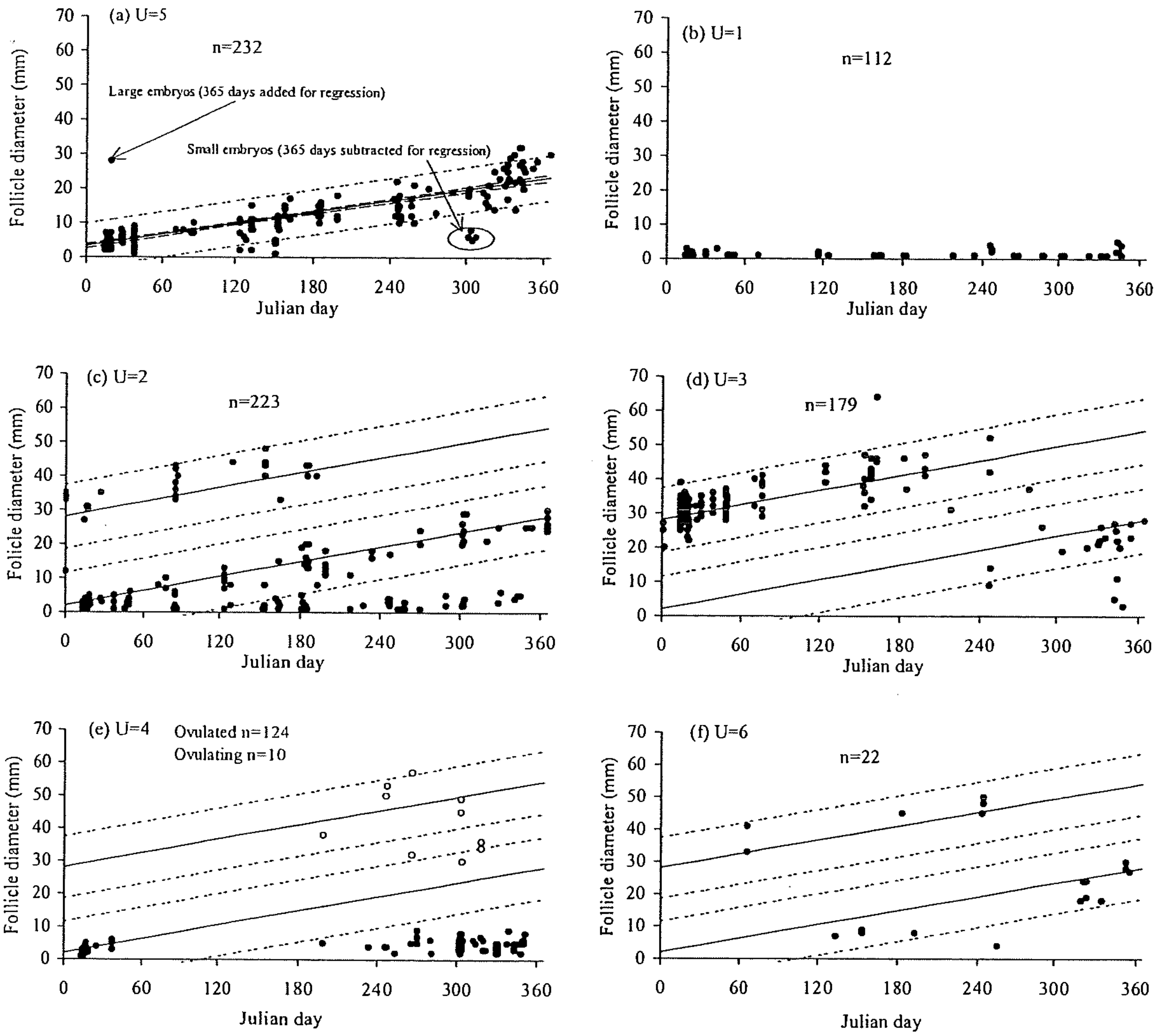


Fig. 5. Ovarian follicle diameter against Julian day by uterus condition

Largest follicle diameter against Julian day for females from Bass Strait and South Australia during 1973–76, 1986–87 and 1998–01 for each of the of six uterus conditions. Mean oocyte diameter (——) with 95% confidence limits (– – – –) and 95% prediction intervals (- - - - -) are presented for pregnant females with *in utero* embryos (U=5) (a), non-pregnant animals (U=1, 2, 3) (b, c and d), pregnant animals with *in utero* eggs (U=4, ovulated (•) and ovulating (o)) (e), and postpartum females (U=6) (f). Values of parameters and statistical quantities for the regression equation o=a'+b't for pregnant females with *in utero embryos* (U=5) are given in the following tabulation:

U	a' (se)	b'(se)	n	r^2	rmse	P
5	3.344 (0.312)	0.0547 (0.0018)	232	0.799	3.270	***
2, 3, 4 (ovulating), 6	2.011 (1.044)	0.0716 (0.0026)	285	0.733	4.695	***

where t is Julian day, o is largest follicle diameter, a' and b' are parameters, n is sample size, r^2 is square of regression correlation coefficient, and rmse is root mean square error for the regression, and P is probability of statistical significance (*P<0.1; **P<0.01; ***P<0.001).

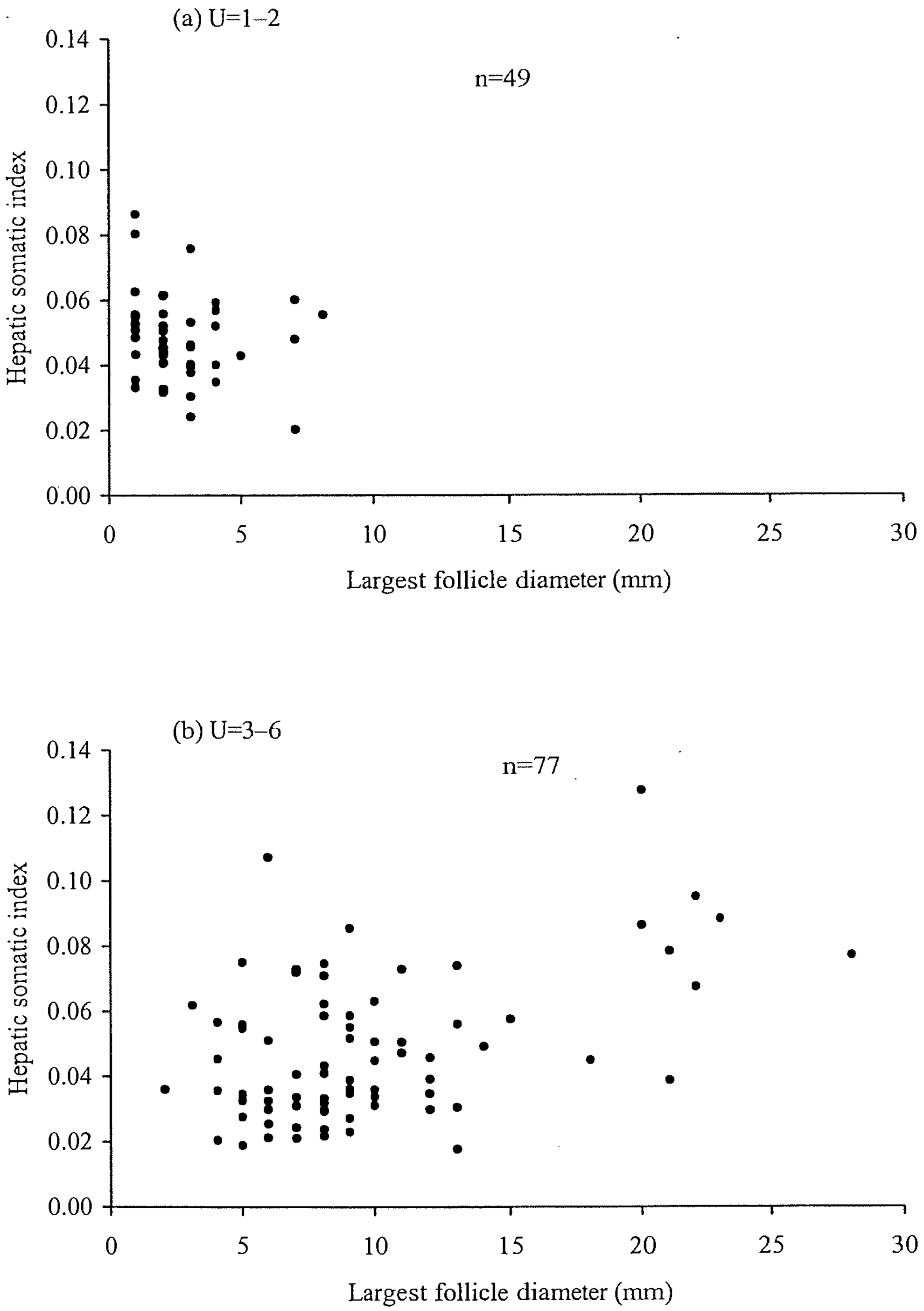


Fig. 7. HSI against largest ovarian follicle diameter

Spearman correlation between hepatic somatic index and ovarian largest follicle diameter is –0.213 (P=0.1409 ns) for U=1–2 animals (a) and 0.321 (P=0.0045 **) for U=3–6 animals (b) (*P<0.1; **P<0.01; ***P<0.001).

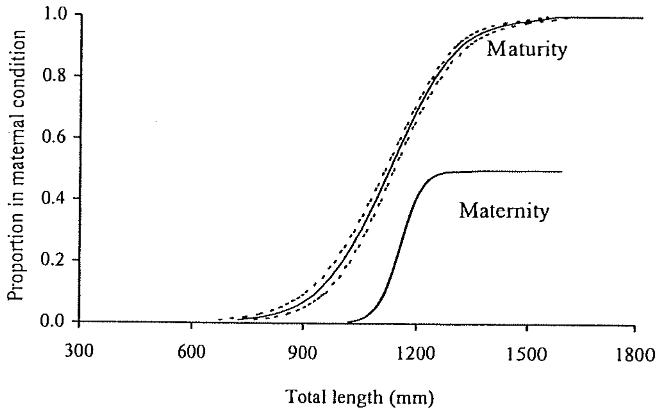


Fig. 8. Comparison of length-at-maternity and length-at-maturity ogives

Proportion of female population classed as being in maternal condition (——) and mature condition against TL during 1986–87 and 1998–01 combined . Values of parameters and statistical quantities for the equation $P_l = P_{max(l)}(1+e^{-\ln(19)(l-l_{50}/l_{95}-l_{50})})^{-1}$ determined from probit analysis are given in the following tabulation:

Variable	l_{50} (CI)	l_{95} (CI)	$P_{max(l)}$	n	N	P
Maturity	1128 (1116–1138)	1383 (1362–1407)	1.00	656	1053	***
Maternity	1156 (1155–1157)	1239 (1238–1241)	0.50	321	1049	***

where l is total length measured in millimetres, P_l is proportion of animals classed as being in maternal condition at TL l, l_{50} and l_{95} are parameters, $P_{max(l)}$ is an asymptotic constant, n is the total number of animals classed as being in maternal condition (adjusted in parentheses), and N is the total number of animals examined (adjusted in parentheses), ML is maximum likelihood, and P is probability of statistical significance (*P<0.1; **P<0.01; ***P<0.001).

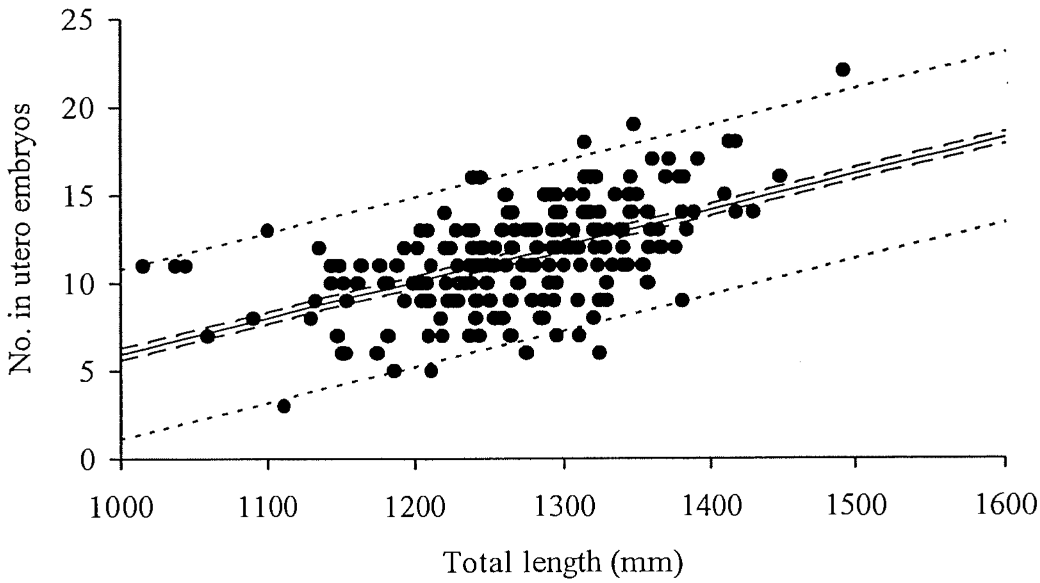


Fig. 9a. Number of *in utero* embryos against maternal total length

Mean number of embryos (——), 95% confidence limits (– – –), 95% prediction intervals (- - - -), and raw data (•) are plotted against maternal total length of pregnant females with macroscopically visible embryos (U=5). Values of parameters and statistical quantitites for the equation p=a'+b'l are given in the following tabulation:

a' (±s.e.)	b' (±s.e.)	n	r^2	rmse	P
–14.52 (±2.79)	0.0205 (±0.0022)	208	0.294	2.434	***

where l is maternal total length measured in millimetres, p is number of *in utero* embryos, a' and b' are parameters, n is sample size, r^2 is square of regression correlation coefficient, rmse is root mean square error, and P is the probability of statistical significance (*P<0.05; **P<0.01; ***P<0.001) for linear regression.

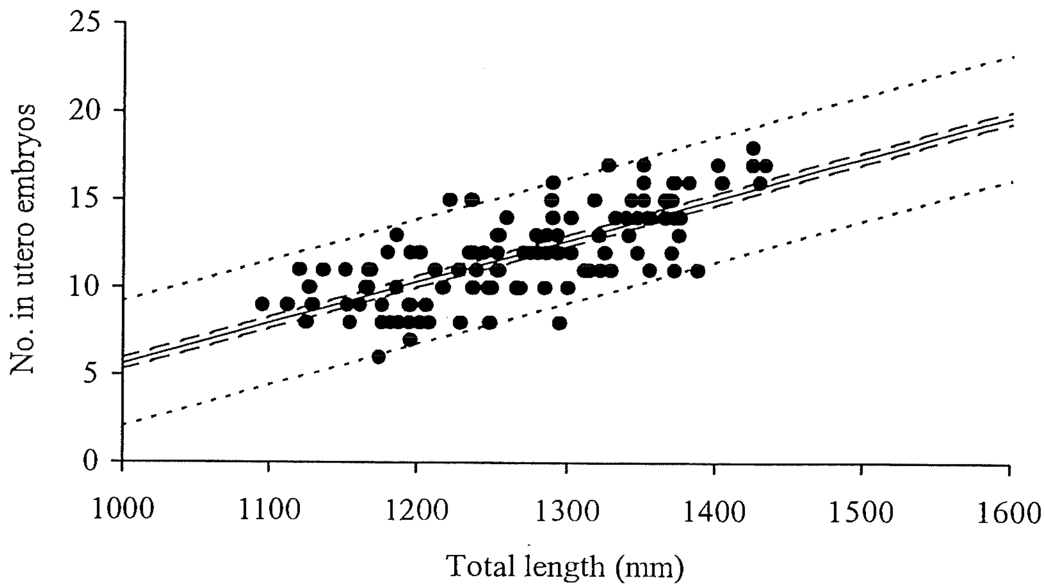


Fig. 9b. Number of *in utero* eggs against maternal total length

Mean number of embryos (——), 95% confidence limits (– – –), 95% prediction intervals (- - - -), and raw data (•) are plotted against maternal total length of pregnant females with macroscopically visible embryos (U=5). Values of parameters and statistical quantitites for the equation p=a'+b'l are given in the following tabulation:

a' (±s.e.)	b' (±s.e.)	n	r^2	rmse	P
−17.87 (±2.57)	0.0235 (±0.0020)	118	0.535	1.790	***

where l is maternal total length measured in millimetres, p is number of *in utero* embryos, a' and b' are parameters, n is sample size, r^2 is square of regression correlation coefficient, rmse is root mean square error, and P is the probability of statistical significance (*P<0.05; **P<0.01; ***P<0.001) for linear regression.

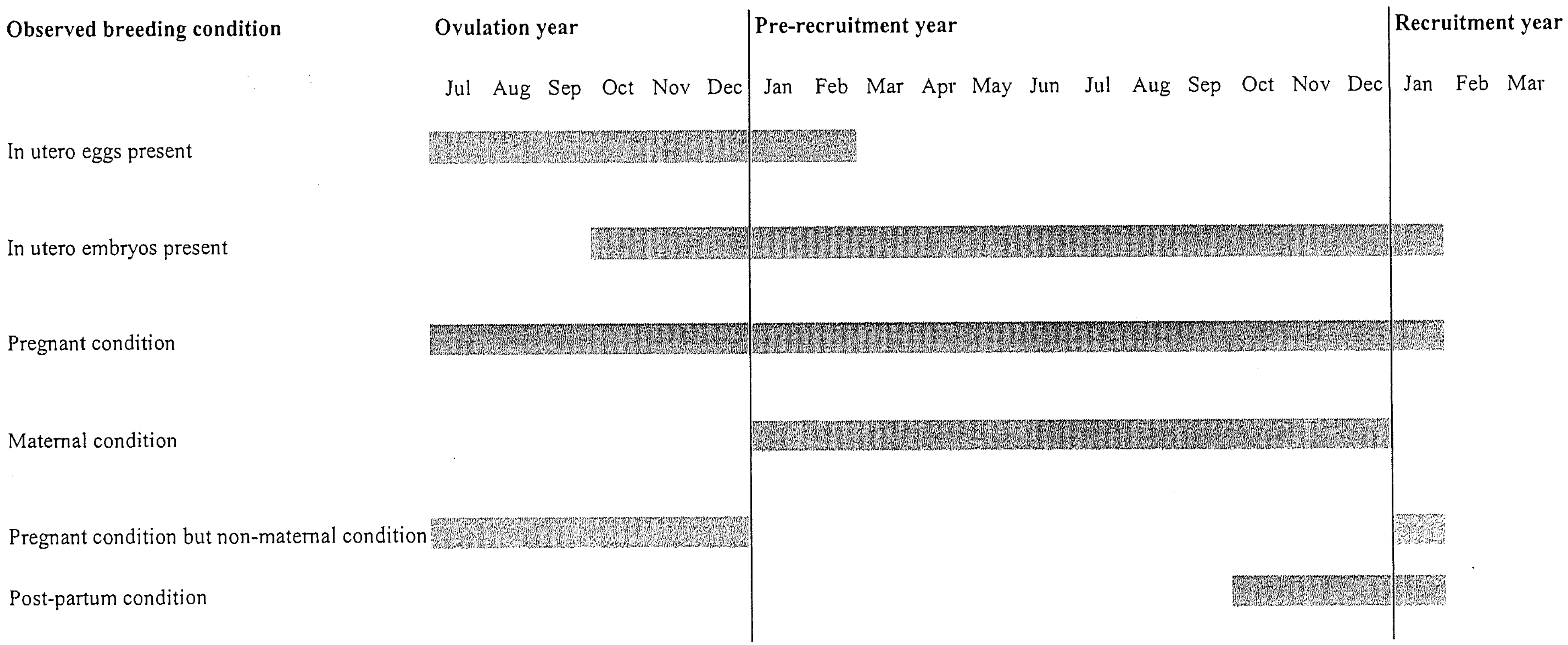


Fig. 10. Synchrony of breeding condition

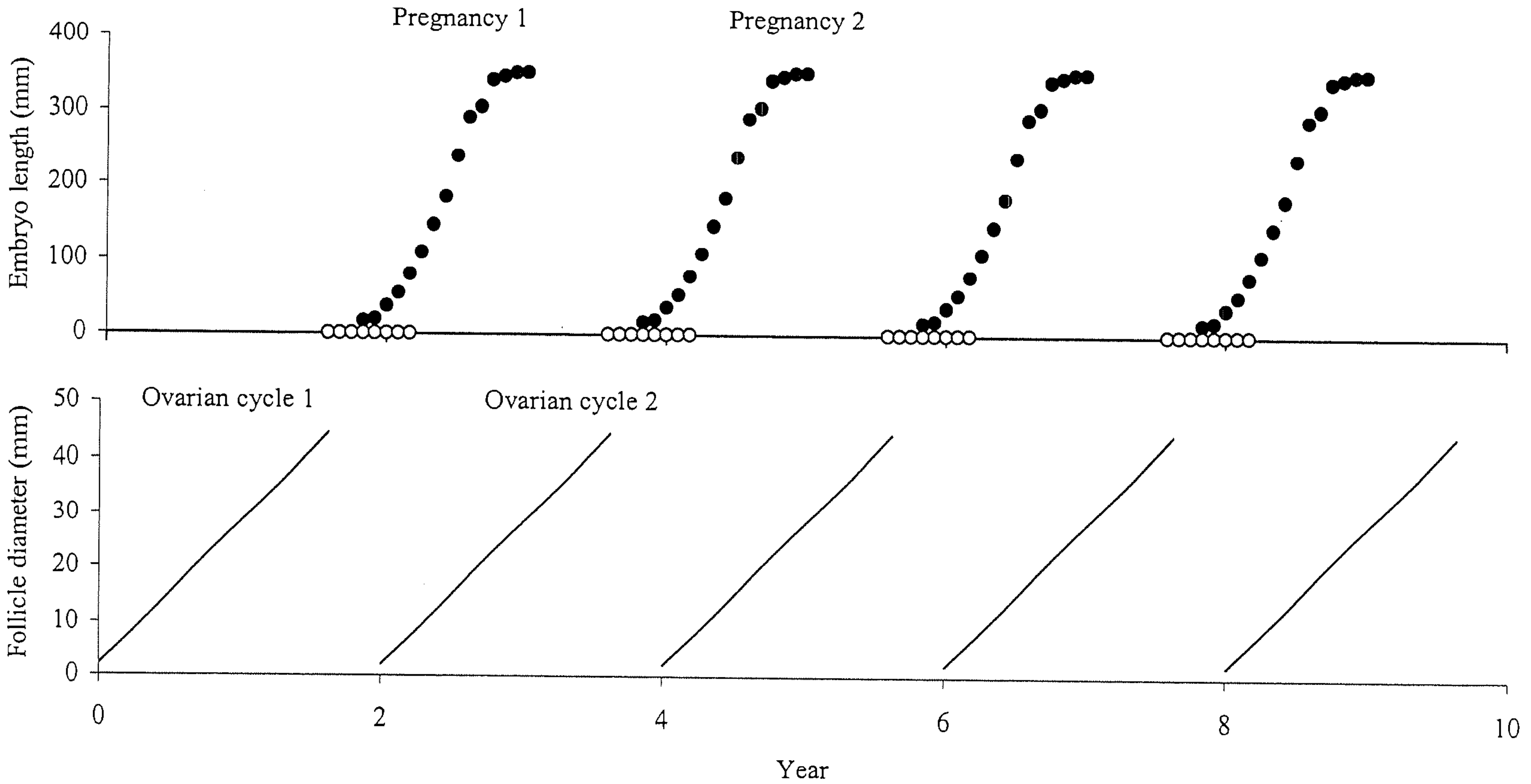


Fig. 11. Periodicity of the ovarian cycle and gestation for mature sharks
The ovarian cycle and frequency of parturition are biennial; 3 years from the beginning of vitellogenesis for a particular follicle to full-term embryo; o, *in utero* egg; •, embryo.

Appendix 3d: Southern sawshark reproduction

This appendix contains a manuscript in preparation, which presents the results of a study of the reproduction of southern sawshark (*Pristiophorus nudipinnis*) required for fishery stock assessment.

Reproductive biology of southern sawshark (*Pristiophorus nudipinnis*) harvested off southern Australia

Contents

Draft manuscript in preparation for submission to a scientific journal for publication (20 July 2005)

Reproductive biology of southern sawshark (*Pristiophorus nudipinnis*) harvested off southern Australia

Terence I. Walker[A,C] and Russell J. Hudson[A,B]

[A]Primary Industries Research Victoria, PO Box 114, Queenscliff, Victoria 3225, Australia
[B]Ocean Grove, Victoria 322X, Australia.
[C]Corresponding author. Email Terry.Walker@dpi.vic.gov.au

Abstract

Breeding female *Pristiophorus cirratus* mostly have synchronous 2-year ovarian and parturition cycles. Ovulation occur mostly during July–mid-January; the gestation period is more than 12 months, with *in utero* embryos observed *in utero* over the 15-month period January–March. Ovarian follicle diameter ranges 28–46 mm at ovulation. Mean wet mass gain is about double from *in utero* egg (~20 g) to full-term embryo (~47 g mean maxium for one litter) at ~300 mm total length. The sex ratio of *in utero* embryos is 1:1. Litter size (6–16 embryos per pregnancy) rises linearly with maternal length. The number of eggs *in utero* give a better indication of litter size than the number of embryos *in utero*, because some pregnant females carrying embryos close to full term are prone to spontaneous abortion on capture. Female length-at-maturity and length-at-maternity do not vary temporally or spatially. Maximum body mass of females is double that of males and, at any length, mean body-mass for pregnant females exceeds that of non-pregnant females, which in turn exceeds that of males.

Key words: Reproduction; Sawshark; Fishery; Australia

Introduction

The southern sawshark (*Pristiophorus nudipinnis*) is distributed at depths to 70 m from the western regions of the Great Australian Bight in South Australia to Eastern Victoria (Last and Stevens 1994). It is one of four species of the family *Pristiophoridae* endemic to Australia. The southern sawshark mothers are matrotrophic as they supplement the yolk nutrients to their embryos with uterine secretions (histotroph) (Hamlett *et al.* 2005) and exhibits aplacental viviparity. It is unknown whether the species forms a single stock or multiple stocks. The species exhibits movement within Bass Strait, but, as indicated by tag release-recapture data, not large-scale movements across southern Australia, and there do not appear to be distinct pupping grounds. The species feeds mainly on small demersal teleost species (PIRVic, unpublished data).

The southern sawshark is sympatric with common sawshark (*Pristiophorus cirratus*), and the two species are taken together as byproduct when fishers target gummy shark (*Mustelus anarcticus*) in the Gillnet Hook and Trap Fishery (GHATF) of southern Australia. Sawsharks are only occasionally targeted. More than 90% of the sawshark catch from the GHATF is taken by fishers operating in Bass Strait with gillnets of 6-inch mesh-size in depths less than 90 m. Since 1974, the annual sawshark catch has exceeded 200 tonnes, peaking during 1995 at 359 tonnes (Walker *et al.* 2003). The reported catches were not separated by species but catches recorded by scientific observers from gillnets of 6-inch mesh-size indicate most of the catch is common sawshark. These data indicate that the ratio of the number of southern sawshark caught to the number common sawshark caught in commercial gillnets is in the ratio 1:4. However, the ratio is approximately 2:3 in a series of eight experimental gillnets constructed with mesh-sizes 2–9 inch mesh-sizes, in steps of 1 inch (Walker *et al.* 2005). This suggests commercial gillnets are more effective at catching common sawshark than southern sawshark. Common and southern sawshark are also taken by demersal otter trawl and Danish seine nets off eastern Victoria and by demersal otter trawl off eastern South Australia. The quantities caught have not been as well monitored in the trawl fisheries as in the GHATF.

We adopt the quantitative approach adopted for school shark (*Galeorhinus galeus*) off southern Australia (Walker 2005) for determining reproductive parameters. This approach provides the basic reproductive parameters required for stock assessment, ecological risk assessment (Walker 2004) or species assessment against IUCN criteria established under each of several risk of extinction categories (Hilton-Taylor 2000).

Materials and Methods

Collection of specimens

Sampling for *Pristiophorus nudipinnis* was undertaken during the three separate periods 1973–76, 1986–87, and 1992–01, mainly in Bass Strait (BS) and waters off South Australia (SA) (Figure 1). During 1973–76, the animals were caught using experimental gillnets of mesh-size ranging 2–9 inches (51–229 mm), in steps of 1 inch (25 mm), and hooks attached to sinking longlines. The sawsharks were captured as a result of fishing at 162 fishing sites mainly in BS (126 sites), but also off eastern Tasmania south of latitude 41° South (20 sites) (grouped with BS samples) and SA (16 sites). During 1986–1987, the animals were caught in experimental gillnets of mesh-size ranging 5–8 inches (127–203 mm), in steps of 1 inch. The sawsharks were captured at 144 fishing sites (60 sites in BS and 84 sites in SA). During 1992–01, the animals were caught at 153 fishing sites (91 sites in BS and 62 sites in SA). All animals were caught by gillnets of 6-inch (152 mm) or 6½-inch (165 mm) mesh-size on board commercial fishing vessels.

Biological sampling

Specimens of *P. nudipinnis* were dissected to investigate their reproductive biology. They were measured to the nearest millimetre as total length (TL); the tail of each animal was first allowed to take a natural position and the upper caudal lobe placed parallel to the body axis. Sex, TL, fullness of the stomach, and several reproductive indices were recorded for each animal. Also recorded, when the sea conditions permitted (mostly on a research vessel during 1973–76), were the mass of total body, liver, left ovary of females, and left testis of males.

For females, at-sea macroscopic inspection of the condition of the paired uteri, oviducal glands, and ovaries was undertaken to investigate breeding condition, litter size, period of gestation, and growth of embryos. Records were made of the diameters of the three largest oocytes and the presence of *corpora atrecia* or *corpora lutea* in the ovary and, for pregnant animals, the number of *in utero* eggs and embryos in each uterus. In addition, the TL, sex, uterus (left or right), and mass (with and without yolk sac) of each embryo and uterus and mass of each *in utero* egg were recorded for many of the pregnant sharks. Indices were adopted for recording the condition of the ovary, oviducal gland, and uteri from rapid visual inspection. Ovary index (O) was based on size and colour of the follicles (O=1–4). Oviducal gland index (G) was based on shape and size of the gland (G=1–3). Uterus index (U) was based on appearance, size and contents of the uteri (U=1–6) (Table 1).

For males, at-sea macroscopic inspection of condition of the testes, seminal vesicles, and claspers was undertaken to investigate maturity by adopting three indices of breeding condition. Testis index (T) was based on shape, size, and relative predominance of testis tissue to epigonal gland tissue (T=1–3). Seminal vesicle index (V) was based on appearance, thickness of the wall, and presence or absence of seminal fluid (V=1–3). The length of left clasper was measured from the basipterygium to the distal end and clasper index (C) was based on appearance and rigidity (C=1–3) (Table 1).

Statistical analysis

Length-mass

The relationship between total body mass, w, and TL, l, was determined using the power curve

$$w = acl^b,$$

adopted commonly for sharks (Olsen 1954) and bony fishes (Ricker 1958) without the constant c, where a and b are parameters determined by linear regression of $\ln(w)$ against $\ln(l)$, and c is a factor correcting for biases caused by natural logarithmic transformation (Beauchamp and Olson 1973).

Period of gestation and growth of embryos

The period of gestation and growth of embryos can be determined by plotting mean TL of embryos observed in pregnant females (U=5 animals) and mean TL values of 0 for *in utero* eggs observed in pregnant females (U=4 animals) against month and then evaluating the seasonal pattern. Mass gain or loss from egg to full-term embryo during gestation was investigated for a sample of pregnant females (U=5 animals). This was undertaken by separately plotting each of four variables against mean embryo TL for U=5 animals. These variables were the mean wet mass of embryos, the mean wet mass of external yolks, the sum of these two quantities, and the mean external yolk wet mass expressed as a proportion of the sum of the two quantities.

Ovarian cycle

The ovarian cycle was investigated by examining the ovary and measuring the diameters of the largest follicles in animals caught throughout the year. The largest follicle diameter (LFD) varied widely between individual animals and varied depending on uterus condition, so seasonal pattern in LFD for each of the six uterus conditions defined in Table 1 was examined separately.

Pregnant females with macroscopically visible *in utero* embryos (U=5 animals) provided the least ambiguous basis for determining seasonal growth rates of follicles and providing a basis for distinguishing between annual, biennial, and longer ovarian cycles. Examination of *in utero* growth of embryos indicate this period covers more than one full year indicating patterns in LFD against Julian day can be adopted for measuring annual rate of follicle growth (Walker 2005).

None of the other five uterus conditions provided such clear information on annual rate of follicle growth. The data indicate that females with uterus condition U=1 exhibit little or no change in LFD over the 12-month period from January to December. Females with uterus conditions U=4 occurred for only several months towards the end of the year or very early in the year, and therefore on their own provide no information on annual growth of follicles. These animals, however, do provide information on the timing of ovulation and on follicle diameter at the time of ovulation.

Animals with uterus conditions U=2, 3, or 6 displayed wide variation in LFD and similar patterns. Some of these animals provide information on follicle diameter immediately prior to ovulation and some with uterus condition U=6 information of follicle diameter during the period immediately following parturition. The uterus condition U=6 was not commonly observed suggests that after parturition the distended uterus contracts to resemble uterus condition U=3. It is generally difficult to distinguish between uterus conditions U=3 and U=6. This implies that animals recorded with uterus condition U=3 might be a mixture of animals approaching first pregnancy (all U=3) and animals between pregnancies (U=6 changing to resemble U=3). Unlike animals with uterus conditions U=5, U=4, and U=6, which can be related to the timing of ovulation and parturition, U=2 and U=3 animals cannot be so reliably related to either of these events.

Annual growth rate for animals of uterus condition U=5 was determined by the linear relationship between LFD, o, and Julian day, t, given by

$$o = a + bt,$$

where a and b are parameters estimated by linear regression. A scattergram of LFD against Julian day for U=5 animals was compare with the U=5 regression and 95% prediction intervals. Similarly, a scattergram LFD against Julian day for each of the U=2, U=3, U=4, and U=6 animals separately was compared with the regression line and 95% prediction intervals for the U=5 animals. Based on clustering of the data points, where it appeared animals data points lay outside the 95% prediction intervals 365 days were added to Julian day. Then a similar regression was undertaken for the U=3, U=4 (ovulating only), and U=6 animals pooled and this regression line was compared with that for the U=5 animals. Differences in these regression lines were tested by comparing slopes and elevations (Kleinbaum *et al.* 1988). These comparisons provided a basis for considering whether the ovarian cycle is annual, biennial, triennial or longer.

Size-at-maturity and size-at-maternity

The proportion of the population of animals mature at any TL can be determined by classing each animal as in mature condition or immature condition and applying logistic regression for females (Mollet *et al.* 2000; Conrath and Musick 2002) and males (Walker 2005) separately. Similarly, for females, the proportion of the population of animals in maternal condition at any TL can be determined by classing each animal as in maternal condition or non-maternal condition and applying logistic regression.

For *Pristiophorus cirratus*, a female was classed as in mature condition if the largest ovarian follicle was >3 mm in diameter (size at first yolking); otherwise it was classed as in immature condition. Given uncertainty of the best indicator of maturity of males, the results from methods based on alternative criteria for assuming the mature condition and the immature condition are compared. Males were classed by testis condition as mature if T=3 and immature if T=1 or T=2. Similarly, they were classed by seminal vesicle condition as mature if V=2 or V=3 and immature if V=1 and by clasper condition as mature if C=2 or C=3 and immature if C=1 (Table 1).

A female was classed in maternal condition at the time of dissection, if it would have given birth to young before or soon after the following 1 January. To implement this criterion, females were classed as in maternal condition if they met any one of three criteria. The first criterion was pregnant with visible embryos (U=5) during January–December except for pregnant females with early-term embryos during October–December or with full-term embryos during January were classed as in non-maternal condition. The second criterion was pregnant with *in utero* eggs (U=4) during January–February, and the third criterion was non-pregnant in post-partum condition with distended uteri (U=6) during October–December. In addition to pregnant females with early-term embryos during October–December or with full-term embryos during January, females were classed as non-maternal if U=1, U=2, U=3, U=4 during July–December, or U=6 during January–September.

Logistic regression was adopted to determine the proportion of females in mature condition, the proportion of males in mature condition, and the proportion of females in maternal condition as a function of TL. Females or males in mature condition were assigned a maturity condition value of 1, whereas those in immature condition were assigned a maturity condition value of 0. Similarly, females in maternal condition were assigned a maternal condition value of 1, whereas females in non-maternal condition were assigned a maternal condition value of 0.

The logistic equation adopted to express P as a function of l is given by

$$P = \frac{c}{(1+e^{-(a+bl)})}$$

where a, b, and c are parameters but to provide parameters that are more biologically meaningful, the equation is reformulated to express P as a function of l by

$$P = P_{\max}\left(1+e^{-\ln(19)\left(\frac{l-l_{50}}{l_{95}-l_{50}}\right)}\right)^{-1},$$

where $P_{\max}$ is the maximum proportion of animals in mature condition or maternal condition, and l_{50} and l_{95} are the lengths at which 50% and 95% of the maximum proportion of animals in mature condition or maternal condition (Walker 2005).

The parameters $P_{\max}$, l_{50} and l_{95}, with 95% confidence intervals, were estimated by the method of maximum likelihood using the probit procedure (Proc Probit) of the computer statistical package SAS (SAS Institute, Cary, North Carolina, USA). This applies a modified Newton–Raphson algorithm for estimation.

The standard error for any length, l, is given by

$$se_l = P_l(1-P_l)/N.$$

The SAS probit procedure sets $1-P_{\max} = 0.000$. This is appropriate for the maturity ogive where all large-sized animals in the population are in mature condition, and hence the proportion of large-sized animals in the population mature is 1.000. Similarly, this is appropriate for the maternity ogive where all of the large-sized animals in the population are in maternal condition, and hence the proportion of large-sized animals in the population in maternal condition is 1.000; parturition frequency is annual. However, this is not appropriate where parturition frequency is biennial, triennial, or some other period.

Application of the SAS probit procedure is more complex to apply to any parturition frequency, γ, other than 1 year. For example, if parturition is biennial where half the population gives birth each year then $\gamma = 0.50$ or if parturition is triennial where one-third the population gives birth each year then $\gamma = 0.33$. This was undertaken for *P. cirratus* by categorising the number of animals in maternal condition and the number of observations into 100-mm length-classes. For parturition frequency, where the ratio of number in maternal condition / number of observations exceeds γ within a 100-mm length-class, the number in mature condition needs to be adjusted to produce the ratio γ. For SAS probit analysis, the number of observations in each 100-mm length-class (or some other selected range) is multiplied by γ. A weight statement was used to weight the values in each length-class by the original number of observations in that length-class. The ogive relationships, with 95% CI, produced by the SAS probit procedure can then by divided by γ to give the required parameters of the maternity ogive, with 95% CI.

Litter size and sex ratio of embryos

Simple mathematical equations can be selected to represent the relationships between the number of macroscopically visible *in utero* embryos, p, and maternal TL, l. The linear relationship (Jones and Ugland 2001; Conrath and Musick 2002) between p and l is given by

$$p = a + bl,$$

where a and b are parameters estimated by linear regression. There is a potential bias in this relationship from embryos being aborted at capture. Hence the linear relationships between the number of *in utero* eggs, e, and maternal TL, l, was also determined.

Results and Discussion

Reproductive biology

A total of 488 male and 467 female *Pristiophorus nudipinnis* were collected and sampled for dissection during the three sampling periods 1973–76, 1986–87, and 1992–01. The longest female P. nudipinnis captured during field operations (1235 mm TL) was much longer than the longest male captured (1095 mm). The highest total body mass of a female recorded (4.3 kg) was almost double the highest mass of a male recorded (2.2 kg).

Total body mass at TL

Statistical comparison of the slopes and intercepts by the Student t-test for selected pairs of straight line $\ln(w)$–$\ln(l)$ relationships determined from linear regression fits indicated that some of the relationships were different. The $\ln(w)$–$\ln(l)$ relationships for pregnant females carrying *in utero* eggs (U=4) and for pregnant females carrying *in utero* embryos (U=5) were significantly different (t-test, t=2.203, d.f.=126, and 0.05>P>0.01 for comparison of slopes and t=2.222, d.f.=126, and 0.05>P>0.01 for comparison of elevations). The $\ln(w)$–$\ln(l)$ relationship for pregnant females (U=4 and U=5 pooled) and the relationship for non-pregnant females (U=1, U=2, U=3, and U=6 pooled) were highly significantly different (t-test, t=4.533, d.f.=205, and P<0.001 for comparison of slopes and t=4.570, d.f.=205, and P<0.001 for comparison of elevations). The $\ln(w)$–$\ln(l)$ relationship for non-pregnant females and the relationship for males were not significantly different (t-test, t=0.335, d.f.=307, and P>0.05 for comparison of slopes, and t=0.269, d.f.=307, and P>0.05 for comparison of elevations). The relationships of total body mass against TL, with 95% confidence limits on the mean curves and 95% prediction intervals are presented separately for pregnant females (Figure 2a), non-pregnant females (Figure 2b), and males (Figure 2c).

The curves for these relationships coincide very closely for the non-pregnant females and males. Surprisingly, the mean body mass is lower for pregnant females than for non-pregnant females and males, for animals of size approaching the maximum TL. Unlike P. cirratus, this is not consistent with increasing mass of the ovaries, *in utero* eggs, and *in utero* embryos in mature and breeding females. This anomaly is likely to be an artefact of fitting to the data where variation in TL among pregnant animals is small compared to that for non-pregnant females and males.

Period of gestation and growth of embryos

Mean TL of embryos (with standard error) from 86 pregnant females (U=5 animals) and assigned mean TL values of 0 mm for eggs *in utero* observed from 131 pregnant females (U=4 animals) are plotted against month (Figure 3). *In utero* embryos were observed during the 15-month period from January one year to March the following year and *in utero* eggs were observed during the 9-month period May–February. Although females can clearly be pregnant during any month of the year, none were observed during the 4-month period March–June. The data indicate a high degree of synchrony in gestation between U=5 animals. This synchrony, together with the long period for the presence of *in utero* eggs, is evidence that the period of gestation exceeds 1 year and the frequency of parturition cannot be annual.

The highest embryo wet mass of 47 g towards the end of gestation observed in one pregnant animal when mean TL of its embryos exceeded 315 mm. This was more than double the mean egg wet mass of 20.2 g (n=260, sd=4.1 g, range 10–43 g) at the beginning of gestation in another animal (Figure 4). Given *P. nudipinnis* is aplacental, this approximately 100% mass gain from egg to full-term embryo suggests the species provides nutrients to the embryos by way of intra-uterine nutrients (histotroph). There is little or no mass gain in the combined mass of embryo and external yolk sac for the first half of gestation, but this increases progressively towards the end of gestation (Figure 4).

Ovarian cycle

The diameter of the largest follicle recorded in the ovary from each of 320 females ranged 1–46 mm. There was little difference between the diameters of the three largest follicles (Table 2), so all statistical analyses of the follicle data were undertaken using only the first measured follicle, which was judged visually to be the largest when measured.

No patterns in plots of the largest follicle diameter (LFD) against Julian day were evident where all the data were pooled; patterns of variation in LFD only became evident when the animals were considered for each uterus condition separately. LFD was consistently small for animals with uterus conditions U=1 (n=21, mean 1.5 mm, s.d. 0.2 mm, range 1–4 mm), a clear indication that all these animals were either immature or at the earliest stages of maturation. LFD varied widely for animals in uterus conditions U=2 (n=31, range 1–40 mm), U=3 (n=45, 14–40 mm), U=4 (n=83, 1–46 mm), U=5 (n=136, 2–31 mm), and U=6 (n=4, 17–33 mm). The U=4 animals were further classed as animals either in the process of ovulating (LFD ranged 28–46 mm) or had completed the process of ovulating (LFD ranged 1–22 mm). Overall the patterns in the plots were consistent with the hypothesis that the ovarian cycle is biennial (Figure 5).

The period of the growth of embryos from being first visible macroscopically to full-term was more than 1 year at 15 months (Jan–Mar). Hence, U=5 animals provided an indication of the annual rate of growth of follicles in mature animals. For 17 animals during January, 1 during February, and 5 during March, which had close to full-term embryos and LFD >15 mm hen captured, it was necessary to adjust Julian day by adding 365 days. Linear regression of LFD against Julian day indicated that mean annual growth in LFD for U=5 animals was 17 mm y^{-1}; predicted mean LFD increased from 2 to 19 mm during 365 days (Figures 5a and 6). During Jan–Mar when gestation was complete or approaching completion, the ovarian follicles were approaching the size when ovulation occurs several months later, as indicated by ovulating U=4 animals (LFD ranged 28–46 mm). These results are consistent with the hypothesis that the ovarian cycle is biennial, and inconsistent with the hypothesis that the ovarian cycle is annual.

Similar regression analysis of LFD against Julian day was undertaken pooling animals with uterus conditions U=2, 3, 4 (ovulating only), and 6. Animals where the follicles were either possibly not growing or undergoing atresia were excluded from the analysis. All U=1 animals (Figure 5b), U=4 animals that had completed ovulation, and U=2, U=3 and U=6 animals where LFD <9 mm were excluded. In addition, from visual inspection of the pattern of clustering of the data points, 365 days were added for U=2, 3 or 6 animals where LFD >20 mm and Julian day <300. The regression indicated that mean annual growth in LFD for these animals was 19 mm y^{-1}. This predicted mean LFD increased from 4 to 23 mm during a 365-day period and from 4 to 42 mm during a 730-day period (Fig. 7). Comparison of the two separate linear regression fits for LFD against Julian day between the U=5 animals and the U=2, 3, 4 (ovulating), 6 animals indicated that these two straight lines had not statistically significantly different slopes (t-test, t=1.242, d.f.=225, and P>0.005) and had not significantly different elevations (t-test, t=0.869, d.f.=225, and P>0.05). These results indicate that the ovarian cycle is biennial, but the growth of the follicles, and hence rate of vitellogenesis, is similar between pregnant females with developing embryos and other vitellinogenic females. These results imply that for females with *in utero* embryos during the first year of the biennial ovarian cycle, the growth rate of follicles is similar between the first and second year.

The animals for each uterus condition U=2, U=3, U=4, and U=6 were then examined to assess whether they were consistent with the hypothesis of a 2-year ovarian cycle. Scattergrams of LFD plotted against Julian day for animals for each of these four uterus conditions were compared with the predicted mean LFD trajectory determined for the non-pregnant animals. The LFD trajectory was extrapolated through a second year and displayed for a 1-year period by presenting the trajectories as parallel lines. On each of the four scattergrams, the mean trajectory and trajectories of the lower and upper 95% prediction intervals were presented for the first year and second year on axes displaying 365 Julian days (Figures 6cdef).

Among the animals with U=2 uterus condition, those where LFD <9 mm are interpreted as recently matured from the U=1 uterus condition. The pattern of LFD against Julian day for the rest of these animals is consistent with a 2-year ovarian cycle (Fig 6c). The pattern for animals with uterus condition U=3 is similar to that for animals with the U=2 uterus condition, except there were no animals with small follicles (LFD <9 mm). The latest U=3 animal capture with enlarged follicles was 19 October (Julian day 292), suggesting these animals begin ovulating at about that date (Figure 6d).

The scattergram for the U=4 animals is also consistent with the 2-year ovarian cycle hypothesis (Figure 5e). These data provided reliable information on the timing of ovulation and on magnitude of LFD at the time of ovulation. The animals were classed as 'ovulating' or 'ovulated' based on LFD, which had two size clusters (ranging 1–22 and 28–46 mm LFD). Animals were classed as ovulating (in the process of ovulation) if they contained eggs *in utero* and ≥25-mm LFD; animals were classed as ovulated (ovulation complete) if they contained eggs *in utero* and <25-mm LFD. Animals found ovulating were captured during the period from 22 August (Julian day 234) to 18 January (Julian day 18). Animals found ovulated were captured during the period from 18 July (Julian day 199) to 27 March (Julian day 86).

For U=6 animals, the individual LFD values have a similar distribution to U=3 animals. They are consistent with the 2-year ovarian cycle with two animals clustered near the lower and two clustered near the upper trajectory (Figure 5f). There is uncertainty distinguishing between the U=6 and U=3 uterus conditions and of the possibility of the pregnant females aborting when captured. Many of those clustered near the upper mean trajectory are likely to be a mix of maturing animals approaching first ovulation and animals having given birth the previous year.

Size-at-maturity and size-at-maternity

Testing for the effects of region, period, and region x period interaction on logistic regression models by backward stepwise elimination of statistically non-significant terms by log-likelihood ratio tests indiacted that none of these terms were significant. Hence, available data were pooled over the three regions EBS, WBS, and SA and three periods 1973–76, 1986–87, and 1998–01.

Total length at which 50% and 95% of the animals matured, with 95% confidence limits (CI), derived from the ogives presented in Figure 7 are tabulated as follows.

Condition	L_{50} (95% CI)	L_{95} (95% CI)	P_{max}	n	N
Maturity	866 (827, 892)	1056 (1037, 1083)	1.00	336	416
Maternity	944	954	0.50	210	1049

The analysis failed to provide confidence intervals for the maternity ogive and the confidence intervals for the maturity ogive are relatively wide and diverge for the small sharks. The results are uncertain and require additional samples of small sharks to better determine the maternity ogive.

Litter size and sex ratio of embryos

A total of 89 pregnant females 880–1130 mm TL were observed to carry 6–16 eggs *in utero* and 82 pregnant females 912–1160 mm TL were observed to carry 7–14 embryos *in utero*. The male:female ratio of embryos in 82 mothers was 0.495:0.505, which is not statistically different from 1:1. The linear relationship between the number of macroscopically visible embryos *in utero* and maternal length (Figure 8a) gives a slightly lower litter size at TL and variance in litter size at TL than the relationship between the number of eggs *in utero* and maternal length (Figure 8b).

In utero	a (s.e.)	b (s.e.)	n	r^2	rmse	P
Embryos	−8.36 (±4.63)	0.0184 (±0.0045)	62	0.160	1.678	***
Eggs	−14.91 (±3.61)	0.0252 (±0.0035)	89	0.362	1.769	***

The difference in the relationships is explained by the occurrence of spontaneous abortion of embryos approaching full-term. Infertile eggs, common in *Mustelus antarcticus* and *Galeorhinus galeus*, were not observed in *P. nudipinnis*. Hence, it can be assumed that all *in utero* eggs are fertile and that their number provides the more reliable indicator of litter size than the number of embryos *in utero*. The second set of parameters is therefore the more appropriate to use for fishery stock assessment and demographic analysis than the first set of parameters.

Synchrony and periodicity of reproductive cycle

Synchrony of the breeding condition and the two-year periodicity of the ovarian cycle and gestation are illustrated in Figures 9 and 10.

Acknowledgments

Acknowledgment is due to a large number of former and present staff of the Primary Industries Reseach Victoria Queenscliff Centre who participated in field sampling of sharks and laboratory processing. Special acknowledgement is due to Anne Gason for advice on statistical analysis of the data and application of the statistical package SAS. The skippers and crews of various shark fishing vessels who kindly provided accommodation, assistance, and hospitality aboard their fishing vessels are also thanked. The work was funded by the Fisheries Research and Development Corporation (FRDC) of Australia as part of FRDC Project 1999/103. Some of the field data were collected opportunitically as part of earlier projects.

References

Beauchamp JJ, Olson JS (1973) Corrections for bias in regression estimates after logarithmic transformation. *Ecology* **54**, 1403–1407.

Conrath CL, Musick JA (2002) Reproductive biology of the smooth dogfish, *Mustelus canis*, in the northwest Atlantic Ocean. *Environment Biology of Fishes* **64**, 367–377.

Hamlett WC, Kormanik G, Storrie MT, Stevens B, Walker TI (2005) Chapter 13. Chondrichthyan parity, lecithotrophy and matrotrophy. pp. 395–434. In 'Reproductive Biology and Phylogeny of Chondrichthyes: Sharks, Rays and Chimaeras'. Ed. WC Hamlett. (Science Publishers, Inc.: Enfield, New Hampshire 03478, USA.)

Hilton-Taylor C (2000) '2000 IUCN Red List of Threatened Species.' (IUCN The World Conservation Union: Gland, Switzerland.)

Jones TS, Ugland KI (2001) Reproduction of female spiny dogfish, *Squalus acanthias*, in the Oslofjord. *Fishery Bulletin* **99**, 685–690.

Kleinbaum DG, Kupper LL, Muller KE (1988) 'Applied regression analysis and other multivariable methods.' (PWS–Kent Publishing Company: Boston, MA.)

Last PR, Stevens JD (1994) 'Sharks and rays of Australia.' (CSIRO Australia: Melbourne.)

Mollet HF, Cliff G, Pratt Jnr HL, Stevens JD (2000) Reproductive biology of the female shortfin mako, *Isurus oxyrinchus* Rafinesque, 1810, with comments on the embryonic development of lamnoids. *Fishery Bulletin* **98**, 299–318.

Olsen AM (1954) The biology, migration, and growth rate of the school shark, *Galeorhinus australis* (Macleay) (Carcharhinidae) in south-eastern Australian waters. *Australian Journal of Marine and Freshwater Research* **5**, 353–410.

Ricker WE (1958) Handbook of computations for biological statistics of fish populations. *Bulletin of Fisheries Research Board of Canada* **119**, 1–191.

Walker TI (2004) Chapter 13. Management measures. pp 285–321. In 'Technical manual for the management of elasmobranchs'. (Eds JA Musick and R Bonfil) (Asia Pacific Economic Cooperation, Secretariat: Singapore.)

Walker TI (2005) 4 Reproduction in fisheries science. pp. 81–127. In 'Reproductive Biology and Phylogeny of Chondrichthyes: Sharks, Rays and Chimaeras'. Ed. WC Hamlett. (Science Publishers, Inc.: Enfield, New Hampshire 03478, USA.)

Walker TI, Hudson RJ, Gason AS (2005) Catch evaluation of target, byproduct, and bycatch species taken by gillnets and longlines in the shark fishery of south-eastern Australia. In 'Proceedings of North Atlantic Fisheries Organisation Symposium. Elasmobranch Fisheries: Managing for Sustainable Use and Biodiversity Conservation'. 11–13 September 2002. Santiago de Compostela, Spain. *Journal of Northwest Atlantic Fishery Science* **35**, 505–530.

Walker TI, Taylor BL, Gason AS (2003) 'Southern shark catch and effort 1970–2002 report to Australian Fisheries Management Authority.' Marine and Freshwater Resources Institute, Queenscliff, Victoria, Australia.

Table 1. Indices adopted for staging reproductive condition

Various assumption made on maturity when analysing the data are also listed.

Organ or tissue	Index	Description	Maturity assumption
Female			
Ovary	O=1	Largest follicles white and of diameter <2 mm	Immature
	O=2	Largest oocytes yolking and of diameter 2–3 mm	Immature
	O=3	Largest oocytes with yellowish yolk and of diameter >3 mm	Mature
	O=4	Yolked oocytes of diameter >3 mm and extensive corpora atretica present	Mature
Oviducal gland	G=1	Indistinct from anterior oviduct	Immature
	G=2	Distinct but only partly formed (Hamlett et al 1998)	Immature
	G=3	Enlarged with ram horn-shaped lobes	Mature
Uterus	U=1	Uniformly thin tubular structure	Immature
	U=2	Thin tubular structure partly enlarged posteriorly	
	U=3	Uniformly enlarged tubular structure	
	U=4	In utero eggs present without macroscopically visible embryos present	Mature
	U=5	In utero embryos macroscopically visible	Mature
	U=6	Enlarged tubular structure distended	Mature
Male			
Testis	T=1	Thin tissue strip with epigonal gland predominant	Immature
	T=2	Thickened strip with epigonal gland tissue extensive	Immature
	T=3	Enlarged and predominant with epigonal gland tissue negligible	Mature
Seminal vescicle	V=1	Thin translucent walls and seminal fluids absent	Immature
	V=2	Thickened opaque walls and seminal fluids present	Mature
	V=3	Thickened opaque walls and seminal fluids absent	Mature
Clasper[A]	C=1	Pliable with no calcification	Immature
	C=2	Partly calcified	Immature
	C=3	Rigid and fully calcified	Mature

[A]Adopted for periods 1986–87 and 1992–01, but not for period 1973–76.

Table 2. Comparison of diameters of three largest ovarian follicles by uterus condition

n, sample size; s.e., standard error

Uterus condition[A]	n	Mean diameter (±s.e.) of ovarian follicle (mm)		
		Oocyte 1	Oocyte 2	Oocyte 3
U=1	21	1.52±0.18	1.48±0.15	1.48±.15
U=2	31	19.45±2.27	19.00±2.23	19.16±2.34
U=3	44	29.07±1.04	28.05±1.04	27.36±1.09
U=4	79	12.87±1.59	11.23±1.49	9.81±1.36
U=5	136	13.03±0.60	12.28±0.59	11.64±0.55
U=6	3	24.67±4.63	23.67±4.81	22.00±4.73
Total	314	15.21±0.66	14.27±0.64	13.54±0.63

[A]Defined in Table 1.

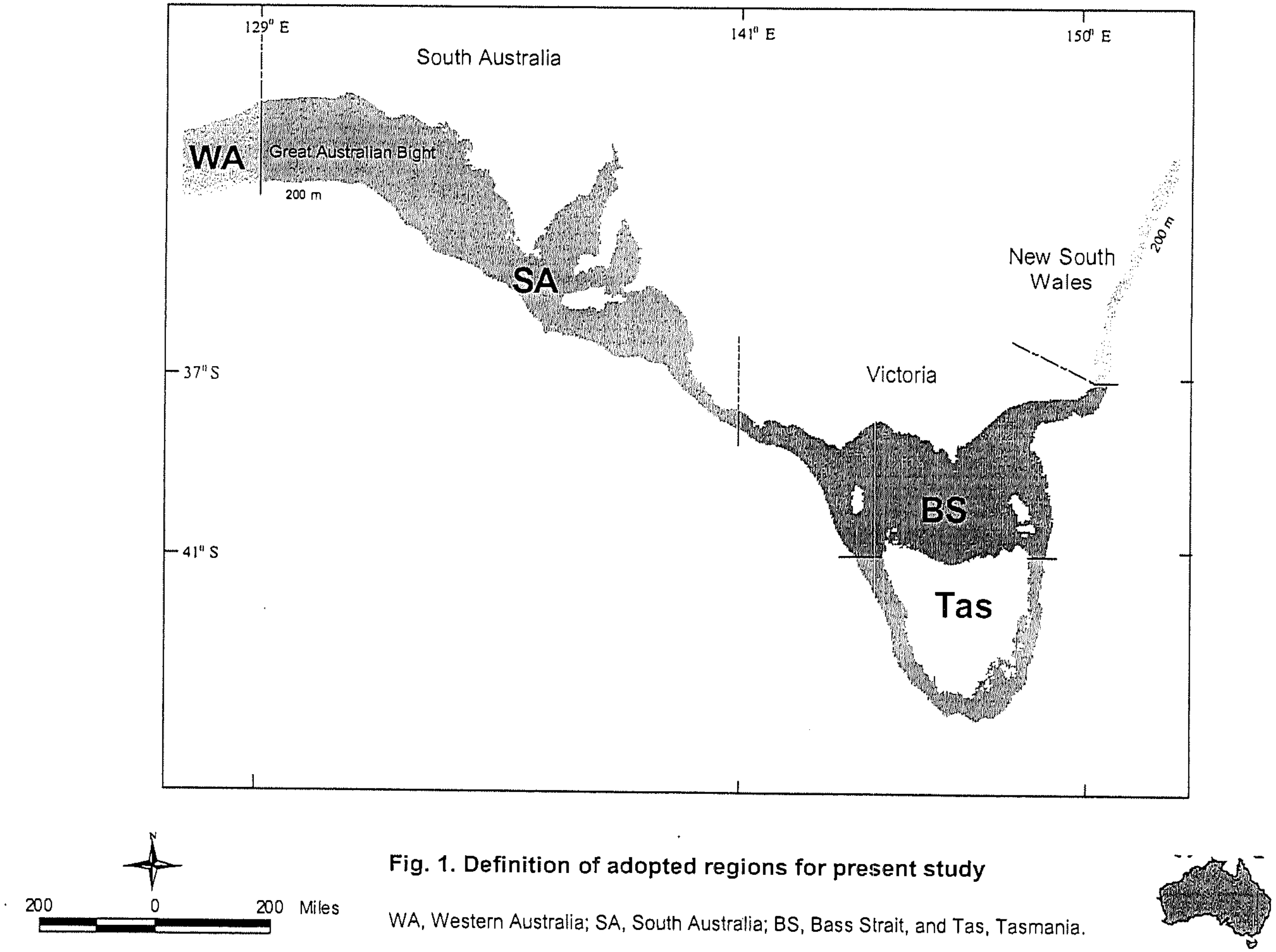


Fig. 1. Definition of adopted regions for present study

WA, Western Australia; SA, South Australia; BS, Bass Strait, and Tas, Tasmania.

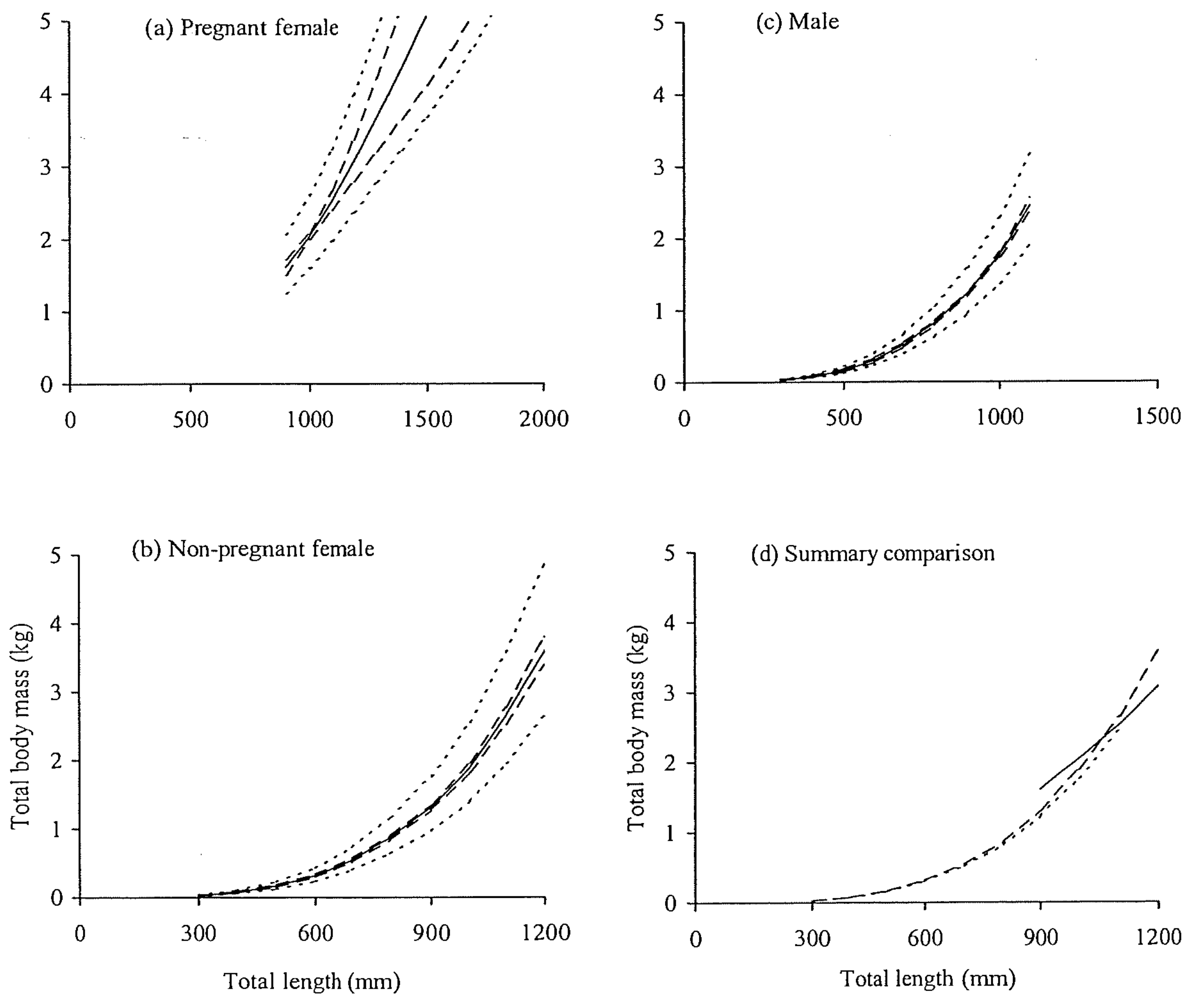


Fig. 2. Relationships between total body mass and total length.

Plots of mean total mass against TL (——), with 95% confidence limits (– – – –) and 95% prediction intervals (- - - - -), for pregnant females (a), non-pregnant females (b), and males (c), and comparison of the mean curves for pregnant females (——), non-pregnant females (– – – –), and males (- - - - -) (d) in southern Australia during the periods 1973–76, 1986–87, and 1998–01 combined. Values for parameters and statistical quantities from linear regression analysis to derive the equation $w=acl^b$ are given in the following tablulation:

Shark category	a (s.e. range) x 10^{-9}	b(se)	c	n	r^2	rmse	P
Pregnant female	354.0 (80.80–1550.0)	2.252 (0.214)	1.007	128	0.407	0.097	***
Non-pregnant female	0.060 (0.034–0.107)	3.498 (0.086)	1.012	79	0.956	0.152	***
Male	0.078 (0.035–0.172)	3.450 (0.115)	1.008	230	0.797	0.130	***

where w is total body mass, l is total length, a and b are parameters, c is the Beauchamp and Olson (1973) correction factor, n is sample size, r^2 is square of correlation coefficient, and rmse is root mean square error for this regression (*P<0.1; **P<0.01; ***P<0.001) for the regression equation ln(w)=a+bln(l).

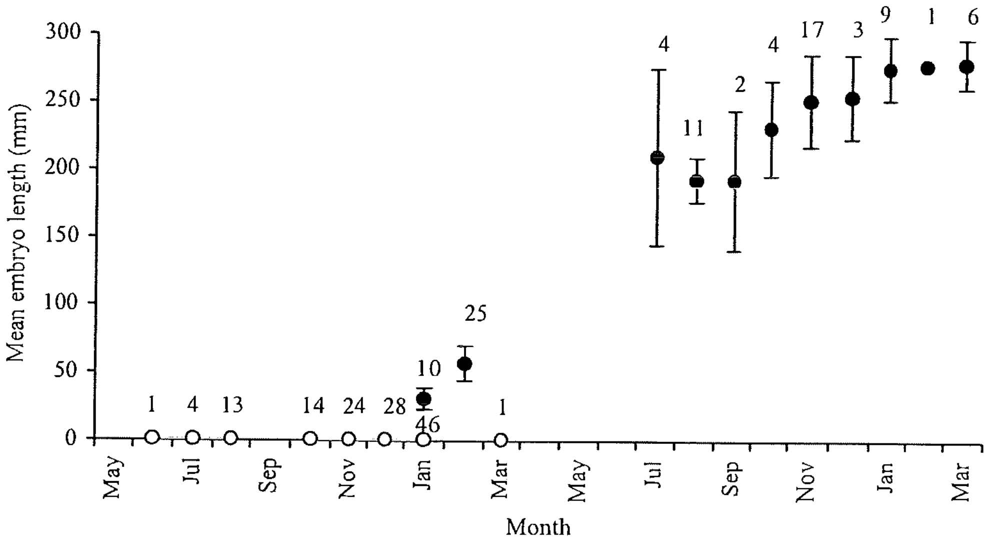


Fig. 3. Mean embryo length against month

Derived from the mean embryo length of the litter from each of 86 pregnant animals with macroscopically visible embryos and of 131 pregnant animals with only *in utero* eggs; •, overall mean; bars, standard deviation .

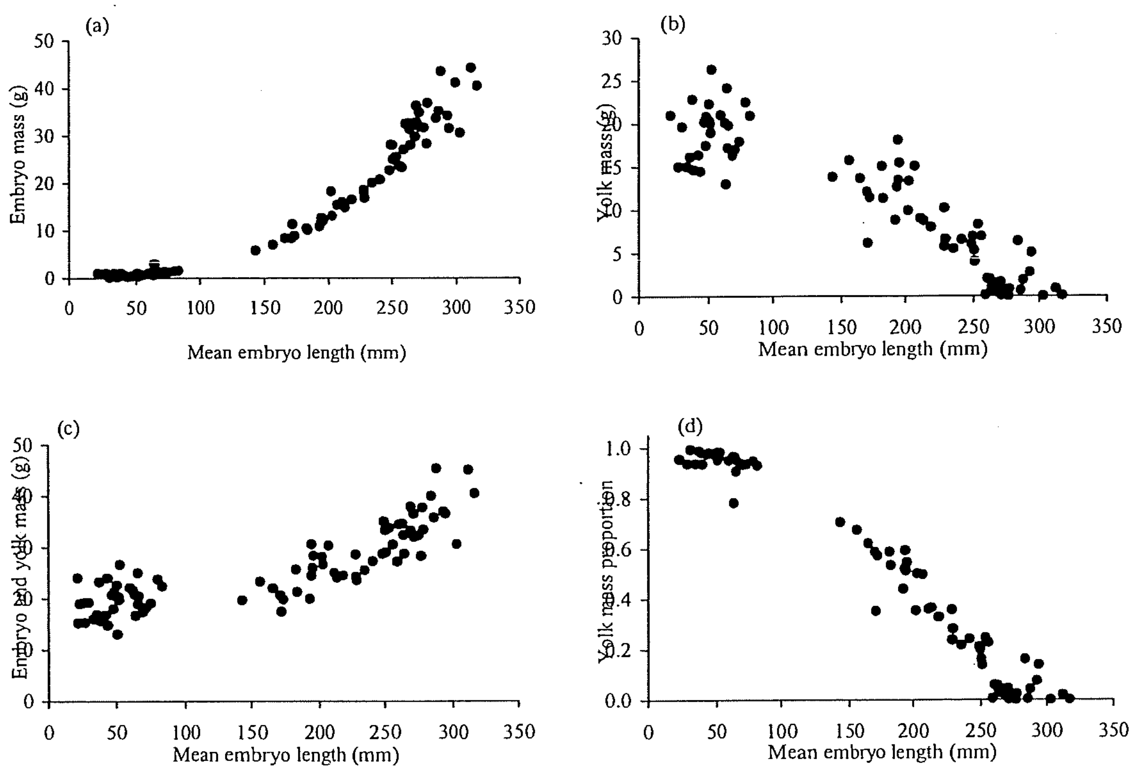


Fig. 4. Mass gain of embryos and mass loss from yolk sac during gestation

Mean mass of embryos (a) mean mass of yolk sacs (b), and yolk sac as a proportion of sum yolk sac and embryo mass (c) against mean embryo length. Each data point is derived from the mean embryo mass, mean yolk mass, and mean embryo length determined for the litter of each of 36 pregnant animals with macroscopically visible embryos. Yolk mass proportion is yolk sac mass/(embryo mass + yolk sac mass).

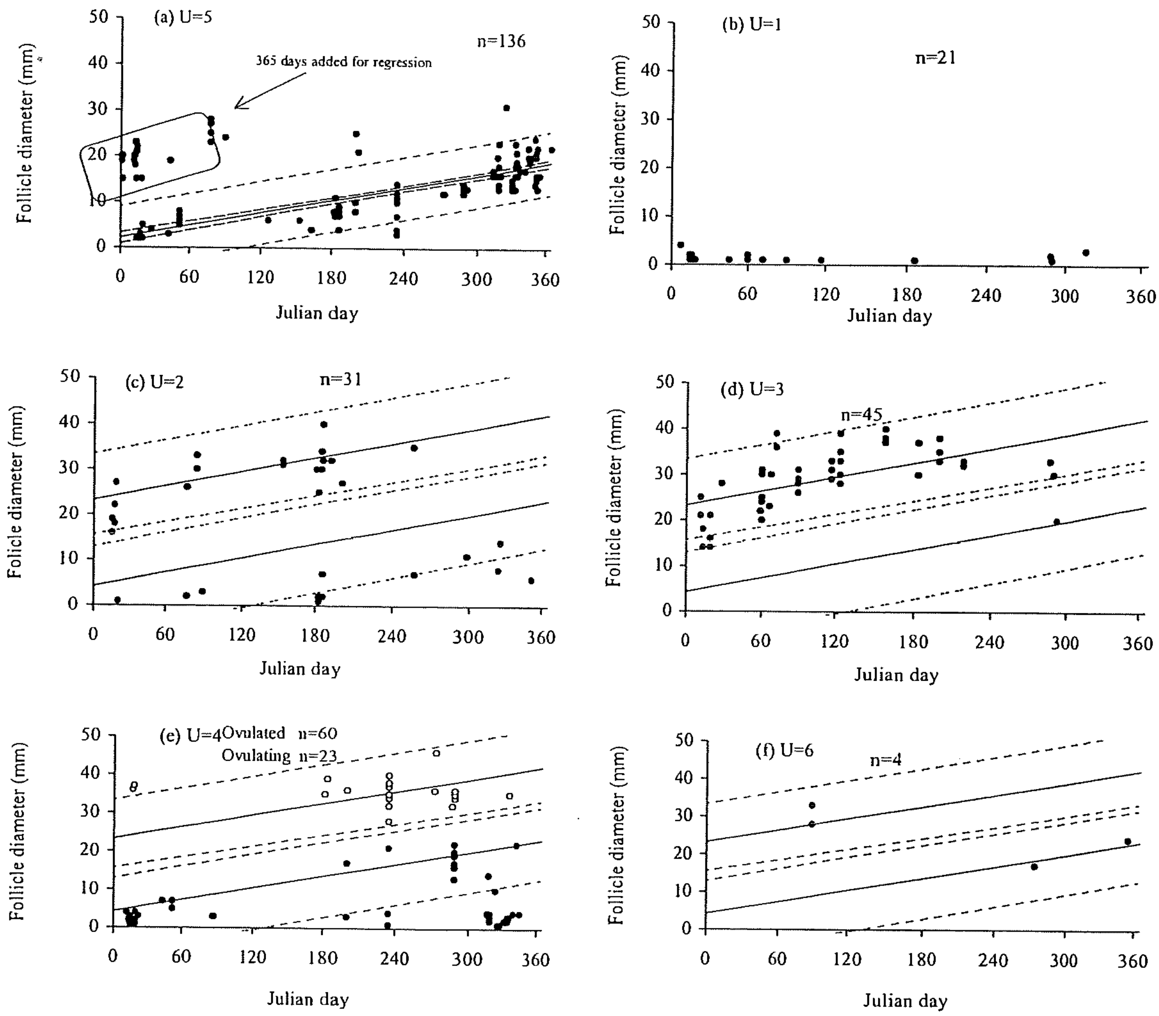


Fig. 5. Ovarian follicle diameter against Julian day by uterus condition

Largest follicle diameter against Julian day for females from Bass Strait and South Australia during 1973–76, 1986–87 and 1998–01 for each of the of six uterus conditions. Mean oocyte diameter (——) with 95% prediction intervals (- - - -) are presented for pregnant females with *in utero* embryos (U=5) (a), non-pregnant animals (U=1, 2, 3) (b, c and d), pregnant animals with *in utero* eggs (U=4, ovulated (•) and ovulating (o)) (e), and postpartum females (U=6) (f). Values of parameters and statistical quantities for the regression equation o=a'+b't for pregnant females with *in utero* embryos (U=5) are given in the following tabulation:

U	a' (se)	b'(se)	n	r^2	rmse	P
5	2.183 (0.601)	0.0456 (0.0022)	136	0.760	3.417	***
2, 3, 4 (ovuluating), 6	4.230 (2.722)	0.0520 (0.0054)	93	0.502	5.048	***

where t is Julian day, o is largest follicle diameter, a' and b' are parameters, n is sample size, r^2 is square of regression correlation coefficient, and rmse is root mean square error for the regression, and P is probability of statistical significance (*P<0.1; **P<0.01; ***P<0.001).

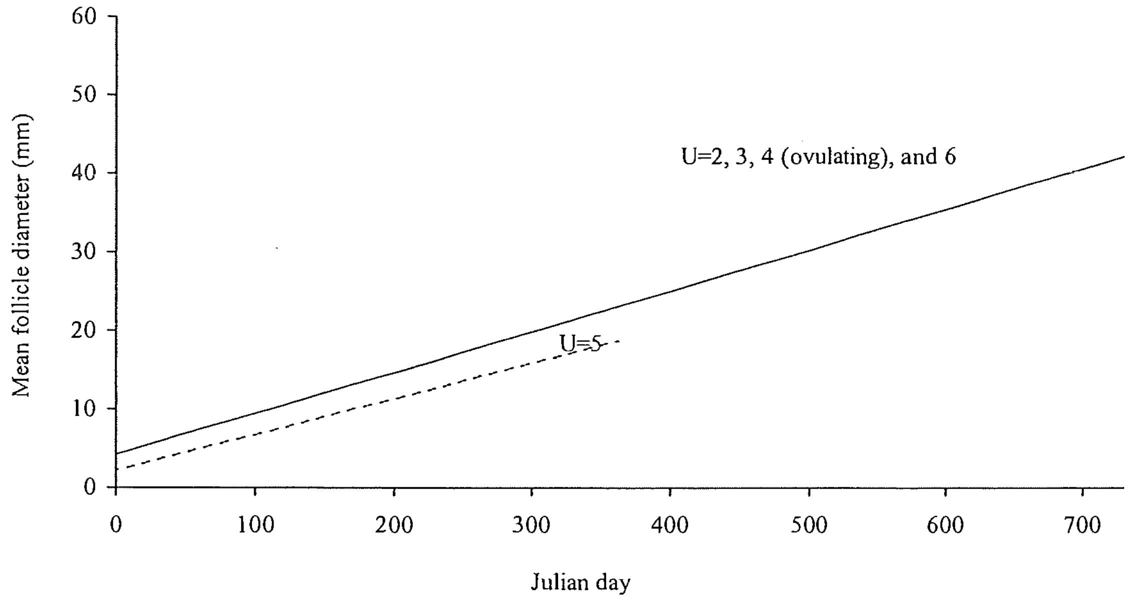


Fig. 6. Comparison of relationships ovarian follicle diameter against Julian day

Mean ovarian follicle diameter against Julian day for females with uterus condition U=5 and with uterus conditions U=2, 3, and 6 collected from Bass Strait (BS) (——) and South Australia (SA) (- - - -) during 1973–76, 1986–87, and 1998–01 combined (from Fig. 5).

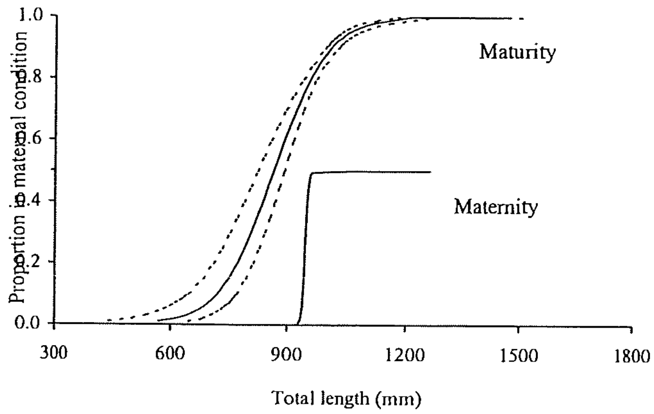


Fig. 7. Comparison of length-at-maternity and length-at-maturity ogives

Proportion of female population classed as being in maternal condition (——) and mature condition against TL during 1986–87 and 1998–01 combined . Values of parameters and statistical quantities for the equation $P_l = P_{max(l)}(1+e^{-\ln(19)(l-l_{50}/l_{95}-l_{50})})^{-1}$ determined from probit analysis are given in the following tabulation:

Variable	l_{50} (CI)	l_{95} (CI)	$P_{max(l)}$	n	N	P
Maturity	866 (827– 892)	1056 (1037–1083)	1.00	321	1049	***
Maternity	944	954	0.50	210	416	***

where l is total length measured in millimetres, P_l is proportion of animals classed as being in maternal condition at TL l, l_{50} and l_{95} are parameters, $P_{max(l)}$ is an asymptotic constant, n is the total number of animals classed as being in maternal condition (adjusted in parentheses), and N is the total number of animals examined (adjusted in parentheses), ML is maximum likelihood, and P is probability of statistical significance (*P<0.1; **P<0.01; ***P<0.001).

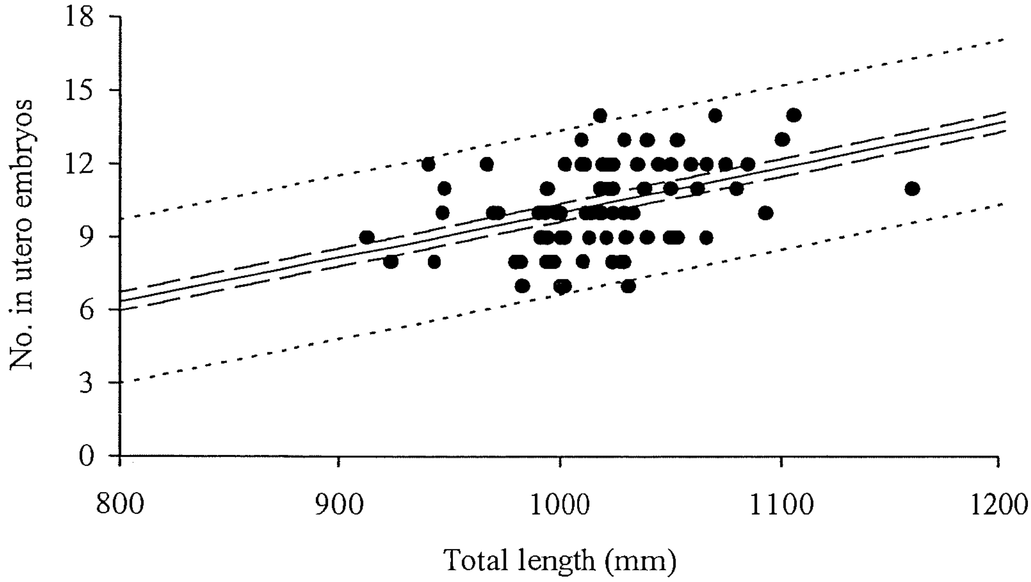


Fig. 8a. Number of *in utero* embryos against maternal total length

Mean number of embryos (——), 95% confidence limits (– – –), 95% prediction intervals (- - - -), and raw data (•) are plotted against maternal total length of pregnant females with macroscopically visible embryos (U=5). Values of parameters and statistical quantitites for the equation p=a'+b'l are given in the following tabulation:

a' (±s.e.)	b' (±s.e.)	n	r^2	rmse	P
–8.36 (±4.63)	0.0184 (±0.0045)	62	0.160	1.678	***

where l is maternal total length measured in millimetres, p is number of *in utero* embryos, a' and b' are parameters, n is sample size, r^2 is square of regression correlation coefficient, rmse is root mean square error, and P is the probability of statistical significance (*P<0.05; **P<0.01; ***P<0.001) for linear regression.

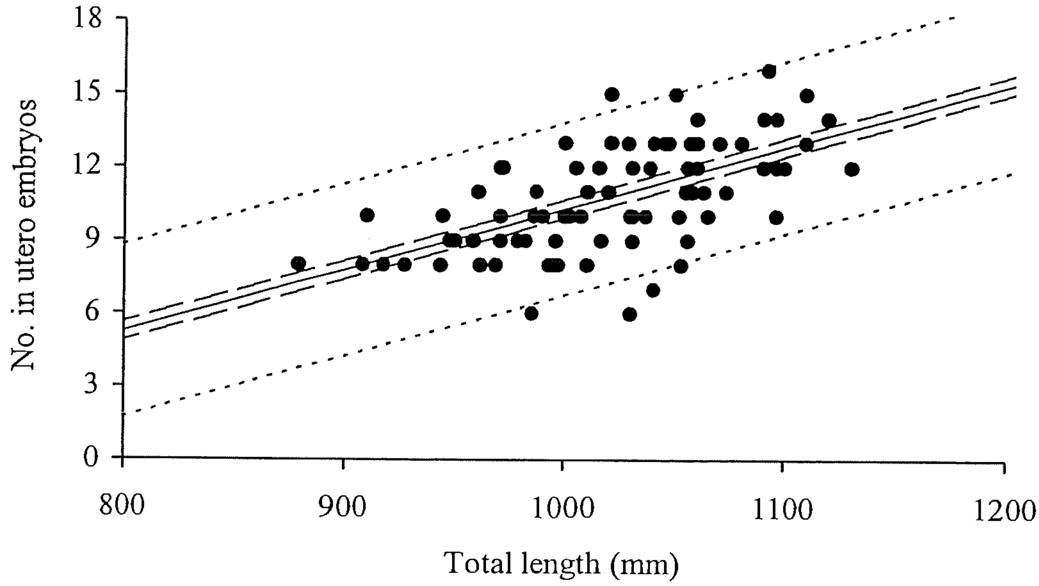


Fig. 8b. Number of *in utero* eggs against maternal total length

Mean number of embryos (——), 95% confidence limits (– – –), 95% prediction intervals (- - - -), and raw data (•) are plotted against maternal total length of pregnant females with macroscopically visible embryos (U=5). Values of parameters and statistical quantitites for the equation e=a'+b'l are given in the following tabulation:

a' (±s.e.)	b' (±s.e.)	n	r^2	rmse	P
−14.91 (±3.61)	0.0252 (±0.0035)	89	0.362	1.769	***

where l is maternal total length measured in millimetres, e is number of *in utero* embryos, a' and b' are parameters, n is sample size, r^2 is square of regression correlation coefficient, rmse is root mean square error, and P is the probability of statistical significance (*P<0.05; **P<0.01; ***P<0.001) for linear regression.

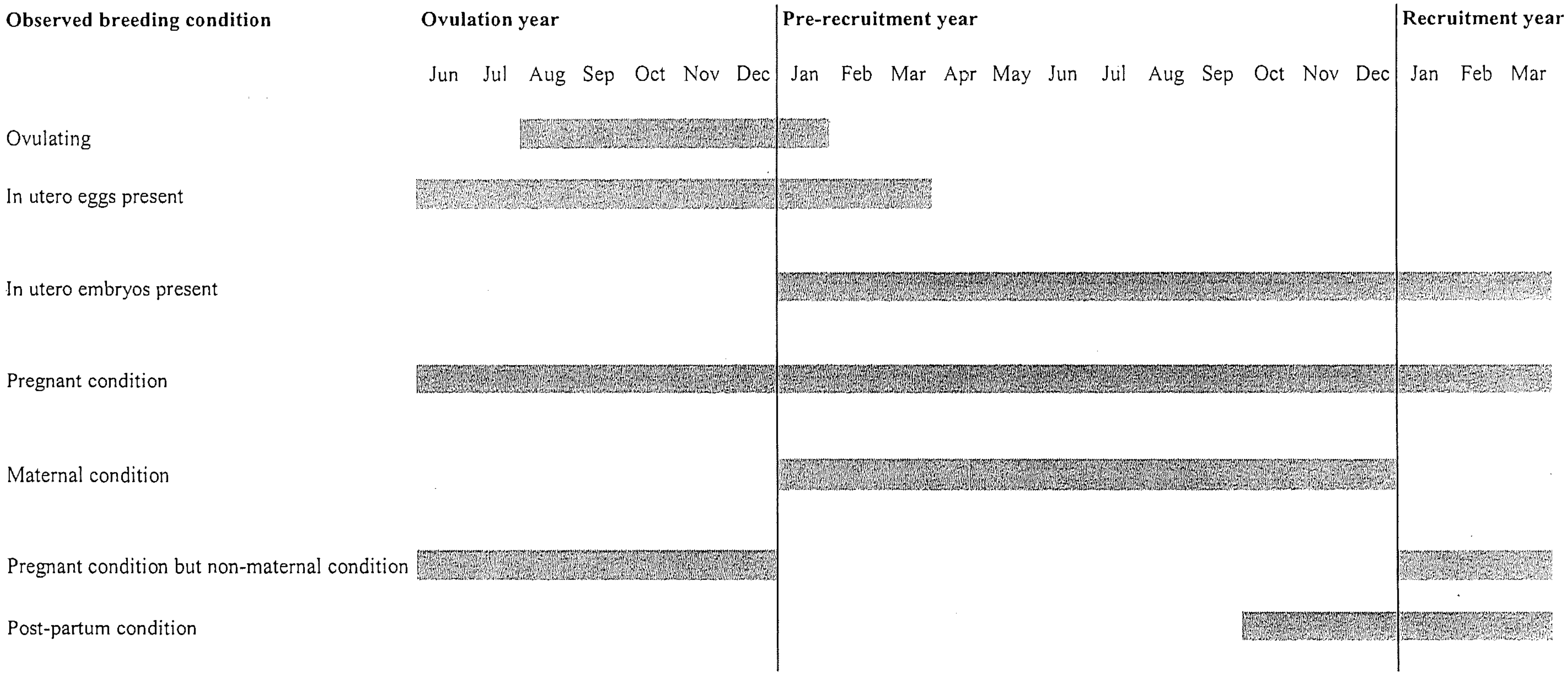


Fig. 9. Synchrony of breeding condition

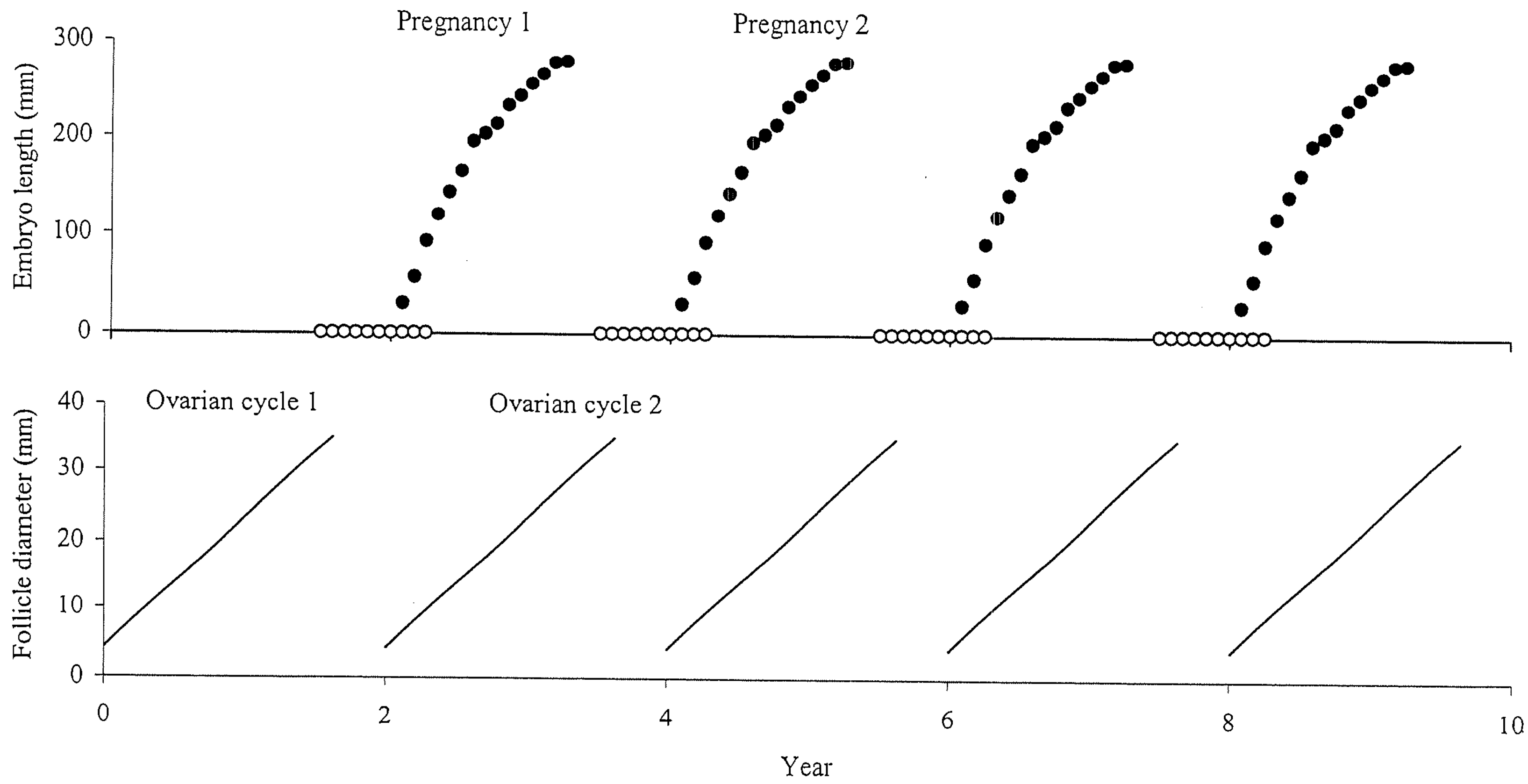


Fig. 10. Periodicity of the ovarian cycle and gestation for a mature sharks

The ovarian cycle and frequency of parturition are biennial; 3 years from the beginning of vitellogenesis for a particular follicle to full-term embryo; o, *in utero* egg; •, embryo.

Appendix 3e: Elephant fish reproduction

This appendix contains a manuscript in preparation, which presents the results of a study of the reproduction of elephant fish (*Callorhynchus milii*) required for fishery stock assessment.

Reproductive biology of elephant fish (*Callorhinchus milii*) harvested off southern Australia

Contents

Early draft manuscript in preparation for submission to a scientific journal for publication (22 July 2005)

Reproductive biology of elephant fish (*Callorhinchus milii*) harvested off southern Australia

Terence I. Walker[A,F], Justin X. Bell[A,C], Russell J. Hudson[A,B], Matthew B. Reardon[A,D], Rachel M. Smith[D], and William C. Hamlett[D,E]

[A]Primary Industries Research Victoria, PO Box 114, Queenscliff, Victoria 3225, Australia.
[B]Ocean Grove, Victoria 322X, Australia.
[C]School of Ecology & Environment, Deakin University, Warrnambool, Victoria 3280 Australia .
[D]Zoology Department, The University of Melbourne, Parkville, Victoria 3052, Australia,
[DE]Department of Anatomy and Cell Biology, South Bend Centre for Medical Education, Indiana University School of Medicine, Note Dame, Indiana 46556, USA.
[F]Corresponding author. Email Terry.Walker@dpi.vic.gov.au

Abstract

Mature female *Callorhinchus milii* mostly have synchronous 1-year ovarian and egg-laying cycles. Ovulation and egg laying occurs during the 4-month period 10 February–8 May and the eggs are laid in coastal and inshore regions on soft fine-grained substrates. Total lengths at 50% and 95% of females in maternal condition are 659 and 888 mm, respectively. Captive studies indicate that the mean annual number of eggs laid per mature female is 19.5 and gestation is 5–10 months. Maximum body mass of females (7.2 kg) is almost triple that of males (2.5 kg), but at any length, mass of females and males are remarkably similar.

Key words: Reproduction; Chimaera; Fishery; Australia

Introduction

The chondrichthyan fishes comprise elasmobranchs (sharks and rays) and holocephalans (chimaeras). The elephant fish (*Callorhinchus milii*) belongs to the holocephalan group, which is the much smaller of the two groups and is much less thoroughly studied than the elasmobranchs.

The elephant fish occurs in southern Australian (Last and Stevens 1994) and New Zealand (Francis 1998) waters and is one of only three described species world-wide in the chimaeroid family (*Callorhynchidae*). These are *Callorhinchus milii*, *C. capensis* (Freer and Griffiths 1993) and *C. callorhynchus* (Di Giácomo and Perier 1994) and are found only in the Southern Hemisphere.

In Australia, the elephant fish is distributed from Esperence in Western Australia to Sydney in New South Wales, including Tasmania at depths mostly <200 m (Last and Stevens 1994). It is uncertain whether the species forms a single stock or multiple stocks. Tag release-recapture data indicate considerable movement within Bass Strait, but not large-scale movements across southern Australia. There are distinct inshore egg-laying grounds and the species feeds mainly on bivalve molluscs (PIRVic, unpublished data).

The elephant fish is taken mainly as byproduct when targeting gummy shark (*Mustelus antarcticus*) in the Gillnet Hook and Trap Fishery (GHATF) of southern Australia. Elephant fish is only occasionally targeted. About 70% of the elephant fish catch from the GHATF is taken in Bass Strait by gillnets of 6-inch mesh-size in depths <90 m. Since 1974, the annual elephant fish catch from the GHATF has been mostly in the range 40–80 tonnes, but peaking at 118 tonnes during 1985. Elephant fish is also caught by demersal otter trawl and Danish seine off eastern Victoria and by demersal otter trawl off eastern South Australia. The quantities caught have not been as well monitored in the trawl fisheries as in the GHATF, but the reported catch was 15 tonnes during 2002 following implementation of quota management (Walker *et al.* 2003).

The elephant fish is oviparous and hence lecithotrophic (Hamlett *et al.* 2005); the young hatch from egg cases at about 100 mm fork length. Recent studies of the microanatomy of spermatophore formation and genital ducts of the male (Hamlett *et al.* 2002; Reardon *et al.* 2002) and the microstructure of the oviducal glands of the female (Smith *et al.* 2004) provided an important grounding for the present study.

The quantitative approach to investigating reproduction of school shark (*Galeorhinus galeus*) off southern Australia (Walker 2005) is followed in the present study. This approach is followed because it provides the basic reproductive parameters required for stock assessment of a chondrichthyan species. These parameters are also required for ecological risk assessment (Walker 2004) and for species assessment against IUCN criteria established under each of several risk of extinction categories (Hilton-Taylor 2000).

Materials and Methods

Collection of specimens

Sampling of *C. milii* was undertaken during four separate periods (1973–76, 1986–87, 1998–01, and 2003–04) mainly in Bass Strait (BS) and waters off South Australia (SA) (Figure 1). During 1973–76, the animals were caught using experimental gillnets of mesh-size ranging 2–9 inches (51–229 mm), in steps of 1 inch (25 mm), and hooks attached to sinking longlines. The animals were captured as a result of sampling at 162 fishing sites mainly in BS (126 sites), but also off eastern Tasmania south of latitude 41° South (20 sites) (grouped with BS samples) and SA (16 sites). During 1986–1987, the animals were caught in experimental gillnets of mesh-size ranging 5–8 inches (127–203 mm), in steps of 1 inch. The animals were captured at 144 fishing sites (60 sites in BS and 84 sites in SA). During 1998–01, most animals were caught at 153 fishing sites (91 sites in BS and 62 sites in SA). All animals were caught by gillnets of 6-inch (152 mm) or 6½-inch (165 mm) mesh-size on board commercial fishing vessels. During 2003–04, mostly mature females were collected from Westernport and Port Phillip Bay in Victoria. Most of these animals were caught by gillnets of ~2½-inch (60 mm), haul seines of 30–59 mm, or rod and line.

Biological sampling

Specimens of *C. milii* were dissected to investigate their reproductive biology; most of the work was undertaken at sea during 1973–76 and 1986–87, but in the laboratory during 1998–01 and 2003–04. The animals were measured to the nearest millimetre as total length (TL) from the tip of the proboscis to the tip of the tail; the tail of each animal was first allowed to take a natural position and the upper caudal lobe placed parallel to the body axis. Sex, TL, fullness of the stomach, and several reproductive indices were recorded for each animal. Also recorded, when the sea conditions permitted, were the mass of total body, liver, left ovary of females, and left testis of males.

For females, at-sea macroscopic inspection of the condition of the paired uteri, oviducal glands, and ovaries was undertaken to investigate breeding condition, litter size, and period of gestation. Also recorded were the diameters of the three largest follicles and the presence of *corpora atrecia* or *corpora lutea* in the ovary and the presence or absence of an *in utero* egg in each uterus. In addition, the uterus (left or right) and mass of each *in utero* egg were recorded for many of the animals carrying eggs. Indices were adopted for recording the condition of the ovary, oviducal gland, and uteri from rapid visual inspection. Ovary index (O) was based on size and colour of the follicles (O=1–4). Oviducal gland index (G) was based on shape and size of the gland (G=1–3). Uterus index (U) was based on appearance, size and presence of eggs *in utero* (U=1–6) (Table 1).

For males, at-sea macroscopic inspection of condition of the testes, seminal vesicles, and claspers was undertaken to investigate maturity by adopting three indices of breeding condition. Testis index (T) was based on shape, size, and relative predominance of testis tissue to epigonal gland tissue (T=1–3). Seminal vesicle index (V) was based on appearance, thickness of the wall, and presence or absence of seminal fluid (V=1–3). The length of left clasper was measured from the basipterygium to the distal end and clasper index (C) was based on appearance and rigidity (C=1–3) (Table 1).

Statistical methods

Total body mass at TL

The relationship between total body mass, w, and TL, l, was determined using the power curve

$$w = acl^b\,,$$

adopted commonly for sharks (Olsen 1954) and bony fishes (Ricker 1958) without the constant c, where a and b are parameters determined by linear regression of $\ln(w)$ against $\ln(l)$, and c is a factor for correcting for biases caused by natural logarithmic transformation (Beauchamp and Olson 1973).

Ovarian cycle

The ovarian cycle was investigated by examining the ovary and measuring the diameters of the three largest follicles in animals caught throughout the year. The diameters of the largest follicles vary widely between individual animals and vary depending on uterus condition, so seasonal patterns of follicle growth for each of the six general uterus conditions defined in Table 1 were examined separately.

In viviparous species, pregnant females with macroscopically visible *in utero* embryos (U=5 animals) provide the least ambiguous basis for determining seasonal growth rates of follicles and for distinguishing between annual, biennial, and longer ovarian cycles. However, elephant fish is an oviparous species and lacks the U=5 stage.

None of the other five uterus conditions provided such clear information on annual rate of follicle growth. The data indicate that females with uterus conditions U=1 exhibit little or no change in follicle diameter over the 12-month period from January to December. Females with uterus conditions U=4 occurred for only several months of the year, and therefore provide no information on annual growth of follicles. These animals, however, do provide information on the timing of ovulation and on follicle diameter at the time of ovulation. Similarly, females with uterus conditions U=6 were few in number, displayed little variation in size, and therefore provided no information on annual growth rate of follicles. Nevertheless, these animals did provide information on follicle diameter at the time of egg laying and during the period immediately following egg laying. That the uterus condition U=6 was not commonly observed at other times of the year suggests that after egg laying the distended uterus contracts to resemble uterus condition U=3. This implies that animals recorded with uterus condition U=3 might be a mixture of animals approaching first egg-laying (all U=3) and animals between egg-laying periods (U=6 changing to resemble U=3). Unlike animals with uterus conditions U=4 and U=6, which can be related to the timing of ovulation and parturition, animals with uterus conditions U=2 and U=3 cannot be so reliably related to either of these events. Elephant fish have only one, two or several large follicles in each ovary at any time during the egg-laying season and can grow these rapidly enough to ovulate about 10 oocytes from each ovary annually.

Size at maturity and size at maternity

The proportion of the population of animals mature at any TL can be determined by classing each animal as in mature condition or immature condition and applying logistic regression for females (Mollet *et al.* 2000; Conrath and Musick 2002) and males (Walker 2005) separately. Similarly, for females, the proportion of the population of animals in maternal condition at any TL can be determined by classing each animal as in maternal condition or non-maternal condition and applying logistic regression.

For *Callorhinchus milii,* a female was classed as in mature condition if the largest ovarian follicle was >3 mm in diameter (size at first yolking); otherwise it was classed as in immature condition. Given uncertainty of the best indicator of maturity of males, the results from methods based on alternative criteria for assuming the mature condition and the immature condition are compared. Males were classed by testis condition as mature if T=3 and immature if T=1 or T=2. Similarly, they were classed by seminal vesicle condition as mature if V=2 or V=3 and immature if V=1 and by clasper condition as mature if C=2 or C=3 and immature if C=1 (Table 1).

A female was classed in maternal condition at the time of dissection, if, had it survived, it would have laid eggs before the following mid-May. To implement this criterion, females were classed as in maternal condition if the largest follicle diameter > 10 mm.

Logistic regression was adopted to determine the proportion of females in mature condition, the proportion of males in mature condition, and the proportion of females in maternal condition as a function of TL. Females or males in mature condition were assigned a maturity condition value of 1, whereas those in immature condition were assigned a maturity condition value of 0. Similarly, females in maternal condition were assigned a maternal condition value of 1, whereas females in non-maternal condition were assigned a maternal condition value of 0.

The logistic equation adopted to express proportion of animals in mature or maternal condition , P_l , as a function of l is given by

$$P = \frac{c}{(1+e^{-(a+bl)})}$$

where a, b, and c are parameters, but to provide parameters that are more biologically meaningful, the equation is reformulated to express P as a function of l by

$$P = P_{\max}\left(1+e^{-\ln(19)\left(\frac{l-l_{50}}{l_{95}-l_{50}}\right)}\right)^{-1},$$

where $P_{\max}$ is the maximum proportion of animals in mature condition or maternal condition, and l_{50} and l_{95} are the lengths at which 50% and 95% of the maximum proportion of animals in mature or maternal condition (Walker 2005).

The parameters $P_{\max}$, l_{50} and l_{95}, with 95% confidence intervals, were estimated by the method of maximum likelihood using the probit procedure (Proc Probit) of the computer statistical package SAS (SAS Institute, Cary, North Carolina, USA). This applies a modified Newton–Raphson algorithm for estimation.

The standard error for any length, l, is given by

$$se_l = P_l(1-P_l)/N.$$

The SAS probit procedure sets $1-P_{\max}=0.000$. This is appropriate for the maturity ogive where all large-sized animals in the population are in mature condition, and hence the proportion of large-sized animals in the population mature is 1.000. As an oviparous species, this is also appropriate for the maternity ogive where all of the large-sized animals in the population are in maternal condition, and hence the proportion of large-sized animals in the population in maternal condition is 1.000. Parturition frequency is annual.

Egg laying, gestation and litter size

The period of gestation was determined by recording the timing of egg laying and hatching of embryos held in captivity in 2–6-m diameter tanks

at the PIRVic Queenscliff Centre with flow-through water from Port Phillip Bay and in the Melbourne Aquarium. Determining sex ratio at birth requires large-scale captive holding operations or field sampling, which was beyond the scope of the present study.

Results and Discussion

A total of 724 male and 1467 female *Callorhinchus milii* were collected and sampled for dissection during the four sampling periods 1973–76, 1986–87, 1992–01, and 2003–04. The longest female *C. milii* captured during field operations (1024 mm TL) was longer than the longest male captured (877 mm). The highest total body mass of a female recorded (7.2 kg) was almost triple the highest mass of a male recorded (2.5 kg).

Total body mass at TL

Statistical comparison of the slopes and intercepts by the Student *t*-test for the straight line ln(*w*)–ln(*l*) relationships determined from linear regression fits indicated that the relationship for females and the relationship for males were highly significantly different (t-test, t=4.681, d.f.=716, and P>0.001 for comparison of slopes and t=4.628, d.f.=716, and P>0.001 for comparison of elevations). The relationships of total body mass against TL, with 95% confidence limits on the mean curves and 95% prediction intervals are presented separately for females (Figure 2a) and males (Figure 2b). The curves for these relationships coincide very closely (Figure 2c).

Ovarian cycle

The diameter of the largest follicle recorded in the ovary from each of 737 females ranged 1–45 mm. There was marked difference between the diameters of the three largest follicles (Table 2), so all statistical analyses of the follicle data were undertaken using only the first measured follicle, which was judged visually to be the largest when measured.

Patterns in plots of the largest follicle diameter (LFD) against Julian day were not clear where all the data were pooled; patterns of variation in LFD became more evident when the animals were considered for each uterus condition separately. LFD was mostly <10 mm for animals with uterus conditions U=1 (n=39, mean 3.8 mm, s.d. 0.7 mm, range 1–23 mm), a clear indication that all these animals were either immature or at the early stages of maturation. LFD varied widely for animals in uterus conditions U=2 (n=236, range 2–45 mm), U=3 (n=275, 4–43 mm), U=4 (n=86, 1–39 mm), and U=6 (n=81, 6–40 mm) (Figure 3).

The distribution of LFD with Julian day does not vary noticeably with uterus condition as found for *Galeorhinus galeus* (Walker 2005), *Pristiophorus cirratus* (Appendix 3d), and *P. nudipinnis* (Appendix 3e). There is a general annual pattern of the follicles being largest during the first half of the year and smallest during the second half of the year. Uterus condition U=4 indicates the presence of eggs *in utero* during the 4-month period 10 February–8 May, which indicates the egg-laying season. Towards the end of this period the ovaries had increasing numbers of progressively smaller atretic follicles. Overall the patterns in the plots were consistent with the hypothesis that the ovarian cycle is annual.

Size at maturity and size at maternity

Of the 785 female *C. milii* examined for maturity, 90% were mature and there were insufficient data to adequately test for the effects of region and sampling period. Hence, all available data were pooled over the three regions EBS, WBS, and SA and four periods 1973–76, 1986–87, 1998–01, and 2003–04.

Total length at which 50% and 95% of the animals were in mature (or maternal) condition, with 95% confidence limits (CI), derived from the ogives presented in Figure 4 are tabulated as follows.

Condition	L_{50} (CI)	L_{95} (CI)	P_{max}	n	N
Maturity	607 (585, 624)	731 (717, 746)	1.00	753	834
Maternity	659 (636, 678)	888 (871, 910)	1.00	635	836

The results for male maturity will be presented in a later version of this manuscript.

Egg laying, gestation and litter size

Four elephant fish were held captive during 12 February–16 April and seven animals during 17 April–7 May. During the entire period 12 February–7 May, 92 eggs were laid for an average holding of 4.7 egg-laying females, giving an average of 19.5 eggs per elephant fish (Bell 2003).

Elephant fish move inshore to egg-laying sites such as the soft-sediment substrates in the south-eastern region of Western Port, in the Geelong Arm of Port Phillip Bay, Swan Bay, and at river mouths. When first laid the eggs are soft, flexible and light brown, which harden and blacken with time (Bell 2003). Depending on conditions the incubation period from egg laying to hatching in captivity varies from 5 months (Justin D. Bell, unpublished data) to 10 months (Gorman 1963).

Acknowledgments

Acknowledgment is due to a large number of former and present staff of the Primary Industries Reseach Victoria Queenscliff Centre who participated in field sampling the elephant fish and laboratory processing. Special acknowledgment is due to Anne Gason for advice on statistical analysis of the data and application of the statistical package SAS. The skippers and crews of various shark fishing vessels who kindly provided accommodation, assistance, and hospitality on-board their fishing vessels are also thanked. The work was funded by the Fisheries Research and Development Corporation (FRDC) of Australia as part of FRDC Project 1999/103. Some of the field data were collected opportunistically as part of earlier projects.

References

Beauchamp, J. J., and Olson, J. S. (1973). Corrections for bias in regression estimates after logarithmic transformation. *Ecology* **54**, 1403–1407.

Bell, J. D. (2003). Fisheries and reproductive biology of the elephant fish, *Callorhinchus milii*, in south-eastern Australia. B.Sc. (Hons) thesis, Deakin University.

Conrath, C. L., and Musick, J. A. (2002). Reproductive biology of the smooth dogfish, *Mustelus canis*, in the northwest Atlantic Ocean. *Environment Biology of Fishes* **64**, 367–377.

Di Giácomo, E. E., and Perier, M. R. (1994). Reproductive biology of the cockfish, *Callorhynchus callorhynchus* (Holocephali: Callorhychidae), in Patagonian waters (Argentina). *Fishery Bulletin* **92**, 531–539.

Francis, M. P. (1998). New Zealand shark fisheries: development, size and management. *Marine and Freshwater Research* **49**, 579–591.

Freer, D. W. L., and Griffiths, C. L. (1993). The fishery for, and general biology of, the St Joseph *Callorhinchus capensis* (Dumeril) of the south-western cape, South Africa. *South African Journal of Marine Science* **13**, 63–74.

Gorman, T. B. S. (1963). Biological and economic aspects of the elephant fish *Callorhinchus milii* in Pegasus Bay and Canterbury Bight. New Zealand Marine Department Fisheries Technical Report No. 8, 54 pp. Wellington, New Zealand).

Hamlett, W. C., Kormanik, G., Storrie, M. T., Stevens, B., and Walker, T. I. (2005). Chapter 13. Chondrichthyan parity, lecithotrophy and matrotrophy. In 'Reproductive Biology and Phylogeny of Chondrichthyes: Sharks, Rays and Chimaeras'. (Ed. Hamlett, W. C.) pp. 395–435. (Science Publishers, Inc.: Enfield, New Hampshire 03478, USA).

Hamlett, W. C., Reardon, M., Clark, J., and Walker, T. I. (2002). Ultrastructure of sperm storage and male genital ducts in a male holocephalan, the elephant fish, *Callorhynchus milii*. *Journal of Experimental Zoology* **292**, 111–128.

Hilton-Taylor, C. (2000). 2000 IUCN Red List of Threatened Species. (IUCN The World Conservation Union: Gland, Switzerland).

Last, P. R., and Stevens, J. D. (1994). Sharks and rays of Australia. (CSIRO Australia: Melbourne).

Mollet, H. F., Cliff, G., Pratt Jnr, H. L., and Stevens, J. D. (2000). Reproductive biology of the female shortfin mako, *Isurus oxyrinchus* Rafinesque, 1810, with comments on the embryonic development of lamnoids. *Fishery Bulletin* **98**, 299–318.

Olsen, A. M. (1954). The biology, migration, and growth rate of the school shark, *Galeorhinus australis* (Macleay) (Carcharhinidae) in south-eastern Australian waters. *Australian Journal of Marine and Freshwater Research* **5**, 353–410.

Reardon, M. B., Walker, T. I., and Hamlett, W. C. (2002). Microanatomy of spermatophore formation and male genital ducts in the holocephalan, *Callorhynchus milii*. In

'Proceedings of Sixth Indo-Pacific Fish Conference'. Special Issue of. *Marine and Freshwater Research* **53**, 591–600.

Ricker, W. E. (1958). Handbook of computations for biological statistics of fish populations. *Bulletin of Fisheries Research Board of Canada* **119**, 1–191.

Smith, R. M., Walker, T. I., and Hamlett, W. C. (2004). Microscopic organisation of the oviducal gland of the holocephalan elephant fish, *Callorhynchus milii*. *Marine and Freshwater Research* **55**, 155–164.

Walker, T. I. (2004). Chapter 13. Management measures. In 'Technical manual for the management of elasmobranchs'. (Eds Musick, J. A. and Bonfil, R.) pp. 285–321. (Asia Pacific Economic Cooperation, Secretariat: Singapore).

Walker, T. I. (2005). 4 Reproduction in fisheries science. In 'Reproductive Biology and Phylogeny of Chondrichthyes: Sharks, Rays and Chimaeras". (Ed. Hamlett, W. C.) pp. 81–127. (Science Publishers, Inc.: Enfield, New Hampshire 03478, USA).

Walker, T. I., Taylor, B. L., and Gason, A. S. (2003). Southern shark catch and effort 1970–2002 report to Australian Fisheries Management Authority. 41. (June 2003). (Marine and Freshwater Resources Institute, Queenscliff, Victoria, Australia.).

Table 1. Indices adopted for staging reproductive condition

Various assumption made on maturity when analysing the data are also listed.

Organ or tissue	Index	Description	Maturity assumption
Female			
Ovary	O=1	Largest follicles white and of diameter <2 mm	Immature
	O=2	Largest oocytes yolking and of diameter 2–3 mm	Immature
	O=3	Largest oocytes with yellowish yolk and of diameter >3 mm	Mature
	O=4	Yolked oocytes of diameter >3 mm and extensive corpora atretica present	Mature
Oviducal gland	G=1	Indistinct from anterior oviduct	Immature
	G=2	Distinct but only partly formed (Hamlett et al 1998)	Immature
	G=3	Enlarged with ram horn-shaped lobes	Mature
Uterus	U=1	Uniformly thin tubular structure	Immature
	U=2	Thin tubular structure partly enlarged posteriorly	
	U=3	Uniformly enlarged tubular structure	
	U=4	In utero eggs present without macroscopically visible embryos present	Mature
	U=5	In utero embryos macroscopically visible	Mature
	U=6	Enlarged tubular structure distended	Mature
Male			
Testis	T=1	Thin tissue strip with epigonal gland predominant	Immature
	T=2	Thickened strip with epigonal gland tissue extensive	Immature
	T=3	Enlarged and predominant with epigonal gland tissue negligible	Mature
Seminal vescicle	V=1	Thin translucent walls and seminal fluids absent	Immature
	V=2	Thickened opaque walls and seminal fluids present	Mature
	V=3	Thickened opaque walls and seminal fluids absent	Mature
Clasper[A]	C=1	Pliable with no calcification	Immature
	C=2	Partly calcified	Immature
	C=3	Rigid and fully calcified	Mature

[A]Adopted for periods 1986–87 and 1992–01, but not for period 1973–76.

Table 2. Comparison of diameters of three largest ovarian follicles by uterus condition

n, sample size; s.e., standard error; na, not applicable.

Uterus condition[A]	n	Mean diameter (±s.e.) of ovarian follicle (mm)		
		Oocyte 1	Oocyte 2	Oocyte 3
U=1	39	3.82±0.73	3.51±0.73	2.90±0.52
U=2	236	16.00±0.57	14.23±0.53	12.81±0.48
U=3	273	26.50±0.51	24.36±0.52	22.33±0.49
U=4	84	25.14±1.11	22.63±1.07	20.95±1.06
U=5	na	na	na	na
U=6	81	28.98±0.79	27.10±0.77	25.49±0.76
Total	713	21.90±0.40	19.97±0.39	18.31±0.37

[A]Defined in Table 1.

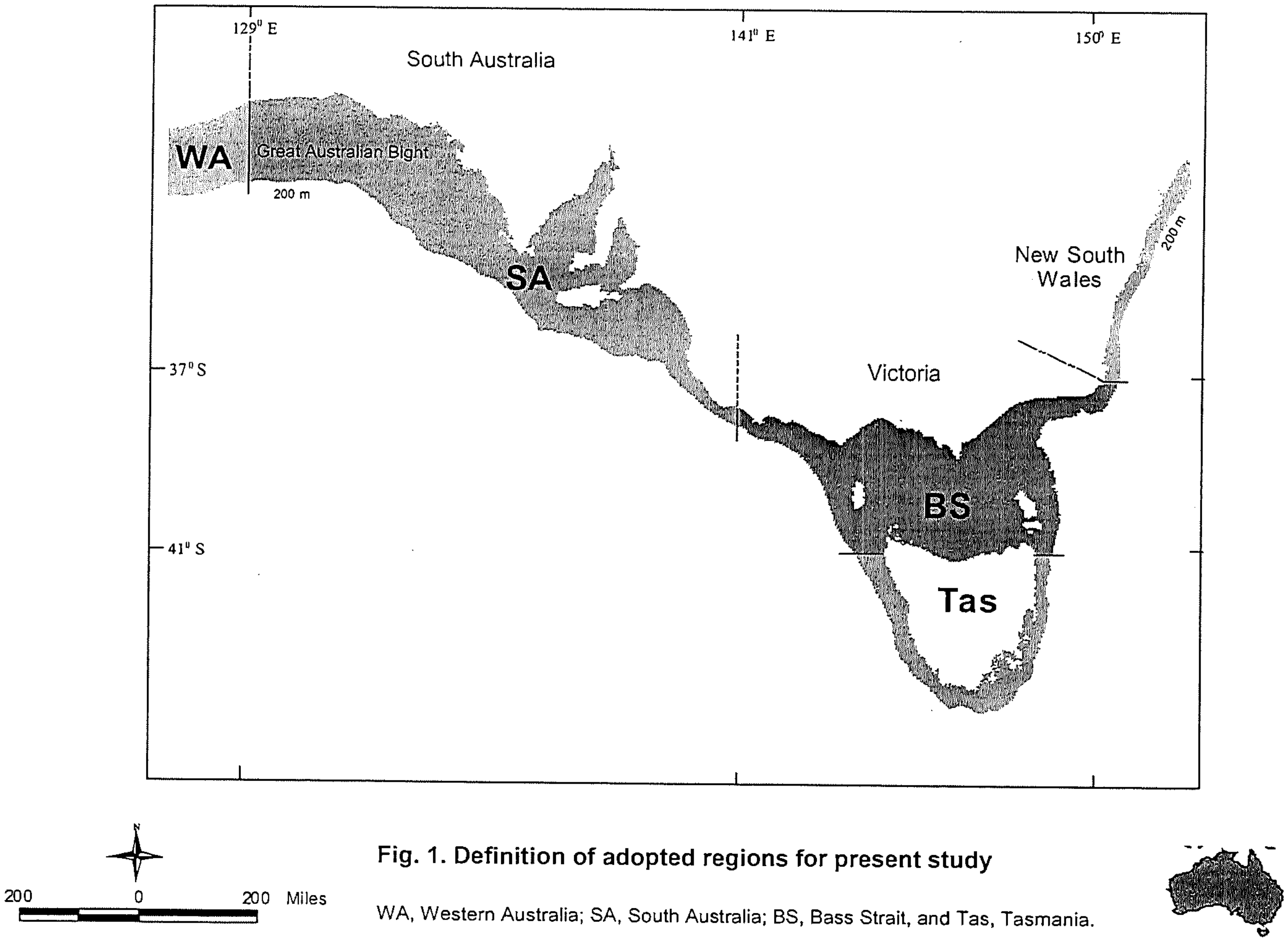


Fig. 1. Definition of adopted regions for present study

WA, Western Australia; SA, South Australia; BS, Bass Strait, and Tas, Tasmania.

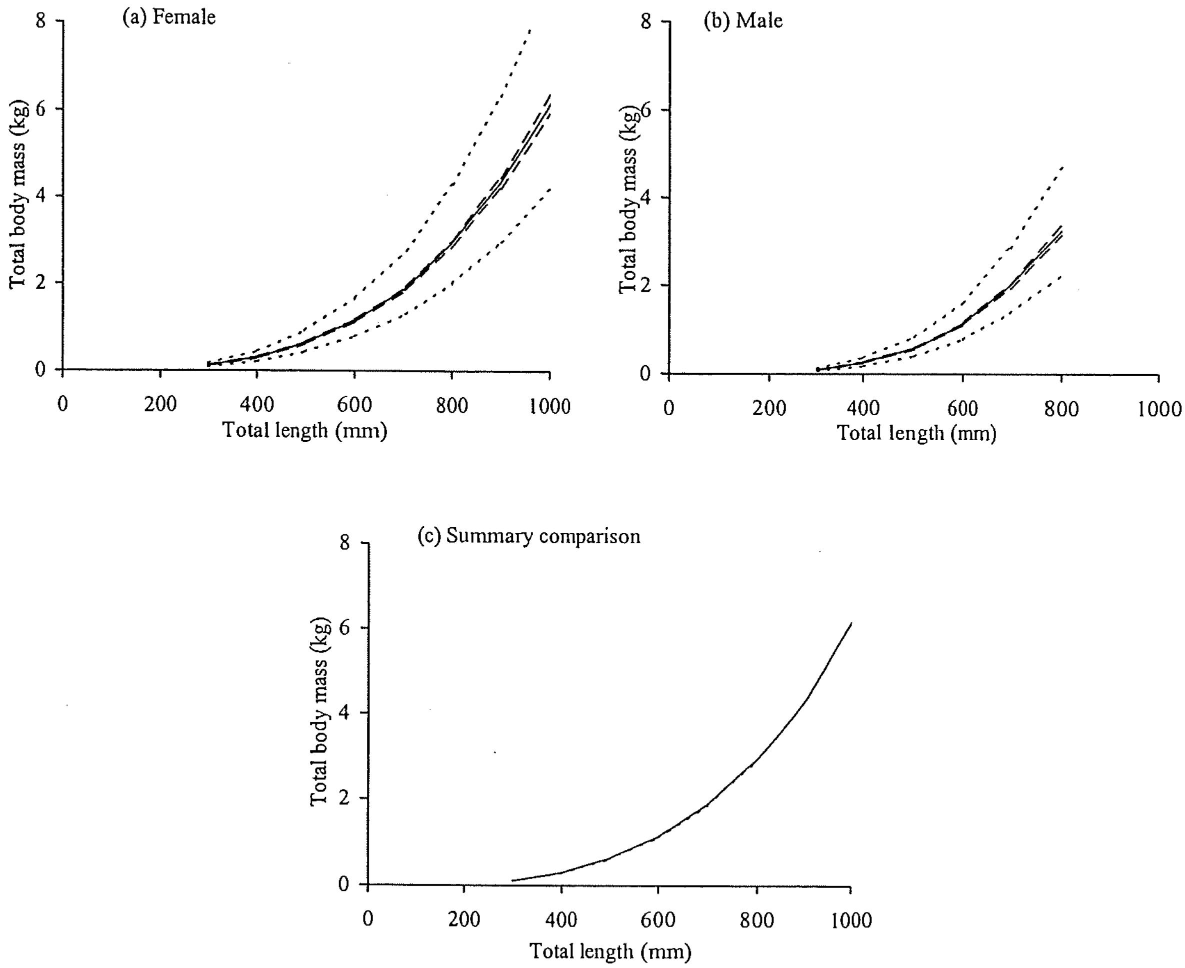


Fig. 2. Relationships between total body mass and total length of elephant fish.

Plots of mean total mass against TL (——), with 95% confidence limits (– – – –) and 95% prediction intervals (- - - - -), for females (a) and males (b), and comparison of the mean curves for females (——) and males (- - - - -) (c) in southern Australia during the periods 1973–76, 1986–87, and 1998–01 combined. Values for parameters and statistical quantities from linear regression analysis to derive the equation $w=acl^b$ are given in the following tablulation:

Shark category	a (s.e. range) x 10^{-9}	b(se)	c	n	r^2	rmse	P
Females	0.754 (0.565–1.010)	3.301 (0.043)	1.019	447	0.926	0.237	***
Males	0.063 (0.044–0.089)	3.688 (0.054)	1.018	271	0.936	0.176	***

where w is total body mass, l is total length, a and b are parameters, c is the Beauchamp and Olson (1973) correction factor, n is sample size, r^2 is square of correlation coefficient, and rmse is root mean square error for this regression (*P<0.1; **P<0.01; ***P<0.001) for the regression equation ln(w)=a+b ln(l).

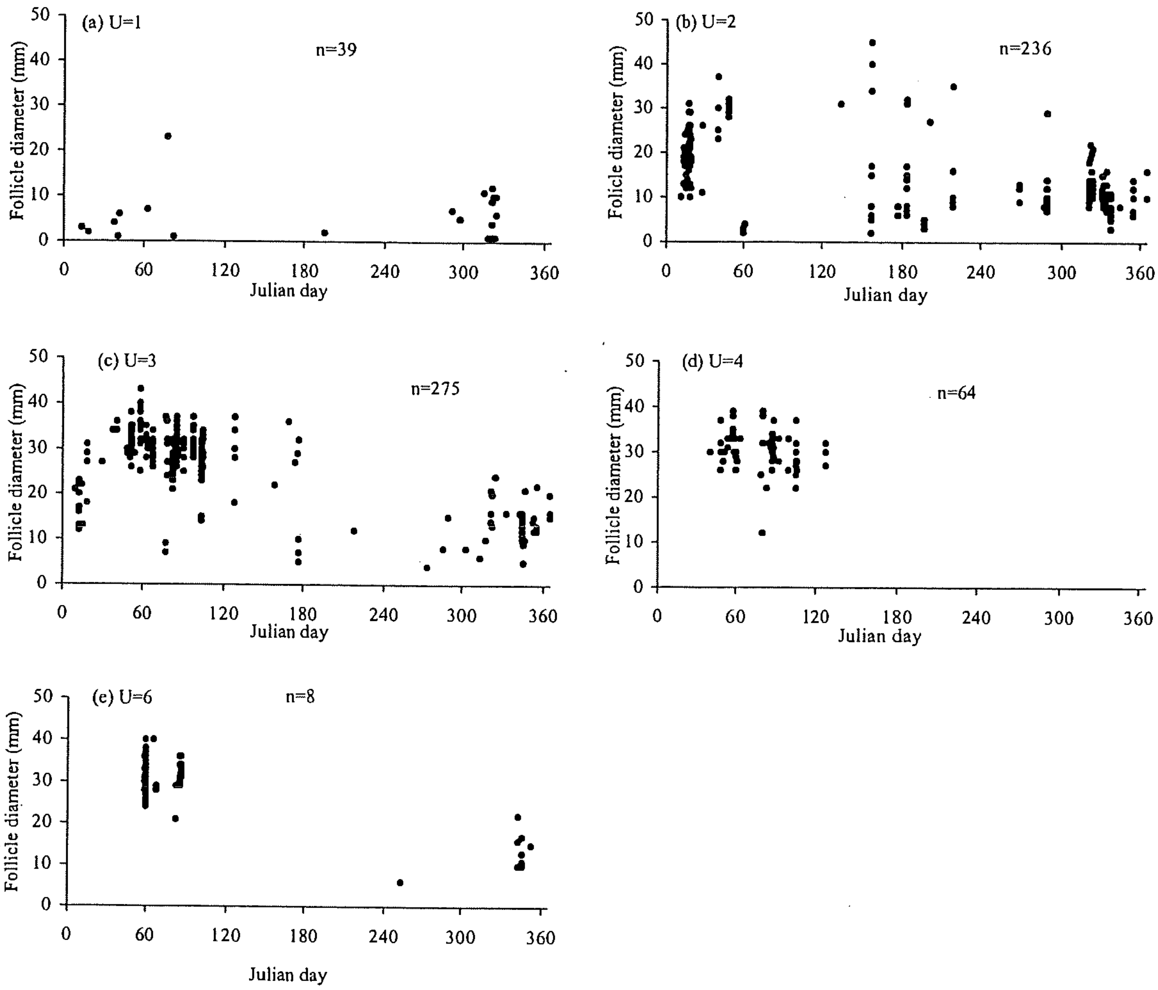


Fig. 3. Ovarian follicle diameter against Julian day by uterus condition

Largest follicle diameter against Julian day for females from Bass Strait and South Australia during 1973–76, 1986–87, 1998–01 and 2003 for each of the of five uterus conditions: non-pregnant animals (U=1, 2, 3) (a, b, and c), pregnant animals with *in utero* eggs (U=4) (d), and postpartum females (U=6) (e).

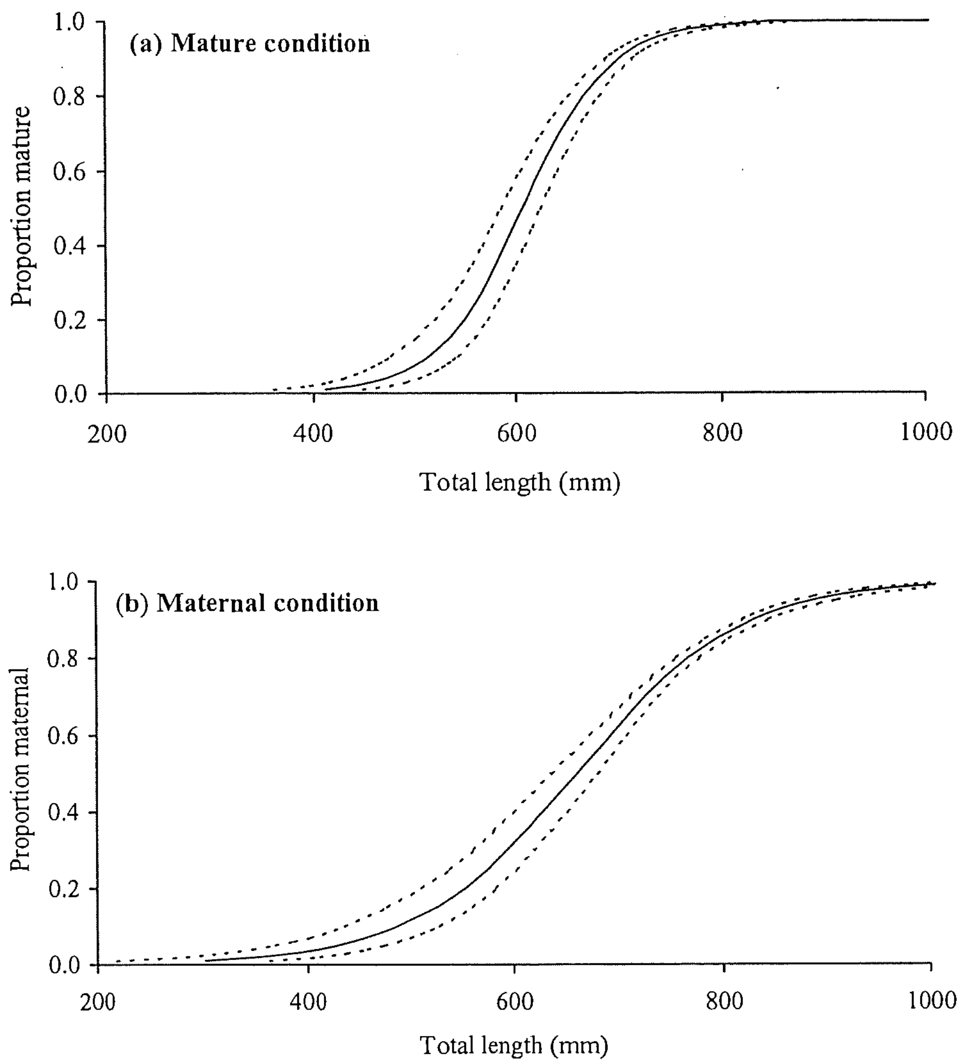


Fig. 4. Comparison of length-at-maternity and length-at-maturity ogives

Proportion of female population classed as in mature (or maternal) condition (——) with 95% confidence intervals (- - - -) against TL for all regions and periods pooled. Values of parameters and statistical quantities for the equation $P_l = P_{max(l)}(1+e^{-\ln(19)(l-l_{50}/l_{95}-l_{50})})^{-1}$ determined from probit analysis are given in the tabulation:

Condition	l_{50} (95% CI)	l_{95} (95% CI)	$P_{max(l)}$	n	N	P
Maturity	607 (585, 624)	731 (717, 746)	1.00	753	834	***
Maternity	659 (636, 678)	888 (871, 910)	1.00	635	836	***

where l is total length measured in millimetres, P_l is proportion of females classed as in mature or maternal condition at TL l, l_{50} and l_{95} are parameters, $P_{max(l)}$ is an asymptotic constant, n is the total number of females classed in mature (or maternal) condition, and N is the total number of females examined, and P is probability of statistical significance (*P<0.1; **P<0.01; ***P<0.001). A female was classed mature if LFD > 3 mm and classed in maternal condition if LFD > 10 mm.

Appendix 3f: Chondrichthyan reproduction

This appendix contains a chapter from a recently published book, which describes the methods required for determining information on reproduction of chondrichthyan species required for fishery stock assessment or ecological risk assessment. In this manuscript, the methods are demonstrated by applying them to school shark (*Galeorhinus galeus*) off southern Australia, using data collected opportunistically during the present project and during earlier projects.

CHAPTER 4

Reproduction in Fisheries Science

Terence I. Walker

4.1 INTRODUCTION

Assessment of populations of chondrichthyan species requires a quantitative approach to the study of reproduction. Measures of reproductive rate, together with mortality rate, are required for stock assessment of species harvested by fisheries and for ecological risk assessment of bycatch species (Walker 2004). Such measures are also required for species assessment against criteria established under each of several risk of extinction categories developed by the Species Survival Commission of the International Union for Conservation of Nature and Natural Resources (IUCN) (Hilton-Taylor 2000).

Population demography is based on simple models for constructing life tables from reproductive and mortality parameters or on age-based models using Leslie matrices. Stock assessment of harvested species for fishery management require more complex model and data requirements to account for density-dependent mechanisms for population regulation (Wood *et al.* 1979; Walker 1992), somatic growth and trends in abundance of the animals, and history of extracted catches by the fishery (Punt and Walker 1998). Some fishery assessment models also account for complex interactions between the animals and the fishing gear, movement of the animals, and stock structuring (Punt *et al.* 2000).

All these types of assessment, irrespective of model complexity, require the same specific parameters for representing three key components of reproduction. The first of these components is the sex ratio at birth, which can be determined by counting embryos or neonates of each sex. The second component is the relationship between the annual number of offspring and maternal age or size of the animals (litter size

Primary Industries Research Victoria, P.O. Box 114, Queenscliff, Victoria 3225, Australia

curve). This can be determined by ageing or measuring a sample of animals in maternal condition and counting the number of their offspring. The third is the relationship between the proportion of the female population contributing to annual recruitment and the age or size of animals (maternity ogive). The third is the most complex component to determine, as it inevitably requires information on the periodicity of each of the ovarian cycle and gestation before estimating values of the parameters of a maternity ogive.

For fisheries assessment, a fourth component is required if the management objectives for a fishery are expressed in terms of mature population number or mature biomass. The fourth requires the relationship between the proportion of the female population in mature condition at any time and the age or size of the animals (maturity ogive); an explicit definition of maturity is essential for this purpose. A maturity ogive might also be required for the males. Maturity ogives are otherwise not needed for determining the dynamics of a population. The female maturity ogive is often used as an approximation to the maternity ogive, if the maternity ogive is not available. However, the model outputs for any species, as demonstrated later in this chapter, is highly dependent on the adopted definition of maturity.

Each of the litter size curve, maternity ogive and maturity ogive are best determined as a function of age. However, they can be expressed as a function of size provided the relationship between size and age of the animals is known. For chondrichthyan species, this is usually required for each sex separately because of differences in their growth. For fisheries assessment, it is also an advantage to have the relationship between size and mass of the animals. Because this relationship can vary depending on the sex and reproductive condition of the animals, methods for determining these relationships are described. Although the relationships between size and age usually vary between females and males, their determination can be complex and are beyond the scope of this volume. The reader is referred to texts on age and growth for determining these relationships.

Chondrichthyan species are renowned for their wide range of reproductive strategies. They are usually categorised as oviparous species or viviparous species, where viviparous species are further categorised as placental and aplacental with several aplacental species exhibiting oophagy and one species exhibiting intra-uterine cannibalism. A simpler categorisation between lecithotrophic species and matrotrophic species has recently been proposed. This dichotomy is based on relative contributions to the mass gain of embryos during gestation either from the initial egg yolk or from nutrients provided by the mother via a placenta, trophonemata, sibling embryos, or continuous ovulation during pregnancy (see Chapter 13 of this volume).

The variation in reproductive mode and differences between species or between populations within species, in the period between successive birth events, or laying of egg clutches, prevents prescriptive procedures for determining maternity ogives. Whereas parturition and ovulation in many species are annual, in other species they are biennial, triennial or possibly longer, and while synchrony of gestation and of the ovarian cycle occur in the populations of many species, in other species they appear to be asynchronous. The periods of gestation and ovarian cycles are particularly difficult to determine for species inhabiting the deep cold waters of the continental slopes and abyssal plains of the world because their duration are of several years. They may also be asynchronous. Determining sex ratio of embryos or period of gestation in oviparous species requires observing eggs in the wild or in captivity.

The methods described in this chapter are demonstrated by presenting the results from analyses of reproductive data available from southern Australia for *Galeorhinus galeus*, a species known to form six separate populations in widely separated regions of the world. This species is selected because of the long period of the ovarian cycle and complexity associated with sampling to determine its maternity and maturity ogives.

Before describing the methods and results for *G. galeus*, a brief description of relevant components of its life history is presented. Also, the three terms mature condition, pregnant condition and maternal condition central to population dynamics are explicitly defined. Brief descriptions of the structure and function of the typical reproductive systems of female and male chondrichthyan animals are presented, adopting preferred terminology, to simplify description of the methods.

Methods for estimating the sex ratio at birth and the parameters of the litter size curve are explained first. Methods are then described for determining the ovarian and gestation periods, which are required to derive the frequency of parturition or egg laying. Next, methods for estimating the parameters of maturity ogives of females and males separately and maternity ogives are described. Finally, methods are described for estimating mass-size relationships for different breeding conditions.

4.2 TERMINOLOGY AND DESCRIPTION OF REPRODUCTIVE SYSTEM

4.2.1 Female Reproductive System

Chondrichthyan fishes typically have paired or single ovaries and paired oviducts. Each oviduct is differentiated into a funnel-shaped ostium, anterior oviduct, oviducal gland with, in some species, an isthmus leading to the uterus, cervix and the urogenital sinus, which is common to both reproductive tracts.

The chondrichthyan ovary is attached to the dorsal wall of the body cavity by the mesovarium mesentery and has three main functions: generate germ cells, accumulate yolk and synthesise and secrete hormones. In mature animals, the ovary consists of small follicles, developing follicles of various sizes, preovulatory follicles undergoing atresia, and corpora lutea, which are all embedded together in a dense stroma of connective tissue. A follicle consists of an oocyte surrounded by granulosa cells and delimited by the basal lamina, the size of which for many depends on the stage of the reproductive cycle. The structure has an oocyte plasmalemma, zona pellicida, follicular epithelium and connective tissue theca. The follicles are small with little or no yolk in juveniles (Hamlett and Koob 1999) but enlarge initially through folliculogenesis as the follicular cells begin to mature. With further development, follicles enlarge as the oocytes accumulate yolk through the process of vitellogenesis, whereby phosvitin and lipovitellin of hepatic origin are deposited in the oocyte (Storrie 2004). Ovulation of the largest oocytes occurs when they attain a particular size. Atretic follicles form by degeneration of preovulatory follicles and resorption of yolk from the oocytes. Atresia of a follicle can occur at any size to form a corpus atretica. Following ovulation, the follicle wall collapses and forms the corpus luteum, consisting of a lipid-filled cell derived from the granulosa cells (Hamlett and Koob 1999).

Following ovulation, ova (ovulated oocytes), moved by cilia in the peritoneal cavity to a single ostium (or paired ostia) that bifurcates into the left and right oviducts. Ova move through the oviducts to the oviducal gland where they are fertilized and encapsulated with egg jelly (Hamlett and Koob 1999). In *Galeorhinus galeus*, initially spherical ova are compressed to an ellipsoid shape by the time they are encapsulated in a brownish-yellow, transparent, flexible egg-case membrane, the free ends of which are spirally twisted, and deposited in the uterus (personal observation).

The uterine walls are thick, spongy and vascular during early gestation but as gestation advances, they become thin, semi-transparent, and further vascularized. As described for *Mustelus manazo* (Teshima and Koga 1973), the external yolk sac of *Galeorhinus galeus* is large during the early embryonic stages but, as the embryo grows, the external yolk sac becomes progressively smaller as the yolk is consumed. As parturition approaches, the contracted yolk-sac and the short stalk by which it is attached appear to be incorporated into the body of the embryo when the yolk is completely absorbed. The egg case membrane remains intact, and unfolds and stretches to accommodate the developing embryo and increasing amounts of enclosed clear fluid (personal observation).

4.2.2 Male Reproductive System

Chondrichthyan fishes typically have external paired claspers (mostly ventrally grooved copulatory appendages), which are extensions of the posterior bases of the pelvic fins. These are calcified and articulate at their bases in mature animals. The internal organs include the testes, genital ducts, Leydig gland and the alkaline gland. The paired genital ducts cover the elongate kidneys embedded in the dorsal abdominal wall, consist of the efferent ductules, epididymis, ductus deferens and seminal vesicle, and are covered by the peritoneum. There are two equally developed elongate testes, each embedded in the anterior portion of the long irregular epigonal gland, which is a lymphomyeloid organ supporting the testis.

The testis has two functions: spermatogenesis (germ cell generation) and steroidogenesis (secretion of steroid hormones). It is packed with spherical spermatocysts consisting of many spermatoblasts (each with a single Sertoli cell and its synchronously developing isogenetic clone germ cells) bounded by a basement membrane. In a particular spermatocyst, all germ cells pass through spermatogenesis as synchronously developing clone cells. Spermatocyst development commences with a single germ cell and a single Sertoli cell and terminates at sperm release when the Sertoli cells fragment to release the spermatozoa and Sertoli cell remnants into the lumen of the efferent ductules. Initially, spermatogonia undergo repeated mitotic divisions to produce 16 germ cells per Sertoli cell. Subsequent meiosis results in 64 spermatozoa per Sertoli cell (Hamlett 1999). Viewed in a transverse histological section, *Galeorhinus galeus* testes are diametric where the germinal zone consists of a strip along the distolateral surface. Development of the spermatocysts occurs diametrically (across the width of the testis towards the efferent ductules located medially). Spermatocyst development was initially described as passing through 18 different stages for the small-spotted catshark (*Scyliorhinus canicula*) (Mellinger 1965) but this was subsequently reduced to 7 stages for the bonnethead shark (*Sphyrna tiburo*) (Parsons and Grier 1992). These stages cover the various mitotic divisions of diploid primary spermatocytes to produce primary spermatocytes, which in turn divide through meiosis to produce haploid secondary spermatocytes. The secondary spermatocytes develop into spermatotids with emerging flagella and then into tightly bundled mature spermatozoa, which are shed into the interstitial spaces of the testis as the spermatocyst disintegrates before passing through the efferent ductules into the epididymis.

Spermatozoa occur throughout the lumina of the epididymis, ductus deferens and seminal vesicles, along with secretions from the genital ducts and from the Leydig gland. The process of spermiogenesis, where spermatids mature into motile sperm, occurs in these genital ducts. Secretions from the Leydig gland facilitate maturation of spermatozoa and matrical material form in the lumena of the seminal vesicles and associate with individual and previously bundled sperm. The sperm are held together by a sticky matrix as either spermatophores (sperm encapsulated) or spermatozeugmata (sperm not encapsulated but tails of peripheral sperm protruding). Ciliated epithelial columnar cells lining the lumen convey the spermatozoa through the genital ducts; only the seminal vesicles have a muscular wall. At copulation and ejaculation, sperm transfer from the seminal vesicle through the urogenital papilla to the dorsal groove of each clasper. Spermatozoa acquire the potential for modest motility while in the terminal regions of the genital ducts but acquire active, robust motility at ejaculation (Hamlett 1999).

4.2.3 Maturity and Ovarian Cycle

Application of demographic models to chondrichthyan populations, originally developed for mammals and other vertebrate groups, without recognising the peculiarities of the reproductive cycles of chondrichthyan species, can lead to bias through implicit assumptions that are not correct. In many reported demographic analyses, female mature condition, pregnant condition and maternal condition are often incorrectly equated. Investigation of the dynamics of a chondrichthyan population requires clear definitions for distinguishing between these three breeding conditions, which relate to the timing and periodicity of ovulation and parturition.

The period from the onset of maturity to the start of first pregnancy for females of most vertebrate species is comparatively short and the reproductive cycle is annual or less, with a few exceptions, particularly among large mammals. For chondrichthyan species, this period can also be annual or less but in other species it is several years. In these other species, the period from the onset of maturity of an animal to first ovulation or the period between successive ovulation cycles can be 2, 3 or, as possibly in deep-sea squalids, more years. Similarly, the period from fertilization to parturition is often more than one year in these other species. Fertilization occurs during the relatively short period following ovulation when the ovum passes through the oviduct and oviducal gland before encapsulation by an egg envelop before entering into the uterus. Storage of sperm in the terminal zone of the oviducal gland, evident in several species (Hamlett *et al.* 2002; Smith *et al.* 2004), ensures a supply of sperm for progressive fertilization of ova released by ovulation over a period of several weeks, or possibly months as in *Galeorhinus galeus*. Sperm storage is probably a mechanism for avoiding the problem of accumulating *in utero* eggs obstructing or retarding fresh sperm transiting the uteri to the oviducal glands.

For an animal experiencing first pregnancy, the period from when an oocyte begins yolking to ovulation, together with the period of gestation for the first pregnancy, is more than one year for most species. However, for subsequent pregnancies, vitellogenesis can proceed concurrently with gestation so parturition can be annual or, as argued for some species, more frequently. For species producing large-sized oocytes, such as *G. galeus* and squalids from coastal waters (~40 mm diameter) (Hanchet 1988) and deep-sea continental slopes (60–87 mm diameter) (Yano and Tanaka 1988; Girard and Du Buit 1999; Guallart and Vincent 2001) the period for vitellogenesis is 2, 3 or possibly more years. For our purpose here, the period of the ovarian cycle is defined as the period from completion of one ovulation to completion of the next, and the period of gestation is defined as the period from fertilization to parturition.

Female maturity in chondrichthyan species is not unambiguously defined but animals are mostly assumed to be mature when the ovarian follicles are enlarged. However, this is arbitrary, subjective and likely to provide unrepeatable results. Less ambiguous criteria that might be adopted for defining maturity relate to levels of selected hormones in the blood, the onset of vitellogenesis, first mating, first sperm storage in the oviducal gland or first ovulation. However, the maturity ogives derived for any species would vary depending on the criterion adopted. For population studies requiring large-scale sampling, the onset of vitellogenesis can be rapidly assessed by macroscopic inspection of the ovaries.

Female maturity was assumed to coincide with the onset of vitellogenesis for *G. galeus*. Animals where the diameter of the largest ovarian follicle was >3 mm were assumed to be vitellogenic animals and in mature condition. Animals where the diameter of the largest follicle was 0–3 mm were assumed to be non-vitellogenic and in immature condition.

4.2.4 Pregnancy, Maternity and Parturition Frequency

The size of a total population depends on birth rate and death rate; immigration and emigration rates can affect the sizes of sub-populations interacting within the total population. Birth rate relates to the number of births for viviparous species or to the number of eggs layed for oviparous species. The number of births or eggs layed can be calculated from the number of females in the population and two mathematical equations. One equation expresses litter size as a function of age (or size) of animal (litter size curve). The other equation expresses the proportion of the female population giving birth or laying eggs by the end of the year to contribute to annual recruitment (0+ year-old cohort) at the beginning of the following year. This is referred to here as the proportion of females in maternal condition, and is expressed as a function of age (or size) of animal. The relationship is referred to as the maternity ogive and year applies to the 12-month period prior to the date (or similar date) for completion of parturition.

In *Galeorhinus galeus* for example, as will be demonstrated later in this chapter, parturition frequency is triennial, which implies about one-third of the female population having reached first ovulation (or maternity) gives birth at the end of any year and contributes to recruitment at the beginning of the following year. As parturition occurs during November–January, the birth dates for all offspring can conveniently be fixed at 1 January. Hence, with respect to maternity, a female observed at any time of the year is in maternal condition if it is in pregnant condition and expected to give birth prior to 1 January or if it is in post-partum condition and recently gave birth prior to 1 January. Any other female observed is in non-maternal condition. Pregnant condition of a female is defined by the presence of *in utero* eggs or embryos; non-pregnant condition of a female is defined by the absence of *in utero* eggs and embryos.

4.3 LIFE HISTORY OF *GALEORHINUS GALEUS*

The triakid *Galeorhinus galeus* occurs as six genetically distinct populations (Ward and Gardner 1997) off western North America, eastern South America, southern Africa, southern Australia and New Zealand, and in the eastern North Atlantic Ocean (Compagno 1984). There is some mixing by a small proportion of the large animals undertaking migrations between New Zealand and Australia (Hurst *et al.* 1999) but genetic studies indicate that there is no interbreeding between these populations. There may be genetic or behavioural sub-structuring within the populations but this remains uncertain.

The coastal semi-pelagic species is presently harvested for its meat, cartilage and fins. The species is particularly long-lived, caught by many fishing methods, and demonstrates how such a low-productivity species can be severely depleted if not adequately managed. A fishery based on *Galeorhinus galeus* for its liver oil in California collapsed during the 1940s (Walker 1999). Recent assessments list the species as endangered off eastern South America, vulnerable off southern Australia and southern Africa, and near threatened off New Zealand. The maximum size and mass of *G. galeus* varies between the six populations suggesting that the reproductive parameters required for population assessments will probably vary between the populations. Information on TL-at-maturity (not maturity ogives), litter size and sex ratio among embryos are reported for California (Ripley 1946), eastern South America (Peres and Vooren 1991; Lucifora 2003), and the eastern North Atlantic (Capapé and Mellinger 1988). Only in Australia (Punt and Walker 1998) are all the required parameters for quantitative assessment reported.

A theory of movement of *Galeorhinus galeus* off southern Australia related to reproductive cycles was first developed from tag data and fishing information. According to this theory, pregnant sharks move into shallow nursery areas in Tasmania and Victoria to give birth and then move to deeper waters. The adults tend to move inshore during summer and offshore, or north to the warmer waters of New South Wales and South Australia, during early winter, before returning south during spring. The neonates and young juveniles tend to remain in the nursery areas before moving to eastern Bass Strait. Older juveniles distribute more widely across southern Australia (Olsen 1954). Archival tagging experiments demonstrating deep-water diurnal feeding patterns off the continental shelf (West and Stevens 2001), information on catch composition from the fishery when it expanded from Bass Strait through South Australia to include the Great Australian Bight, and reproductive data presented in this chapter, generally support this theory. Pregnant females at most stages of gestation are observed in waters of the Great Australian Bight, including the eastern region on the south coast of Western Australia, where they occur for much of the period of gestation, before returning to eastern Bass Strait and Tasmania to give birth (Fig. 4.1).

As will be discussed further, spatial and temporal variations in size, breeding condition and sex ratio of each population and the use of gillnets for harvesting these populations can cause biases in parameter estimates caused by non-representative sampling and possibly length-selective fishing mortality.

4.4 SAMPLING *GALEORHINUS GALEUS*

4.4.1 Capture of Sharks

Sampling to investigate the population biology of *Galeorhinus galeus* was undertaken during three separate periods (1973–76, 1986–87, and 1992–01) mainly in Bass Strait (BS) and waters off South Australia (SA) (Fig. 4.1).

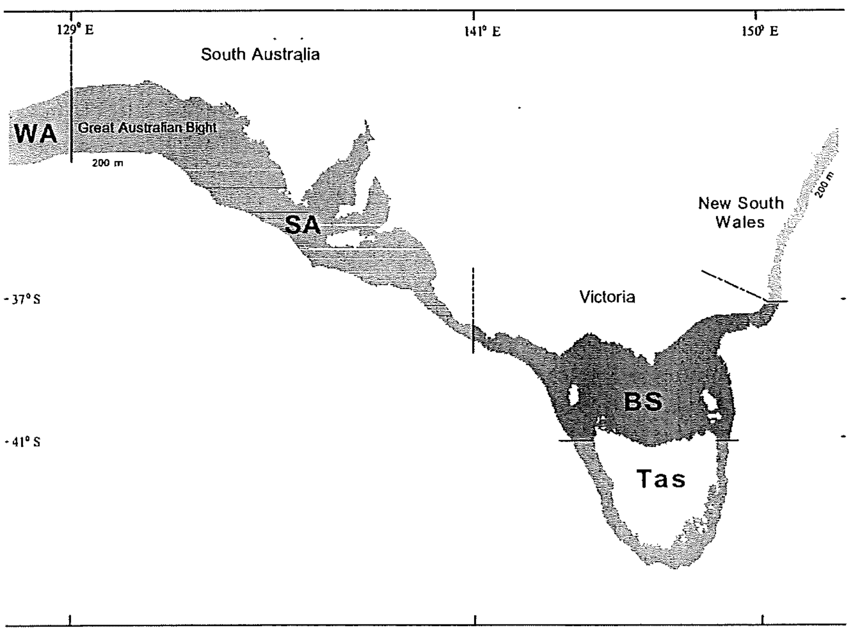


Fig. 4.1 Definition of adopted regions for *Galeorhinus galeus*
WA, Western Australia; SA, South Australia; BS, Bass Strait, and Tas, Tasmania

During 1973–76, the animals were caught using experimental gillnets of mesh-size ranging 2–9 inches (51–229 mm), in steps of 1 inch (25 mm), and hooks attached to sinking longlines. The animals were captured at 162 sites mainly in BS (126 sites) but also off eastern Tasmania south of latitude 41° South (20 sites) (grouped with BS samples) and SA (16 sites). During 1986–1987, the animals were caught in experimental gillnets of mesh-size ranging 5–8 inches (127–203 mm), in steps of 1 inch. The animals were captured at 144 sites (60 sites in BS and 84 sites in SA). During 1992–01, the sampling was opportunistic with most animals caught during 1998–01 at 153 fishing sites (91 sites in BS and 62 sites in SA) by gillnets of 6-inch (152 mm) or 6½-inch (165 mm) mesh-size on board commercial fishing vessels.

4.4.2 Biological Sampling

Specimens of *Galeorhinus galeus* were dissected to investigate their reproduction and other aspects of their biology. They were measured to the nearest millimetre as total length (TL); the tail of each animal was first allowed to take a natural position and the upper caudal lobe was then placed parallel to the body axis. Recorded data included sex, TL, fullness of the stomach and several reproductive indices for each animal. Also recorded, when the sea conditions permitted (mostly on a research vessel during 1973–76), were the wet mass of total body, liver, ovary of females and left testis of males.

For females, at-sea macroscopic inspection of the condition of the paired uteri, paired oviducal glands, and single ovaries was undertaken to investigate breeding condition, litter size, period of gestation and the growth of embryos. Records were made of the diameters of the three largest oocytes and the presence of *corpora atretica* or *corpora lutea* in the ovary and, for pregnant animals, the number of *in utero* eggs and embryos in each uterus. In addition, the TL, sex, uterus (left or right) and wet mass (with and without yolk sac) of each embryo and uterus and wet mass of each *in utero* egg were recorded for many of the pregnant sharks. Indices were adopted for recording the condition of the ovary, oviducal gland, and uteri from rapid visual inspection. Ovary index (O) was based size and colour of the follicles (O = 1–4). Oviducal gland index (G) was based on shape and size of the gland (G = 1–3). Uterus index (U) was based on appearance, size and contents of the uteri (U = 1–6) (Table 4.1).

For males, at-sea macroscopic inspection of condition of the testes, seminal vesicles, and claspers was undertaken to investigate maturity by adopting three indices of breeding condition. Testis index (T) was based on shape, size and relative predominance of testis tissue to epigonal gland tissue (T = 1–3). Seminal vesicle index (V) was based on appearance, thickness of the wall, and presence or absence of seminal fluid (V = 1–3). The length of left clasper was measured from the basipterygium to the distal end and clasper index (C) was based on appearance and rigidity (C = 1–3) (Table 4.1). A histological approach to determining male maturity

Table 4.1 Indices adopted for staging reproductive condition.
Assumptions on mature and immature conditions made for *Galeorhinus galeus* are also listed.

Organ	*Index*	*Description*	*Maturity assumption*
Female			
Ovary	O = 1	Largest follicles white and of diameter <2 mm	Immature
	O = 2	Largest oocytes yolking and of diameter 2–3 mm	Immature
	O = 3	Largest oocytes with yellowish yolk and of diameter >3 mm	Mature
	O = 4	Yolked oocytes of diameter >3 mm and extensive corpora atretica present	Mature
Oviducal gland	G = 1	Indistinct from anterior oviduct	Immature
	G = 2	Distinct but only partly formed	Immature
	G = 3	Enlarged and heart-shaped	Mature
Uterus	U = 1	Uniformly thin tubular structure	Immature
	U = 2	Thin tubular structure partly enlarged posteriorly	Uncertain
	U = 3	Uniformly enlarged tubular structure	Uncertain
	U = 4	In utero eggs present without macroscopically visible embryos present	Mature
	U = 5	In utero embryos macroscopically visible	Mature
	U = 6	Enlarged tubular structure distended	Mature
Male			
Testis	T = 1	Thin tissue strip with epigonal gland predominant	Immature
	T = 2	Thickened strip with epigonal gland tissue extensive	Immature
	T = 3	Enlarged and predominant with epigonal gland tissue negligible	Mature
Seminal vescicle	V = 1	Thin translucent walls and seminal fluids absent	Immature
	V = 2	Thickened opaque walls and seminal fluids present	Mature
	V = 3	Thickened opaque walls and seminal fluids absent	Mature
Clasper[A]	C = 1	Pliable with no calcification	Immature
	C = 2	Partly calcified	Immature
	C = 3	Rigid and fully calcified	Mature

[A]Adopted for periods 1986–1987 and 1992–01, but not for period 1973–76.

was adopted during 1973–76. Two or three pieces of testis tissue (4–8 mm thick) were removed at-sea by transverse section from the left testis and stored temporarily in Bouin's solution. The Bouin's solution was renewed every 12 h for about 36 h and then replaced with 10% neutralised formalin, for subsequent laboratory processing.

4.5 STATISTICAL METHODS

The equations for the litter size curve and the maturity and maternity ogives are expressed as a function of TL rather than age for the statistical analyses undertaken for *Galeorhinus galeus* in Australia. This allows for more robust parameter estimates for *G. galeus* because sample sizes of available data are much larger with TL than with age. Relationships equating TL and age for population assessment of *G. galeus* in Australia are available (Grant *et al.* 1979; Moulton *et al.* 1992). Alternative parameters for the same or similar equations presented as a function of TL in the following can be also expressed as a function of age.

4.5.1 Determining Litter Size and Sex Ratio of Embryos

Simple mathematical equations can be selected to represent the relationships between the number of macroscopically visible *in utero* embryos, p, and maternal TL, l. The linear relationship (Conrath and Musick 2002; Jones and Ugland 2001) between p and l is given by

$$p = a + bl$$

where a and b are parameters estimated by linear regression. One example of a curvilinear relationship between p and maternal l is given by

$$p = ce^{a+bl}$$

where a and b are parameters estimated by linear regression after reformulation to $\ln(p) = a + bl$, and c is a constant to correct for bias caused by logarithmic transformation of p for the regression (Beauchamp and Olson 1973). The linear relationship of *in utero* embryos against TL was adopted for *Galeorhinus galeus* in the present study and a curvilinear relationship was adopted for *Mustelus antarcticus* in southern Australia (Walker 1983; Lenanton *et al.* 1990) from inspection of scattergrams of the data.

For *Galeorhinus galeus*, the effects of factors region and period, the region × period interaction term, and covariate TL on the linear relationship between p and l were statistically tested by analysis of covariance (ANCOVA) through initially including all three terms in the generalized linear model

$$p = \text{Region Period Region} \times \text{Period TL}$$

and then sequentially excluding the term with the highest statistically non-significant P value ($P > 0.05$), until only significant terms were present (stepwise backward elimination). The ANCOVAs were undertaken using the general linear modelling procedure (Proc GLM) of the computer statistical package SAS (SAS Institute, NC, USA).

Paired t-tests were applied to pregnant females with *in utero* eggs ($U = 4$) or macroscopically visible embryos ($U = 5$ animals) to test two hypotheses. (1) The sex ratio of *in utero* embryos is 1 : 1. (2) The number of *in utero* embryos and infertile eggs in the left uterus equals the number in the right uterus. The SAS means procedure (Proc Means) was used to compute the required statistics. The statistics were the differences in the means, the standard errors of the difference in the means, the values of the Student t statistic, and the probabilities that the differences are statistically significant (Cody and Smith 1997).

4.5.2 Determining Period of Gestation and Growth of Embryos

The period of gestation and growth of embryos can be determined by plotting mean TL of embryos observed in pregnant females (U = 5 animals) and mean TL values of 0 for *in utero* eggs observed in pregnant females (U = 4 animals) against month and then evaluating the seasonal pattern. In addition, for *Galeorhinus galeus*, mass gain or loss from egg to full-term embryo during gestation was investigated for a sample of pregnant females (U = 5 animals). This was undertaken by separately plotting each of four variables against mean embryo TL for U = 5 animals. These variables were the mean wet mass of embryos, the mean wet mass of external yolks, the sum of these two quantities and the mean external yolk wet mass expressed as a proportion of the sum of the two quantities.

4.5.3 Determining Period of Ovarian Cycle

The ovarian cycle can be investigated by examining the ovary and measuring the diameters of the largest follicles in animals caught throughout the year. The diameters of the largest follicles vary widely between individual animals and vary depending on uterus condition, so seasonal patterns of follicle growth for each of the six uterus conditions defined in Table 4.1 need to be examined separately.

In *Galeorhinus galeus*, pregnant females with macroscopically visible *in utero* embryos (U = 5 animals) provide the least ambiguous basis for determining seasonal growth rates of follicles and for distinguishing between annual, biennial and longer ovarian cycles. The earliest observations of small *in utero* embryos were during late February and the latest observations of near-term embryos were during January. This provides a period, close to one full year, that can be adopted for measuring annual rate of follicle growth.

None of the other five uterus conditions provided such clear information on annual rate of follicle growth. The data indicate that females with uterus conditions U = 1 and U = 2 exhibit little or no change in follicle diameter over the 12-month period from January to December. Females with uterus conditions U = 4 were few in number and occurred for only several months at the end of the year or very early in the year, and therefore provide no information on annual growth of follicles. These animals, however, do provide information on the timing of ovulation and on follicle diameter at the time of ovulation. Similarly, females with uterus condition U = 6 were few in number, displayed little variation in size, and therefore provided no information on annual growth rate of follicles. Nevertheless, these animals did provide information on follicle diameter at the time of parturition and during the period immediately following parturition. That the uterus condition U = 6 was not commonly observed at other times of the year suggests that after parturition the distended uterus contracts to resemble the uterus condition U = 3. This implies that animals recorded with uterus condition U = 3 might be a mixture of animals approaching first pregnancy (all U = 3) and animals between pregnancies (U=6 changing to resemble U = 3). Unlike animals with uterus conditions U=5, U = 4, and U = 6, which can be related to the timing of ovulation and parturition, animals with uterus condition U = 3 cannot be so reliably related to either of these events. Hence, these animals are possibly less reliable for determining annual growth rate of follicles.

Pooling data from BS and SA, annual growth rate for animals of uterus condition U = 5 was determined by the linear relationship between follicle diameter, *o*, and Julian day, *t*, given by

$$o = a'' + b''t$$

where a'' and b'' are parameters estimated by linear regression. For each of BS and SA separately, the regression line for U = 5 animals was then compared with the scattergrams of follicle diameter against Julian day for each of the U = 3, U = 4 (ovulating), and U = 6 animals separately. A similar regression was undertaken for the U = 3, U = 4 (ovulating), and U = 6 animals combined and compared with that for the U = 5 animals and the scattergrams. These comparisons provided a basis for considering whether the ovarian cycle is annual, biennial, triennial or longer.

4.5.4 Determining Size-at-maturity and Size-at-maternity

The proportion of the population of animals mature at any TL can be determined by classing each animal as in mature condition or immature condition and applying logistic regression for females (Mollet *et al.* 2000; Conrath and Musick 2002) and males separately. Similarly, for females, the proportion of the population of animals in maternal condition at any TL can be determined by classing each animal as in maternal condition or non-maternal condition and applying logistic regression.

For *Galeorhinus galeus*, a female was classed as in mature condition if the largest ovarian follicle was >3 mm in diameter (size at first yolking); otherwise it was classed as in immature condition. Given uncertainty of the best indicator of maturity of males, the results from methods based on alternative criteria for assuming the mature condition and the immature condition are compared. Males were classed by testis condition as mature if T = 3 and immature if T = 1 or T = 2. Similarly, they were classed by seminal vesicle condition as mature if V = 2 or V = 3 and immature if V = 1 and by clasper condition as mature if C = 3 and immature if C = 1 or C = 2 (Table 4.1). There were too few data available to apply a fourth method based on stages of spermatogenesis.

A female was classed as in maternal condition at the time of dissection, if it would have given birth to young before or soon after the following 1 January. To implement this criterion, females were classed as in maternal condition if they met any one of three criteria. These criteria were pregnant with visible embryos (U = 5) during February–December, pregnant with *in utero* eggs (U = 4) during January–May, non-pregnant, or in post-partum condition with distended uteri (U = 6) during November–December. All other females were classed as in non-maternal condition; that is U = 1, U = 2, U = 3, U = 4 during June–December, U = 5 during January with full-term embryos, or U = 6 during January–October.

Logistic regression was adopted to determine the proportion of females in mature condition, the proportion of males in mature condition, and the proportion of females in maternal condition as a function of TL. Females or males in mature condition were assigned a maturity condition value of 1, whereas those in immature condition were assigned a maturity condition value of 0. Similarly, females in maternal condition were assigned a maternal condition value of 1, whereas females in non-maternal condition were assigned a maternal condition value of 0.

These proportions are given by P as a function of TL, l, where P is determined by logistic regression analysis. P is given by a random dichotomous variable y taking the value of 1 with a probability of φ for the mature or maternal condition and the value of 0 with a probability of $1-\varphi$ for the immature or non-maternal condition. This point-binomial variable has a probability distribution such that

$$P = P^y(y;\varphi) = \varphi^y(1-\varphi)^{(1-y)}$$

where $y = 0,1$

The likelihood function, $L(y;\varphi)$, takes the form

$$L(y;\varphi) = \prod_{j=1}^{N} \{\varphi_j y_j (1-\varphi_j)^{(1-y_j)}\}$$

where φ_j represents the probability that individual j in a random sample of N animals from the shark population which is judged to be in mature condition or, alternatively, maternal condition at the time of sampling. The logistic equation adopted to express P as a function of l is given by

$$P = \frac{c'''}{(1 + e^{-(a''' + b''' l)})}$$

where a''', b''' and c''' are parameters (Walker 1994) but to provide parameters that are more biologically meaningful, the equation is reformulated as

$$P = P_{\max}\left(1 + e^{-\ln(19)\left(\frac{l-l_{50}}{l_{95}-l_{50}}\right)}\right)^{-1}$$

where P_{max} is the maximum proportion of animals in mature condition or maternal condition (equivalent to c'''), l_{50} and l_{95} and are the lengths at which 50% and 95% of the maximum proportion of animals in mature condition or maternal condition (Punt and Walker 1998).

The parameters a''', b''' and c''', P_{max}, l_{50} and l_{50}, with 95% confidence intervals, were estimated by the method of maximum likelihood using the probit procedure (Proc Probit) of the computer statistical package SAS (SAS Institute, Cary, North Carolina, USA). This applies a modified Newton-Raphson algorithm for estimation.

The standard error for any length, l, is given by

$$se_l = P_l(1-P_l)/\mathrm{N}$$

For *Galeorhinus galeus*, the effects of factors region and period and the region x period interaction term on the logistic relationships between P and l were statistically tested by initially including all three terms in the logistic regression model

$$P=\left(\frac{n}{N}\right)=\text{Region Period Region x Period TL}$$

where n is the number of mature or maternal animals, N is the total number of animals included by SAS is the analysis. SAS assigns the data to length-classes automatically, unless specified by the user, and discards data where there is more than one length-class with values of only 0 and more than one length-class with only values of 1.

For *Galeorhinus galeus*, the model was then run repeatedly and the least statistically non-significant factor or interaction term was sequentially deleted by stepwise backward elimination until only statistically significant terms ($P < 0.05$) remained in the model. The logistic relationships between P and l were tested for the effects of the two regions BS and SA, the three sampling periods 1973–76, 1986–87, and 1998–01, and the region x period interactions. These tests were undertaken by applying the logistic procedure (Proc Logistic) of the computer statistical package SAS. The terms were tested by the χ^2 likelihood-ratio test (Rao 1973; Silvey 1975).

The SAS probit procedure sets $1-c''' = 0.000$. This is appropriate for the maturity ogive where all large-sized animals in the population are in mature condition, and hence the proportion of large-sized animals in the population mature is 1.000. Similarly, this is appropriate for the maternity ogive where all of the large-sized animals in the population are in maternal condition, and hence the proportion of large-sized animals in the population in maternal condition is 1.000; parturition frequency is annual. However, this is not appropriate where parturition frequency is biennial, triennial or some other period.

Application of the SAS probit procedure is more complex to apply to any parturition frequency, γ, other than 1 year. For example, if parturition is biennial where half the population gives birth each year then $\gamma = 0.50$ or if parturition is triennial where one-third the population gives birth each year then $\gamma = 0.33$. This was undertaken for *G. galeus* by categorising the number of animals in maternal condition and the number of observations into 100-mm length-classes. For parturition frequency, where the ratio of number in maternal condition / number of observations exceeds γ within a 100-mm length-class, the number in mature condition needs to be adjusted to produce the ratio γ. For SAS probit analysis, the number of observations in each 100-mm length-class (or some other selected range) is multiplied by γ. A weight statement was used to weight the values in each length-class by the original number of observations in that length-class. The ogive relationships, with 95% CI, produced by the SAS probit procedure can then be divided by γ to give the required parameter values of the maternity ogive, with 95% CI.

4.5.5 Determining Total Body Mass at Size

The relationship between total body mass, w and TL, l, can be determined using the power curve

$$w = a'''' \, c'''' l^{b''''}$$

adopted commonly for sharks (Olsen 1954) and bony fishes (Ricker 1958) without the constant c'''', where a'''' and b'''' are parameters determined by linear regression of $ln(w)$ against $ln(l)$, and c'''' is a factor for correcting for biases caused by natural logarithmic transformation (Beauchamp and Olson 1973).

For *Galeorhinus galeus*, linear regression relationships were determined separately for males, non-pregnant females, pregnant females with *in utero* eggs, and pregnant females with *in utero* embryos. These four relationships were determined separately because females grow larger than males and because pregnant animals weigh more than non-pregnant animals at any length. The Student *t*-test was applied to test for differences between the slopes (parallelism) and intercepts (elevation) for selected pairs of straight lines determined from the four $ln(w)$–$ln(l)$ regression fits (Kleinbaum *et al.* 1988).

4.6 APPLICATION OF METHODS TO *GALEORHINUS GALEUS*

4.6.1 Litter Size and Sex Ratio of Embryos

Macroscopically visible *in utero* embryos were examined in 63 pregnant *G. galeus* (U = 5 animals); there were 22 U = 5 animals from BS and 41 from SA. ANCOVA testing for effects of factors region and period, region x period interaction, and covariate maternal TL by stepwise backward elimination indicated that only maternal TL was statistically significant (Table 4.2). This allowed data from the two regions (BS and SA) and three periods (1973–76, 1986–87 and 1998–01) to be pooled to provide a single relationship between the number of *in utero* embryos and maternal TL determined from linear regression. These animals ranged in size 1429–1680 mm TL and carried 15–43 *in utero* embryos (Fig. 4.2).

Table 4.2 Hypothesis testing for females with in utero embryos

Analyses of covariance (ANCOVA) testing for the effects of region (Bass Strait and South Australia), period (1973–76, 1986–87 and 1998–01), and region x period interaction on the number of in utero embyos against total length for pregnant females with macroscopically visible embryos (U = 5) by stepwise backward elimination of non-significant factors. d.f., degrees of freedom; M.S., mean square; ns, not significant; P is probability of statistical significance (*P < 0.05; **P < 0.01; ***P < 0.001).

Source of variation		*d.f.*	*M.S.*	*P*	
Step 1					
	Region	1	20.814	0.6108	ns
	Period	2	5.604	0.8749	ns
	Region x period	2	88.443	0.1516	ns
	Maternal length	1	529.230	0.0008	***
	Residual	56	41.847		
Step 2					
	Region	1	71.307	0.1971	ns
	Period	2	56.334	0.2751	ns
	Maternal length	1	530.626	0.0008	***
	Residual	57	42.664		
Step 3					
	Region	1	106.569	0.0932	ns
	Maternal length	1	603.990	0.0004	***
	Residual	59	43.128		

The overall mean number (±s.e.) of *in utero* embryos and infertile eggs in the 63 pregnant females with macroscopically visible embryos was 31.24 (±0.88) where 30.18 (±0.92) were embryos and 1.06 (±0.20) were infertile eggs indicating 96.6% of *in utero* eggs develop as embryos. The mean number of embryos in the left uterus (14.71±0.53) was not significantly different from the mean number of embryos in the right uterus (15.46±0.46). Similarly, the mean number of infertile eggs in the left uterus (0.60±0.12) was not different from the mean number of infertile eggs in the right uterus (0.46±0.13). The mean number of male embryos (14.56±0.62) was not significantly different from the mean number of female embryos (14.76±0.62); 2.8% of the embryos were too small to confidently identify their sex (0.86±0.54) (Tables 4.3 and 4.4). Of the 63 U = 5 animals, 28 animals had zero infertile eggs (44.4% of animals), 19 had one (30.2%), 11 had two (17.4%), two had three (3.2%), one had four (1.6%), one had six (1.6%), and 1 had ten (1.6%) infertile eggs. In addition to counting the

Table 4.3 Mean number (±se) of *in utero* embryos and eggs in pregnant females
s.e., standard error for 63 pregnant females with macroscopically visible embryos (U = 5 animals).

Mean (±s.e.) of number of embryos and infertile eggs											
Left uterus			Right uterus			Embryos			Total		
Embryos	Eggs	Total	Embryos	Eggs	Total	Male	Female	Unknown	Embryos	Eggs	Total
14.71±0.53	0.60±0.12	15.32±0.51	15.46±0.46	0.46±0.13	15.92±0.44	14.56±0.62	14.76±0.62	0.86±0.54	30.18±0.92	1.06±0.20	31.24±0.88

Table 4.4 Testing sex ratio and number of *in utero* and infertile eggs embryos between left and right uteri
Statistical paired t-tests comparing the number of *in utero* embryos and eggs between the left and right uteri and between male and female embryos in 63 pregnant females with macroscopically visible embryos (U = 5 animals). ns, not significant; P is the probability of statistical significance (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

Comparison	*Mean difference*	*s.e. of difference*	*t value*	*P*	
Left uterus embryos & eggs – right uterus embryos & eggs	–0.603	0.355	1.70	0.0946	ns
Left uterus embryos – right uterus embryos	–0.746	0.375	1.99	0.0512	ns
Left uterus eggs – right uterus eggs	0.143	0.139	1.03	0.3088	ns
Male embryos – female embryos^{A}	–0.210	0.719	0.29	0.7715	ns

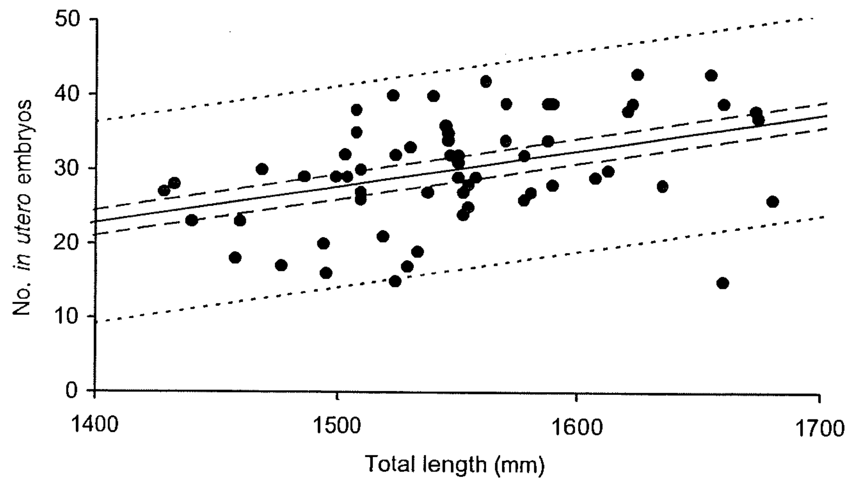


Fig. 4.2 Number of *in utero* embryos against maternal total length
Mean number of embryos (—), 95% confidence limits (– –), 95% prediction intervals (- - -), and raw data (•) are plotted against maternal total length of pregnant females with macroscopically visible embryos (U = 5). Values of parameters and statistical quantitites for the equation $p = a' + b'l$ are given in the following tabulation:

a' (±s.e.)	b' (±s.e.)	n	r^2	*rmse*	P
−46.0 (±22.2)	0.0491 (±0.0143)	63	0.148	6.724	***

where l is maternal total length measured in millimetres, p is number of in utero embryos, a' and b' are parameters, n is sample size, r^2 is square of regression correlation coefficient, *rmse* is root mean square error, and P is the probability of statistical significance (*P < 0.05; **P < 0.01; ***P < 0.001) for linear regression.

embryos and infertile eggs in these 63 U = 5 animals, eggs were counted in four pregnant females with only *in utero* eggs (U = 4 animals). Based of the simultaneous presence of large follicles in the ovary and eggs *in utero*, these animals were all judged to be in the process of ovulation (Table 4.5).

4.6.2 Period of Gestation and Growth of Embryos

Mean TL of embryos (with standard error) measured in each of 54 pregnant females (U = 5 animals) and an assigned mean TL value of 0 mm for *in utero* eggs observed in 65 pregnant females (U = 4 animals) are plotted against month (Fig. 4.3). *In utero* embryos were observed during the eight-month period May–January and *in utero* eggs were observed during the seven-month period June–January. *In utero* embryos were not observed during the three-month period during February–April; pregnant females with early-stage embryos (U = 5 animals) occur in the Great Australian Bight and more easterly regions in SA (anecdotal information from fishers), and possibly in oceanic waters away from the continental shelf. These regions were not sampled at that time of the year. The data for the period May–January indicate a high degree of synchrony in gestation between

Table 4.5 Number of *in utero* eggs in ovulating pregnant females
TL, total length; LFD, largest follicle diameter in four U = 4 animals.

Animal	*Date*	*TL (mm)*	*LFD (mm)*	*No. in utero eggs*		
				Left uterus	*Right uterus*	*Total*
1	Oct-92	1580	52	4	4	8
2	Dec-92	1600	53	22	22	44
3	Nov-95	1480	47	5	5	10
4	Nov-98	1570	34	6	6	12

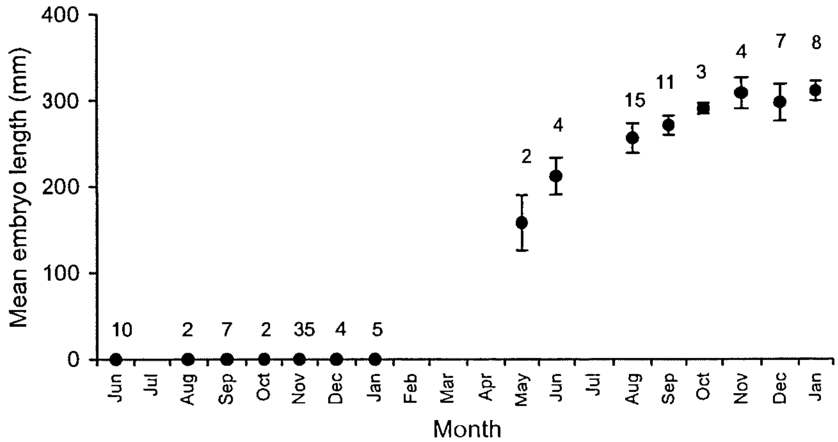


Fig. 4.3 Mean embryo length against month
Derived from the mean embryo length of the litter from each of 54 pregnant animals with macroscopically visible embryos and 65 pregnant animals with only *in utero* eggs; •, overall mean; bars, standard deviation .

U = 5 animals. This synchrony, together with the long period for the presence of *in utero* eggs, is evidence that the period of gestation exceeds one year and the frequency of parturition cannot be annual.

The highest mean embryo wet mass of 149 g towards the end of gestation observed in one pregnant animal when mean TL of its embryos exceeded 300 mm was about double the highest mean egg wet mass of 80 g at the beginning of gestation in another animal (Fig. 4.4). Given *Galeorhinus galeus* is aplacental, this approximately 100% mass gain from egg to full-term embryo suggests *G. galeus* provides nutrients to the embryos by way of intra-uterine nutrients (histotroph), although the mass gain might be from hydration. There is little or no mass gain in the combined mass of embryo and external yolk sac for the first half of gestation but this increases progressively towards the end of gestation (Fig. 4.4).

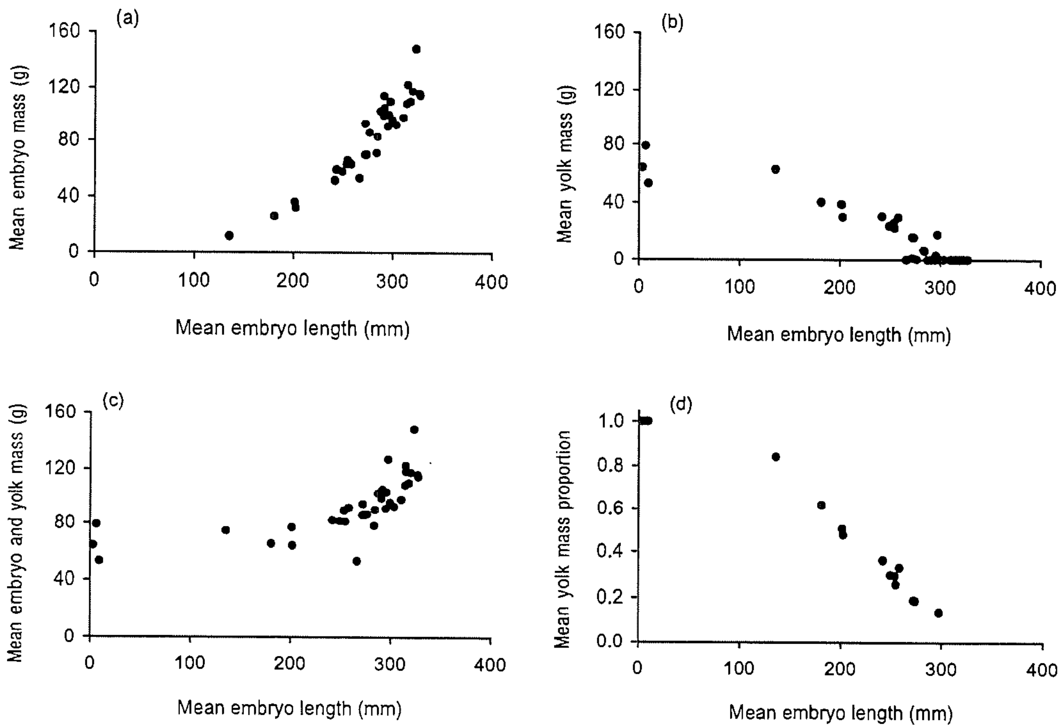


Fig. 4.4 Mass gain of embryos and mass loss from yolk sac during embryonic development
Mean mass of embryos (a), mean mass of yolk sacs (b), mean mass of embryo and yolk (c) and yolk sac as a proportion of sum yolk sac and embryo mass (d) against mean embryo length. Each data point is derived from the mean embryo mass, mean yolk mass and mean embryo length determined for the litter of each of 36 pregnant animals with macroscopically visible embryos. Yolk mass proportion is yolk sac mass/(embryo mass + yolk sac mass).

4.6.3 Ovarian Cycle

The diameter of the largest follicle recorded in the ovary from each of 715 females (230 from BS and 485 from SA) ranged 1–56 mm. There was little difference between the diameters of the three largest follicles (Table 4.6), so all statistical analyses of follicle data were undertaken using only the first measured follicle, which was judged visually to be the largest when measured.

Table 4.6 Comparison of diameters of three largest ovarian follicles for each uterus condition n, sample size; s.e., standard error.

Uterus condition[A]	*n*	*Mean diameter (±s.e.) of three largest ovarian follicles (mm)*		
		Follicle 1	*Follicle 2*	*Follicle 3*
U = 1	226	1.40±0.06	1.38±0.06	1.36±0.06
U = 2	238	2.08±0.12	2.08±0.12	2.07±0.12
U = 3	94	14.68±1.26	14.39±1.24	14.22±1.23
U = 4	16	34.06±3.95	34.25±4.07	31.44±4.20
U = 5	72	9.63±0.36	9.33±0.33	9.15±0.32
U = 6	24	13.58±0.65	13.83±0.79	13.50±0.66

[A]Defined in Table 4.1

Patterns in plots of largest follicle diameter (LFD) against Julian day were not evident where all the data were pooled; patterns in LFD against Julian day were evident only when the animals were considered for each uterus condition separately. LFD was consistently small for animals with uterus conditions U = 1–2, a clear indication that these animals were immature or at early stages of folliculogenesis or vitellogenesis. There was little variation in LFD among U = 1 animals (n = 232, mean 1.4 mm, s.d. 1.0 mm, range 1–9 mm) or among U = 2 animals (n = 244, mean 2.1 mm, s.d. 1.8 mm, range 1–15 mm) (Fig. 4.5). LFD varied widely among animals with uterus conditions U = 3 (1–56 mm), U = 4 (7–53 mm), U = 5 (3–19 mm) and U = 6 (4–19 mm). From uterus condition alone, these animals can be classed as pregnant or post-partum (U = 4–6) or as mature and approaching ovulation (U = 3). The patterns in plots of LFD against Julian day are generally similar between BS and SA, apart from the lack of U = 4 and U = 5 animals in BS prior to Julian day 274 (October) (Fig. 4.6).

For pregnant females with macroscopically visible embryos (U = 5 animals), linear regression of LFD against Julian day for animals from BS and SA pooled indicated that annual growth of LFD is 10.2 mm y^{-1}. At the end of the year when gestation is complete or approaching completion, the ovarian follicles are much too small for ovulation. The predicted mean LFD increased from 1.9 to 12.1 mm during one year, evidence that the ovarian cycle, and hence frequency of parturition, exceeds one year (Figs 4.5, 4.6).

The U = 3, U = 4 and U = 6 animals were then examined to assess whether they conformed to the hypothesis of a two-year, three-year or longer ovarian

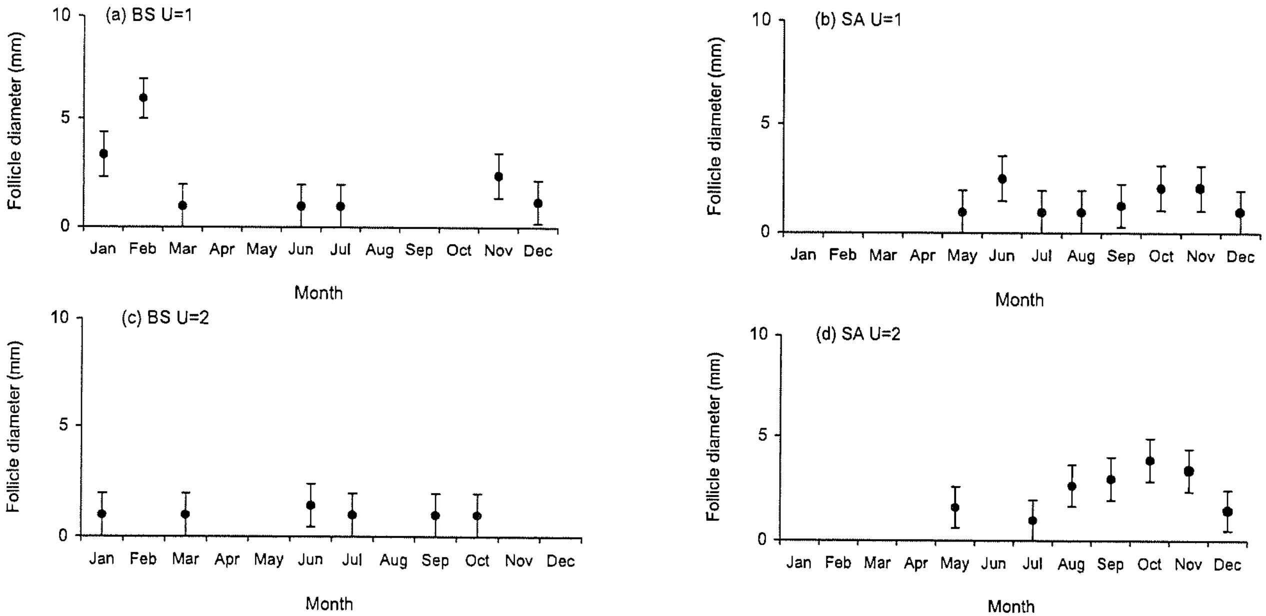


Fig. 4.5 Ovarian follicle diameter against month by region for uterus conditions U = 1–2
Mean oocyte diameter (±s.d.) plotted against month for each of the two uterus conditions U=1 (a and b) and U=2 (c and d) from Bass Strait (BS) (left) and South Australia (SA) (right) during 1973–76, 1986–87 and 1998–01. Sample size (n) and annual mean follicle diameter are given in the following tablulation:

Fig.	*U*	*Region*	*n*	*Mean (±s.d.) (mm)*	*Range (mm)*
(a)	1	BS	113	1.2 (1.0)	1– 7
(b)	1	SA	119	1.6 (1.0)	1– 9
(c)	2	BS	65	1.1 (0.5)	1– 3
(d)	2	SA	179	2.5 (1.9)	1–15

cycle. For BS and SA separately, scattergrams of LFD plotted against Julian day for animals in each of these three uterus conditions showed that LFD values were in three broad clusters (<18 mm, 18–33 mm and 34–56 mm). This provides evidence in favour of the three-year ovarian cycle, consistent with the three-year ovarian cycle hypothesised for the stock of *G. galeus* occurring off eastern South America (Peres and Vooren 1991). Growth of the largest ovarian follicles was described by determining the linear relationship between LFD and adjusted Julian day for the 3-year period. Adjusted Julian day for the three-year period was calculated by adding to Julian day 365 days if LFD ranged 18–33 mm or 730 days if LFD ranged 34–56 mm; Julian day was unadjusted if LFD <18 mm. The predicted mean LFD trajectory for the three-year ovarian cycle was determined by linear regression pooling data from BS and SA and then plotted on each of the three scattergrams of uterus condition for each of BS and SA separately. The U = 4 animals where ovulation was complete (LFD <30 mm) were excluded from the regression analysis. The LFD trajectory was extrapolated through a second and third year but displayed for a 1 year period by presenting the trajectory as three parallel lines, one for each year. The linear relationship for U = 3, U = 4 (ovulating only), and U = 6 animals and the linear relationship for U = 5 animals were not significantly different (t-test, $t = 1.906$, d.f. = 228 and $P > 0.05$ for comparison of slopes and $t = 1.045$, d.f. = 228, and $P > 0.05$ for comparison of elevations) (Figs 4.6, 4.7).

Among most U = 3 animals, the individual LFD values were consistent with the hypothesis for a three-year ovarian cycle in both BS and SA (Fig. 4.6). The animals are distinct between the second and third years but are less distinct between the first and second years.

Among U = 4 animals, the individual LFD values were also consistent with the hypotheses for a three-year ovarian cycle, particularly in SA. These data provide reliable information on the timing of ovulation and on magnitude of LFD at the time of ovulation. The animals were classed as ovulating or ovulated on the basis of LFD, which were variously clustered in each of BS (ranging 8–30 mm LFD) and SA (ranging 7–11 mm, one 34 mm, and 42–53 mm LFD). Animals were classed as ovulating (in process of ovulation) if they contained eggs *in utero* and ≥34-mm LFD; animals were classed as ovulated (ovulation complete) if they contained eggs *in utero* and <34-mm LFD. No U = 4 animals were found ovulating in BS but four ovulated animals were captured during the period from 13 October (Julian day 286) to 14 November (Julian day 318). With the exception of one ovulated animal captured on 21 June (Julian day 172), all U = 4 animals captured in SA during the period from 21 June (Julian day 172) to 4 December (Julian day 338) were ovulating (Fig. 4.6).

Among most U = 6 animals, the individual LFD values have a similar distribution to those of the U = 5 animals; they are clustered near the trajectory for the first year. The single LFD value near the mean LFD trajectory at the end of the first year in each of BS and SA were likely to

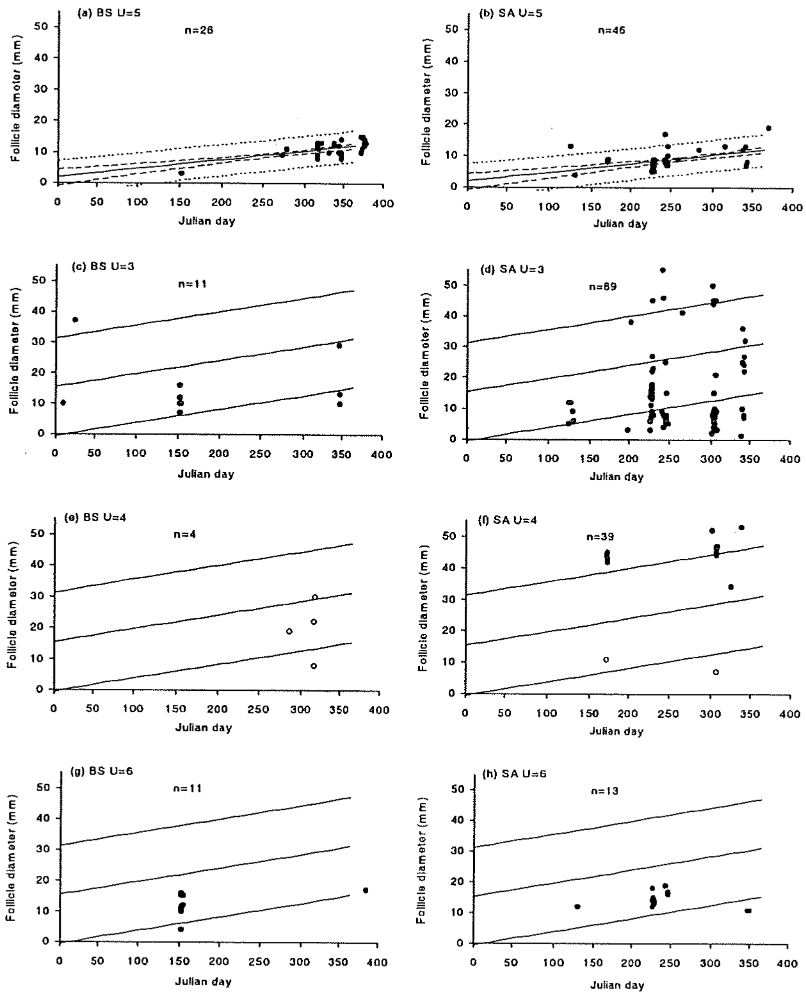


Fig. 4.6 Largest follicle diameter against Julian day by region for uterus conditions U = 3–6
Follicle diameter against Julian day for females from Bass Strait (BS) (left) and South Australia (SA) (right) during 1973–76, 1986–87 and 1998–01 combined for each of the of four uterus conditions (U = 3–6). Mean follicle diameter (—) with 95% confidence limits (– –) and 95% prediction intervals (- - -) are presented for pregnant females with *in utero* embryos (U = 5) (a and b), non-pregnant animals (U = 3) (c and d), pregnant animals with *in utero* eggs (U = 4, ovulating (•) and ovulated (○)) (e and f), and postpartum females (U = 6) (g and h). Values of parameters and statistical quantities for the regression equation o = a'+$b't$ for U = 5 animals and for U = 3, U = 4 (ovulating) and U = 5 animals pooled from BS and SA are given in the following tablulation:

U	*a' (±se)*	*b'(±se)*	*n*	r^2	*rmse*	*P*
5	1.93 (1.25)	0.0280 (±0.0044)	72	0.354	2.467	***
3, 4 (ovulating), 6	−0.56 (0.73)	0.0436 (±0.0011)	160	0.903	5.166	***

Fig. 4.6 *Contd. ...*

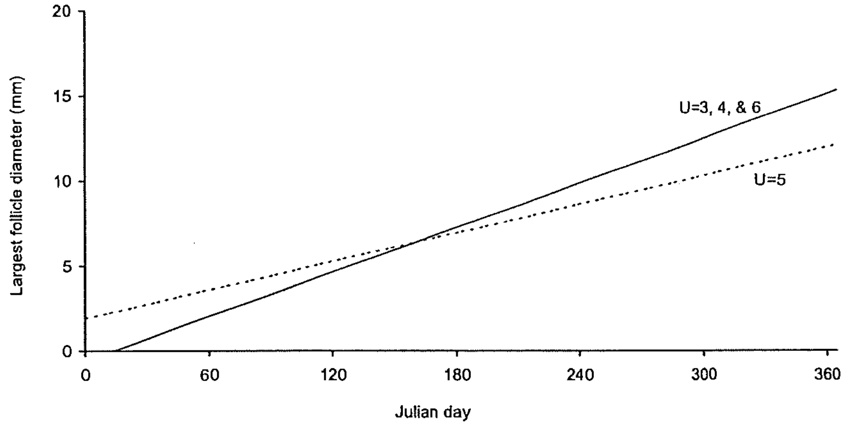


Fig. 4.7 Comparison of LFD against Julian days for U = 5 condition with U = 3, 4 (ovulating) and 6 conditions

The regression line of largest follicle diameter (LFD) against Julian day for females of uterus condition U = 5 (——) is compared with the regression line for uterus conditions U = 3, 4 (ovulating only), and 6 (- - -). The data are pooled from animals collected from Bass Strait and South Australia during 1973–76, 1986–87 and 1998–01.

be animals that had recently given birth. The other animals clustered near the trajectory for the first year during earlier months are inconsistent with the three-year ovarian cycle in BS and SA (Fig. 4.6); they are most likely U = 3 animals incorrectly classed as U = 6.

Ovary mass was available for only 43 animals. Ovary mass was available for U = 1 animals (n = 11, mean 13.0 g, s.d. 11.2 g, range 1–32 g), U = 3 animals (n = 2, mean 70.5 g, s.d. 3.5 g, range 68–73 g), U=4 animals (n = 7, mean 568.3 g, s.d. 660.0 g, range 48–1880 g), and U = 5 animals (n = 23, mean 169.1 g, s.d. 244.1 g, range 44–910 g). Ovary mass was not taken for U = 2 or U = 6 animals.

Percentage GSI was available for 41 animals. GSI was available for U = 1 animals (n = 11, mean 0.15, s.d. 0.09, range 0.02–0.28), U = 3 animals (n = 2, mean 0.42, s.d. 0.01, range 0.41–0.42), U = 4 animals (n = 7, mean 2.58, s.d. 2.76, range 0.28–7.23), and U = 5 animals (n = 21, mean 0.70, s.d. 0.21, range 0.22–3.88). Percentage GSI was not taken for U = 2 or U = 6 animals.

Percentage HSI was available for 64 animals. Percentage HSI was available for U = 1 animals (n = 25, mean 5.47, s.d. 1.35, range 3.32–9.00), U = 3 animals (n = 3, mean 6.46, s.d. 5.05, range 2.75–12.22), U = 4 animals (n = 12, mean 10.48, s.d. 2.52, range 5.69–14.15), and U = 5 animals (n = 24,

---

Fig. 4.6 *Contd. ...*

where *t* is Julian day (for up to 3 years), *o* is follicle diameter, *a'* and *b'* are parameters, *n* is sample size, r^2 is square of regression correlation coefficient, and rmse is root mean square error for the regression, and *P* is probability of statistical significance (*P < 0.1; **P < 0.01; ***P < 0.001).

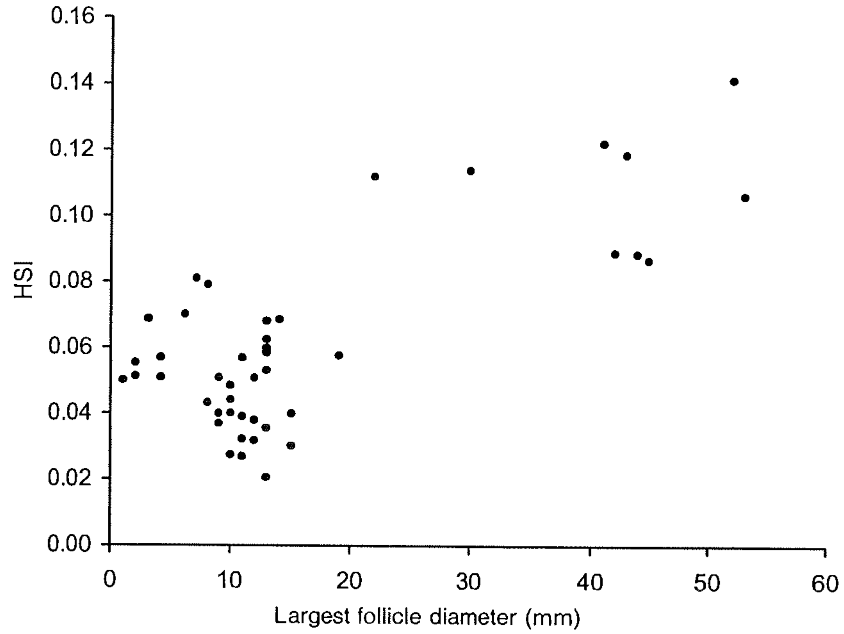


Fig. 4.8 HSI against largest ovarian follicle diameter
Spearman correlation between hepatic somatic index (HSI) and ovarian largest follicle diameter is 0.397 (P = 0.0076 **) for U = 1–6 animals where *P* is probability of statistical significance (*P < 0.1; **P < 0.01; ***P < 0.001).

mean 4.65, s.d. 1.51, range 2.06–8.24). Percentage HSI was not taken for U = 2 or U = 6 animals. HSI tends to rise with LFD (Fig. 4.8.), suggesting the liver mass increases concurrently with the process of vitellogenesis.

4.6.4 Female Maturity Ogives

For *Galeorhinus galeus*, hypothesis testing for the effects of region, period and region x period interaction on logistic regression models was undertaken by stepwise backward elimination of statistically non-significant terms by log-likelihood ratio tests. For the full model, only the effect of period (P = 0.0040) was statistically significant; the effects of region and region x period interaction were not significant. Hence, further hypothesis testing was undertaken for only the effect of period; the two regions BS and SA were pooled. The effect of period between 1973–76 and 1986–87 (P = 0.0006) and between 1973–76 and 1998–01 (P=0.0032) was highly significant but was not significant between 1986–87 and 1998–01 (P = 0.2415) (Table 4.7). Hence, the data were pooled for the periods 1986–87 and 1998–01 for subsequent probit analysis (Table 4.8). Separate figures are therefore presented for the 1973–76 period and for the 1986–87 and 1998–01 periods combined (Fig. 4.9). The large difference in sample size between 1973–76 (56 mature and 104 immature animals) and the combined periods

Table 4.7. Hypothesis testing for female maturity based on largest follicle diameter

Testing for the effects of region (Bass Strait and South Australia), period (1973–76, 1986–87, and 1998–01) and region x period interaction on logistic regression of the proportion of the animals in the population mature against TL. Only final model in stepwise backward elimination to show effect of region is presented. An animal is classed as mature where the largest follicle diameter is >3 mm. χ^2, *Chi* square; ns, not significant; P is probability of statistical significance (*P < 0.05; **P < 0.01; ***P < 0.001).

Source of variation	*Number of animals*		χ^2	*P*	
	Mature	*Immature*			
Region x Period	375	491			
Intercept			148.557	<0.0001	***
Period			8.274	0.0040	**
TL			153.393	<0.0001	***
Between 1973–76 and 1986–87	222	423			
Intercept			86.070	<0.0001	***
Period			11.784	0.0006	***
Total length			102.721	<0.0001	***
Between 1973–76 and 1998–01	212	172			
Intercept			62.411	<0.0001	***
Period			8.678	0.0032	**
Total length			64.105	<0.0001	***
Between 1986–87 and 1998–01	316	387			
Intercept			125.823	<0.0001	***
Period			1.372	0.2415	ns
Total length			128.154	<0.0001	***
Between 1973–76 and 1986–87, 1998–01	375	491			
Intercept			0.010	0.9201	ns
Period			13.362	0.0003	***
Total length			154.284	<0.0001	***

1986–87 and 1998–01 (245 mature and 458 immature animals) accounts for the wide difference between the magnitude of the 95% confidence intervals for these two periods. The l_{50} value (with 95% CI) of 1349 (1339, 1358) mm for mature condition during 1986–87 and 1998–01 combined is larger than the value of 1244 (1184, 1304) mm for 1973–76 (Fig. 4.9), suggesting an actual or apparent increase in the size-at-maturity. An apparent change in size-at-maturity could occur from the effects of sampling different age classes between the two periods or from the effects of length-selective fishing mortality. As demonstrated for *Mustelus antarcticus* (Walker *et al.* 1998), gillnets of 6–7-inch mesh-size used in the intensive shark fishery of southern Australia have the effects of selectively removing large young animals and small old animals from the population. This can give rise to the "phenomenon of apparent change of growth rate" reflected in apparent rather than actual changes in the shape of growth curves (Lee 1920; Ricker 1969). Such changes in a population could also affect maturity ogives.

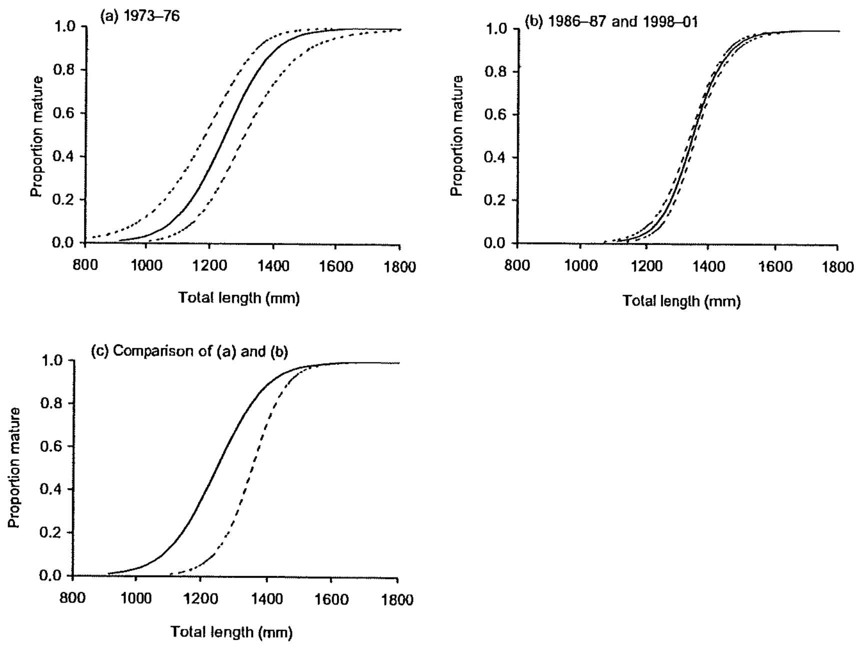


Fig. 4.9 Female length-at-maturity ogives

Proportion of population mature against TL (——), with 95% confidence intervals (- - -), for females sampled during 1973–76 (a), 1986–87 and 1990–01 combined (b), and comparison of the mean ogives for 1973–76 (——) and 1986–87 and 1998–01 (- - -). An animal is defined as mature if the largest ovarian follicle diameter is >3 mm. Values of parameters and statistical quantities for the equation $P = P_{max}(1+e^{-\ln(19)(l-l_{50})/(l_{95}-l_{50})})^{-1}$ determined from probit analysis are given in the following tabulation:

Period	l_{50} *(CI)*	l_{95} *(CI)*	P_{max}	*n*	*N*	*ML*	*P*
1973–76	1244 (1184, 1304)	1458 (1385, 1587)	1.000	59	163	–22.622	***
1986–87, 1990-91	1349 (1339, 1358)	1502 (1487, 1521)	1.000	316	703	–443.147	***

where l is total length measured in millimetres, P is proportion of animals at TL l, l_{50} and l_{95} are parameters, P_{max} is an asymptotic constant, *n* is the total number of animals classed as mature, and *N* is the total number of animals selected in statistical procedure, *ML* is maximum likelihood, and *P* is probability of statistical significance (*P < 0.1; **P < 0.01; ***P < 0.001).

4.6.5 Female Maternity Ogives

Pregnant female *Galeorhinus galeus* tend to aggregate in discrete regions at certain times of the year, so it was important to have wide spatial and temporal sampling for maternal condition. Because the maturity ogive for 1973–76 period was statistically different from the maturity ogive for the 1986–87 and 1998–01 periods combined and because the sample size for maternity was small during 1973–76, only data for the combined periods were used for determining a maternity ogive.

Table 4.8 Number of females sampled in each length-class for maturity condition
An animal is classed as mature where the largest follicle diameter is >3 mm.

Period	*Length-class (mm)*	*No. of animals* Immature	Mature	Total	*Proportion mature*
1973–76	<700	46		46	0.000
	700–799	9		9	0.000
	800–899	17		17	0.000
	900–999	12		12	0.000
	1000–1099	10		10	0.000
	1100–1199	7	1	8	0.125
	1200–1299	3	2	5	0.400
	1300–1399		4	4	1.000
	1400–1499		10	10	1.000
	1500–1599		26	26	1.000
	1600–1699		13	13	1.000
	Total	104	56	160	
1986–87 and 1992–01 combined	<700	9		9	0.000
	700–799	46		46	0.000
	800–899	60		60	0.000
	900–999	54		54	0.000
	1000–1099	52		52	0.000
	1100–1199	43		43	0.000
	1200–1299	71		71	0.000
	1300–1399	67	35	102	0.343
	1400–1499	53	97	150	0.647
	1500–1599	3	86	89	0.966
	1600–1699		24	24	1.000
	Total	458	245	703	

In preparation for probit analysis the females were classed as in either maternal or non-maternal condition for each 100-mm TL-class, which indicated that about one-third of the females in the largest TL-classes were in maternal condition. These data are consistent with the results for the ovarian cycle, which indicate that most animals ovulate every third year. Before weighting the total number in each TL-class by a factor of 3, the total number of animals in the 1500–1599 mm TL-class needed to be adjusted from 90 to 93, so as to adjust the proportion in maternal condition from 0.344 to 0.333 (Table 4.9). This is because each value after weighting for probit analysis must be 1.000 or less.

The probit analysis produced a curve and 95% confidence intervals (CI), which had maximum values of 1.000. These curves were then divided by 3 for the purpose of readjusting the curve back to a maximum value of 0.333. After adjustment, TL at which 50% (l_{50}) and 95% (l_{95}) of the animals were in maternal condition, with 95% CI, and P_{max} derived from the ogives presented in Fig. 4.10 are tabulated as follows.

l_{50} (95% CI) (mm)	l_{95} (95% CI) (mm)	P_{max}
1421 (1423, 1423)	1488 (1484, 1492)	0.333

Table 4.9 Number of females samples in each length-class for maternal and non maternal condition

Animals were classed as in maternal condition if U = 4 (Jan–May), U = 5 (Feb–Dec), or U = 6 (Nov–Dec); otherwise they were classed as in non-maternal condition. For probit analysis the observed value of 90[A] animals in the 1500–1599 mm length-class was increased to 93 animals to prevent the ratio of the number of maternal animals / total number of animals within that length-class from exceeding 0.333.

Length-class	*No. of females for each uterus index*[B]						*No. of females for each condition*			*Proportion in*
(mm)	*1*	*2*	*3*	*4*	*5*	*6*	*Non-maternal*	*Maternal*	*Total*	*maternal condition*
<700	25	7					32	0	32	0.000
700–799	25	47					72	0	72	0.000
800–899	67	13					80	0	80	0.000
900–999	34	32					66	0	66	0.000
1000–1099	42	29					71	0	71	0.000
1100–1199	17	26					43	0	43	0.000
1200–1299	40	33					33	0	33	0.000
1300–1399	32	59	10		1	1	102	1	103	0.010
1400–1499	9	53	57	11	11	9	137	13	150	0.087
1500–1599		2	30	16	29	13	59	31	90[A]	0.344
≥1600			6	6	8	4	16	8	24	0.333
Total	291	301	103	33	49	27	751	53	804	

[B]Defined in Table 4.1

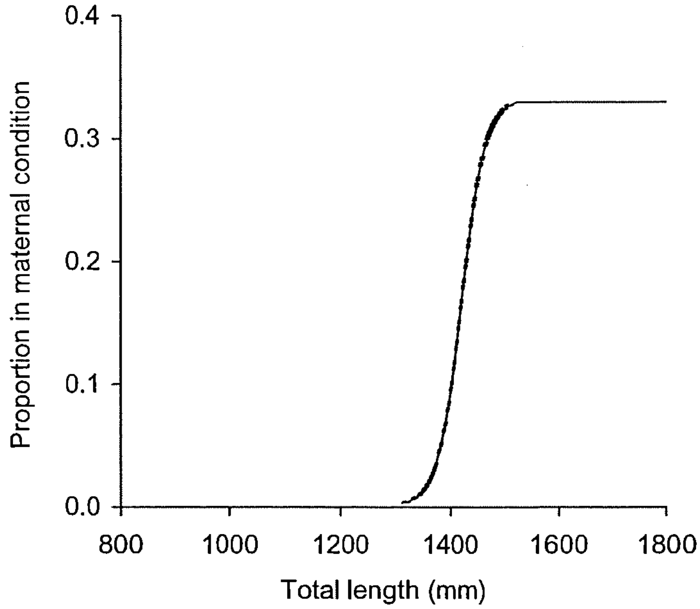


Fig. 4.10 Proportion of female population in maternal condition versus TL
Proportion of female population in maternal condition against TL (——) with 95% con-fidence intervals (- - -) for females during 1986–87 and 1998–01 combined. Values of parameters and statistical quantities for the equation $P_l = P_{max}(1+e^{-\ln(19)(l-l_{50})/(l_{95}-l_{50})})^{-1}$ determined from probit analysis are given in the following tabulation:

l_{50} (*CI*)	l_{95} (*CI*)	P_{max}	*n*	*N*	*ML*	*P*
1421 (1420, 1423)	1488 (1484, 1492)	0.333	53	269	–4953.89	***

where *l* is total length measured in millimetres, P_l is proportion of animals at TL, *l*, l_{50} and l_{95} are parameters, P_{max} is an asymptotic constant, n is the total number of animals classed as in maternal condition, and *N* is the total number of animals selected in statistical procedure, *ML* is maximum likelihood, and *P* is probability of statistical significance (*P < 0.1; **P < 0.01; ***P < 0.001). Animals were classed as being in maternal condition if U = 4 (Jan–May), U = 5 (Feb–Dec), or U = 6 (Nov–Dec).

The TL-at-maternity is considerably larger than TL-at-maturity (Fig. 4.11). If the common practice of weighting the maturity ogive by the frequency of parturition (0.333 for *Galeorhinus galeus*) was adopted the proportion of animals in maternal condition (Fig. 4.12) would be markedly over-estimated. This in turn would create a major bias if applied in a population model by overestimating the number of births. For *G. galeus*, there is a three-year lag between first maturity defined as LFD >3mm and maternal condition, during which time the animals would have undertaken considerable growth. It follows that the greater the time period between maturity and maternity the greater the bias caused by growth of the animals. For this reason it is necessary to estimate the maternity ogive completely independently of the maturity ogive.

Table 4.10 Hypothesis testing for proportion of female population in maternal condition

Logistic regression testing for the effects of period (1973–76, 1986–87 and 1998–01) within the Bass Strait region and effects of region (Bass Strait and South Australia) during 1998–01. Animals were classed as in maternal condition if U = 4 (Jan–May), U = 5 (Feb–Jan) or U = 6 (Nov–Dec); otherwise they were classed as in non-maternal condition. d.f., degrees of freedom; χ^2, chi square; ns, not significant; *P < 0.05; **P < 0.01; ***P < 0.001.

Source of variation	*Number of animals*		χ^2	*P*	
	Maternal	*Non-maternal*			
Between 1973–76, 1986–87, and 1998–01	85	770			
Intercept			1405.916	<0.0001	***
Period			585.460	<0.0001	***
Total length			1725.837	<0.0001	***
Bass Strait between 1973–76 and 1986–87	72	573			
Intercept			862.998	<0.0001	***
Period			97.850	<0.0001	***
Total length			1293.626	<0.0001	***
Bass Strait between 1973–76 and 1998–01	42	328			
Intercept			263.358	<0.0001	***
Period			394.760	<0.0001	***
Total length			400.119	<0.0001	***
Bass Strait between 1986–87 and 1998–01	56	639			
Intercept			1350.949	<0.0001	***
Period			389.728	<0.0001	***
Total length			1619.825	<0.0001	***

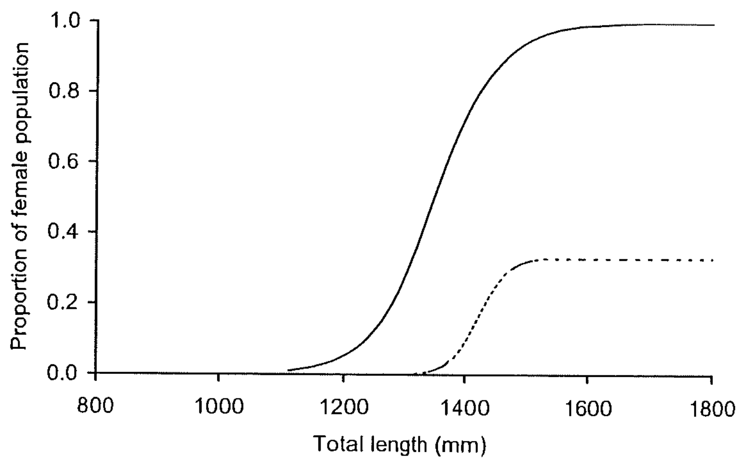


Fig. 4.11 Comparison of maturity and maternity ogives

Proportion of female population in mature condition (——) or maternal condition (- - -) against TL for females from southern Australia during 1986–87 and 1998–01.

4.6.6 Male Maturity Ogives

The results from indices of maturity based on testis, seminal vesicle and clasper condition were compared. Testing for spatial and temporal effects was avoided and the data were pooled between BS and SA and between 1973–76, 1986–87 and 1998–01 (Table 4.11). The reason for pooling the data across all three sampling periods is that the maturity indices require subjective judgement by the field observers and different observers collected the data between the three periods. The reason for pooling the data between the regions is that males are highly migratory and form part of the one population irrespective of where they are captured.

Based on TL at which 50% (l_{50}) and 95% (l_{95}) of the animals were in mature condition, with 95% CI, presented in Fig. 4.13, the testis condition and seminal vesicle condition are in reasonable agreement. The value of l_{50} is 1291 (1276, 1306) mm for testis condition and 1260 (1244, 1276) mm for seminal vesicle condition. The value of l_{50} of 1312 (1301, 1322) mm for clasper condition is larger than the values for the other two methods and the ogive has a different shape (Fig. 4.13). All three methods require subjective judgement but clasper condition is considered the most subjective and the least reliable indicator of maturity for *G. galeus*.

4.6.7 Total Body Mass at Size

The largest female *Galeorhinus galeus* sampled during field operations (1745 mm TL) was longer than the largest male sampled (1628 mm). The highest total body mass of a female recorded (32.3 kg) was more than 50% above the highest mass of a male recorded (21.0 kg).

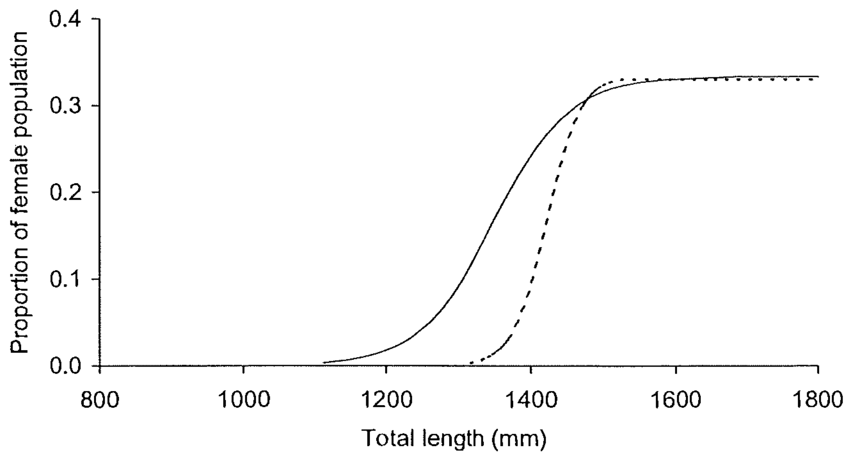


Fig. 4.12 Bias in one-third maturity ogive compared with maternity ogive
Proportion of female population in maternal condition (- - -) and one-third of female population in mature condition (——) against TL for females from southern Australia during 1986–87 and 1998–01.

Table 4.11 Number of males sampled in each length-class by method for maturity
Males were classed as immature for T = 1 or T = 2 and mature for T = 3 testis condition, as immature for V = 1 and mature for V = 2 or V = 3 seminal vesicle condition, and immature for C = 1 or C = 2 and mature for C = 3 clasper condition.

Method	*Length-class (mm)*	*No. of animals for each index*[A]			*No. of animals*			*Proportion mature*
		1	*2*	*3*	*Immature*	*Mature*	*Total*	
Testis condition	<600	35	0	0	35	0	35	0.000
	600–699	39	0	0	39	0	39	0.000
	700–799	59	0	0	59	0	59	0.000
	800–899	74	0	0	74	0	74	0.000
	900–999	65	0	1	65	1	66	0.015
	1000–1099	60	2	1	62	1	63	0.016
	1100–1199	44	14	2	58	2	60	0.033
	1200–1299	18	26	23	44	23	67	0.343
	1300–1399	3	15	51	18	51	69	0.739
	1400–1499	0	1	76	1	76	77	0.987
	1500–1599	0	1	41	1	41	42	0.976
	1600–1699	0	0	3	0	3	3	1.000
	Total	397	59	198	456	198	654	
Seminal vescicle condition	<600	36	0	0	36	0	36	0.000
	600–699	43	0	0	43	0	43	0.000
	700–799	65	0	0	65	0	65	0.000
	800–899	76	0	0	76	0	76	0.000
	900–999	67	1	0	67	1	68	0.015
	1000–1099	62	2	0	62	2	64	0.031
	1100–1199	60	1	1	60	2	62	0.032
	1200–1299	34	25	1	34	26	60	0.433
	1300–1399	6	52	8	6	60	66	0.909
	1400–1499	2	71	10	2	81	83	0.976
	1500–1599	1	42	5	1	47	48	0.979

	1600–1699	0	4	0	0	4	4	1.000
	Total	452	198	25	452	223	675	
Clasper condition	<600	1	0	0	1	0	1	0.000
	600–699	6	0	0	6	0	6	0.000
	700–799	34	13	0	47	0	47	0.000
	800–899	28	1	0	29	0	29	0.000
	900–999	45	3	0	48	0	48	0.000
	1000–1099	37	2	0	39	0	39	0.000
	1100–1199	44	5	0	49	0	49	0.000
	1200–1299	25	19	8	44	8	52	0.154
	1300–1399	5	13	49	18	49	67	0.731
	1400–1499	0	0	59	0	59	59	1.000
	1500–1599	0	0	21	0	21	21	1.000
	1600–1699	0	0	2	0	2	2	1.000
	Total	225	56	139	281	139	420	

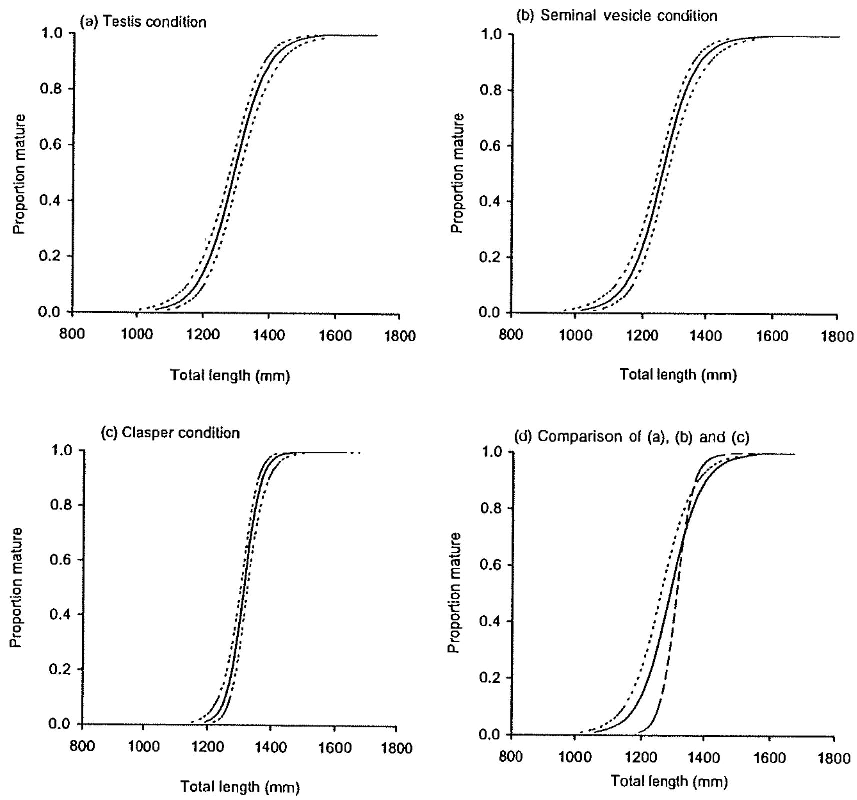


Fig. 4.13 Maturity of males based on testis, seminal vesicle and clasper condition
Proportion of population mature against TL (——) with 95% confidence intervals (- - -) for males determined from testis condition (a), seminal vesicle condition (b), clasper condition (c) and comparison of mean ogives for testis (——), seminal vesicle (- - -) and clasper condition (– – –) (d). Males were classed as immature for T = 1 or T = 2 and mature for T = 3 testis condition, as immature for V = 1 and mature for V = 2 or V = 3 seminal vesicle condition, and immature for C = 1 or C = 2 and mature for C = 3 clasper condition. Values of parameters and statistical quantities for the equation $P = P_{max}(1+e^{-\ln(19)(l-l_{50})/(l_{95}-l_{50})})^{-1}$ determined from probit analysis are given in the following tabulation:

Method	l_{50} (*CI*)	l_{95} (*CI*)	P_{max}	*n*	*N*	*ML*	*P*
Testis	1291 (1276, 1306)	1439 (1413, 1473)	1.000	198	566	−146.841	***
Seminal vesicle	1260 (1244, 1276)	1415 (1389, 1449)	1.000	223	585	−141.998	***
Clasper	1312 (1301, 1322)	1388 (1372, 1413)	1.000	139	357	−80.727	***

where *l* is total length measured in millimetres, *P* is proportion of animals at TL *l*, l_{50} and l_{95} are parameters, P_{max} is an asymptotic constant, *n* is the total number of animals classed as mature, and *N* is the total number of animals selected in statistical procedure, *ML* is maximum likelihood, and *P* is probability of statistical significance (*P < 0.1; **P < 0.01; ***P < 0.001).

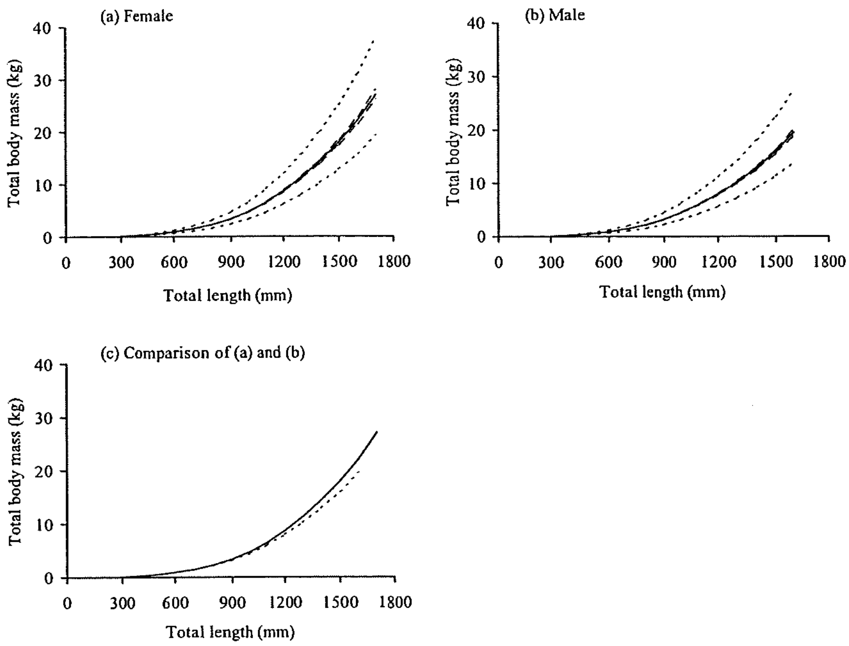


Fig. 4.14 Relationships between total body mass and total length.
Plots of mean total mass against TL (——), with 95% confidence limits (– – –) and 95% prediction intervals (- - -), for females (a), males (b), and comparison of the mean curves for females (——) and males (- - -) (c) in southern Australia during the periods 1973–76, 1986–87 and 1995–01 combined. Values for parameters and statistical quantities from linear regression analysis to derive the equation $w = acl^b$ are given in the following tablulation:

Sex	*a* (*s.e. range*) x 10^{-9}	*b*(*se*)	*c*	*n*	r^2	*rmse*	*P*
Females	0.699 (0.592–0.825)	3.276 (0.024)	1.015	401	0.979	0.172	***
Males	1.810 (1.530–2.150)	3.129 (0.024)	1.015	355	0.979	0.173	***

where *w* is total body mass, *l* is total length, *a* and *b* are parameters, *c* is the Beauchamp and Olson (1973) correction factor, *n* is sample size, r^2 is square of correlation coefficient and *rmse* is root mean square error, and P is probability of statistical significance (*P < 0.1; **P < 0.01; ***P<0.001) for the regression equation ln(*w*) = a+bln(*l*).

Statistical comparison of the slopes and intercepts by the Student *t*-test for selected pairs of straight line ln(*w*)–ln(*l*) relationships determined from linear regression fits indicate that two of the relationships tested were significantly different. The ln(*w*)–ln(*l*) relationship for pregnant females carrying *in utero* eggs (U = 4) and the relationship for pregnant females carrying *in utero* embryos (U = 5) were not significantly different (t-test, $t = 1.410$, d.f. = 58, and $P > 0.05$ for comparison of slopes and $t = 1.405$, d.f. = 58, and $P > 0.05$ for comparison of elevations). Similarly, the ln(*w*)–

$\ln(l)$ relationship for pregnant females (U = 4 and U = 5 pooled) and the relationship for non-pregnant females (U = 1, U = 2, U = 3, and U = 6 pooled) were not significantly different (t-test, $t = 0.958$, d.f. = 399, and $P > 0.05$ for comparison of slopes and $t = 0.987$, d.f. = 399, and $P > 0.05$ for comparison of elevations). The $\ln(w)$–$\ln(l)$ relationship for females and the relationship for males were highly significantly different (t-test, t=4.285, d.f. = 754, and $P < 0.001$ for comparison of slopes, and $t = 4.030$, d.f. = 754, and $P < 0.001$ for comparison of elevations). Hence, the relationships of total body mass against TL, with 95% confidence limits on the mean curves and 95% prediction intervals are presented separately for females and males. These curves indicate that for a given length, the mean body mass is higher for females than for males (Fig. 4.14).

It is common among chondrichthyan species for females to attain higher TL and total body mass than males (Klimley 1987; Jakobsdóttir 2001; Simpfendorfer *et al.* 2001). For *Galeorhinus galeus*, the curves for these relationships coincide for low TL but diverge with increasing TL. In some species, for a given TL in the size range for mature animals, the mean body mass is higher for pregnant females than for non-pregnant females and is higher for non-pregnant females than for males (unpublished data). This is consistent with increasing mass of the ovaries and perhaps liver in mature females and the presence of *in utero* eggs and embryos in pregnant females.

4.7 CONCLUSIONS FOR *GALEORHINUS GALEUS*

Several important conclusions can be drawn from application of the methods described in this chapter to *Galeorhinus galeus*.

1. The results obtained for maturity and maternity are dependent on explicit definitions of terms. A definition of female maturity based on the diameter of the largest ovarian follicle provides an objective criterion unlikely to be affected by the field observer. Similarly, a definition of maternity based on uterus condition provides objective criteria unlikely to be affected by the field observer, other than occasional uncertainty distinguishing between the U = 3 and U = 6 conditions. A definition of male maturity is more problematic; maturity based on seminal vesicle, testis, and clasper condition all require a degree of judgement.
2. Pregnant condition of a female is defined by the presence of *in utero* eggs or embryos; non-pregnant condition of a female is defined by the absence of *in utero* eggs and embryos. A female observed at any time of the year is in maternal condition if it is in pregnant condition and expected to give birth prior to 1 January or if it is in post-partum condition and recently gave birth prior to 1 January (November–December). Any other female observed is in non-maternal condition (Fig. 4.15).

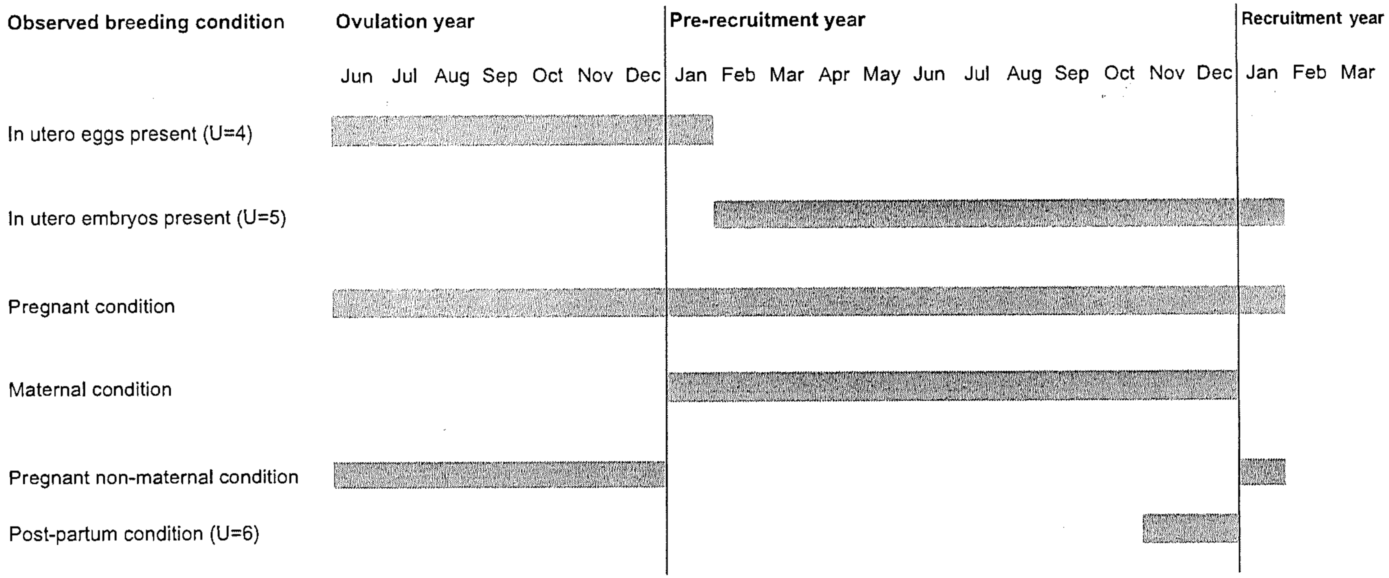


Fig. 4.15 Synchrony of breeding condition in *Galeorhinus galeus*
The U = 6 condition can occur at any time but only Nov–Dec is presented when they are in maternal condition.

3. Females attain a higher TL (1745 mm) and body mass (32.3 kg) than do males (1628 mm, 21.0 kg) and, for a given TL, the mean body mass is higher for females than for males; mass is not significantly affected by breeding condition.
4. Ovarian follicles have diameters ranging 1–57 mm and ovary mass can reach 1880 g in a pregnant animal during the early stages of ovulation and 910 g in a pregnant animal with close to full-term embryos. Ovary mass is mostly less than 73 g. A correlation between hepatic somatic index (liver mass/total body mass) (HSI) and largest follicle diameter indicates liver mass increases during the process of vitellogenesis. The highest HSI occurs in ovulating animals, which suggests liver mass and presumably its lipid content increase prior to pregnancy.
5. The ovarian cycle in most mature females has a period of three years. It is expected that the ovarian cycle of an individual is synchronous with one-third of the population of mature females; another one-third of the population is out of phase and ahead by one year and the other one-third is out of phase and ahead by two years (Fig. 4.16).
6. *In utero* eggs without macroscopically visible embryos are present during the eight-month period June–January; most animals observed in this condition are ovulating, suggesting that the process of ovulation in an individual is several months duration.
7. Macroscopically visible embryos are present during the 12-month period February–January and most attain a size of more than 300 mm TL at full-term; wet mass gain from egg to full-term is about 100%.
8. The number of *in utero* embryos range 15–43 and increases linearly with maternal length; this relationship is not affected by sampling region or sampling period.
9. The sex ratio of embryos is 1:1, the number *in utero* embryos in the left uterus is not significantly different from the number in the right uterus, and 3.4% of oocytes ovulated remain infertile in the uterus during pregnancy.
10. Parturition frequency for an individual female is mostly triennial. It is expected that an individual is synchronous with about one-third of the female population having ovulated at least once (having reached maternity); another one-third of the population is out of phase by one year and the other one-third is out of phase by two years (Fig. 4.16). Natural mortality can be expected to marginally increase the proportion in the population in maternal condition.
11. Female TL-at-maturity determined from diameter of the largest ovarian follicle appears to have increased between 1973–76 and 1986–87 with no change between 1986–87 and 1998–01.
12. The TL-at-maternity (l_{50} = 1421 mm, l_{95} = 1488 mm, and P_{max} = 0.333) is considerably larger than TL-at-maturity (l_{50} = 1349 mm, l_{95} = 1502 mm, and P_{max} = 1.000) for the periods 1986–87 and 1998–01 combined.

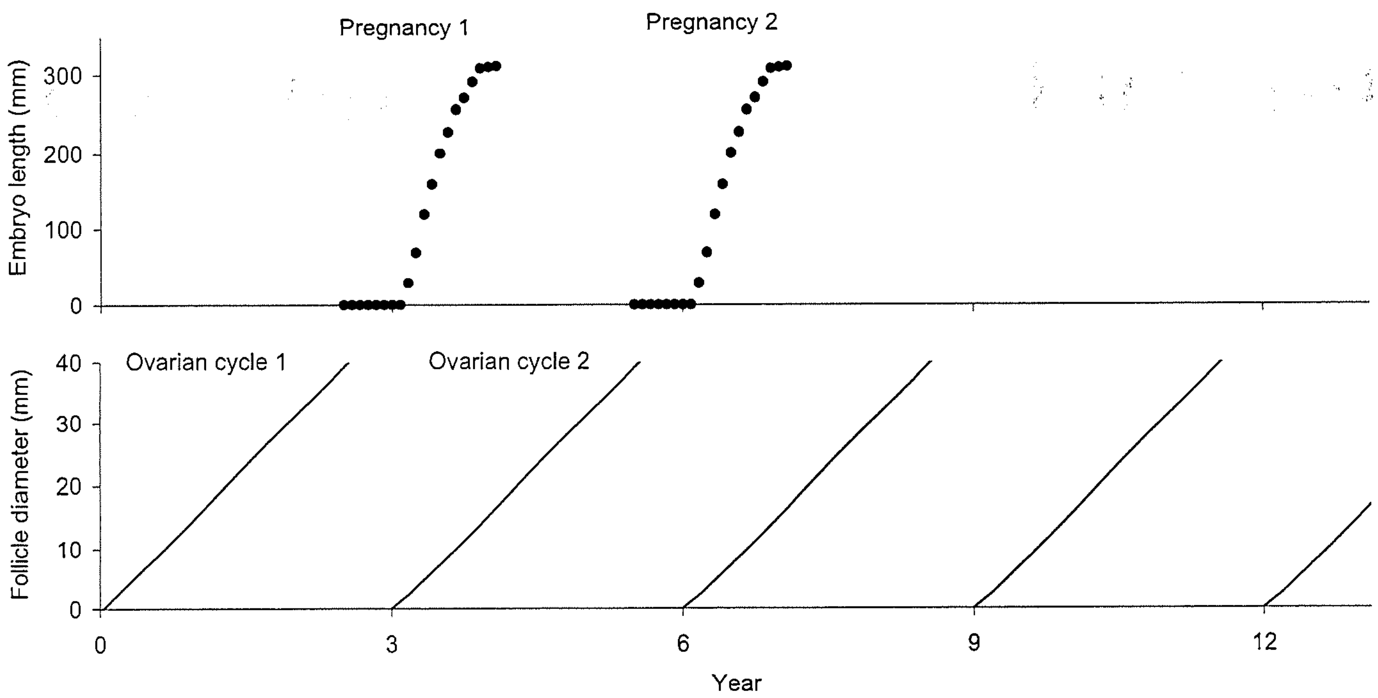


Fig. 4.16 Periodicity of the ovarian cycle and gestation for a mature animal of *Galeorhinus galeus*
The ovarian cycle and frequency of parturition are triennial; it is 4 years from the beginning of vitellogenesis for a particular follicle to full-term embryo.

Weighting the maturity ogive by the frequency of parturition (0.333 for *G. galeus*), as is commonly practised, would markedly over-estimate recruitment in any population dynamics model. It is essential to estimate the maternity ogive completely independently of the maturity ogive.

13. Male TL-at-maturity determined from indices of maturity based on macroscopic inspection of testis condition, seminal vesicle condition and clasper condition is highest for clasper condition (l_{50} = 1312 mm, l_{95} = 1388 mm, and P_{max} = 1.000). The shape of the TL-at-maturity ogives for testis condition (l_{50} = 1291 mm, l_{95} = 1439 mm, and P_{max} = 1.000) is similar but higher than for seminal vesicle condition (l_{50} = 1260 mm, l_{95} = 1415 mm, and P_{max} = 1.000). The data are pooled across regions and sampling period because males are highly migratory, often aggregate by size and breeding condition, and methods of determining maturity are subjective and likely to vary between observers.

4.8 ACKNOWLEDGMENTS

Acknowledgment is due to Lauren P. Brown, Russell J. Hudson, Dr Ian A. Knuckey, Peter L. Moulton, and Stephen R. Saddlier, formerly or presently of Primary Industries Research Victoria (PIRVic), who participated in field sampling of sharks and laboratory processing. Anne S. Gason of PIRVic provided advice on statistical analysis of the data and application of the computer statistical package SAS. Professor William C. Hamlett of the Indiana University School of Medicine is thanked for valuable discussions. The Fisheries Research and Development Corporation in Australia contributed most of the funding through several projects.

4.9 LITERATURE CITED

Beauchamp, J. J. and Olson, J. S. 1973. Corrections for bias in regression estimates after logarithmic transformation. Ecology 54: 1403–1407.

Capapé, C. and Mellinger, J. 1988. Nouvelles données sur la biologie de la reproduction du milandre, *Galeorhinus galeus* (Linné, 1778), (Pisces, Triakidae) des côtes tunisiennes. Cahiers de Biologie Marine 29: 135–146.

Cody, R. P. and Smith, J. K. 1997. *Applied Statistics and the SAS Programming Language*. Prentice Hall, Upper Saddle River, NJ. 445 pp.

Compagno, L. J. V. 1984. FAO species catalogue. Vol. 4. Sharks of the world. An annotated and illustrated catalogue of shark species known to date. Part 2. Carcharhiniformes. FAO Fisheries Synopsis 125: 251–655.

Conrath, C. L. and Musick, J. A. 2002. Reproductive biology of the smooth dogfish, *Mustelus canis*, in the northwest Atlantic Ocean. Environment Biology of Fishes 64: 367–377.

Girard, M. and Du Buit, M.-H. 1999. Reproductive biology of two deep-water sharks from the British Isles, *Centroscymnus coelolepis* and *Centrophorus squamosus*

(Chondrichthyes: Squalidae). Journal of the Marine Biological Association of the United Kingdom 79: 923–931.

Grant, C. J., Sandland, R. L. and Olsen, A. M. 1979. Estimation of growth, mortality and yield per recruit of the Australian school sharks, *Galeorhinus australis* (Macleay), from tag recoveries. Australian Journal of Marine and Freshwater Research 30: 625–637.

Guallart, J. and Vincent, J. J. 2001. Changes in composition during embryo development of the gulper shark, *Centrophorus granulosus* (Elasmobranchii, *Centrophoridae*): An assessment of maternal-embryonic nutritional relationships. Environmental Biology of Fishes 61: 135–150.

Hamlett, W. C. 1999. Male reproductive system. Pp. 444–470. In W. C. Hamlett (ed.), *Sharks, Skates and Rays: The Biology of Elasmobranch Fishes*. The Johns Hopkins University Press, Baltimore, MD.

Hamlett, W. C. and Koob, T. J. 1999. Female reproductive system. Pp. 398–443. In W. C. Hamlett (ed.), *Sharks, Skates and Rays: The Biology of Elasmobranch Fishes*. The Johns Hopkins University Press, Baltimore, MD.

Hamlett, W. C., Musick, J. A., Hysell, C. K. and Sever, D. M. 2002. Uterine epithelial-sperm interaction, endometrial cycle and sperm storage in the terminal zone of the ovicucal gland in the placental smoothhound, *Mustelus canis*. Journal of Experimental Zoology 292: 129–144.

Hanchet, S. 1988. Reproductive biology of *Squalus acanthias* from the east coast, South Island, New Zealand. New Zealand Journal of Marine and Freshwater Research 22: 537–549.

Hilton-Taylor, C. 2000. *2000 IUCN Red List of Threatened Species*. IUCN The World Conservation Union, Gland, Switzerland. 61 pp.

Hurst, R. J., Bagley, N. W., McGregor, G. A. and Francis, M. P. 1999. Movement of the New Zealand school shark, *Galeorhinus galeus*, from tag returns. New Zealand Journal of Marine and Freshwater Research 33: 29–48.

Jakobsdóttir, K. B. 2001. Biological aspects of two deep-water squalid sharks: *Centroscyllium fabricii* (Reinhardt, 1825) and *Etmopterus princeps* (Collett, 1904) in Icelandic waters. Fisheries Research 51: 247–265.

Jones, T. S. and Ugland, K. I. 2001. Reproduction of female spiny dogfish, *Squalus acanthias*, in the Oslofjord. Fishery Bulletin 99: 685–690.

Kleinbaum, D. G., Kupper, L. L. and Muller, K. E. 1988. *Applied regression analysis and other multivariable methods*. PWS–Kent Publishing Company, Boston, MA. 718 pp.

Klimley, A. P. 1987. The determinants of sexual segregation in the scalloped hammerhead shark, *Sphyrna lewini*. Environmental Biology of Fishes 18: 27–40.

Lee, R. M. 1920. A review of the methods of age and growth determination in fishes by means of scales. Fishery Investigations Ser. 2 , 4: 1–32.

Lenanton, R. C. J., Heald, D. I., Platell, M., Cliff, M. and Shaw, J. 1990. Aspects of the reproductive biology of the gummy shark, *Mustelus antarcticus* Günther, from waters off the south coast of Western Australia. Australian Journal of Marine and Freshwater Research 41: 807–822.

Lucifora, L. O. 2003. Ecología y conservación de los grandes tiburones costeros de Bahía Anegarda, Provincia de Buenos Aires, Argentina. Ph.D. Thesis, Universidad Nacional de Mar del Plata, Mar del Plata, Argentina. 406 pp.

Mellinger, J. 1965. Stades de la spermatogenese cher *Scyliorhinus caniculus* (L.): description, donnees histochimigues, variations normales et experimentales. Zeitschrift fur Zellforschung 67: 653–673.

Mollet, H. F., Cliff, G., Pratt Jnr, H. L. and Stevens, J. D. 2000. Reproductive biology of the female shortfin mako, *Isurus oxyrinchus* Rafinesque, 1810, with comments on the embryonic development of lamnoids. Fishery Bulletin 98: 299–318.

Moulton, P. M., Walker, T. I. and Saddlier, S. R. 1992. Age and growth studies of gummy shark, *Mustelus antarcticus* Günther, and school shark, *Galeorhinus galeus* (Linnaeus), from southern-Australian waters. Australian Journal of Marine and Freshwater Research 43: 1241–1267.

Olsen, A. M. 1954. The biology, migration, and growth rate of the school shark, *Galeorhinus australis* (Macleay) (Carcharhinidae) in south-eastern Australian waters. Australian Journal of Marine and Freshwater Research 5: 353–410.

Parsons, G. R. and Grier, H. J. 1992. Seasonal changes in shark testicular structure and spermatogenesis. Journal of Experimental Zoology 261: 173–184.

Peres, M. B. and Vooren, C. M. 1991. Sexual development, reproductive cycle, and fecundity of the school shark *Galeorhinus galeus* off southern Brazil. Fishery Bulletin 89: 655–667.

Punt, A. E., Pribac, F., Walker, T. I., Taylor, B. L. and Prince, J. D. 2000. Stock assessment of school shark, *Galeorhinus galeus*, based on a spatially-explicit population dynamics model. Marine and Freshwater Research 51: 205–220.

Punt, A. E. and Walker, T. I. 1998. Stock assessment and risk analysis for the school shark (*Galeorhinus galeus*) off southern Australia. Marine and Freshwater Research 49: 719–731.

Rao, C. R. 1973. *Linear Statistical Inference and Its Applications*. John Wiley and Sons, New York, NY. 265 pp.

Ricker, W. E. 1958. Handbook of computations for biological statistics of fish populations. Bulletin of Fisheries Research Board of Canada 119: 1–191.

Ricker, W. E. 1969. Effects of size-selective mortality and sampling bias on estimates of growth, mortality, production, and yield. Journal of the Fisheries Research Board of Canada 26: 479–541.

Ripley, W. E. 1946. The soupfin shark and the fishery. California Division of Fish and Game Fish Bulletin 64: No. 64, 7–37.

Silvey, S. D. 1975. *Statistical Inference*. Chapman and Hall, London, UK. 191 pp.

Simpfendorfer, C. A., Goodreid, A. B. and McAuley, R. B. 2001. Size, sex and geographic variation in the diet of the tiger shark, *Galeocerdo cuvier*, from Western Australian waters. Environmental Biology of Fishes 61: 37–46.

Smith, R. M., Walker, T. I. and Hamlett, W. C. 2004. Microscopic organization of the oviducal gland of the holocephalan elephant fish, *Callorhynchus milii*. Marine and Freshwater Research 55: 155–164.

Storrie, M. T. 2004. Microscopic modifications of the reproductive tissues of the female gummy shark (*Mustelus antarcticus*) throughout maturation and gestation. Ph.D. Thesis, Deakin University, Warrnambool, Victoria, Australia. 153 pp.

Teshima, K. and Koga, S. 1973. Studies on sharks. V. Taxonomic characteristics of reproductive organs in Japanese *Mustelus*. Marine Biology 23: 337–341.

Walker, T. I. 1983. Investigations of the gummy shark, *Mustelus antarcticus* Günther, from south-eastern Australian waters, Report to Fishing Industry Research Committee. June 1983. Pp. 1–94. In A. E. Caton (ed.), *Proceedings of the Shark Assessment Workshop, South East Fisheries Committee Shark Research Group*. 7–10 March 1983. Melbourne. Department of Primary Industry, Canberra, ACT, Australia.

Walker, T. I. 1992. A fishery simulation model for sharks applied to the gummy shark, *Mustelus antarcticus* Günther, from southern Australian waters. Australian Journal of Marine and Freshwater Research 43: 195–212.

Walker, T. I. 1994. Fishery model of gummy shark, *Mustelus antarcticus*, for Bass Strait. Pp. 422–438. In I. Bishop (ed.), *Resource Technology '94 New Opportunities Best Practice*. 26–30 September 1994. University of Melbourne, Melbourne. The Centre for Geographic Information Systems & Modelling, The University of Melbourne, Melbourne, Australia.

Walker, T. I. 1999. *Galeorhinus galeus* fisheries of the world. In 'Case studies of management of elasmobranch fisheries'. FAO Fisheries Technical Paper 378/2: 728–773.

Walker, T. I. 2004. Chapter 13. Management measures. Pp. 285–321. In J. A. Musicki and R. Bonfil (eds), *Technical Manual for the Management of Elasmobranchs*. Asia Pacific Economic Cooperation Secretariat, Singapore.

Walker, T. I., Taylor, B. L., Hudson, R. J. and Cottier, J. P. 1998. The phenomenon of apparent change of growth rate in gummy shark (*Mustelus antarcticus*) harvested off southern Australia. Fisheries Research 39: 139–163.

Ward, R. D. and Gardner, M. G. 1997. Stock structure and species identification of school and gummy sharks in Australasian waters. Project FRRF 93/11 and FRDC 93/64. February 1997. CSIRO Marine Research, Hobart, Tasmania, Australia. 92 pp.

West, G. J. and Stevens, J. D. 2001. Archival tagging of school shark, *Galeorhinus galeus*, in Australia: Initial results. Environmental Biology of Fishes 60: 283–298.

Wood, C. C., Ketchen, K. S. and Beamish, R. J. 1979. Population dynamics of spiny dogfish (*Squalus acanthias*) in British Columbia waters. Journal of the Fisheries Research Board of Canada 36: 647–656.

Yano, K. and Tanaka, S. 1988. Size at maturity, reproductive cycle, fecundity and depth segregation of the deep sea squaloid sharks *Centroscymnus owstoni* and *C. coelolepsis* in Surga Bay, Japan. Nippon Suisan Gakkaishi 54: 167–174.

Appendix 3g: Elephant fish papers

This appendix contains three papers published in scientific journals, which provide valuable descriptions of the reproductive structures of male and female elephant fish to better understand their structure and function.

Mar. Freshwater Res., 2002, **53**, 591–600

Microanatomy of spermatophore formation and male genital ducts in the holocephalan, *Callorhynchus milii*

Matt B. Reardon[A], *Terence I. Walker*[B], *and William C. Hamlett*[C]

[A]Department of Zoology, University of Melbourne, Parkville, Vic. 3052, Australia
[B]Marine and Freshwater Resources Institute, PO Box 114, Queenscliff, Vic. 3225, Australia
[C]South Bend Center for Medical Education, Indiana University School of Medicine, Notre Dame, IN 46556, USA

Abstract. The present study is the first to consider male genital tract histology and spermatophore development among the holocephalans. Spermatogenesis in *Callorhynchus milii* occurs in a spermatocyst containing sperm laterally embedded in the apex of Sertoli cells. Individual sperm occur in the proximal tubule of the epididymis where they commence to associate into laterally aligned bundles. The Leydig gland, a modified anterior mesonephros, lies laterally to the genital ducts and supplies a seminal matrix to the epididymis and ductus deferens. The epididymis consists of a thin-walled large lumen with a smooth epithelium of simple ciliated columnar cells and secretory pyriform cells. The lumen contains a flocculent granular seminal matrix with a peripheral area of hyalin material. Sperm are present in the ductus deferens as bundles embedded in an ovoid-to- elongate spermatophore matrix with a distinct cortex and medulla. The epithelium of the ductus deferens consists of simple ciliated columnar cells and secretory pyriform cells. Fully formed spermatophores occur in the seminal vesicle where laterally aligned sperm are embedded in a matrix. The development of spermatophores as a mode of sperm storage may reflect a breeding strategy that is both opportunisitic and competitive.

Introduction

The structure of the reproductive tracts of male chondrichthyans is of interest to evolutionary biologists and comparative anatomists, since it presents an opportunity to study arguably archetypal traits. Chondrichthyans have an evolutionary line separate from that of other vertebrates, extending some 400 million years, and with many apparently conserved features (Tricas *et al.* 1997), thus allowing us to make inferences about the evolution of reproductive modes in other vertebrate groups.

There is, however, a dearth of knowledge about the morphology and function of these tracts in chondrichthyans, particularly holocephalans. Most studies of reproduction in male chondrichthyans have involved elasmobranchs; furthermore, testis structure and spermatogenesis have been the focus of the majority of these studies (Bols *et al.* 1980; Callard 1991*a*, 1991*b*; Parsons and Grier 1992; Rossouw 1995). Little attention has been given to the diversity of sperm maturation, distribution and storage in the post-testicular ducts. Studies of these reproductive structures may contribute to an understanding of why chondrichthyans have been such a success in evolutionary terms.

Sperm are formed in chondrichthyans through seven stages of spermatogenesis that occur within independent, migratory cysts, called spermatocysts. These spermatocysts, each containing a cohort of a Sertoli cell and germ cells, migrate from the germinal zone to the terminal zone of the testis, where spermatids are generally released into the epididymis via efferent ductules and the Sertoli cell is lost. This contrasts to the strategy employed in mammals, amphibians, reptiles and birds where spermatogenesis occurs within seminiferous tubules that release free spermatozoa into their lumen at spermiation, with the Sertoli cell being conserved for multiple cohorts of spermatozoa (Callard 1991).

The epididymis in mammals is generally divided into a head, midpiece and tail region, each of which is responsible for a different contribution to sperm maturation and storage. The degree of specialization of each zone is much less in chondrichthyans, where the epididymis is responsible for initial stages of sperm maturation, with maturation continuing through the ductus deferens, before sperm is stored in the seminal vesicle (Jones 1998).

Male chondrichthyans display a wide variety of modes of sperm aggregation, storage and dispersal (Pratt and Tanaka 1994); the aggregates include single-layered or compound spermatozeugmata, in which the sperm tails protrude from the matrix, and spermatophores, in which clumped sperm are embedded in a medulla with a distinct cortical region.

 10.1071/MF01130 1323-1650/02/020591

The formation of spermatophores in chondrichthyans is not understood. A study on the basking shark, *Cetorhinus maximus*, suggests that the secretions of the epithelium of the genital ducts and associated Leydig gland are responsible for the matrix of the spermatophores, with the shape being determined by physical action within the seminal vesicle (Matthews 1950).

The formation and structure of spermatophores have not previously been described in holocephalans. An early study of *Callorhynchus antarcticus* (local synonym for *C. milii* (Last and Stevens 1994)) indicated that sperm are stored in spermatophores (Parker and Haswell 1897). The authors noted the presence of spermatophores, without explanation of their structure or formation. Spermatozeugmata in *Hydrolagus colliei* are the only sperm aggregates to have been described in holocephalans (Pratt and Tanaka 1994).

Glycogen stores in the midpiece of sperm are quite large in *H. colliei* compared with those in elasmobranchs (Stanley 1983). This, coupled with sperm aggregation and sperm storage in females, may reflect an opportunistic breeding strategy in populations that may be sexually stratified. The present study was undertaken to determine what particular strategy is employed by the elephant fish, *Callorhynchus milii*, in its sperm development, storage and dispersal, and what regions of the post-testicular reproductive tract contribute to this process.

Materials and methods

Fish collection

Three male *C. milii* were obtained for detailed histological study. Two caught by commercial gill-net vessels in Bass Strait (39–41°S,144–148°E) in October 2000 were kept live in tanks on board the fishing vessels and killed by blunt trauma to the chondrocranium after the vessel reached port. The urogenital tracts were immediately removed and placed in 4% neutral buffered formaldehyde (NBF). A third fish had previously been preserved in 4%NBF at MAFRI,

Light microscopy

Tissues from the testis, epididymis, ductus deferens, seminal vesicle and Leydig gland were washed in fresh water, dehydrated through a series of progressively higher ethanol concentrations and embedded in Paraplast (paraffin/plastic polymer) in an LX 120 Tissue Processor. Embedded tissue was sectioned at 3 υ with a Microm HM325 microtome. Tissue was also sectioned at 1 υ on a Reichert Ultracut S ultra-microtome. Sections were fixed to glass slides by drying for 12 h in an incubator at 50°C. Sections were stained with Harris haemotoxylin and eosin (H&E) to show nuclei, or with Mallory's Triple Stain (MTS) for connective tissue, or with combined Periodic Acid–Schiff and Alcian Blue stain (PAS/AB) for mucopolysaccharides, or with non-sulfated acid glycosaminoglycans and toluidine blue (TB) for contrast on the 1 υ sections. Cover-slips were affixed with DPX mounting agent. Sections were photographed on an Olympus BX50 microscope (B201) with an Olympus PM-C35DX camera attachment, using an Olympus PM-30 Exposure Control Unit.

Scanning electron microscopy (SEM)

Tissues from the epididymis, ductus deferens, seminal vesicle and Leydig gland were stored in 4% NBF, washed in fresh water, sectioned with a razor blade, dehydrated through an ethanol series, dried with hexamethyldisilazane (HMDS), mounted on stubs with SPI high-purity silver paint, sputter coated with gold under vacuum, then scanned with a Philips SEM 505 scanning electron microscope. Images from the SEM were captured with 'Spectrum' PC software.

Results

Urogenital tract

The urogenital tract of a male *C. milii* is illustrated in Fig. 1. The paired elongate testes are suspended from the dorsal abdominal cavity by a visceral peritoneum, the mesorchium. Sperm proceed through the testis via a spermatocyst containing laterally aligned sperm bundles (Fig. 2). Efferent ductules course through the mesorchium to the epididymis, but it was not possible to dissect these ducts owing to their small size.

The epididymis, lying laterally to the testis and covered by a visceral peritoneum, is highly convoluted in mature males, with a relatively small luminal diameter and no apparent regionalization into head, body and tail. It is confluent with the ductus deferens.

The ductus deferens has a larger diameter than the epididymis, and is less convoluted; it narrows abruptly to form a connective tubule that extends to the seminal vesicle. This connective tubule is indistinct from mid winter through early spring, but becomes apparent when the seminal vesicle enlarges as it is filled with seminal fluid and spermatophores (Reardon, unpublished). The seminal vesicle has a large diameter, and the walls have a thick smooth-muscle layer. The vesicle stores mature spermatophores suspended in highly viscous seminal fluid. Mesonephric ducts secrete urine into the genital ducts in immature males, but when the male matures they are modified into Leydig gland tubules and contribute to the seminal matrix. The epididymis and ductus deferens are laterally embedded in the Leydig gland proximal to the testis, and, distally, the seminal vesicle is embedded in the kidney. The epididymis and ductus deferens receive secretions from the Leydig gland via secretory tubules.

Leydig gland

The Leydig gland (Fig. 3) is a continuous small-diameter tubule. The epithelium is smooth with secretory simple columnar cells interspersed with ciliated pyriform cells with distinct basal bodies. Microvilli are present at the apex of both cell types. Fig. 3(*D*) shows a continuous folding tubule in which the extent of secretory activity is variable throughout the length of the tubule. All sections of the tubule show secretory cells, but these have an activity cycle; some have euchromatic chromosomes (a period of protein synthesis and secretion), and others have heterochromatic chromosomes (latency during recovery). Supranuclear secretory vesicles are evident. Secretions of the Leydig gland are distinctly PAS+. Similar PAS+ material is seen in the lumen of the epididymis and ductus deferens.

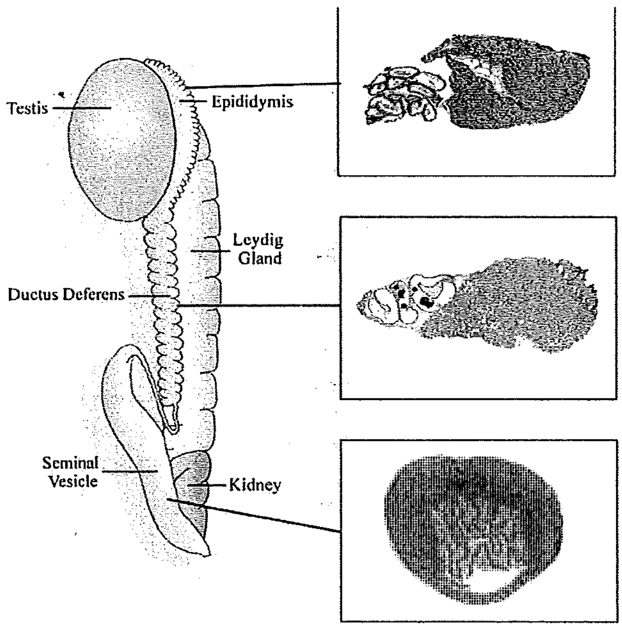


Fig. 1. Diagrammatic representation of the urogenital duct, with cutaway sections showing the internal structure of the major regions of the ducts.

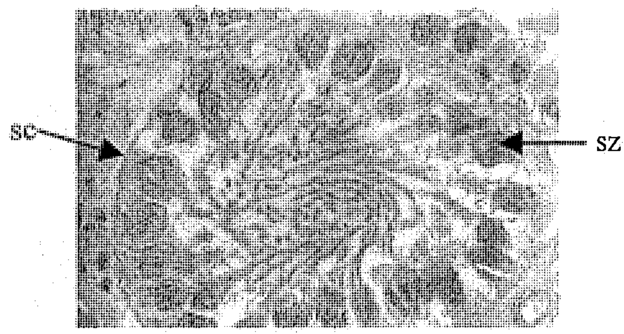


Fig. 2. Spermatocyst (sc) at stage 7, containing spermatozoa (sz) prior to release at spermiation (H&E).

Epididymis

The epididymis (Fig. 4) consists of a narrow, highly convoluted tubule with a smooth unfolded epithelium. A thin sheath of collagen with interstitial fibroblasts surrounds the tubule. The epithelium consists of simple columnar cells interspersed with ciliated pyriform cells. Microvilli are present at the apex of all cell types. There are distinct apical secretory vesicles present in the epithelium, indicating that the epididymis contributes to the luminal matrix. Nuclei of

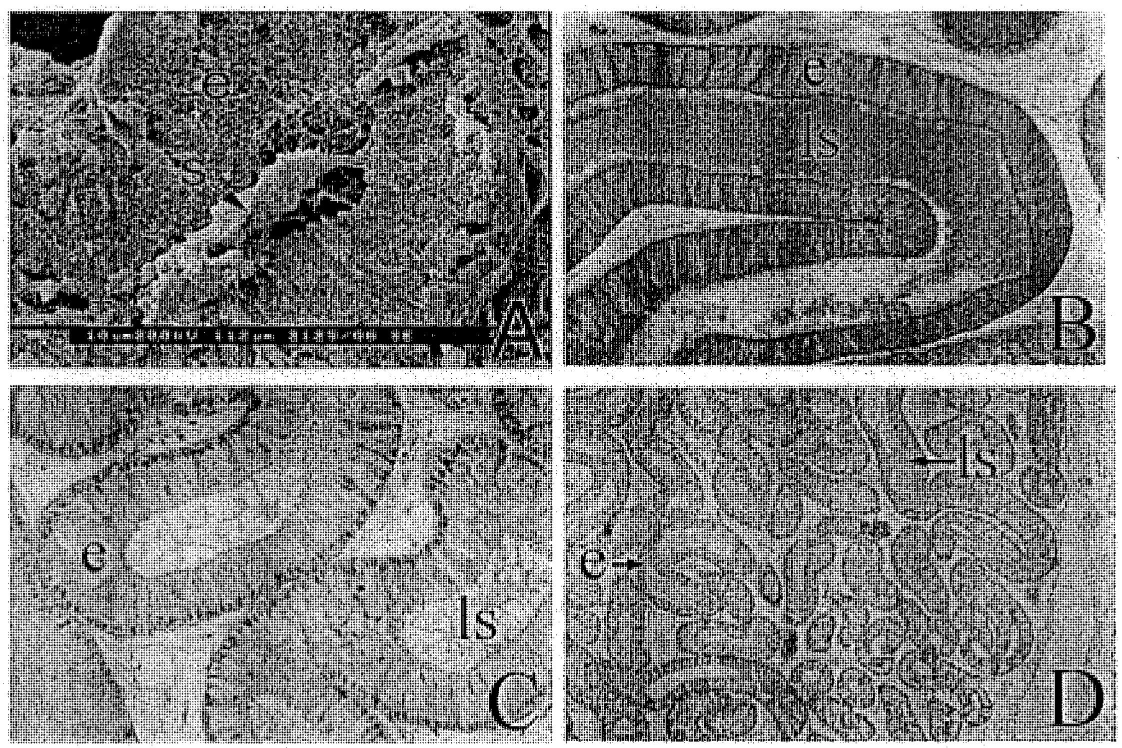


Fig. 3. Tubules of the Leydig gland in *C. milii.* *(A)* SEM: secretion (s) of the epithelium (e) into the lumen. *(B)* PAS+ Leydig gland secretions (ls) in the lumen of the tubule contributes to the seminal matrix of the genital ducts (PAS/AB). *(C)* Period of latency (PAS/AB). *(D)* Variation in secretory activity; differing secretion density in different regions of the tubules (PAS/AB).

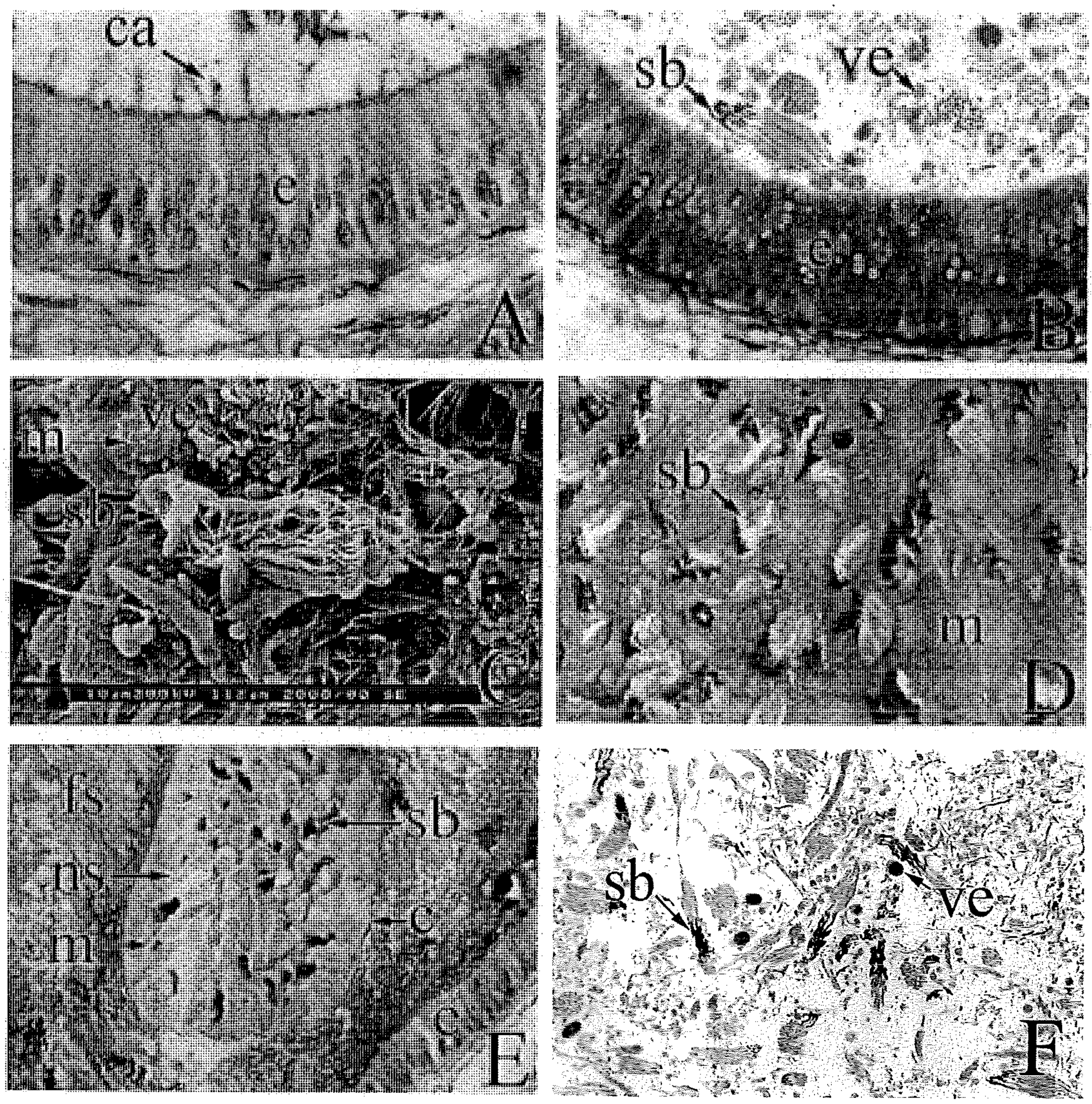


Fig. 4. Epididymis of *C. milii*. *(A)* Simple columnar epithelium (e) interspersed with ciliated (ca) pyriform cells (PAS/AB). *(B)* Lumen containing laterally aligned sperm bundles (sb) and vesicular elements (ve) (TB). *(C)* SEM: sperm bundle (sb) in the lumen with associated vesicular elements (ve) and matrix (m). *(D)* Sperm tightly bundled into laterally aligned arrays within the seminal matrix (PAS/AB). *(E)* Nascent spermatophore (ns) showing sperm bundles (sb) in the medullary region (m) surrounded by a forming cortex (c), with free sperm (fs) still present in the lumen indicating that spermatophore formation begins in the epididymis (PAS/AB). *(F)* Free sperm bundles (sb) in the lumen with vesicle elements (ve) (TB).

the secretory columnar cells are basal with a supranuclear Golgi apparatus.

The lumen contains flocculent granular PAS+ matrix with a peripheral AB+ hyaline component indicating chemically distinct regionalization of the nascent spermatophores observed here. Sperm are found singly and aligned laterally in bundles surrounded proximally by an unstained area. Also present in the lumen are vesicular elements. These are mostly spherical, of variable size, and containing smaller vesicles. No sperm tails protrude through the cortex of the spermatophore, thus distinguishing it from a spermatozeugma. The forming spermatophores appear to be ≤1 mm.

Ductus deferens

The epididymis widens posteriorly into the ductus deferens (Fig. 5), which is slightly infolded. The duct is surrounded by collagenous connective tissue with interstitial fibroblasts. The epithelium consists of simple columnar cells interspersed with ciliated pyriform cells. Supra-nuclear secretory vesicles are present in the simple columnar cells,

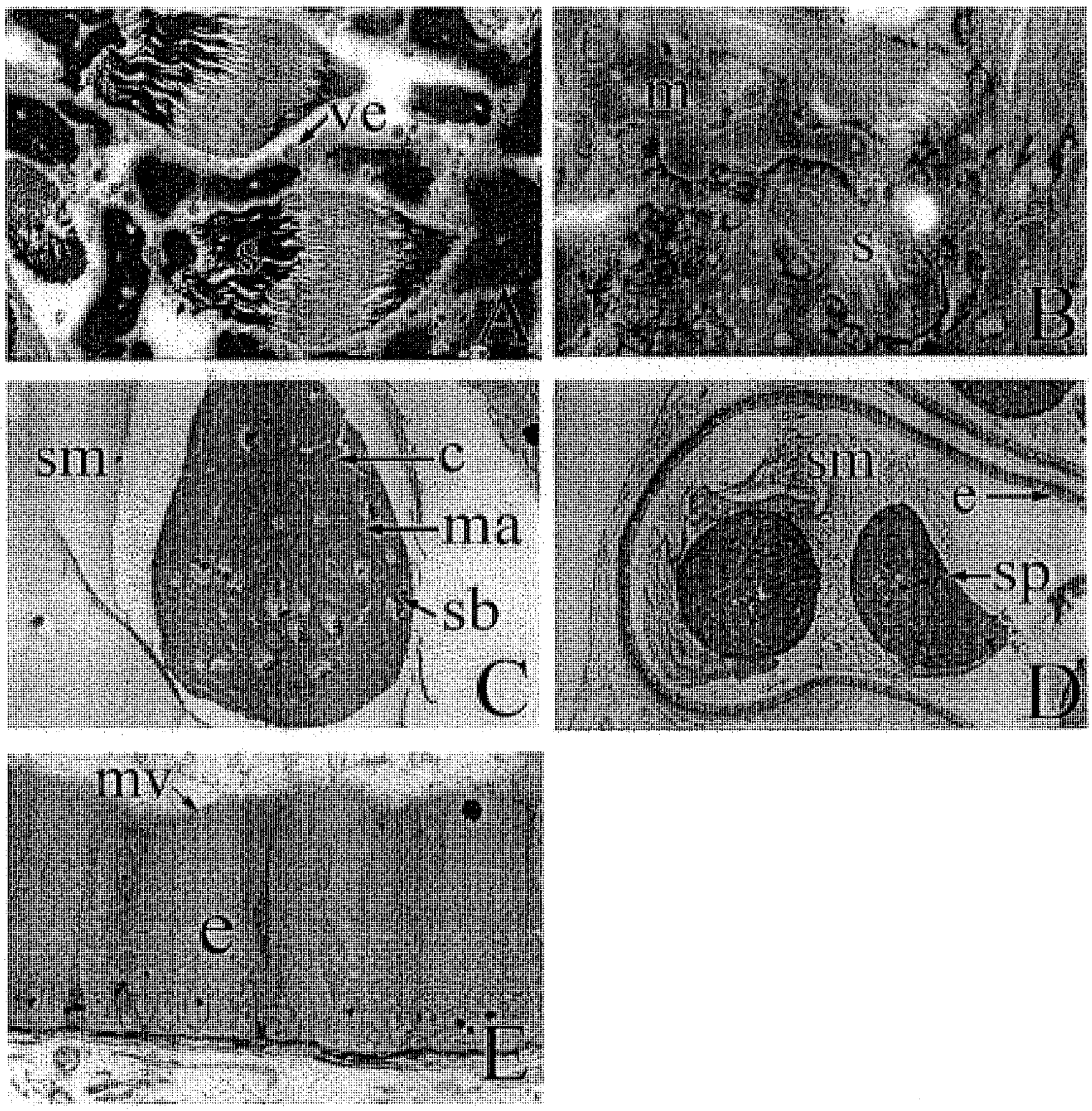


Fig. 5. Ductus deferens of *C. milii*. *(A)* Sperm (s) within developing spermatophore, tightly bundled and surrounded by vesicular elements (ve) (TB). *(B)* Sperm (s) within spermatophore matrix (m) (MTS). *(C, D)* Developing spermatophore: sperm bundles (sb) within a medulla (ma) surrounded by a cortex (c); the spermatophore is surrounded by a supporting matrix (sm) (PAS/AB). *(E)* Epithelium (e): simple columnar arrangement with interstitial pyriform cells, and microvilli (mv) (TB).

with the nucleus occupying a basal position. Microvilli are present on all cell types within the epithelium.

The lumen contains no free sperm; all sperm are bound into nascent spermatophores. The spermatophores are separate from one another, and a distinct cortical and medullary region is seen within each spermatophore. The sperm are tightly bundled in a laterally aligned array, with each bundle being surrounded by a clear region and separated from other bundles by the structural matrix of the spermatophore. The heterogeneous spermatophore matrix contains vesicular elements. Although Fig. 5(*B*) does not show specific chemical moieties, it does show heterogeneity. The red zones correspond to the PAS + material shown in the PAS\AB stained sections. The adjunct blue zones correspond to AB + matrix with some faint PAS + material also evident. Although most of the medulla is PAS +, the presence of AB + material indicates that the region is not clearly delineated from the PAS + and AB + cortex. The spermatophore is <1 mm.

Seminal vesicle

The ductus deferens is continuous via a thin connecting tubule with the larger-diameter seminal vesicle (Fig. 6). The connecting tubule is indistinct in immature and non-breeding

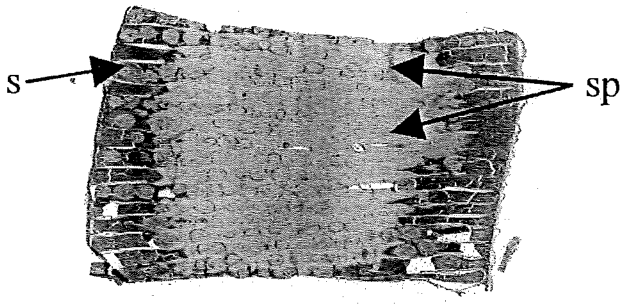


Fig. 6. Longitudinal section of seminal vesicle, showing spermatophores (s) between successive septa (sp) (MTS).

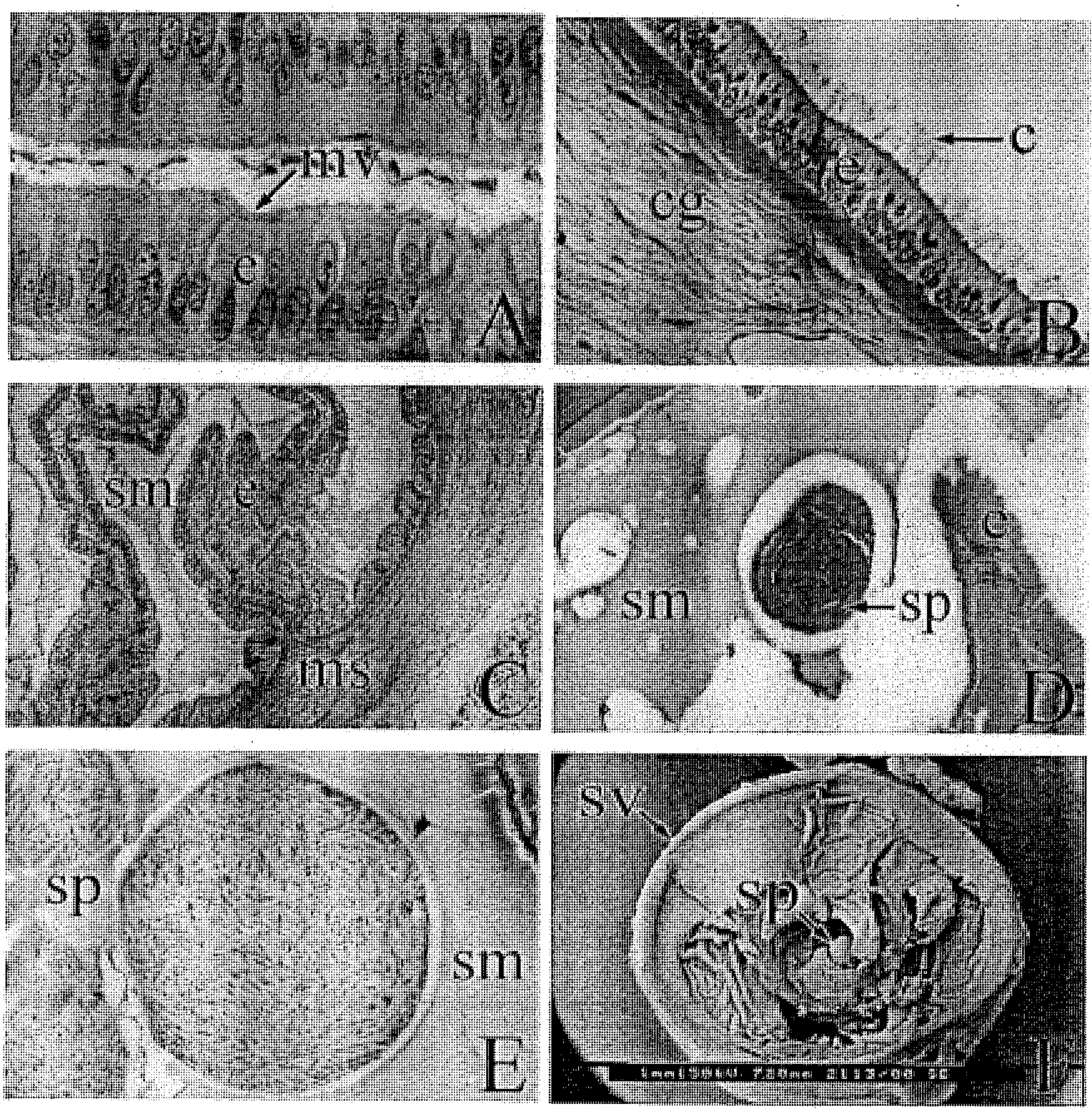


Fig. 7. Seminal vesicle of *C. milii*. *(A)* Epithelium (e): simple columnar cells interspersed with pyriform cells; microvilli (mv)(TB). *(B)* Cilia (c) on pyriform cells of the epithelium; collagen (cg) provides support between layers of epithelium (PAS/AB). *(C)* Highly folded epithelium (e), supporting matrix in the lumen (sm), and a smooth muscle sheath (ms) encircling the vesicle (PAS/AB). *(D)* Fully developed spermatophore (sp) within supporting matrix (sm) (MTS). *(E)* Distinct medullary and cortical regions within the spermatophore (sp), with sperm bundles in the medulla (MTS). *(F)* SEM: septa and an individual spermatophore (sp).

males but becomes detached laterally from the Leydig gland when the seminal vesicle is distended with semen (Reardon, unpublished). A thick sheath of collagen with interstitial fibroblasts surrounds the seminal vesicle. Internal to the sheath is a distinct lamina of smooth muscle with interstitial collagen fibres. The lumen of the seminal vesicle is segmented by internal diaphragm-like spiral septa perpendicular to the length of the vesicle, with an eccentric aperture that is less than one-half the diameter of the seminal vesicle itself. In channels between successive septa, spermatophores were observed (Fig. 7).

The majority of epithelial cells are simple columnar with basal nuclei and no visible secretory vesicles. This suggests that the seminal vesicle makes no contribution to the seminal fluid, but acts as a supportive storage structure. Interspersed between the columnar cells are pyriform cells with relatively long cilia and no secretory vesicles. The cilia have basal bodies distinguishing them from stereocilia. Distinct independent spermatophores are present. The spermatophore, ≤1 mm, has an AB+ cortical region and a predominantly PAS+ medulla, although a slight gradation between the two areas indicates regional heterogeneity. Vesicular elements are incorporated into the spermatophore, with none present in the surrounding fluid.

Discussion

The present study is the first to consider male genital tract histology and spermatophore development in any species of the sub-class holocephali. There was considerable variation between sperm-specific basic proteins in the holocephalan *H. colliei* and the shark *S. caniculus* (Saperas *et al.* 1993). This is consistent with the theory of early divergence between holocephalans and elasmobranchs in the evolutionary history of chondrichthyans, 350–400 million years ago, while the structural unit of spermatogenesis – the spermatocyst – is a conserved feature (Long 1995; Rossouw 1995; Tricas *et al.* 1997).

The presence of spermatophores in *Callorhynchus antarcticus* (a synonym of *C. milii* (Last and Stevens 1994)) has been previously noted, albeit briefly, with no details of their histology and formation (Parker and Haswell 1897). The Leydig gland tissue of *C. antarcticus* is also incorrectly labelled as being that of kidney tissue. The formation of spermatophores is described for the basking shark, *Cetorhinus maximus* (Matthews 1950) and other elasmobranchs such as the bigeye thresher, *Alopius superciliosus*, and the sand tiger shark, *Odontaspis taurus* (Pratt and Tanaka 1994). Pratt and Tanaka (1994) present a scanning electron micrograph of a sperm aggregate in *Hydrolagus colliei* and refer to it as a spermatozeugma. The micrograph does not show the perimeter of the mass and no light microscopy is presented, hence the nature of the sperm packaging requires re-examination. The presence of spermatophores is noted in *Callorhynchus callorhynchus*, and it is suggested that they are formed via agglutination in the green glands (seminal vesicles) (Di Giacomo and Perier 1994). No histology is presented to confirm this, so it is possible that the mechanism of spermatophore formation in *C. callorhynchus* is similar to that described here in *C. milii*.

The term spermatophore used here describes a type of sperm aggregation in which laterally aligned bundles of sperm are embedded in a matrix to form spherical to ovoid aggregates. These aggregates are <1 mm, whereas those of the basking shark can reach 30 mm (Matthews 1950). The sperm bundles lie within a medullary region of the spermatophore that is continuous with the cortex. Sperm tails do not protrude through the cortex; thus, this method of sperm storage is distinct from spermatozeugmata (Pratt and Tanaka 1994). The only study into the process of sperm aggregation into spermatozeugmata is on the clearnose skate, *Raja eglanteria* (Hamlett *et al.* 1999).

In the present study nascent spermatophores with a distinctive ovoid shape were found in the epididymis of *C. milii*, along with free sperm, laterally aligned sperm, vesicular elements, Sertoli cell cytoplasts and Sertoli cell bodies; this indicates that the process of spermatophore formation begins in this region. Spermatozeugma formation in *R. eglanteria* also begins in the epididymis (Hamlett *et al.* 1999). However, observations of *C. milii* contrast with observations on *C. maximus*, for which it was assumed that secretions from the genital ducts, combined with a 'tumbling mill' effect of the compartments found in the seminal vesicle, gave the spermatophore its shape and composition (Matthews 1950). It is most likely that the *C. milii* spermatophore has a spherical to ovoid shape because a sphere presents the lowest-energy form, and also the smallest ratio of surface area to volume; this minimizes sperm loss to the environment.

Although the PAS + and AB + cortical region of the *C. milii* spermatophore differs from the predominantly PAS + medullary region, the presence of AB + material in the medulla does suggest some homogeneity, and the cortex may form through interactions with the surrounding media. The mucopolysaccharide composition of the matrix may render it more gel-like and result in a firmer cortex that then assumes a low-energy state as it is washed through the genital ducts, as well as being propelled by ciliary action. Muscular contraction of the seminal vesicle is responsible for the propulsive force at ejaculation.

The formation of sperm bundles, and the capacity for sperm motility were observed in the epididymis of the Port Jackson shark, *Heterodontus portusjacksonii*, suggesting that post-testicular sperm maturation occurs in the epididymis (Jones *et al.* 1984). Protein secretions from the Leydig gland, and changes in the potassium:sodium ratio, are assumed to play a significant part in the maturation of sperm in the epididymis (Jones *et al.* 1984). The human epididymis is also responsible for the synthesis and secretion of post-testicular proteins involved in sperm maturation. Although the functions of several of the proteins are unknown, it is clear that both androgen levels and temperature are principal factors modulating their expression (Kirchhoff *et al.* 1998). Future studies of hormone levels and epididymal secretion of proteins in *C. milii* may determine how these factors are correlated with the dynamics of sperm maturation and aggregation.

The need for sperm storage in the male may be understood in the context of the life history of the animal being studied. The extent of the maturation and storage of sperm in the epididymis, or in other regions of the genital duct, is related to such factors as the allometric limitations on testis size, and the metabolic rate of the animal, as well as the reproductive strategy of the female; these factors affect the rate of sperm production (Jones 1998). It has also been observed that an epididymis supporting sperm maturation and storage is coincident with the practice of internal fertilization in elasmobranch fishes, reptiles, birds, monotremes and eutherians (Bedford 1979). In an ocean environment, mature females may not be readily available at predictable times. It is therefore advantageous for a male to have a ready supply of sperm to take advantage of chance infrequent mating events, and to successfully outcompete other males. This is supported by a review of seasonality in spermatogenesis in chondrichthyans (Parsons and Grier 1992), which shows that the rate of sperm production does not necessarily coincide with mating activity, suggesting that sperm storage is required to achieve peak amounts of sufficiently mature sperm for mating. Sperm storage also occurs in the terminal zone of the oviducal gland in female elasmobranchs (Pratt and Tanaka 1994) and *C. milii* (R. Smith, personal communication), suggesting that a single mating may be sufficient to fertilize all ova in a female.

Changes in the protein composition of the sperm plasma membrane are considered essential for further maturation in mammalian sperm. In mammals, the epididymal control of ion concentration allows peripheral proteins closer contact with the membrane, facilitating re-organization of protein in the plasma membrane; both processes are required for sperm motility. The epididymis may also mediate these changes in plasma membrane architecture through the secretion of lipid-binding proteins (Hoskins *et al.* 1979; Jones *et al.* 1983; Cooper 1998). The zonation of the epididymis has been shown to correspond with the nature of the protein secretion, and thus the stages of sperm maturation, in the boar and the echidna (Djakiew and Jones 1983; Dacheux *et al.* 1998). The contribution of the epididymis is an important consideration in sperm maturation; it may reflect a particular reproductive strategy in terms of the storage capacity of the epididymis, and the rate at which sperm can mature.

No zonation was observed in the epididymis of *C. milii*. It is likely that the seminal vesicle, not the epididymis, is involved in the storage of sperm, considering the density of spermatophores in this segment, as also seen in *C. maximus* (Matthews 1950). The structure of the epididymal epithelium is of a secretory nature, as is that of the Leydig gland, and PAS + (mucopolysaccharide) secretions from the Leydig gland were observed in the lumen of the epididymis. It is therefore likely that sperm maturation is completed by secretions of the epididymal epithelium and the Leydig gland, in the proximal segments of the epididymis.

The ductuli efferentes that lead from the testis to the epididymis have also been implicated in the net fluid resorption and active solute transport that are essential in the subsequent milieu of the epididymis (Clulow *et al.* 1998). The ductuli efferentes of *C. milii* were not examined, so it was not possible to determine whether the epithelium has a secretory nature, and thus have a role in the process of sperm maturation.

The ductus deferens of *C. milii* contained fully formed spermatophores with a distinct cortex and medulla and no other luminal contents apart from a lightly staining PAS + fluid, and vesicular elements, surrounded by a clear fluid. This clear fluid possibly aids in the transport of the viscous spermatophore-supporting medium throughout the length of the genital ducts. The ductus deferens had more extensive folding than the epididymis, possibly for further fluid resorption through increased surface area. Supranuclear secretory vesicles were observed in the epithelium, although it is unclear what contribution they make to the spermatophore, as it appears to be fully formed at this stage. Other histological studies of the reproductive tract of several species of shark and of *R. eglanteria* show a similarly structured ductus deferens, and conclude that this part of the duct also contributes to the seminal matrix in some way as yet undescribed (Botte *et al.* 1963; Hamlett *et al.* 1999).

The seminal vesicle of *C. milii* contained cells with no apparent secretory vesicles. This, coupled with its chambered structure, suggests that fully formed spermatophores are stored here until ejaculated by contractions of the smooth muscle sheath enclosing it. Spermatophores have been described in several species across disparate family groups within the Chondrichthyes (Matthews 1950; Pratt and Tanaka 1994) as well as in such diverse groups as Platyhelminthes, Mollusca, Cephaloda, Annelida, Insecta, Crustacea and Arachnida (Mann 1984). Clearly, the use of spermatophores as a means of sperm transport is evolutionarily successful.

To analyse the advantage conveyed to a species by the development and deployment of spermatophores, the environment of that species must be put in context. For chondrichthyans in general, the conservation of sperm by minimizing loss through leakage may be one advantage of spermatophores as a mode of sperm transfer in an aqueous environment (Matthews 1950). The issue of sperm nutrition must also be raised, as sperm may be stored for long periods before mating, and the opportunity to mate may be infrequent in dispersive species (Pratt and Tanaka 1994). The presence of vesicular elements within spermatophores in *C. milii* suggests that they are, in part, fulfilling the role of sperm nutrition.

It has been proposed that spermatophores provide a barrier to re-insemination, thus serving as a competitive device against other males, and that spermatophores may also be implicated in the stimulation of oogenesis and in oviposition in other genera (Mann 1984). Mature adults of *C. milii* appear to be sexually segregated (R Hudson, personal communication). In a sexually segregated species, competition may be fierce when the opportunity to mate does arise; thus, any process to increase the likelihood of paternity bestows a selective advantage on a mature male. Although sperm plugs have been observed in *C. milii* (R. Smith, personal communication) it is unclear whether spermatophores play any role in the stimulation of the female reproductive cycle. Further study of the biochemical properties of spermatophores, and the nature of the contribution the genital ducts make to their formation, will help to complete the picture of how and why spermatophores form in members of some genera but not in others.

Acknowledgments

The authors acknowledge Russell Hudson (Marine and Freshwater Resources Institute), Amy Beck, Rachel Smith, Bruce Abaloz, Joan Clark and David Paul (University of Melbourne) and Emma Goeman for their assistance in data collection and analysis. M.B.R. acknowledges Dr Rob Day who co-supervised the Honours project of which this study was a part. This research was supported by an Enhancing International Science Networks Visiting Fellowship (Department of Natural Resources and Environment, Melbourne Australia) and a Visiting Research Scholar Travel Grant, 2000 (The University of Melbourne, Parkville, Victoria, Australia) to W.C.H.

References

Bedford, J. M. (1979). Evolution of sperm maturation and storage function of the epididymis. In 'The Spermatozoon. Maturity, Motility, Surface Properties and Comparative Aspects'. (Eds D. W. Fawcett and J. M. Bedford.) (Urban and Schwarzenberg: Baltimore/ Munich.)

Bols, N. C., Boliska, S. A., Rainville, J. B., and Kasinsky, H. E. (1980). Nuclear basic protein changes during spermiogenesis in the longnose skate and the spiny dogfish. *Journal of Experimental Zoology* **212**, 423–33.

Botte, V., Giovanni, C., and Stanley, H. P. (1963). Histological and histochemical observations on the male reproductive tract of *Scyliorhinus stellaris, Torpedo marmorata* and *T. torpedo. Publiccazione di Statione Zoologico di Napoli*. 33, 224–42.

Callard, G. V. (1991*a*). Spermatogenesis. In 'Vertebrate Endocrinology: Fundamentals and Medical Implication'. Vol. 4A. (Eds P. K. T. Pang and M. P. Schreibman.) (Academic Press: New York.)

Callard, G. V. (1991*b*). Reproduction in male elasmobranch fishes. In 'Oogenesis, Spermatogenesis and Reproduction'. (Ed. R. K. H. Kinne.) *Comparative Physiology* **10**, 104–54. (Karger: Basel.)

Clulow, J., Jones, R. C., Hansen, L. A., and Man, S. Y. (1998). Fluid and electrolyte resorption in the ductuli efferentes testis. *Journal of Reproduction and Fertility* Supplement **53**, 1–14.

Cooper, T. G. (1998). Interactions between epididymal secretions and spermatozoa. *Journal of Reproduction and Fertility* Supplement **53**, 119–36.

Dacheux, J.-L., Druart, X., Fouchecourt, S., Syntin, P., Gatti, J.-L., Okamura, N., and Dacheux, F. (1998). Role of epididymal secretory proteins in sperm maturation with particular reference to the boar. *Journal of Reproduction and Fertility* Supplement **53**, 99–107.

Di Giacomo, E. E., and Perier, M. R. (1994). Reproductive biology of the cockfish, *Callorhynchus callorhynchus* (Holocephali: Callorhynchidae), in Patagonian waters (Argentina). *Fishery Bulletin* **92**, 531–9.

Djakiew, D., and Jones, R. C. (1983). Sperm maturation, fluid transport, and secretion and absorption of protein in the epididymis of the echidna, *Tachyglossus aculeatus. Journal of Reproduction and Fertility* **68**, 445–56.

Hamlett, W. C., Hysell, M. K., Rozycki, T., Brunette, N., Tumilty, K., Henderson, A., and Dunne, J. (1999). Sperm aggregation and spermatozeugmata formation in the male genital ducts in the clearnose skate, *Raja eglantaria*. In 'Proceedings of the 5th Indo-Pacific Fish Conference Noumea. (1997)'. (Eds B. Seret and J-Y. Sire.) (French Ichthyological Society/ORSTOM: Paris.)

Hoskins, D. D., Johnson, D., Brandt, H., and Acott, T. S. (1979). Evidence for the role for a forward motility protein in the epididymal development of sperm motility. In 'The Spermatozoon: Maturation, Motility, Surface Properties and Comparative Aspects'. (Eds D. W. Fawcett and J. M. Bedford.) (Urban and Schwarzenburg: Baltimore/Munich.)

Jones, R. C. (1998). Evolution of the vertebrate epididymis. *Journal of Reproduction and Fertility* Supplement **53**, 163–81.

Jones, R. C., Jones, N., and Djakiew, D. (1984). Luminal composition and maturation of spermatozoa in the male genital ducts of the Port Jackson shark, *Heterodontus portusjacksoni. Journal of Experimental Zoology* **230**, 417–26.

Kirchhoff, C., Osterhoff, C., Pera, I., and Schroter, S. (1998). Function of human epididymal proteins in sperm maturation. *Andrologia* **30**, 225–32.

Last, P. R., and Stevens, J. D. (1994). 'Sharks and Rays of Australia.' (CSIRO: Australia.) 513 pp.

Long, J. (1995). 'The Rise of Fishes.' (UNSW Press: Sydney.)

Mann, T. (1984). 'Spermatophores: Development, Structure, Biochemical Attributes and Role in the Transfer of Spermatozoa.' (Ed. D. S. Farner.) (Springer: New York.)

Matthews, L. H. (1950). Reproduction in the basking shark, *Cetorhinus maximus* (Gunner). *Philosophical Transactions of the Royal Society of London* **234**, 247–16.

Parker, T. J., and Haswell, W. A. (1897). 'A Textbook of Zoology.' (Macmillan: London.) 181 pp.

Parsons, G. R., and Grier, H. J. (1992). Seasonal changes in shark testicular structure and spermatogenesis. *Journal of Experimental Zoology* **261**, 173–84.

Pratt, H. L., and Tanaka, S. (1994). Sperm storage in male elasmobranchs: a description and survey. *Journal of Morphology* **219**, 297–08.

Rossouw, G. J. (1995). Spermatogenesis in *Callorhynchus callorhynchus* (Linnaeus) (Pisces : Holocephali) from Chile. *Revista Chilena de Historia Natural* 68, 101–5.

Saperas, N., Chiva, M., Bols, N. C., Kulak, D., and Kasinsky, H. E. (1993). Sperm specific basic proteins in the holocephalan fish *Hydrolagus colliei* (Chondrichthyes, Chimaeriformes) and comparison, with protamines from an elasmobranch. *Biological Bulletin* **185**, 186–96.

Stanley, H. P. (1983). The fine structure of spermatozoa of *Hydrolagus collei* (Chondrichthyes, Holocephali). *Journal of Ultrastructural Research*. **83**, 184–94.

Tricas, T. C., Deacon, K., Last, P., McCosker, J. E., Walker, T. I., and Taylor, L. (1997). 'Sharks and Rays.' (Ed. L. Taylor.) (Reader's Digest: Surry Hills, Australia.)

Manuscript received 21 May 2001; revised and accepted 3 February 2002

JOURNAL OF EXPERIMENTAL ZOOLOGY 292:111–128 (2002)

Ultrastructure of Sperm Storage and Male Genital Ducts in a Male Holocephalan, the Elephant Fish, *Callorhynchus milii*

WILLIAM C. HAMLETT,[1,2,3*] MATTHEW REARDON,[2] JOAN CLARK,[2] AND TERENCE I. WALKER[3]
[1]*Department of Anatomy and Cell Biology, South Bend Center for Medical Education, Indiana University School of Medicine, Notre Dame, Indiana 46556*
[2]*Department of Zoology, The University of Melbourne, 3010, Victoria, Australia*
[3]*Marine and Freshwater Resources Institute, Department of Natural Resources and Environment, Queenscliff, Victoria, Australia*

ABSTRACT In chondrichthyes, the process of spermatogenesis produces a spermatocyst composed of Sertoli cells and their cohort of associated spermatozoa linearly arrayed and embedded in the apical end of the Sertoli cell. The extratesticular ducts consist of paired epididymis, ductus deferens, isthmus, and seminal vesicles. In transit through the ducts, spermatozoa undergo modification by secretions of the extratesticular ducts and associated glands, i.e., Leydig gland. In mature animals, the anterior portion of the mesonephros is specialized as the Leydig gland that connects to both the epididymis and ductus deferens and elaborates seminal fluid and matrix that contribute to the spermatophore or spermatozeugmata, depending on the species. Leydig gland epithelium is simple columnar with secretory and ciliated cells. Secretory cells have periodic acid-Schiff positive (PAS+) apical secretory granules. In the holocephalan elephant fish, *Callorhynchus milii*, sperm and Sertoli cell fragments enter the first major extratesticular duct, the epididymis. In the epididymis, spermatozoa are initially present as individual sperm but soon begin to laterally associate so that they are aligned head-to-head. The epididymis is a highly convoluted tubule with a small bore lumen and an epithelium consisting of scant ciliated and relatively more secretory cells. Secretory activity of both the Leydig gland and epididymis contribute to the nascent spermatophores, which begin as gel-like aggregations of secretory product in which sperm are embedded. Fully formed spermatophores occur in the ductus. The simple columnar epithelium has both ciliated and secretory cells. The spermatophore is regionalized into a PAS+ and Alcian-blue-positive (AB+) cortex and a distinctively PAS+, and less AB+ medulla. Laterally aligned sperm occupy the medulla and are surrounded by a clear zone separate from the spermatophore matrix. Grossly, the seminal vesicles are characterized by spiral partitions of the epithelium that project into the lumen, much like a spiral staircase. Each partition is staggered with respect to adjacent partitions while the aperture is eccentric. The generally nonsecretory epithelium of the seminal vesicle is simple columnar with both microvillar and ciliated cells. *J. Exp. Zool. 292:111–128, 2002.* © 2002 Wiley-Liss, Inc.

Because of their key phylogenetic position, chondrichthyes offer a unique opportunity to study the anatomy, physiology, and biochemistry of reproduction in an ancient group. As chondrichthyes are the oldest extant gnathostomes, having branched from the main evolutionary line some 400 million years ago, we can use extant species to elucidate highly conserved and successful reproductive mechanisms and make inferences about evolution. The internal organs of the male include the testes, genital ducts (including the efferent ductules, epididymis, ductus deferens, isthmus, and seminal vesicle), Leydig's gland, and the alkaline gland. Peritoneum covers the genital ducts and the elongate kidneys.

Grant sponsor: Indiana University School of Medicine; Grant sponsor: Department of Natural Resources and Environment, Melbourne, Australia; Grant sponsor: Department of Zoology, The University of Melbourne, Parkville.
*Correspondence to: Dr. William C. Hamlett, Professor of Anatomy & Cell Biology, South Bend Center for Medical Education, Indiana University School of Medicine, B-10 Haggar Hall, Notre Dame, Indiana 46556. E-mail: hamlett.1@nd.edu
Received 14 September 2001; Accepted 14 September 2001

DOI 10.1002/jez.1148

There is considerable variation regarding terminology of genital ducts in chondrichthyes (Borcea, '06; Matthews, '50; Botte, et al., '63; Stanley, '63; Hamlett, '99). From the testis, spermatozoa and Sertoli cell fragments move through the efferent ductules located in the anterior end of the mesorchium.

Most authors refer to the small bore, highly coiled initial segment of the extratesticular ducts as the epididymis. This segment is continuous with the broader diameter, sinuous ductus deferens, also called the vas deferens or Wolffian duct. The ductus deferens is continuous as the very large diameter seminal vesicle, also referred to as the ampulla, of the ductus deferens. Jones and Jones ('82); Jones et al. ('84); and Jones and Lin ('93) refer to the derivative of the mesonephric duct as the ductus epididymis rather than the ductus deferens, the term used by most authors. We have chosen to use the most widely held terminology (e.g., epididymis, ductus deferens, and seminal vesicle).

Both the epididymis and ductus deferens receive a viscous fluid produced by the adjacent Leydig gland. In sexually immature specimens, the ductus is a thin straight tube but, in sexually mature males, it is coiled and covers most of the kidney. The two broad seminal vesicles receive no ducts from the Leydig. The seminal vesicles unite to form the single urogenital sinus that ends as the urogenital papilla emptying into the cloaca. The cloaca is common to both sexes and is located between the pelvic fins and receives material from the digestive, urinary, and reproductive tracts.

Stanley ('63) studied urogenital morphology, including light microscopy in the chimaeroid fish, *Hydrolagus collei*. The present study was undertaken to detail the normal ultrastructure of the male genital ducts and spermatophore formation in the holocephalan elephant fish, *C. milii*. Results will allow comparisons to be made with male genital ducts in the sister group, the elasmobranchs. Some light microscopic observations have been previously reported but not yet published (Reardon, 2001). This report is the first to present ultrastructural analysis of the male ducts in any holocephalan.

MATERIALS AND METHODS

Male elephant fish, *Callorhynchus milii*, were obtained on board commercial gill net vessels operating in Australia's Southern Shark Fishery of Victoria. Fishing gear consisted of 2,600 meters of monofilament gillnet with a mesh size of 152 mm. The gillnet was set twice within a 24 hour period, before dawn and in the early afternoon. The fishing gear was hauled late morning and late night, respectively, to give an average fishing time of 8–10 hours at a depth range of 20 to 60 meters. Samples were kept live in tanks on board and sacrificed just before dissection and fixation. Animals were sacrificed by blunt trauma to the chondrocranium on board a fishing vessel in San Remo whilst in port. They were dissected via a longitudinal ventral incision, and the genital ducts removed and placed in 10% neutral buffered formalin for light microscopy (LM) or 2.5% glutaraldehyde-2.0% paraformaldehyde in 0.1M sodium cacodylate buffer to which had been added picric acid for transmission electron microscopy (TEM). Following dehydration, the tissue was transferred through three changes of propylene oxide of 5 min each at room temperature. Tissues were infiltrated in a 1:1 volumetric ratio of propylene oxide to catalyzed Poly/Bed 812-Araldite overnight. All samples were then transferred to pure catalyzed Poly/Bed 812-Araldite and embedded. Samples were cured at 39°C for 12 hr, 45°C for 12 hr, and 60°C for 12 hr under vacuum. Blocks of tissue were sectioned either with glass or diamond knives on a Reichert Ultracut S Ultramicrotome. One- to two-micrometer-thick sections were cut for light microscopy and stained with toluidine blue. Silver or gray sections were picked up on acid-cleaned copper grids. Sections on grids were stained with uranyl acetate and lead citrate.

Tissues from the epididymis, ductus deferens, isthmus, seminal vesicle, and Leydig gland taken for light microscopy were washed in fresh water and embedded in Paraplast (paraffin with plastic polymer) in an LX 120 Tissue Processor. Embedded tissue was cross sectioned at 3 μm with a Micron HM325 microtome. Sections were affixed to glass slides by drying for 12 hours in an incubator at 50°C. Separate sections were stained with each of Harris Hematoxylin and Eosin, Mallory's Triple connective tissue stain, and combined periodic acid-Schiff (PAS)-Alcian Blue (AB) stain. Sections were mounted on glass slides and cover slipped with DPX mounting medium. Sections were photographed on a Nikon Coolpix 990 digital camera attached to a Nikon Optiphot-2 research grade light microscope.

Tissues for scanning electron microscopy (SEM) from the epididymis, ductus deferens, seminal vesicle, and Leydig gland were stored in 10% neutral buffered formalin, washed in fresh water, sectioned with a razor blade, and dehydrated with

HMDS (Hexamethyldisilazane). Dehydrated tissue was mounted on stubs with SPI High Purity Silver Paint and sputter coated with gold-palladium. Specimens were examined with a Philips SEM 505. Images from the SEM were captured as JPEG files using the "Spectrum" Windows-based PC program.

RESULTS

Gross anatomy

Testes are paired, elongate, cylindrical structures suspended from the dorsal body wall by the mesorchium. Efferent ductules course through the mesorchium to convey spermatozoa and remnants of Sertoli cells to the epididymis. Due to their small size and technical difficulties in dissecting them, the efferent ductules were not included in this study.

The epididymis is a highly convoluted tubule with a small bore lumen and outer diameter. It is confluent with the wider diameter ductus deferens (Figs. 1–2). Whereas the epididymis is highly convoluted, the ductus is more sinuous and serpentine. Both the epididymis and ductus rest in an adjacent position to the Leydig gland. The ductus narrows to a connective isthmus that is confluent with the much larger and broader seminal vesicles. The seminal vesicles are large and robust in mature animals and are filled with spermatozoa embedded in seminal matrix to form definitive spermatophores. The seminal vesicles rest on the paired kidneys.

Leydig gland

In mature males, the Leydig gland is a specialization of the anterior portion of the mesonephros. It is a branched tubular gland that produces secretions in the form of seminal fluid and matrix that contribute to the spermatophore. The epithelium is simple columnar with two distinct cell types, i.e., secretory and ciliated (Figs. 3–6). The epithelium rests on a basement membrane with subjacent connective tissue stroma (Fig. 3). Secretory cells show evidence of synthetic and secretory cycles in that not all portions of all tubules are synchronous regarding the stage of synthesis and secretion. Some cells have PAS+ secretory vesicles in an apical position, while others are devoid of the vesicles but show amplified regions adjacent to the nucleus that are accumulations of rough endoplasmic reticulum, as well as supranuclear clear areas that are elements of the Golgi complex. The secretory cells outnumber the ciliated cells and have a basal nucleus. The ciliated cells are pyriform in shape with a truncated base, nucleus in the mid-portion of the cell, and a wider apex with cilia.

Secretory product is a mixture of PAS+ and AB+ material. The apex of all cells stain distinctly PAS+, but there are homogeneous concentrated regions of secretory product that are AB+. Luminal secretory product occurs initially as frothy material surrounded by more gel-type material that is PAS+ (Fig. 4). As secretory activity continues, more vesicular elements and homogeneous matrix make their appearance (Fig. 5) and eventually PAS+ vesicles fill the lumen (Fig. 6).

Epididymis

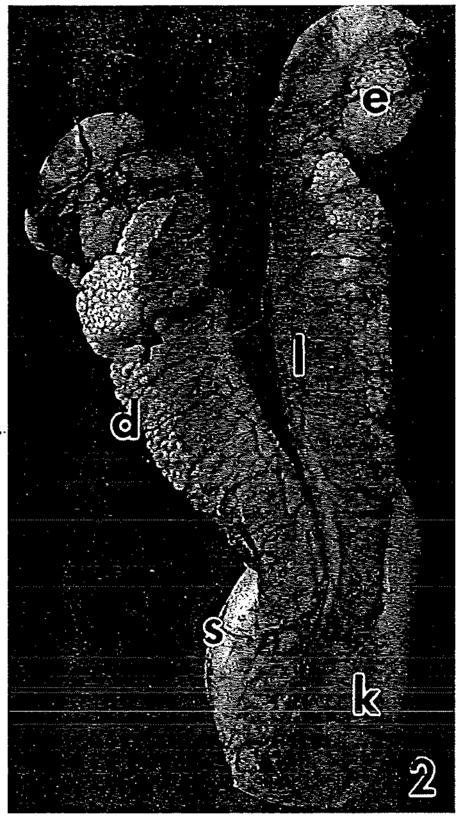
Scanning electron microscopy (Fig. 7) reveals the proximity of the epididymis to Leydig gland tubules. Nascent spermatophores begin as gel-like aggregations of secretory product in which spermatozoa occur (Fig. 8) surrounded by seminal fluid.

Light microscopy of epoxy-resin-embedded material sectioned at 1 μm and stained with toluidine blue O demonstrates the vascularized connective tissue stroma and the columnar epithelium of the epididymis (Fig. 9). Columnar epithelial cells are joined by classic terminal bar complexes, and secretory cells contain heterogeneous profiles of secretory vesicles, as well as lipid inclusions. Sperm are released from the testis as individuals but immediately begin to laterally align in the epididymis. The spiraled heads of spermatozoa align with the midpiece and tail in tandem (Fig. 9).

A light micrograph of a paraffin-embedded specimen stained with Mallory's triple stain reveals heterogeneity in the seminal matrix and fluid (Fig. 10). Laterally aligned sperm aggregates are evident immediately, surrounded by a clear zone. Peripheral to the clear zone, seminal matrix occurs that contains distinct vesicular elements.

Transmission electron microscopy (Figs. 11–18) shows the secretory epithelium to have ovoid nuclei, a prominent nucleolus, and the majority of the chromatin in the form of euchromatin. There is abundant rough endoplasmic reticulum adjacent to the nucleus in the base of the cells and supranuclear clear areas forming secretory vesicles that correspond to the region of the Golgi complex. Apical secretory vesicles with heterogeneous contents are common, as are lipid inclusions. Microvilli and cilia occur on the luminal aspect of the epithelial cells.

Prominent heterogeneous vesicular elements (Figs. 14, 17–18) occur in the lumen of the epid-



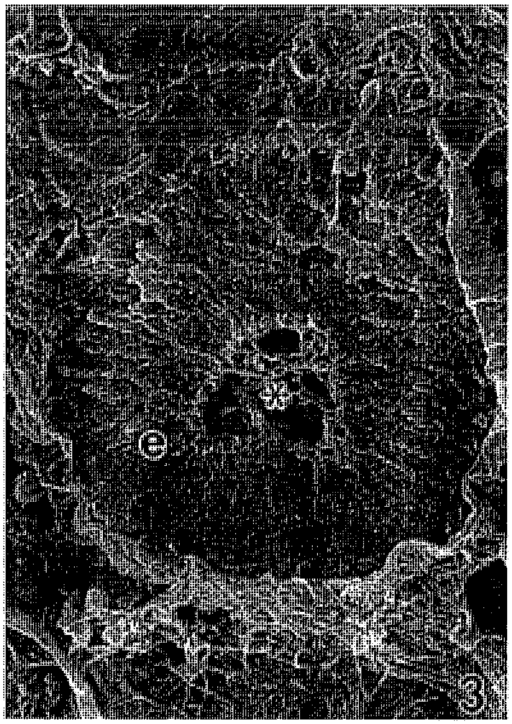
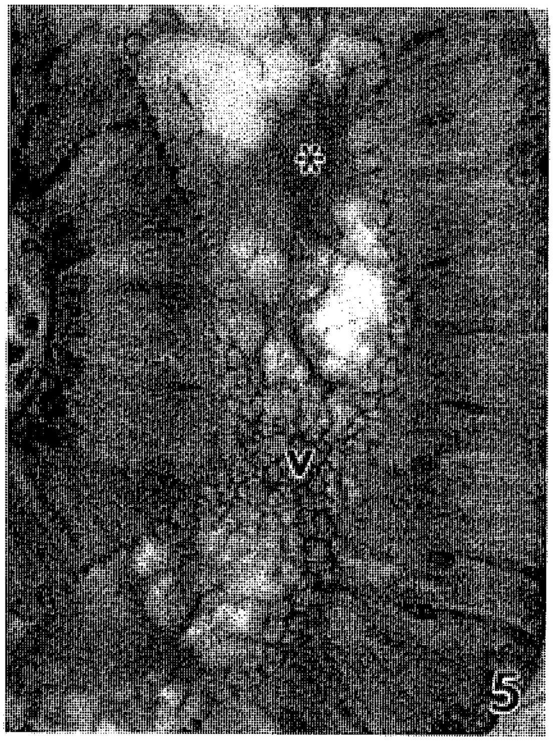


Fig. 1. Ventral view of male reproductive ducts with testis removed. e = epididymis, d = ductus deferens, i = isthmus, s = seminal vesicle.

Fig. 2. Dorsal view of male reproductive ducts and accessory organs. e = epididymis, l = Leydig gland, d = ductus deferens, s = seminal vesicle, k = kidney.

Fig. 3. Scanning electron micrograph of Leydig gland tubule. e = simple columnar epithelium, asterisk = luminal secretory product. 400×.

Fig. 4. Light micrograph of Leydig gland tubule illustrating luminal secretory product (asterisk), ciliated cells (c), and secretory cells with basal nuclei (s). 600×.



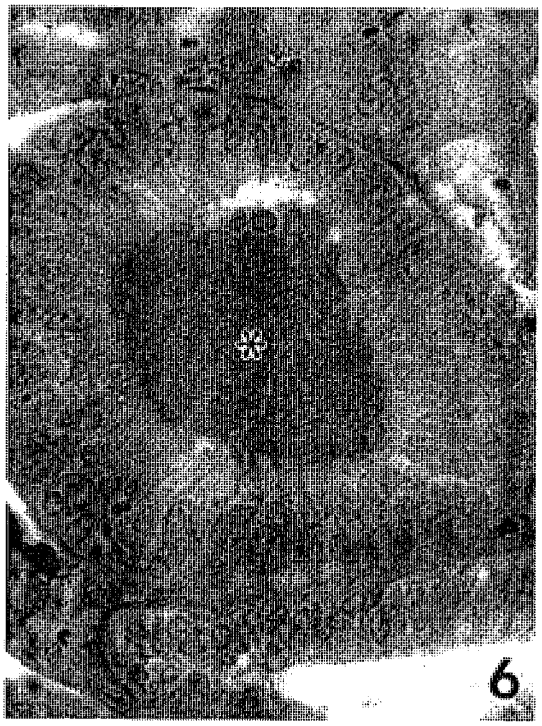
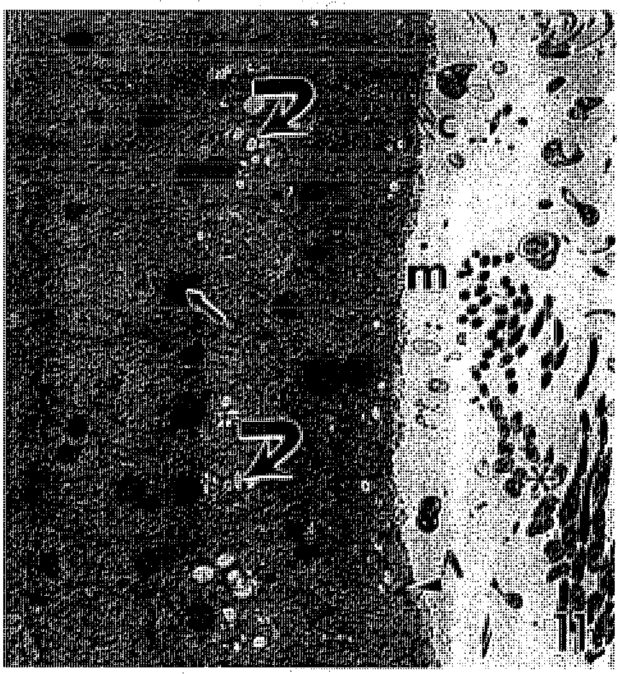


Fig. 5. Light micrograph of Leydig gland tubule illustrating luminal secretory product in two configurations, vesicular bodies (v) and homogeneous matrix (asterisk). 600×.

Fig. 6. Light micrograph of Leydig gland tubule illustrating luminal secretory product packaged into homogeneous masses. 600×.

Fig. 7. Scanning electron micrograph of Leydig gland (l) and epididymal tubules (e) containing nascent spermatophores (asterisks). 50×.

Fig. 8. Scanning electron micrograph of epithelial wall of epididymis (e) and nascent spermatophore (asterisk), separated by a space filled with fluid (f). 200×.



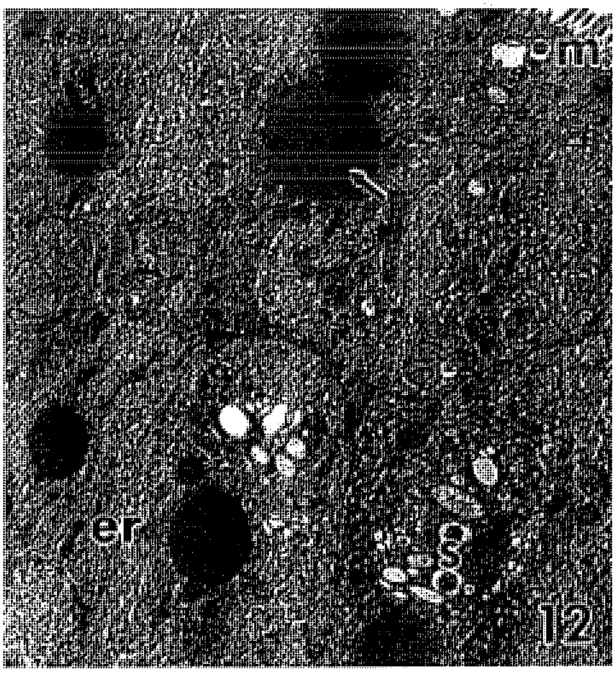
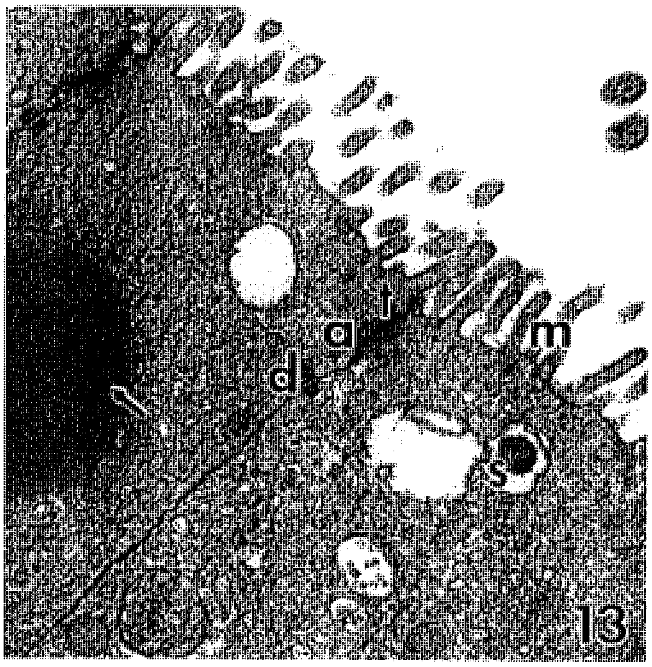


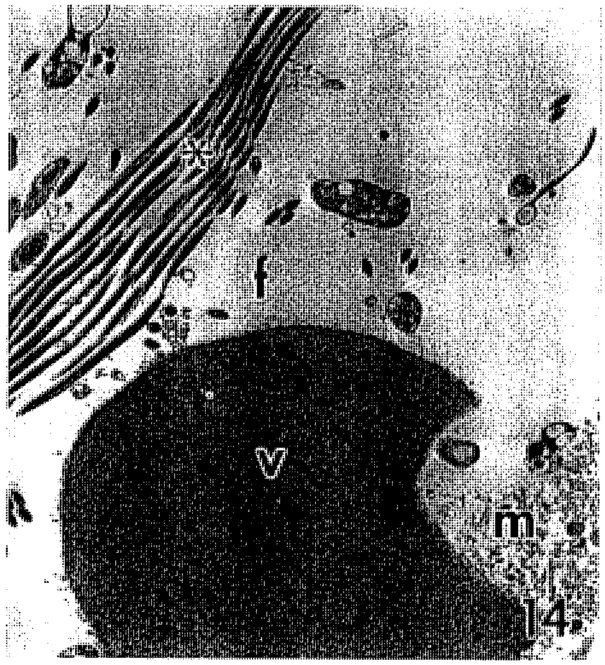
Fig. 9. Light micrograph of 1 μm-thick section of epoxy-embedded epididymis stained with toluidine blue O. Epididymal epithelial cells (e) are secretory (s) with apical secretory vesicles (curved arrow) and lipid inclusions (arrow). A connective tissue (ct) core supports the epithelium, while individual (circle) and bundled sperm (asterisk) occur in the lumen. Arrowhead = terminal bars. 600×.

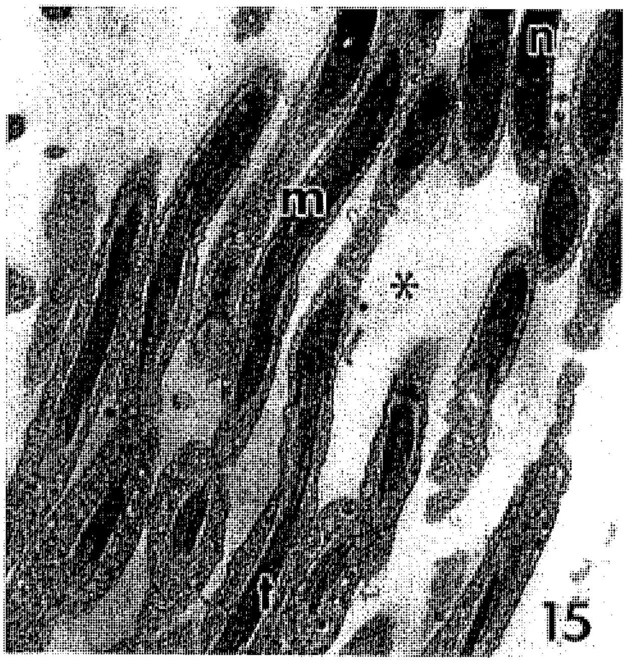
Fig. 10. Light micrograph of paraffin-embedded section of epididymis. Epithelium (e) borders the lumen that contains bundled sperm (asterisk) and vesicular elements (v) suspended in seminal matrix. 600×.

Fig. 11. Transmission electron micrograph of epididymis showing microvilli (m), scant cilia (c), sperm (asterisk), terminal bars (arrowhead), lipid inclusions (arrow), and secretory vesicles (curved arrows). 1,650×.

Fig. 12. Transmission electron micrograph of epididymis showing microvilli (m), lipid inclusions (arrow), rough endoplasmic reticulum (er) and secretory vesicles (s). 5,200×.







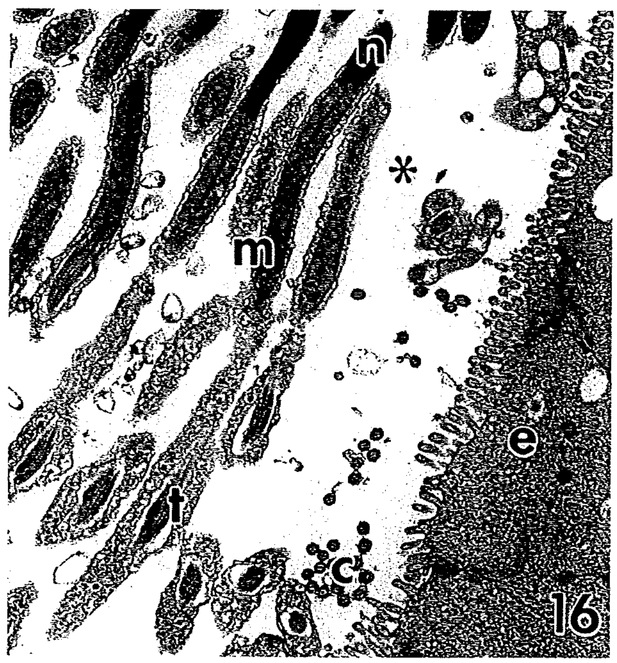


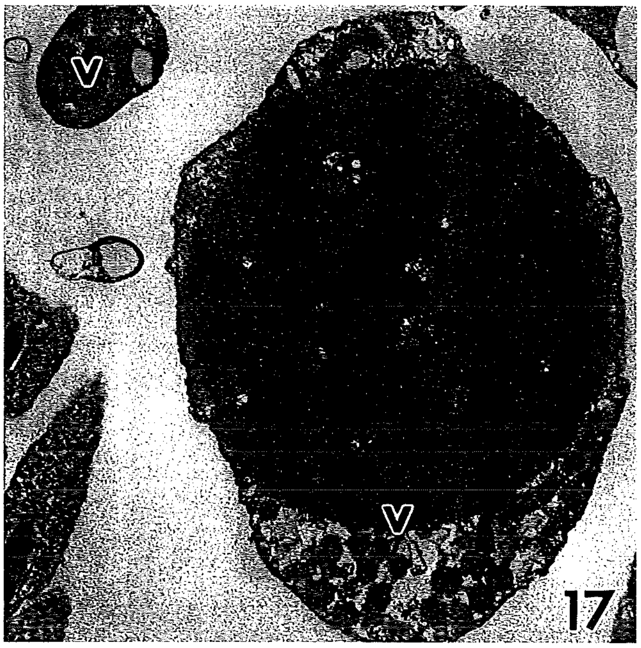
Fig. 13. Transmission electron micrograph of epididymis with microvilli (m), zonula occludens or tight junction (t), zonula adherens (a), and macula adherens or desmosome (d), lipid inclusions (l), and secretory vesicles (s). 15,500×.

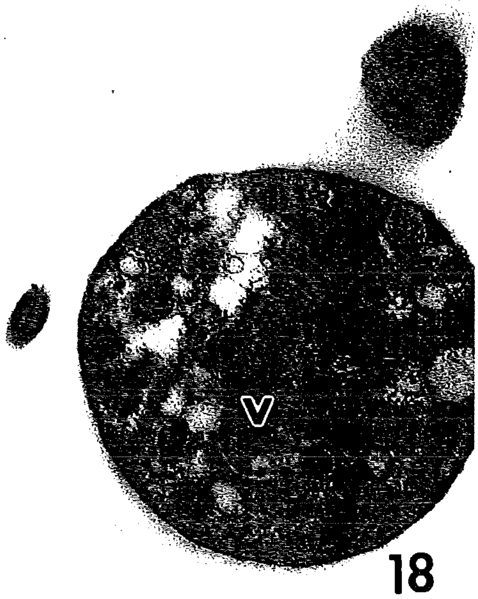
Fig. 14. Transmission electron micrograph of luminal contents of epididymis with bundled sperm (asterisk) and vesicles (v) suspended in seminal matrix (m) and fluid (f). 3,900×.

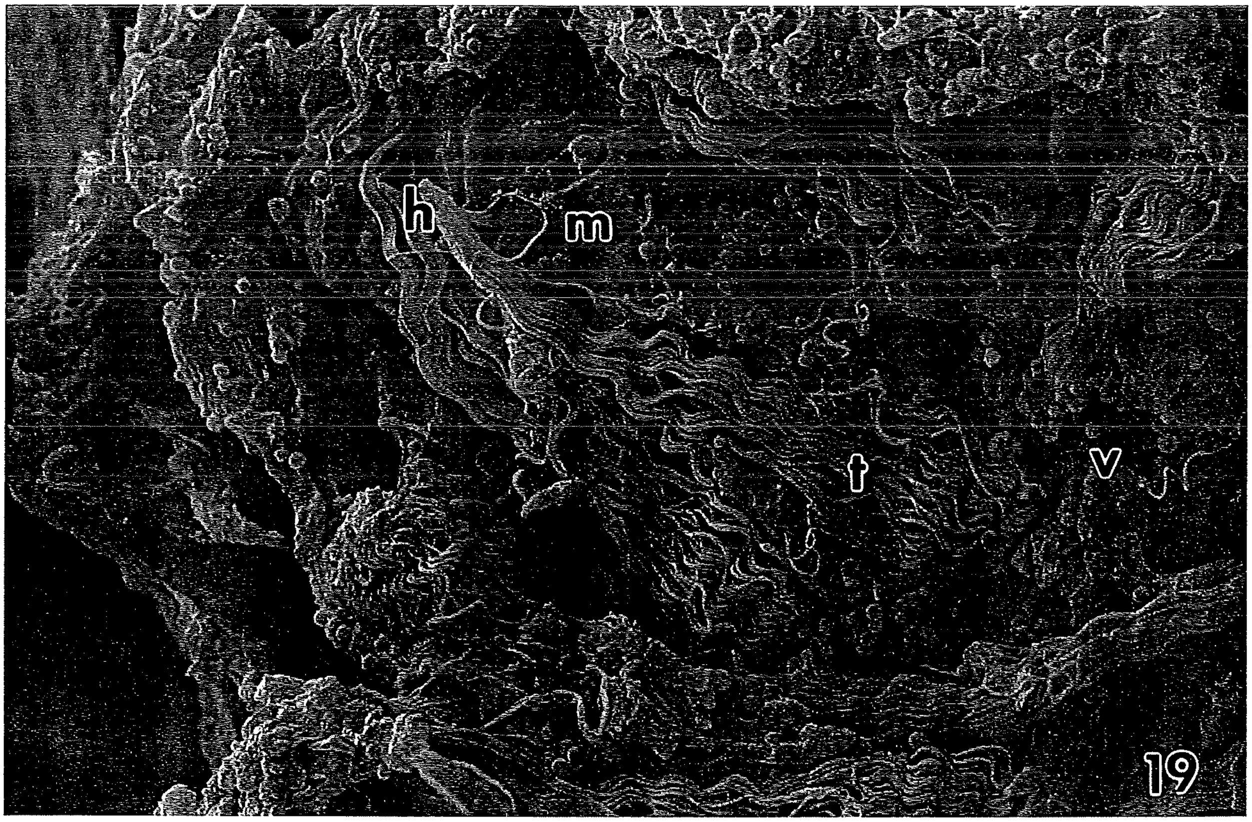
Figs. 15–16. Transmission electron micrographs of epididymal sperm with nucleus (n), midpiece with mitochondria (m), and tail (t) with microtubules suspended in seminal fluid (asterisks). e = epithelium. Fig. 15 = 8,900×. Fig. 16 = 6,610×.

idymis. Some of the vesicles have a homogeneous content (Fig. 14), while others contain smaller vesicles with electron-lucent to electron-dense contents (Figs. 17–18).

The classic terminal bar complex of light microscopy is revealed by TEM to consist of a luminal tight junction or zonula occludens, a zonula adherens and desmosome (Fig. 13). Sperm pref-







Figs. 17–18. Transmission electron micrographs of vesicular elements (v) in the epididymis. Fig. 17 = 8.900V. Fig. 18 = 21,000×.

Fig. 19. Scanning electron micrograph of luminal contents of the ductus deferens. Sperm are bundled at the heads (h), while the tails (t) are not compacted. Vesicular elements (v) are suspended in seminal matrix (m). 1,600×.

erentially align with heads, midpieces, and tails laterally opposed. There are no morphological junctional specializations apparent via TEM that are responsible for the alignment.

Ductus deferens

Fully formed spermatophores occupy the lumen of the ductus deferens (Figs. 19–22). The number of groups of laterally aligned spermatozoa has increased over that observed in the epididymis. Groups of spermatozoa of approximately the same size are separated from each other by spermatophore matrix. The matrix is organized into cortical and medullary regions. The AB+ and PAS+ cortex has an abundance of vesicular elements (Fig. 22) associated with it, while the PAS+ medulla is primarily composed of a homogeneous matrix in which are suspended vesicular elements and laterally aligned sperm bundles. A distinct clear zone (Figs. 20–21) immediately surrounds the sperm bundles separate from the medullary matrix.

Light microscopy discloses the epithelium to be simple columnar with two cell types, i.e., ciliated and secretory (Fig. 22). Both cell types are joined by the classic tripartite terminal bar complex, consisting of luminal zonula occludens with subjacent zonula adherens and desmosome (Fig. 24). Both cell types have ovoid basal nuclei. Ciliated cells also have a small component of microvilli (Fig. 24). Basal bodies have standard striated rootlet fibers associated with them. Secretory cells have a supranuclear Golgi complex and an array of rough endoplasmic reticulum adjacent to the nucleus. Secretory vesicles occupy the apical cytoplasm (Figs. 23–25), as well as scant lipid inclusions.

Transmission electron microscopy of spermatophores within the ductus deferens shows tightly bundled spermatozoa (Figs. 26–27, 29) immediately surrounded by a loosely organized matrix that grades to a denser homogeneous matrix more peripheral to the sperm (Figs. 26, 28–29). Suspended in the matrix are vesicular bodies (Figs. 26, 28–29).

Isthmus

Light microscopy of the isthmus between the ductus and seminal vesicle shows the epithelium to be simple ciliated columnar. When stained by Mallory's triple method (Fig. 30), abundant circumferential collagen lamellae are observed. When stained with hematoxylin and eosin, nuclei of smooth muscle cells (Fig. 31) are seen to be intertwined with the collagen. Modest epithelial folds project into the lumen of the connection.

Seminal vesicles

Scanning electron microscopy of a cross section of the seminal vesicle reveals spiral partitions that project into the lumen, much like a spiral staircase (Fig. 32). Each partition is staggered in relation to the adjacent projections while the aperture is eccentric (Fig. 32). Spermatophores with no projecting sperm tails occur and are supported by the partitions (Figs. 32–33).

Light microscopy of sections of seminal vesicle stained by Mallory's triple method show a distinct lamina interna of circumferential collagen, a broader lamina media of collagen interspersed with smooth muscle fibers (Fig. 34), and an outer collagenous lamina externa. The epithelium is simple columnar with both ciliated and microvillar cells (Fig. 34). Spermatophores have a PAS+ and AB+ cortex and a predominantly PAS+ medulla; although the medulla is less PAS+ than in the ductus. Vesicular elements continue to populate the cortex and medulla (Figs. 35–36) with none in the surrounding fluid, while sperm occupy a loosely organized clear zone within the medulla.

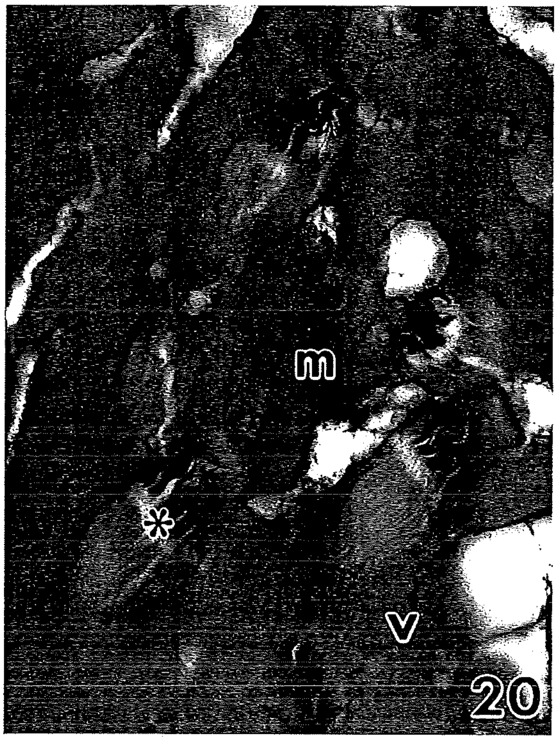
Transmission electron microscopy depicts the epithelium of the seminal vesicles to have both ciliated and microvillar cells (Figs. 37–39). There is scant evidence of secretory activity from either cell type. Peripheral to the definitive spermatophores are areas of matrix and clear zones presumably representing fluid (Figs. 37–39).

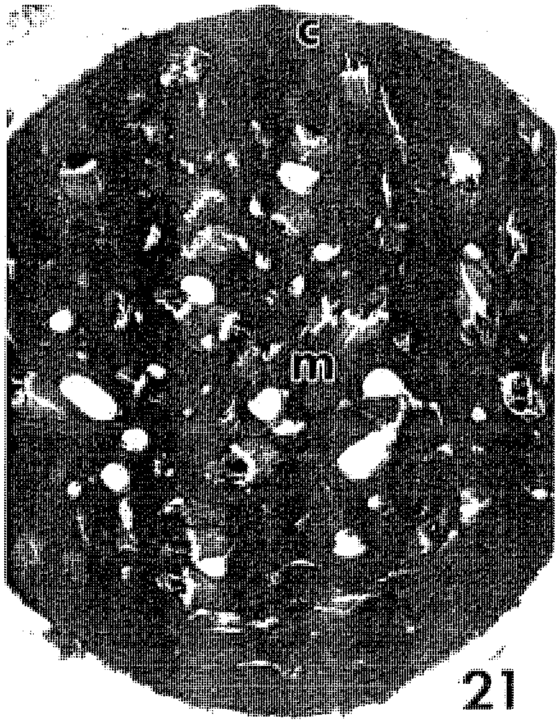
DISCUSSION

The structure and function of the male genital ducts in chondrichthyes has only previously been studied in two species: the Port Jackson shark, *Heterodontus portusjacksoni* (Jones and Jones, '82; Jones et al., '84; Jones and Lin, '93) and the clearnose skate, *Raja eglanteria* (Hamlett et al., '99a). Recently, Jones and Hamlett (2001) used lectin histochemistry to investigate glycosylation of the male genital ducts and spermatozeugmata formation in *R. eglanteria*. The present study is the first to document the ultrastructure of spermatophores and the male genital ducts and Leydig gland in any holocephalan fish.

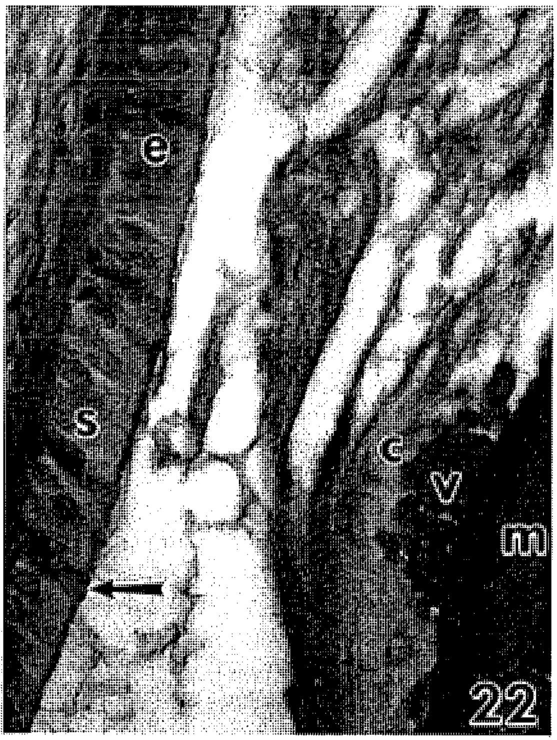
The process of spermatogenesis in the holocephalan, *H. collei*, is essentially the same as in elasmobranchs (Stanley, '63). However, Saperas et al. ('93) showed variation between sperm-specific basic proteins in a comparison between *H. collei* and the shark, *Squalus acanthias*.

Spermatophores have been described in the basking shark, *Cetorhinus maximus* (Matthews, '50) and other elasmobranchs (Pratt and Tanaka,



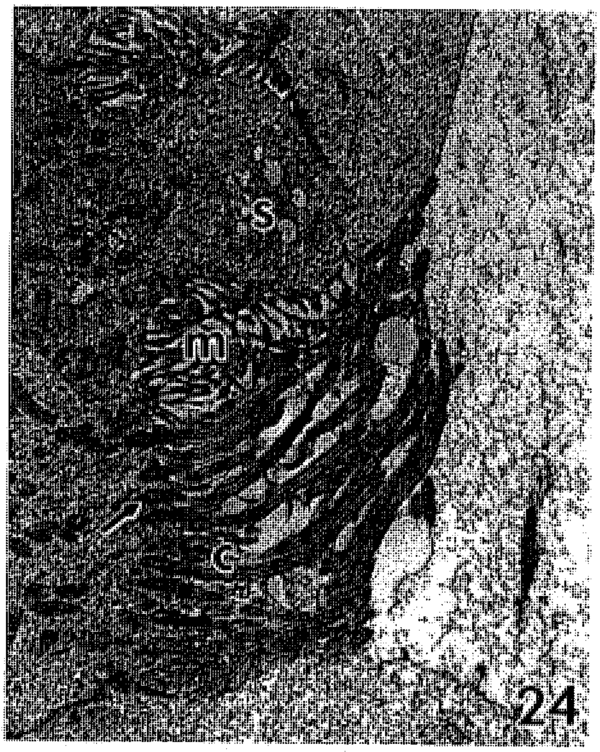


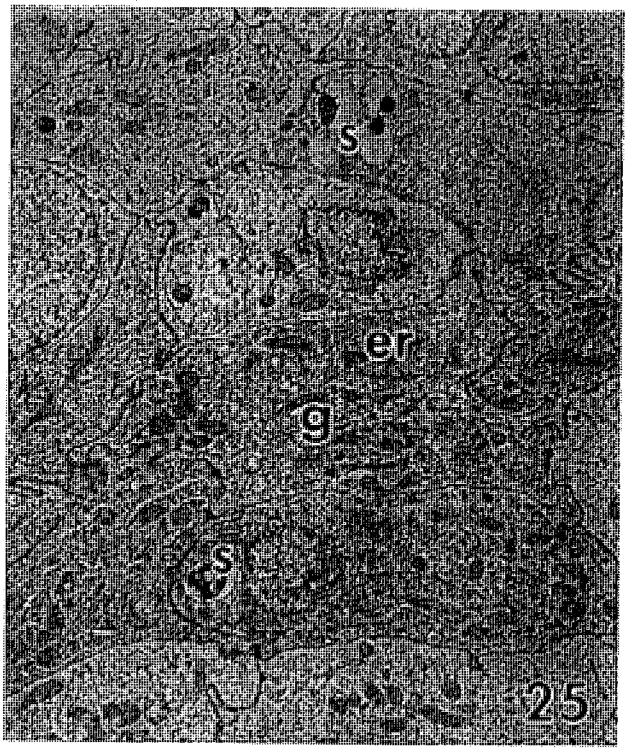
21



Figs. 20–22. Light micrographs of spermatophore in ductus deferens. Bundled sperm (asterisk) occur in the medulla (m) of the spermatophore along with vesicular elements (v). Ductus epithelium (e) consists of secretory cells (s) and ciliated cells (arrow), while some vesicular elements occur in the cortex (c). Figs. 20 and 22 = 600×. Fig. 21 = 200×.

Fig. 23. Transmission electron micrograph of ductus deferens epithelium showing cilia (c), secretory vesicles (s), and luminal secretory product (asterisks). 3,000×.





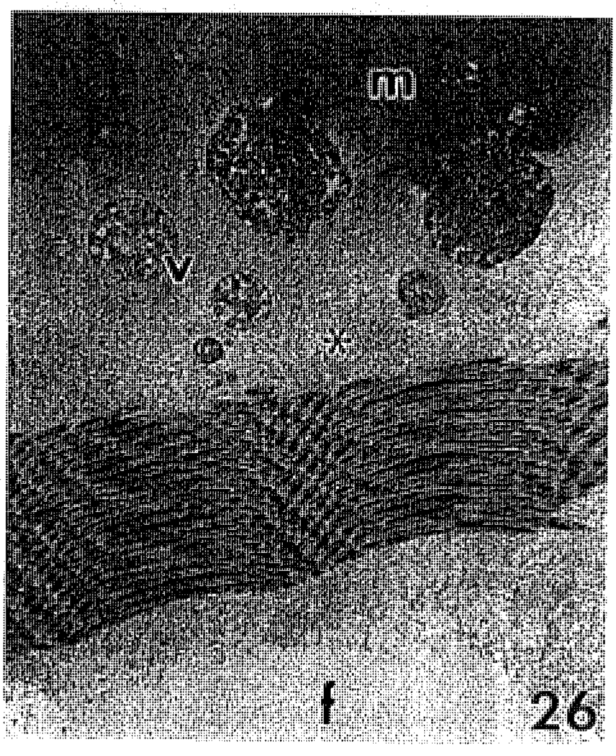
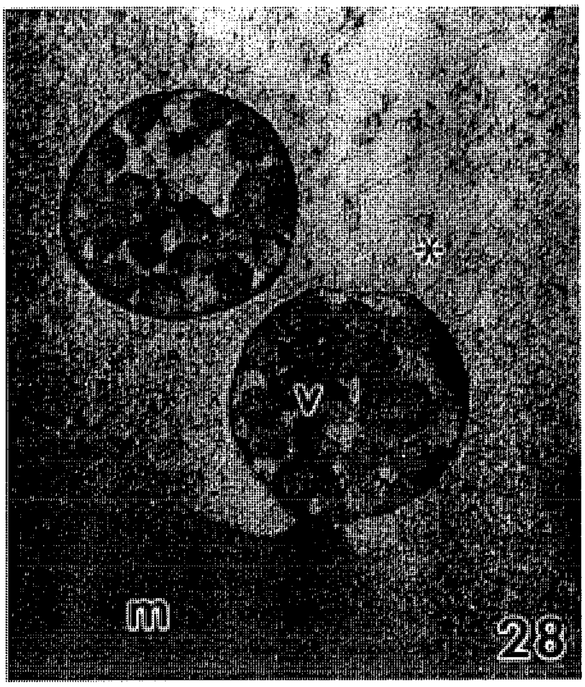
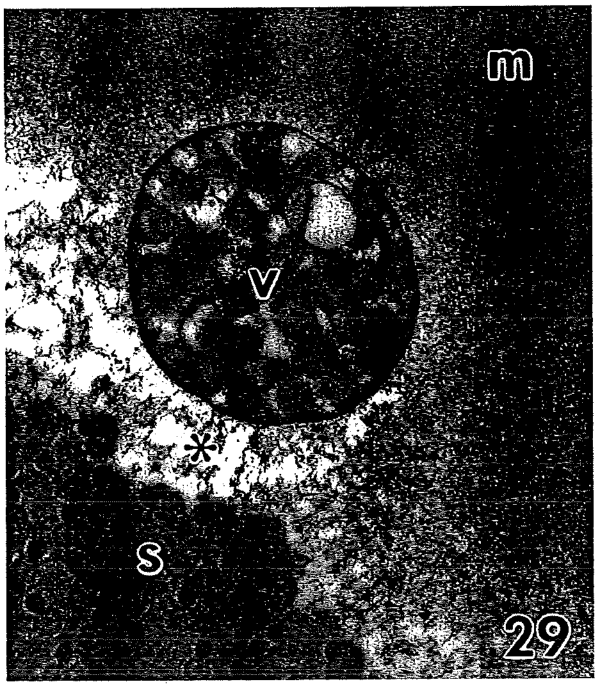


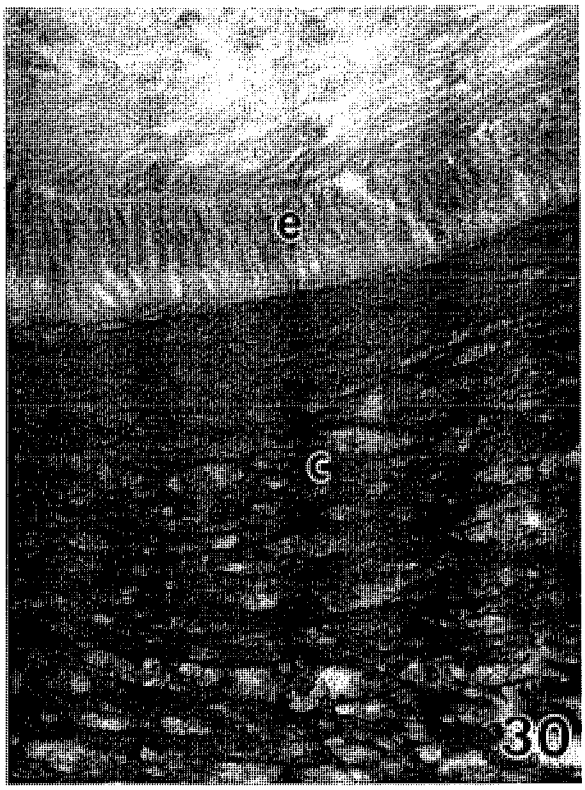
Fig. 24. Transmission electron micrograph of ductus deferens epithelium with cilia (c), basal bodies (arrow), microvilli (m), and secretory vesicles (s). 6,610×.

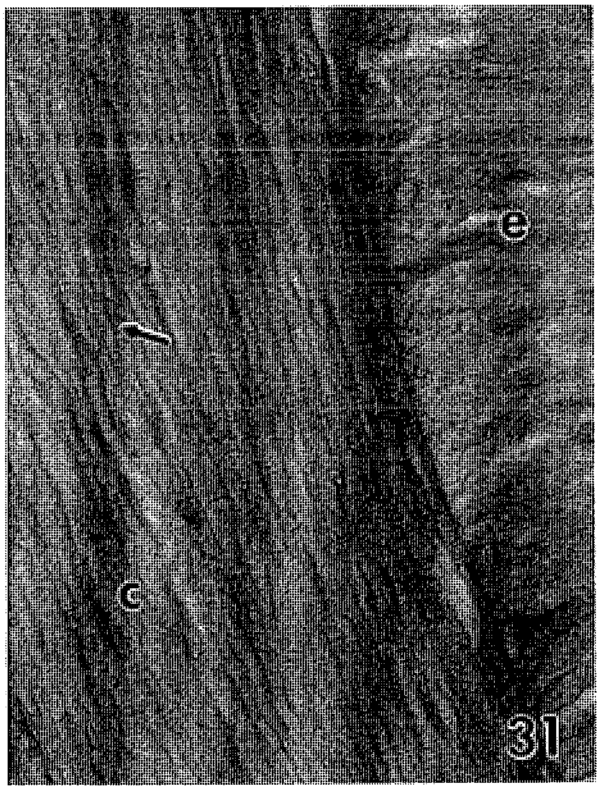
Fig. 25. Transmission electron micrograph of obliquely sectioned ductus epithelial cell showing rough endoplasmic reticulum (er), Golgi complex (g), and secretory vesicles (s). 6,610×.

Figs. 26–27. Transmission electron micrographs of luminal sperm bundles and vesicular elements (v) in the ductus suspended in homogeneous seminal matrix (m), more loosely organized matrical material (asterisk), and seminal fluid (f). Fig. 26 = 2,950×. Fig. 27 = 2,500×.





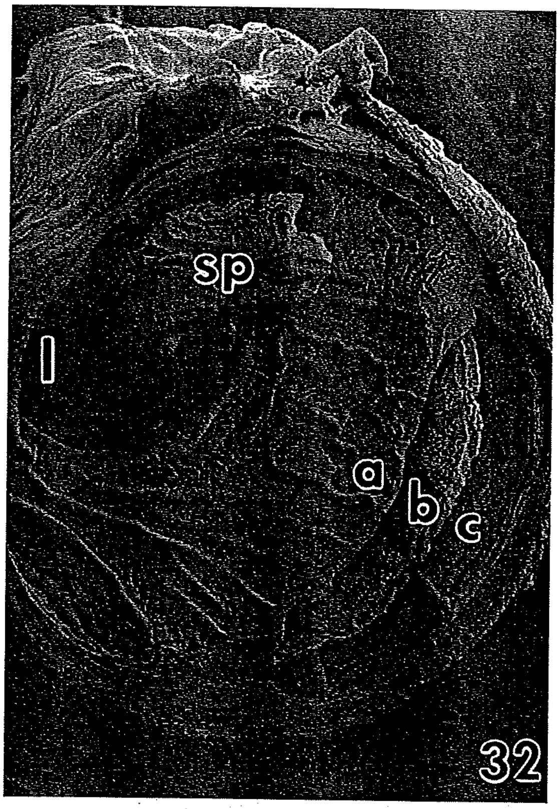


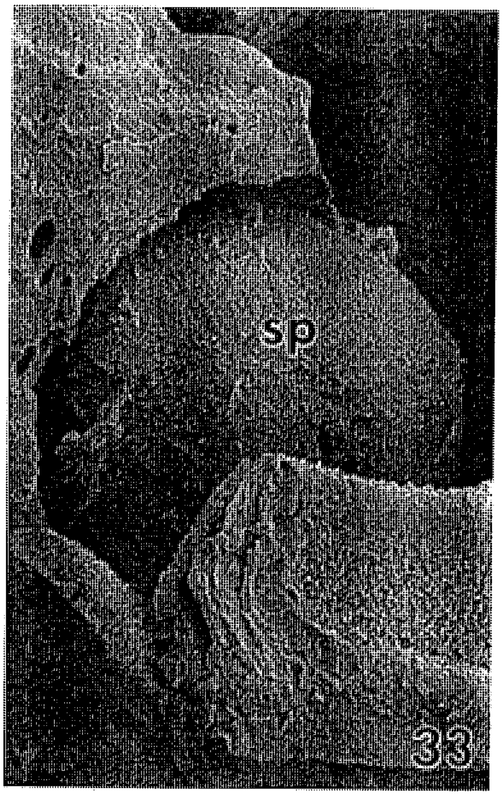


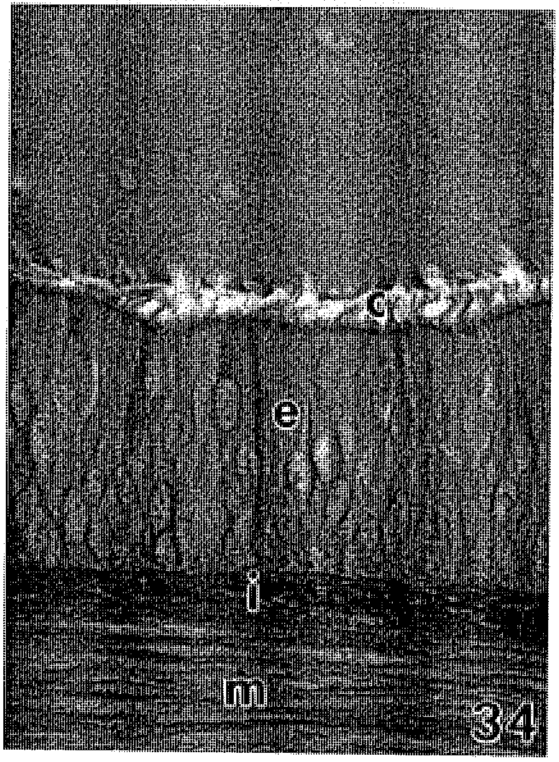
Figs. 28–29. Transmission electron micrographs of ductus deferens luminal contents consisting of bundled sperm (s) and vesicular elements (v) suspended in spermatophore medulla consisting of homogeneous matrix (m) that grades to more loosely organized matrix (asterisk). Fig. 28 = 15,500×. Fig. 29 = 15,500×.

Fig. 30. Light micrograph of paraffin-embedded isthmus between the ductus deferens and seminal vesicle stained with Mallory's triple stain. The columnar epithelium (e) is surrounded by a thick ensheathment rich in circumferential collagen bundles (c). 600×.

Fig. 31. Light micrograph of paraffin-embedded isthmus between the ductus deferens and seminal vesicle stained with hematoxylin and eosin to show the columnar epithelium (e) and smooth muscle nuclei (arrow) interspersed between collagen bundles (c). 600×.







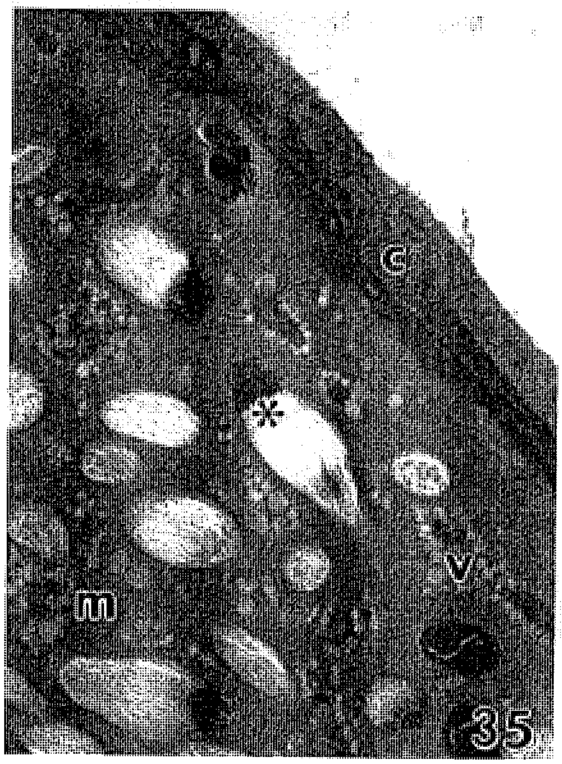


Fig. 32. Scanning electron micrograph of transversely sectioned seminal vesicle showing spermatophore (sp) within eccentrically placed lumen (l) created by spiral partitions (a, b, c) of the epithelium. 50×.

Fig. 33. Scanning electron micrograph of spermatophore (sp) in seminal vesicle. 150×.

Fig. 34. Light micrograph of seminal vesicle stained with Mallory's triple stain shows a ciliated (c) columnar epithelium (e), distinct lamina interna (i) of circumferential collagen, and a broader lamina media (m) of collagen interspersed with smooth muscle fibers. 600×.

Fig. 35. Light micrograph of spermatophore from the seminal vesicle reveals bundled sperm (asterisk), vesicular elements (v) suspended in medullary matrix (m), and the spermatophore cortex (c). 600×.

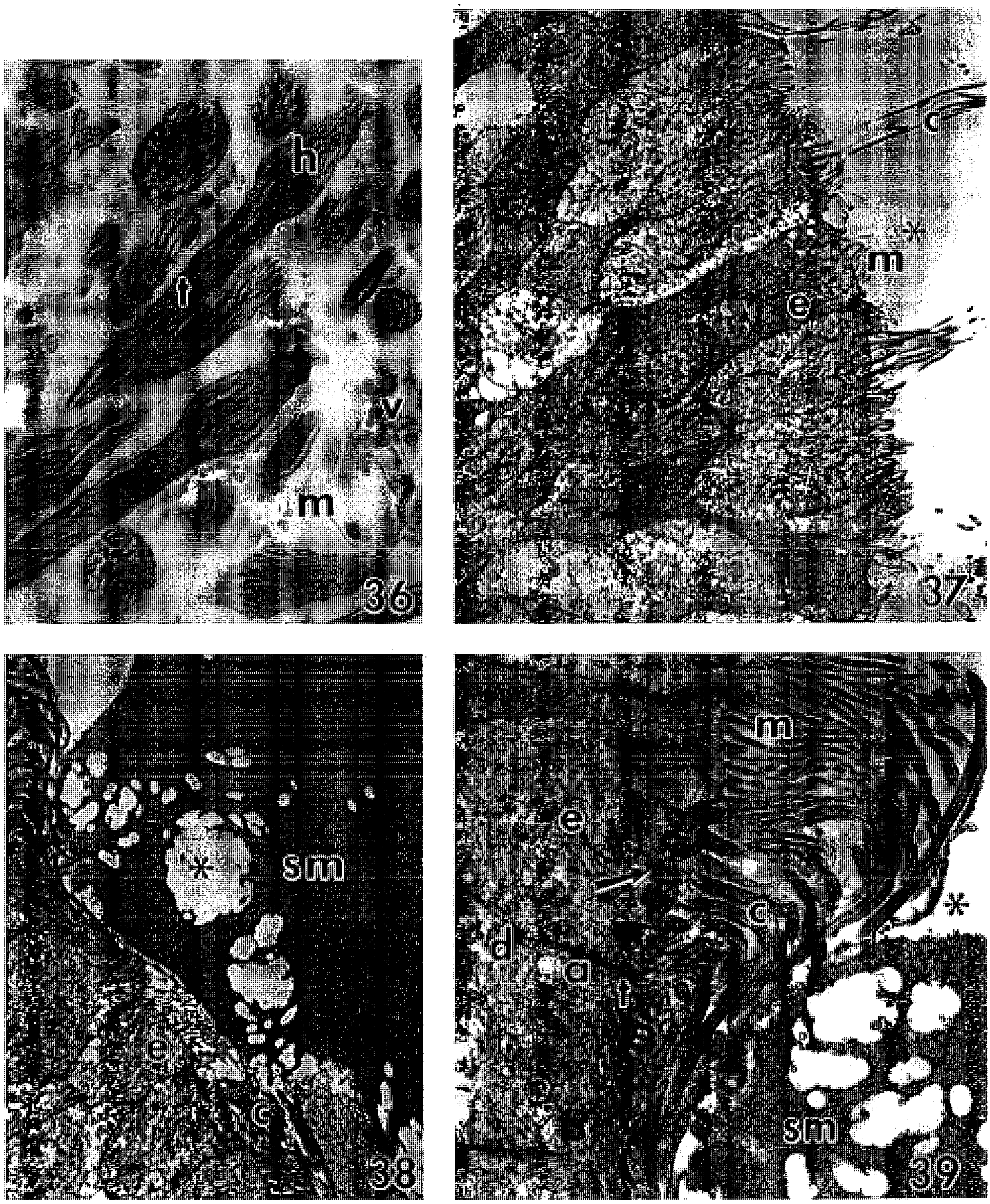


Fig. 36. Light micrograph of bundled sperm in seminal vesicle showing sperm heads (h) and tails (t) surrounded by seminal matrix (m) with vesicular bodies (v). 600×.

Figs. 37–39. Transmission electron micrographs of seminal vesicle epithelium (e) with cilia (c) and microvilli (m) and joined by luminal zonula occludens or tight junctions (t), subjacent zonula adherens (a), and macula adherens or desmosomes (d). Homogeneous seminal matrix (sm) and more fluid components (asterisk) contribute to the spermatophore environment. Fig. 37 = 2,500×. Fig. 38 = 4,000×. Fig. 39 = 10,000×.

'94). Pratt and Tanaka ('94) showed a SEM of a sperm aggregate in *H. collei* and called it a spermatozeugmatum. The edge of the sperm mass was not shown, and no histology was presented; hence, the nature of the sperm packaging requires verification.

The term spermatophore describes sperm aggregation in which laterally aligned bundles of sperm are embedded in matrix to form ovoid to spherical aggregates. Sperm bundles occur only in the medulla. Sperm tails do not protrude from the spermatophore and do not pierce the cortex, thus these are distinct from spermatozeugmata in which sperm tails protrude from the matrix (Pratt and Tanaka, '94).

Various suggestions have been as to why sperm aggregation in male elasmobranchs may be advantageous. Matthews ('50) suggested that spermatophores in the basking shark helped to prevent loss of semen during copulation when the semen must traverse the clasper to the female tract. A turgid spermatophore may be more hydraulically suited to passage to the female without undue loss than a fluid semen. Pratt and Tanaka ('94) mention that spermatozeugmata of carcharhinids and sphyrnids are porous and may provide a mechanism to ensure adequate nourishment reaches the sperm while still packaging them for transport at copulation. The clear zone immediately surrounding sperm bundles in the medulla of *C. milii* appear to be filled with an aqueous medium which may aid in gas and nutrient delivery.

The folds found in the seminal vesicles may increase surface area for nutrient exchange and for physical support (Pratt and Tanaka, '94). Matthews ('50) thought that the septa may play a role in spermatophore formation. He believed that epithelial secretions of the epididymis, ductus deferens, and the Leydig gland mix with spermatozoa. In the upper portion of the seminal vesicle, secretions form globules by action of cilia in the septal bays. The "tumbling mill" action increases the size of spermatophores. Pratt and Tanaka ('94) point out that sperm aggregates occur throughout the seminal vesicle in the short fin mako and doubt the "tumbling mill" theory is active in this species.

In *C. milii*, nascent spermatophore formation begins in the epididymis, although large robust spermatophores are found in the ductus deferens and seminal vesicle. In the clearnose skate, *Raja eglanteria*, the process of sperm aggregation to form spermatozeugmata also starts in the epididymis, but fully formed spermatozeugmata are found in the seminal vesicles (Hamlett et al., '99a). Matthews ('50) described a "tumbling mill" action on forming spermatophores as contributing to their particular shape. This process does not seem to occur in the elephant fish as spermatophores are seen in the ductus deferens. Alternatively, the ovoid shape of the spermatophore in *C. milii* may be a consequence of the fact that a sphere presents the lowest energy form and the smallest surface area to volume ratio, so sperm loss is minimized. Histochemical results show the medulla to be predominantly PAS+, while the cortex is a mixture of PAS+ and AB+ material. The mucopolysaccharide composition of the medulla may make it more gel-like, and the cortex may assume a firmer configuration. Nascent spermatophores are moved through the epididymis and ductus deferens by fluid pressure from the Leydig gland and secretory activity of the epididymis and ductus deferens in concert with ciliary activity. The isthmus and seminal vesicles have smooth muscles to affect spermatophore movement, as well as cilia.

Testicular spermatozoa are immotile but acquire motility after traversing the ductus deferens in species with internal fertilization such as reptiles, birds, mammals, and elasmobranchs (Bedford, '79). In sharks, at copulation and ejaculation, sperm are transferred from the seminal vesicle through the urogenital papilla and out the clasper groove by seawater expelled by the siphon sac (Matthews, '50). Spermatozoa acquire the potential for modest motility while within the terminal regions of the genital ducts but acquire active, robust motility at ejaculation. In *Triakis scyllia*, Minamikawa and Morisawa ('96) measured organic and inorganic constituents, pH, and osmotic pressure in uterine fluid, artificial uterine fluid (300mM NaCl, 8mM KCl, 5.5 mM $CaCl_2$, 2mM $MgCl_2$, 350 mM urea, 20 mM HEPES at pH 7.8), blood plasma, and seminal plasma and correlated the effects on sperm motility. *Triakis* has aplacental viviparity of the yolk sac type. They conclude that ionic levels and hexose have important roles in initiation of sperm motility and may be involved in the maintenance of sperm in the female reproductive tract.

Elasmobranchs maintain high blood plasma levels of urea, which accounts for a considerable proportion of the plasma osmotic pressure (Holmes and Donaldson, '69). *Triakis* blood plasma also had high urea concentrations. Inorganic and organic components of uterine fluid were similar to blood plasma. Na^+ levels in seminal plasma was lower than in blood and seminal K^+ was much higher than in blood. Sperm showed little motility in nonelectrolyte solutions at all concentrations but moved vigorously in NaCl or KCl solutions at a concentration of 500 mM and osmolarity 1,000 mOsm/kg. Sperm mixed with seawater from the siphon sac and the uterine fluid showed osmolarity of 1,000 mOsm/kg. It is likely that proper osmolarity and ion concentrations are a necessary prerequisite for initiation of sperm motility, although other factors such as siphon sac secretions may help to trigger the changes.

Concentration of glucose was 13-fold higher in uterine fluid than seminal fluid, and sperm motility was high in both uterine fluid and blood plasma containing high concentrations of glucose. Sperm motility decreased markedly in media lacking glucose but with the same ionic composition as uterus fluid. It is reasonable to conclude that hexose, especially glucose, has a role in maintenance of sperm motility in the female reproductive tract.

The factors that mediate lateral sperm alignment are unknown. Potential candidates include nonjunctional contacts. Cells migrating in vertebrate embryos generally do not involve organized intercellular junctional complexes, yet the interacting plasmalemmae often come within 10–20 nm (Alberts et al., '94). As several known transmembrane proteins extend above the plasmalemma by 10–20 nm, two cell surface proteins could interact directly to mediate adhesion.

Nonjunctional substances that bind sperm to sperm and sperm clumps to matrix may include integrins, Ig family members, or selectins. Integrins are transmembrane heterodimers, and some integrins bind to only one matrix molecule while others bind to more than one. Binding of integrins to ligands depends on extracellular divalent cations Ca^{2+} or Mg^{2+}, depending on the integrin. The relationship between sperm-matrix binding and sperm motility has yet to be investigated in any cartilaginous fish.

Spermatozoa form spherical bundles as they pass through the seminal vesicle in the Port Jackson shark (Jones and Jones, '82; Jones et al., '84). Bundle formation has also been described in other species including *Spinax niger*, *S. acanthias*, *S. stellaris*, *Chimaera monstrosa*, *T. marmorata* and *T. torpedo* (Redenz and Belonoschkin, '29; Botte et al., '63). In contrast, sperm initially begin to aggregate in the epididymis in both *R. eglanteria* (Hamlett et al., '99a) and *C. milii*. In the Port Jackson shark, post-testicular sperm maturation appears to occur in the epididymis (Jones et al. '84). Leydig gland proteins and changes in potassium:sodium ratio are assumed to play significant roles in sperm maturation. The human epididymis is responsible for secretion of post-testicular proteins involved in sperm maturation, although the precise functions of these proteins is unknown. It is clear, however, that both androgen levels and temperature are factors in modulating their expression (Kirchoff et al., '98). Further studies of hormone levels, lectin histochemistry, and biochemical analysis of spermatophore matrix components in *C. milii* are needed to determine how these factors correlate with sperm aggregation and motility.

The extent of maturation and sperm storage in the genital ducts is related to the life history of the animal and includes such factors as testis size, metabolic rate, and reproductive mode of the female (Jones, '98). Epididymal sperm maturation and storage have been noted in animals that practice internal fertilization, including chondrichthyans, reptiles, birds, monotremes, and eutherians (Bedford, '79). In an oceanic environment, mature males and females may not be available at predictable times; hence, it is advantageous for males to store sperm to take advantage of infrequent matings. Parsons and Grier ('92) showed that the rate of sperm production does not necessarily coincide with mating activity, suggesting that male sperm storage is necessary for optimal reproduction. Sperm storage also occurs in the terminal zone of the oviducal gland in several chondrichthyans (Hamlett et al., '99b,c). Hence, the dialog between male and female sperm storage maximizes the chance of successful propagation.

It seems that secretory activity of the Leydig gland, epididymis, and ductus deferens all contribute to the luminal milieu of the genital ducts. These secretions, no doubt, perform functions related to sperm maturation with storage occurring in the seminal vesicle. Further studies of luminal solute concentrations in the genital ducts are needed. The walls of the ductus deferens are much more folded than the epididymis and might be involved in fluid resorption through increased surface area, as well as by an abundance of apical mitochondria. The seminal vesicle enlarges greatly during and after sperm formation. It is clear that the seminal vesicle is involved with sperm storage and perhaps maintenance. The smooth muscle investiture is likely responsible for ejaculation, although actual mating has not been studied in *C. milii*.

The role of the multitude of vesicular bodies incorporated into the spermatophore matrix remains uncertain. The exact origin of all components of the vesicular bodies is unclear. Some of the vesicular bodies appear to be remnants of Sertoli cells, as organelles are observed in them, but for the most part they appear to be derived from the Leydig gland. The fact that patterns of commercial fish catches indicates sexual segregation in this species indicates that mating may be infrequent and it is important for males to have a ready supply of sperm stored in their seminal vesicles. The large number of vesicular bodies cannot be accounted for by Sertoli cell origin, so the conclusion is that contributions primarily from the Leydig gland form these elements. They may serve as an energy source for the sperm or perform some as yet unelucidated function. Insights into their function await biochemical analysis.

Of considerable interest is the role played by the matrix in which sperm are carried when the sperm is delivered to the female. Analysis of the sperm matrix is needed to ascertain its role beyond that of simply serving as a transport medium. How does the matrix and the sperm interact with the female tract and its secretions? What is the role of the male matrix in sperm activation, motility, nutrition, etc.? These and other questions remain to be answered. Chondrichthyans provide a unique opportunity for the study of cell and molecular mechanisms of sperm-male and sperm-female interactions.

Much remains unknown regarding the reproductive biology of *C. milii*. They have unique features not found in other chondrichthyes, including an accessory head appendage and prepelvic claspers in the male and a "sperm pouch" adjacent to the cloaca in the female. Mating behavior is unknown, and copulation has never been documented. A so-called "sperm plug" resides in the female sperm pouch and not in the cloaca, as the common name indicates. Histological examination of sperm plugs (Smith, 2001) indicates they contain sperm bundles, but it is undetermined if the sperm are viable. It is also unknown if more than one male contributes to the plug. The role of the head appendage, prepelvic claspers, and sperm plug remain to be investigated.

ACKNOWLEDGMENTS

This study was supported by funds provided by Indiana University School of Medicine. Portions of the work were accomplished while W.C. Hamlett was on sabbatical leave in the Department of Zoology, The University of Melbourne, Parkville, Victoria, Australia. During this time, support was provided by Enhancing International Science Networks Visiting Fellowship from the Department of Natural Resources and Environment, Melbourne, Australia, and a Visiting Research Scholar Travel Grant from The University of Melbourne, Parkville, Victoria, Australia. We wish to thank Prof. Marilyn Renfree, Chair of the Department of Zoology, The University of Melbourne, for arranging research space, the collection of specimens, logistical and technical support, and a supportive and collegial research environment.

LITERATURE CITED

Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson JD. 1994. Molecular biology of the cell. New York, NY: Garland Pub. Inc.

Bedford JM. 1979. Evolution of the sperm maturation and storage functions of the epididymis. In Fawcett DW, Bedford JM, editors. The spermatozoon: Maturation, motility, surface properties and comparative aspects. Baltimore, MD: Urban and Schwarzenburg. p 7–21.

Borcea J. 1906. Systeme uro-genital des elasmobranches. Arch Zool Exper Gen 4:119–484.

Botte V, Chieffi G, Stanley HP. 1963. Histological and histochemical observations on the male reproductive tract of *Scyliorhinus stellaris*, *Torpedo marmorata* and *T. torpedo*. Publ Sta Zool, Napoli 33:224–242.

Hamlett WC. 1999. Male reproductive system. In: Hamlett WC, editor. Sharks, skates, and rays: the biology of elasmobranch fishes. Baltimore, MD: The Johns Hopkins Univ Press. p 444–470.

Hamlett WC, Hysell MK, Rozycki T, Brunette N, Tumilty K, Henderson A, Dunne J. 1999a. Sperm aggregation and spermatozeugmata formation in the male genital ducts in the clearnose skate, *Raja eglanteria*. In: Seret B and Sire J-Y, editors. 5th Indo-Pacific Fish Conference Noumea. 1997 ed. Paris: French Ichthyological Society and ORSTOM. p 281–291.

Hamlett WC, Hysell MK, Jezior M, Rozycki T, Brunette N, Tumilty K. 1999b. Fundamental zonation in elasmobranch oviducal glands. In: Seret B and Sire J-Y, editors. 5th Indo-Pacific Fish Conference Noumea. 1997 ed . Paris: French Ichthyological Society and ORSTOM. p 271–280.

Hamlett WC, Knight DP, Koob TJ, Jezior M, Luong T, Rozycki T, Brunette N, Hysell MK. 1999c. Survey of oviducal gland structure and function in elasmobranchs. J Exp Zool 282: 399–420.

Holmes WN, Donaldson EM. 1969. Fish Physiology, Vol. I. New York, NY: Academic Press. p 1–89.

Jones CJP, Hamlett WC. 2001. Glycosylation of the male genital ducts and spermatozeugmata formation in the clearnose skate, *Raja eglanteria*. Proc XVI Internat Symp Morphol Sci, Sun City, South Africa. p. 11.

Jones N, Jones RC. 1982. The structure of the male genital system of the Port Jackson shark, *Heterodontus portusjacksoni*, with particular reference to the genital ducts. Aust J Zool 30:523–541.

Jones RC, Jones N, Dakiew D. 1984. Luminal composition and maturation of spermatozoa in the male genital ducts of the Port Jackson shark, *Heterodontus portusjacksoni.* J Exp Zoo. 230: 417–426.

Jones RC, Lin M. 1993. Structure and functions of the genital ducts of the male Port Jackson shark, *Heterodontus portusjacksoni.* Environ Bio Fish 38:127–138.

Jones RC. 1998. Evolution of the vertebrate epididymis. J Repro Fertil Suppl 53:163–181.

Kirchoff C, Osterhoff C, Pera I, Schröter. 1998. Function of human epididymal proteins in sperm maturation. Andrologia 30:225–230.

Matthews HL. 1950. Reproduction in the basking shark, *Cetorhinus maximus.* Phil Trans Royal Soc London, Biol 234:247–316.

Minamikawa S, Morisawa M. 1996. Acquisition, initiation and maintenance of sperm motility in the shark, *Triakis scyllia.* Comp Biochem Physiol 113A:387–392.

Parsons GR, Grier J. 1992. Seasonal changes in shark testicular structure and spermatogenesis. J Exp Zool 261: 173–184.

Pratt L, Tanaka S. 1994. Sperm storage in male elasmobranchs: a description and survey. J Morph 219:297–308.

Reardon M. 2001. Seasonality and microanatomy of spermatophore formation in a holocephalan, the elephant fish, *Callorhynchus milii.* Honors thesis, Dept. Zool. The University of Melbourne, Parkville, Victoria, Australia. 44 p.

Redenz E, Belonoschkin B. 1929. Form und funktion des nebendens einiger elasmobranchier. Zellforsch Mikrosk Anat 9:663–682.

Saperas N, Chiva M, Bols NC, Kulak D, Kasinsky HE. 1993. Sperm specific basic proteins in the holocephalan fish *Hydrolagus colliei* (chondrichthyes, chimaeraformes) and comparison with protamines from an elasmobranch. Biol Bull 185:186–196.

Smith R. 2001. The reproductive biology of the female elephant fish, *Callorhynchus milii,* with particular reference to the oviducal gland. Honors thesis, Dept. Zool. The University Melbourne, Parkville, Victoria, Australia. 60 p.

Stanley H. 1963. Urogenital morphology in the chimaeroid fish *Hydrolagus collei* (Lay and Bennett). J Morphol 112: 99–128.

CSIRO PUBLISHING

www.publish.csiro.au/journals/mfr
Marine and Freshwater Research, 2004, 55, 155–164

Microscopic organisation of the oviducal gland of the holocephalan elephant fish, *Callorhynchus milii*

Rachel M. Smith[A,B,E], *Terence I. Walker*[C] *and William C. Hamlett*[A,D]

[A]Department of Zoology, The University of Melbourne, Victoria 3010, Australia.
[B]4 Bloomfield Road, Ascot Vale, Victoria 3032, Australia.
[C]Primary Industries Research Victoria, PO Box 114, Queenscliff, Victoria 3225, Australia.
[D]Department of Anatomy & Cell Biology, Indiana University School of Medicine, South Bend Center for Medical Education, Notre Dame, IN 46530, USA.
[E]Corresponding author. Email: rachel.smith@csl.com.au

Abstract. The study of chondrichthyan reproductive biology has a long history, but the structure and function of the holocephalan oviducal glands (OG) is poorly known; these organs are a vital component in the understanding of chondrichthyan life history. Histochemical techniques revealed that a fundamental zonation was evident in the OG of *Callorhynchus milii*, similar to most elasmobranchs. In sexually mature females, the following zones occurred (anterior to posterior): (*1*) club zone, periodic acid–Schiff positive (PAS+), indicating glycoprotein or any mucus substance containing neutral sugars, and Alcian blue positive, pH 2.5 (AB+), indicating the presence of sulfated and unsulfated acid glycosaminoglycans and sialoglycoproteins; (*2*) papillary zone (AB+); (*3*) baffle zone (PAS−, AB−); and (*4*) terminal zone (AB++). Using histological and histochemical techniques not used previously with the holocephalan group, we demonstrated that the structure and function of the OG zones were equivalent between oviparous elasmobranchs and *C. milii*, even though their final egg capsule morphologies differed. It was also evident that the club and papillary zones produce the egg jelly that surrounds the egg and the baffle zone formed the multilaminate egg capsule. Furthermore, the terminal zone had functions associated with sperm storage and the production of fine hairs that decorate the surface of the egg capsule.

Introduction

The cartilaginous chondrichthyan fish comprise two subclasses, namely the elasmobranchs (sharks and rays) and the holocephalans (chimaeras). All use internal fertilisation and females display several diverse reproductive modes, including oviparity (egg laying) and several types of viviparity (giving birth to living young; Hamlett and Koob 1999). All chondrichthyans have a typical repertoire of vertebrate hormones that mediate sexual cycles and gonadal maturation (Callard and Koob 1993). All skates, all holocephalans, and approximately 40% of all sharks are oviparous (Hamlett and Koob 1999).

Most chondrichthyans possess a specialised region of the oviduct termed the oviducal gland (OG; Hamlett *et al.* 1998, 1999), which transfers eggs to the uterus, produces egg jelly, forms the tertiary egg envelope, and stores sperm. The primary egg envelope is produced by the oocyte, the secondary envelope is produced by the follicle or granulosa cells and the tertiary egg envelope is produced by the OG. In oviparous species, the tertiary egg envelope is often termed the egg capsule.

The elasmobranch OG has distinct regions or zones, characterised by different histological organisation and staining affinities. The zones are named based on the histological profile of the surface mucosa, as seen in longitudinal section when viewed with a light microscope. The most anterior is the club zone, which bears a resemblance to an Indian club. The next is the papillary zone, which presents the profile of a small cone or papilla. Both the club and papillary zones produce egg jelly that surrounds the egg. The baffle zone is the region that produces the multilaminate egg capsule and is named due to the presence of baffle plates that direct the stream of nascent egg capsule secretion as it emerges from secretory ducts (Knight *et al.* 1996). The most posterior zone is the terminal zone, named for its position. It functions in sperm storage and the production of fine hairs that adorn the exterior of the egg capsule in species that have hairs (Metten 1939; Prasad 1945*a*; Hamlett *et al.* 1998).

Most of the published literature on the structure and function of the OG in chondrichthyans has focused on the oviparous small-spotted catshark *Scyliorhinus canicula* (Metten 1939; Threadgold 1957; Rusaouën 1976; Knight *et al.* 1996). Analysis of the OG in this species revealed histochemical differences between the various zones (Threadgold 1957; Rusaouën 1976), indicating a separate function for each zone. Only basic morphological descriptions of the

10.1071/MF01078 1323-1650/04/020155

holocephalan OG have been published (Dean 1895, 1906, 1912; Prasad 1948; Stanley 1963) and there has been no histochemical analysis to allow comparison with the elasmobranch OG.

The suggestion that the OG may function as a sperm storage device was first made by Lo Bianco (1909). Sperm were found in the lumen of tubules that secrete the egg capsule, but no spermatozoa were observed in the region that secretes egg jelly (Metten 1939). Prasad (1945*a*) identified spermatozoa in the egg capsule-secreting region in the OG of five elasmobranch species and Pratt (1993) described sperm storage in several elasmobranch species. Hamlett *et al.* (1998) presented comparative studies on the structure and function of the OG in a select group of elasmobranch fish displaying a variety of reproductive modes and concluded that sperm storage occurred exclusively in the terminal zone. What had been reported previously as sperm 'storage' in the egg capsule-producing zone was interpreted as 'incidental' occurrence resulting from filling of the tubules at insemination. Egg capsule-producing tubules were subsequently emptied with the elaboration of the egg capsule, thereby flushing sperm from the tubules. Dean (1906) found sperm in the upper reaches of the oviduct of the holocephalan *Hydrolagus colliei*, but did not suggest its presence constituted sperm storage.

Materials and methods

Female elephant fish (*C. milii*) were caught by commercial fishers using gill-nets in the south-eastern areas of Australia of Port Phillip Bay (February–June 2000), Western Port (February–March 2001) and Bass Strait (March 2000–January 2001). Animals were killed by blunt trauma to the cranium, measured, and dissected.

The right urogenital tract was isolated and fixed in 10% neutral buffered formalin. Oviducal glands were cut in sagittal section and the oviduct and uterus sliced (4–8 mm). Tissue samples were placed in processing cassettes or wrapped in gauze. A 15% formic acid solution was applied to decalcify the samples before dehydration through a series of alcohols and embedding in paraffin wax (Paraplast Wax, Z Corporation, Burlington, MA, USA; Stevens 1990). Sections were stained with Mallory's triple stain (Humason 1972), hematoxylin and eosin (Stevens 1990) or combined Alcian blue (AB) pH 2.5 and periodic acid–Schiff (PAS; Cook 1990). Coverslips were mounted with adhesive (DPX, Electron Microscopy Sciences, Hatfield, PA, USA).

Sections were photographed on an Olympus BX50 microscope (Olympus Optical Co. Ltd, Tokyo, Japan), equipped with an Olympus PM30 camera attachment, using slide film (Fujichrome Velvia ISO 40; Fuji Photo Film USA, Cypress, CA, USA). Slides were scanned digitally using a slide scanner (Nikon DX2000; Maxwell Optical Industries, Sydney, Australia).

For scanning electron microscopy, egg capsule and tissue samples were cut to appropriate size and fixed in a glutaraldehyde solution (10% paraformaldehyde, 25% glutaraldehyde and picric acid in 0.1 M cacodylate buffer). Samples were washed three times in distilled water for 10 min each and dehydrated through a graded series of alcohols (30%–100%) for 10 min each. They were placed in two changes of 100% ethanol and one change of 100% dry ethanol for 30 min each and in a 1 : 1, followed by a 1 : 2, parts solution of ethanol to hexamethyldysilidane (HMDS) and, finally, by 100% HMDS for 5 min each. Excess HMDS was pipetted off and the dry samples were placed in a desiccator. Specimens were mounted on stubs with silver dag and sputter coated with gold in an atmosphere of argon. Images were digitally collected on a Philips 505 scanning electron microscope (Philips Industries, Eindhoven, The Netherlands) and viewed using Spectron software (The University of Melbourne, Australia).

Results

Egg capsule

Tanned egg capsules of *C. milii* (Fig. 1) have two distinct surfaces, dorsal and ventral. The capsule is laid with the ventral surface in contact with the substrate, usually sand or mud. The ventral surface is smooth with a raised area to accommodate the egg and lateral flanges with grooves. The dorsal surface is convex with a broad dome and lateral flanges decorated with fine hairs (Figs 1, 2). Oviducal glands have the shape of a crescent, with the outer curve pointed anteriorly and the tips directed posteriorly (Fig. 3).

Oviduct

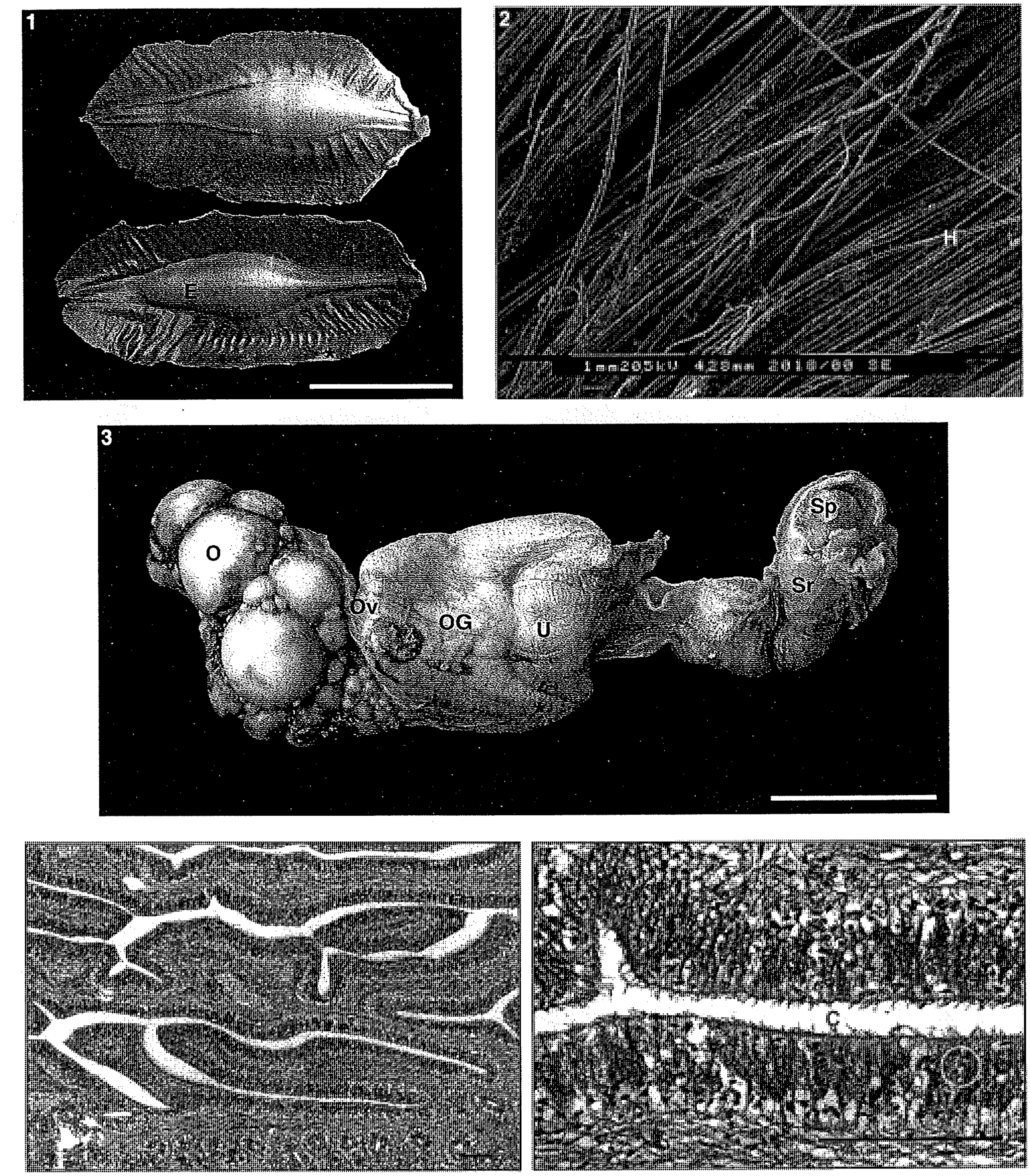
The anterior oviduct is characterised by ciliated longitudinal folds surrounded by a thin band of smooth muscle (Fig. 4). The epithelium comprises two cell types, mucous and simple ciliated columnar cells (Fig. 5). Ciliated cells are pyriform in shape, with nuclei situated near the lumen. Mucous cells are simple columnar with PAS+ apical secretory vesicles and basal nuclei. Ciliary activities, in concert with smooth muscle contractions, provide the force to move the ova through the oviduct.

Oviducal gland

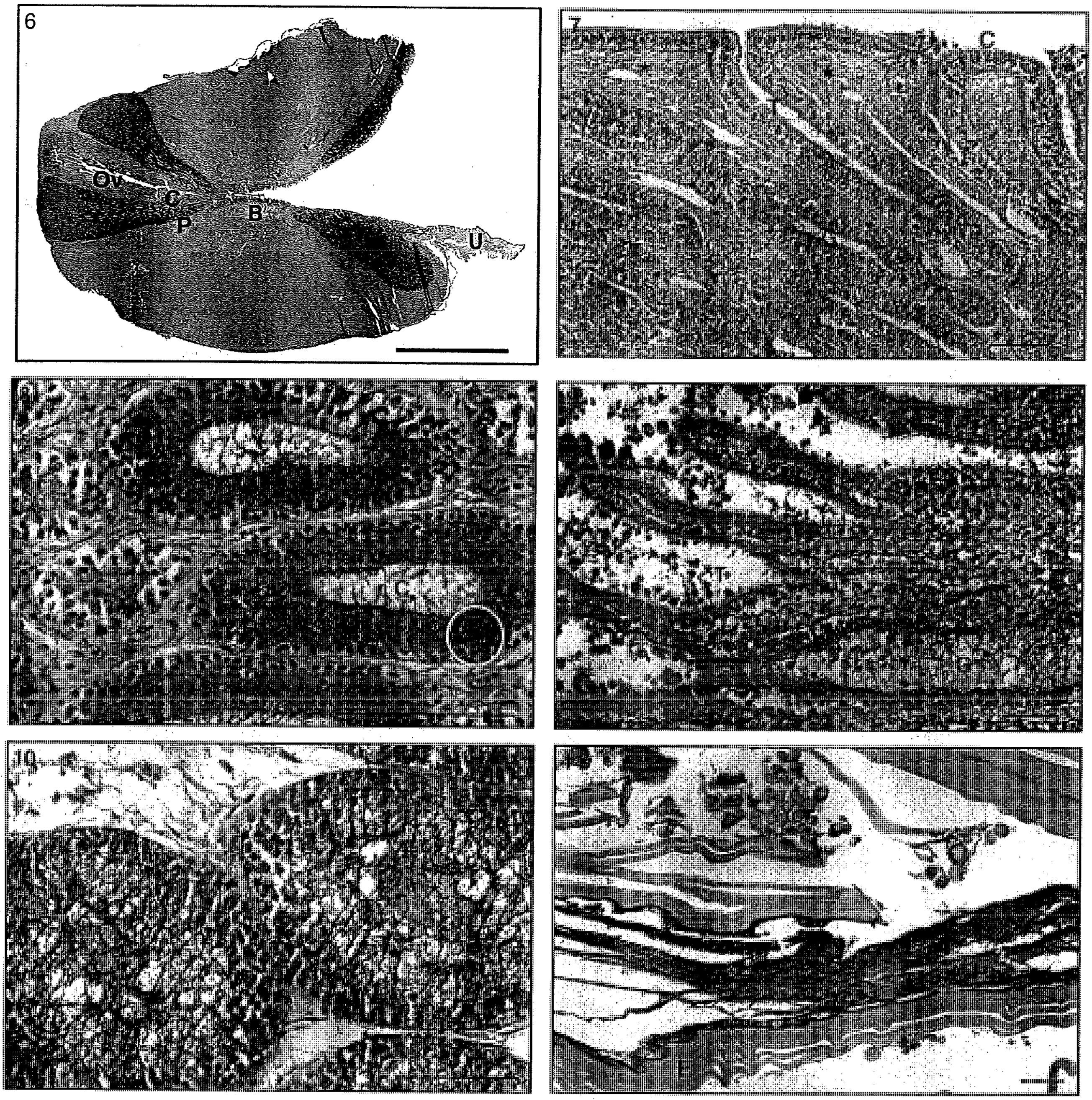
The same four fundamental zones described in most elasmobranch OG are present in the OG of *C. milii* (Fig. 6). Oviducal glands are characterised by four distinct zones (Fig. 6) based on the shape of the epithelial surface folds or lamellae. These four zones are the club, papillary, baffle and terminal, each responsible for a unique function, including the coating of the ova with egg jelly, encapsulation of ova, formation of surface hairs, and sperm storage. On the basis of AB–PAS staining, each different zone displays unique staining affinities. During the egg-laying season, the OG become larger as the secretory tubules expand and dilate as they engorge with secretory products.

Club zone

Club zone lamella are truncated basally and expand luminally to resemble an Indian club (Fig. 7) when viewed in longitudinal section via light microscopy. The surface epithelium of the lamellae are simple ciliated columnar. Adjacent lamellae are separated by transverse grooves. Simple tubular glands deposit secretory product into the transverse grooves. Simple tubular glands are composed of ciliated columnar cells with basal nuclei and secretory cells that contain a mixture of AB+ and PAS+ secretory material (Fig. 8). A series of adjacent tubules elaborate their secretions in synchrony into



Figs 1–5. (*1*) Egg capsules of *C. milii*; the lateral flanges (*) contain grooves. The top capsule shows the dorsal surface, which is covered in hairs. The bottom capsule shows the ventral surface; (E) denotes the compartment that houses the fertilised egg and egg jellies (Scale bar = 110 mm). (*2*) A scanning electron micrograph of the external egg capsule showing the hairs (H) that cover the dorsal surface of the egg capsule. (*3*) External view of the female reproductive tract of *C. milii*. Ovary (O) with enlarged ova. The oviduct (Ov) is confluent with the oviducal gland (OG). The expanded lateral margins of the OG (*) merge with the uterus (U). The sperm receptacle or pouch (Sr) contains a sperm plug (Sp) that has been deposited by the male at copulation (Scale bar = 75 mm). (*4*) Longitudinal section of the anterior oviduct, consisting of longitudinal folds of simple ciliated epithelium (Scale bar = 10 μm). Stains throughout are combined Alcian blue–periodic acid–Schiff unless otherwise noted. (*5*) Epithelium of a longitudinal fold of oviduct, composed of two cell types; ciliated cells (C) with the nuclei located towards the luminal aspect of the cells (circled) and secretory cells (S) with basally located nuclei (Scale bar = 10 μm).



Figs 6–11. (*6*) A Nikon scan of a microscope slide stained with combined Alcian blue–periodic acid–Schiff of a longitudinal section of an OG of *C. milii* showing the oviduct (Ov), leading to the club (C), papillary (P), baffle (B) and terminal (T) zones which merge with the uterus (U) (Scale bar = 10 mm). (*7*) Club shaped surface folds of lamellae (*) have ciliated (C) epithelium and are separated by transverse grooves (T). Tubular glands (G) release secretory material into the OG lumen, between adjacent club projections (Scale bar = 10 μm). (*8*) A club zone gland tubule with ciliated cells (C) and secretory cells (circled), where half the tubule is staining for PAS+ and the other half is staining for AB+, indicating the heterogeneous mixture secreted by the club zone (Scale bar = 10 μm). (*9*) Adjacent papillae (*) when viewed in longitudinal section, have transverse grooves (T), where tubular glands (G) empty their AB+ secretory material (Scale bar = 10 μm). (*10*) A longitudinal section of a gland tubule in the papillary zone consisting of secretory cells (circled) secreting the AB+ secretory material (*) and ciliated sustentacular cells (Scale bar = 10 μm). (*11*) Secreted egg capsule and jellies moving down the OG lumen. The egg capsule material is composed of many layers (E). The AB+, PAS+ egg jelly material secreted by the club zone (C) is surrounded by the AB+ egg jelly from the papillary zone (P). The bonding layer (*) secreted by the caudal region of the papillary zone, is thought to bond the capsule and jellies together. Yolk platelets (Y) that have shifted during sectioning (Scale bar = 10 μm).

a common transverse groove, thereby producing one jelly layer. The secretory product of the anterior-most club zone produces jelly that is in contact with the egg. Each more posterior segment of the club zone adds a peripheral jelly coat. The additive result is an ever-thickening jelly coat. Subjacent to the epithelium, the connective tissue stroma is characterised by collagen bundles, fibroblasts, and a rich vasculature.

Papillary zone

The papillary zone is characterised by lamellae that take the form of small cones or papillae when viewed in longitudinal section (Fig. 9). The epithelium is simple ciliated columnar. As in the club zone, lamella alternate with transverse grooves. Simple tubular glands discharge their AB+ secretory product into the transverse grooves (Fig. 9). Tubular glands have two cell types: supportive or sustentacular and secretory cells (Fig. 10). Supportive cells are pyriform in shape and have nuclei located near the lumen. Secretory cells are simple columnar with basal nuclei and are AB+. They are more vacuolated than those in the club zone and the secretory vesicles elaborate an AB+ egg jelly peripheral to that of the club zone (Fig. 10). Secretory cells of the caudal-most papillary tubules, adjacent to the baffle zone, produce a more intensely AB++ secretory material than the remainder of the papillary zone. These caudal-most papillary tubules secrete a bonding layer that attaches the peripheral-most aspect of the jelly layer to the inside of the egg capsule (Fig. 11). In an egg capsule containing ova coated with egg jelly, the peripheral-most component of the jelly adheres to the interior of the egg case.

The morphological appearance of gland tubules in the club and papillary zones is similar at the light microscopic level, but the gland tubules in the two zones display distinct morphological differences at the ultrastructural level (W. C. Hamlett, unpublished observations). At the light microscopic level, the difference between the zones is in the morphology of the surface lamellae and the type of secretory products produced. Egg jelly secreted by the club and papillary zones emerges from gland tubules as elongated strands that expand and become hydrated as they move to the gland lumen and into the partially secreted egg capsule (Fig. 12).

Baffle zone

The baffle zone consists of apically flattened lamellae, alternating with transverse grooves that extend across the width of the gland (Fig. 13). The lamellae are narrow at the base, but expand to form a flattened surface plateau of densely ciliated columnar epithelium. Simple tubular glands are comprised of two cell types: sustentacular, consisting of ciliated columnar cells with nuclei adjacent to the lumen, and secretory cells with apical secretory vesicles and basal nuclei (Fig. 14). The AB– and PAS– secretory material is elaborated by simple tubular glands. Nascent egg capsule material then enters a secretory duct leading to the spinneret region. The spinneret is composed of a pair of flattened baffle plates situated at the mouth of the secretory duct. The baffle plates are coated with cilia and, as secretory product emerges from the secretory duct, it is extruded between the paired baffle plates. Secretory products from adjacent spinnerets merge together in the common transverse groove to form one lamina of the multilaminate egg capsule. Therefore, each more posterior transverse groove adds another peripheral lamina to the capsule.

Terminal zone

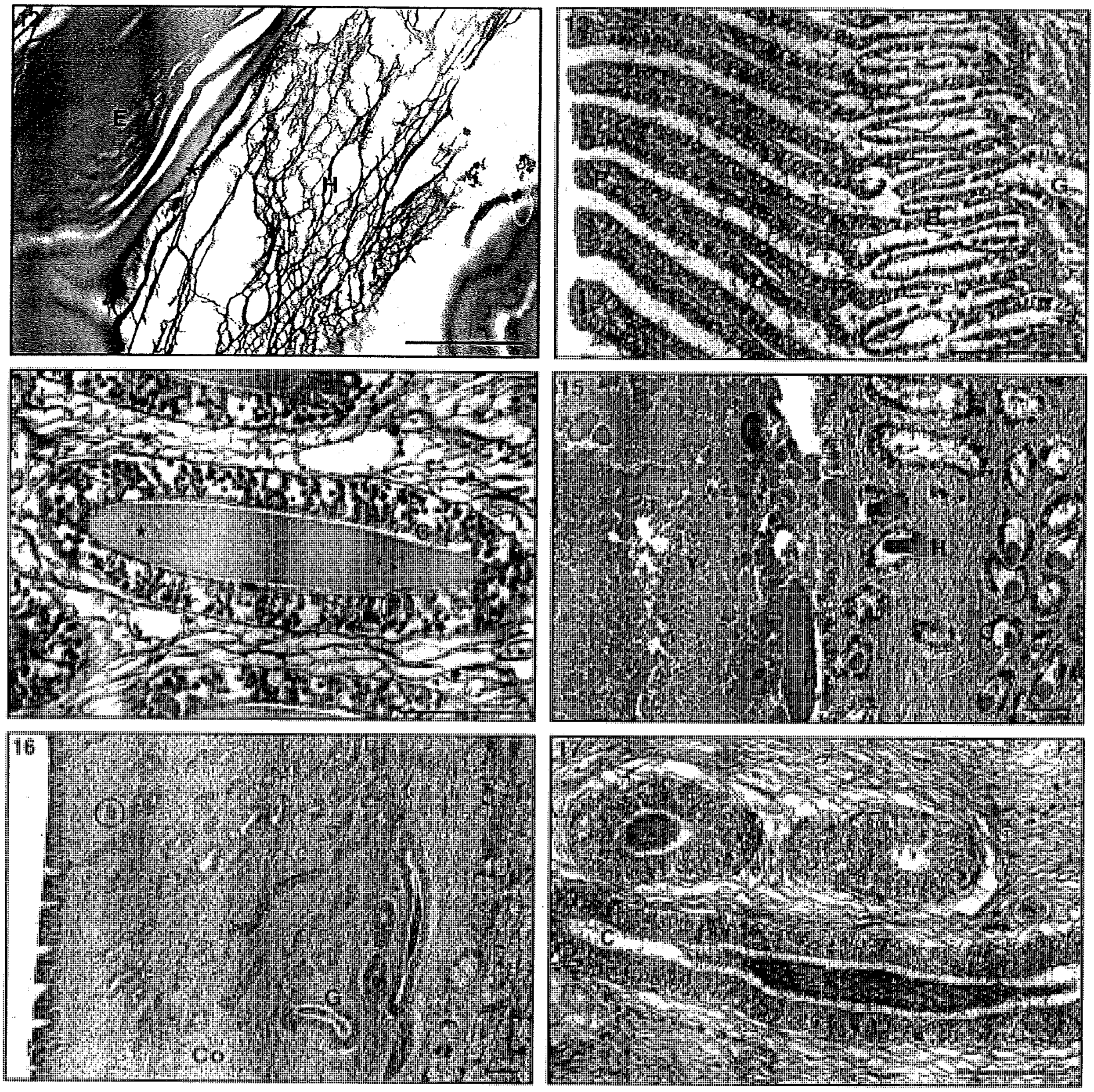
The terminal zone is broader and more expansive than the others. It is not organised into lamella but consists of isolated, scattered tubules. Hair-forming tubules consist of simple glands; the base of each tubule is composed of serous cells that are microscopically identical to secretory cells of the baffle zone (Fig. 15). The luminal aspect of the tubules is populated by AB+ mucous cells that elaborate a coating on each hair. Hairs have the same staining affinity (AB–, PAS–) as egg capsule material secreted by the baffle zone. The hairs remain as small, discrete individual units that decorate the dorsal surface of the egg capsule and do not form sheets as in the baffle zone (Fig. 15).

Sperm storage occurs in simple tubules scattered in the terminal zone. These tubules do not contain hair-forming cells, but are strictly composed of short columnar cells. Large aggregations of loosely aggregated sperm are harbored in the deepest recess of the tubules (Figs 16, 17) characterised by AB++ secretory and ciliated cells. Adjacent to the sperm storage areas are dilated vascular sinusoids separated from the tubules by a loosely organised connective tissue with abundant fibroblasts and collagen (Fig. 16).

In mature animals actively producing egg capsule, the terminal zone and the immediately adjacent portion of the uterus dilate dramatically and become edematous. This region is characterised by a loose, hydrated connective tissue matrix containing large blood sinuses and little muscle.

Discussion

Oviducal glands, also termed shell or nidamental glands (Prasad 1945*a*, 1945*b*; Knight *et al.* 1993), are discrete, specialised regions of the anterior portion of the oviduct in most chondrichthyans. Few exceptions exist, including the absence of traditional club, papillary, baffle, and terminal zones in the highly modified OG of the yellow spotted ray *Urolophus jamaicensis*. This animal has two zones in its OG, but the tubules do not secrete any jelly or egg capsule materials and there are no baffle plates (Hamlett *et al.* 1998). A manuscript is in preparation detailing the ultrastructure of this highly unusual gland (W. C. Hamlett, unpublished observations). Members of the family *Narcinidae*, the numbfishes, are reported to have no OG at all (Prasad 1945*a*).



Figs 12–17. (*12*) Layers of egg capsule (E) further down the lumen, where papillary egg jelly is becoming hydrated (H), note the bonding layer (*) (Scale bar = 10 μm). (*13*) AB–, PAS– material is secreted by the tubular glands (G) in the baffle zone, to the spinneret region (line), where paired baffle plates (B) manipulate the material into the transverse grooves (T) between adjacent plateau projections (P) (Scale bar = 10 μm). (*14*) A longitudinal section of a baffle gland tubule secreting the nascent AB–, PAS– egg capsule material (*). The tubule is composed of ciliated cells (C) and secretory cells (circled) (Scale bar = 10 μm). (*15*) The hair-secreting region in the terminal zone has tubular glands (G) that secrete hairs (H) which stain AB–, PAS–. The gland tubules coat the hairs with an AB+ mucus before they are moved into the lumen. Yolk platelets (Y) of the ova as it is seen moving down the lumen (Scale bar = 10 μm). (*16*) The sperm storage region in the terminal zone has connective tissue (Co) interspersed with blood sinuses (circled) and tubular glands (G). The tubular glands function in sperm storage (*) and AB+ mucous secretion. Hematoxylin and eosin (Scale bar = 10 μm). (*17*) Connective tissue (Co) is interspersed with tubules comprised of simple columnar epithelium with cilia (C), which contain loosely aggregated bundles of sperm (*). Hematoxylin and eosin (Scale bar = 10 μm).

The overall structure of OG varies with the reproductive mode of the particular animal. Oviparous species, such as the catshark *S. canicula* (Knight *et al.* 1993) and the skates *Raja erinacea* (Knight *et al.* 1996) and *R. eglanteria* (Hamlett *et al.* 1999), share the same design principles. In all these species, the club, papillary, and baffle zones are virtually identical; however, the morphology of the egg capsule differs. The terminal zone of these oviparous species is broad and extensive. Instead of possessing lamellae, simple tubular glands are scattered throughout the terminal zone where they perform two functions, namely sperm storage and the production of fine hairs that decorate the outside of the egg capsule. The deepest regions of the hair-forming tubules have secretory cells that are virtually identical microscopically to capsule-forming cells of the baffle zone. Cells near the surface of the tubules are mucous. The apparent difference in the secretory product of the baffle zone tubules and hair-forming tubules of the terminal zone is that the lamellar architecture of the baffle zone results in a continuous sheet of egg capsule being formed. Secretion of each tubule emerges as a liquid crystal polymer that then blends with secretions of adjacent tubules to form a complete sheet (Knight *et al.* 1996). In the hair-forming tubules of the terminal zone, the hairs never merge but remain separate. We suggest the surface mucous segment allows the hairs to remain separate as they emerge. The chemical basis of this phenomenon remains unresolved.

Observations of the OG of holocephalans are limited, but there are early reports of sperm in the upper oviduct of a *Chimaera* (Dean 1895, 1906) and a description of the morphology of the egg capsules (Dean 1912). An early description of the OG in *Hydrolagus collei* includes an anterior albumen zone that secretes into cranial transverse bands and a few mucous lamellae between the albumen and shell zones (Prasad 1948). The shell zone was divided into a cranial region with baffle plates and a caudal shell zone with a few mucous tubules. Our results for *C. milii* verify and extend these observations, but we adopt current terminology based on the morphological characteristics of the zones (Hamlett *et al.* 1998) rather than the purported nature of the secretions. Because there are presently no biochemical studies of the composition of the secretions, the terminology of 'albumen' and 'mucus' is not warranted and the types of secretions may not be consistent across the reproductive modes. Subtle variations in the type of secretions undoubtedly occur in the production of different types of egg coverings, whereas the basic morphological zonation persists. Our club zone is synonymous with the earlier described albumen zone, our papillary zone with the earlier middle mucous zone, our baffle zone with the earlier cranial shell zone, and our terminal zone with the earlier caudal shell zone. In addition, the present study detected sperm storage, not previously documented for a holocephalan species.

In *C. milii*, the club and papillary zones elaborate egg jelly that coats the fertilised egg, initially filling the lumen of the egg capsule. It has been suggested that the function of the egg jelly must be critical to the developing embryo for at least the early stages of its development (Hamlett *et al.* 1998). The egg jelly in *R. erinacea* functions as a structural device that supports the egg and developing embryo hydrodynamically and the jellies from various regions have differing carbohydrate compositions. The egg jelly supports the embryo during the fragile period of embryogenesis and while it has external gill filaments. The jelly is progressively liquefied as the embryo grows. There is currently no evidence that the jelly layers are nutritive for the embryo (Koob and Straus 1998).

Adequate histochemical examinations of the secretions produced by the club and papillary zones have been performed for only one species, namely the oviparous catshark *S. canicula*. The secretions of the club zone were initially reported to be as PAS+ carbohydrate (Threadgold 1957), but the secretions were subsequently reported as both PAS+, AB+ polysaccharide (Rusaouën 1976), whereas the club zone secretions in *C. milii* stained both PAS+ and AB+.

The secretory material of the papillary zone in *C. milii* is AB+ with more intense staining in the caudal-most papillary tubules. The exact chemical composition of papillary secretions has yet to be determined, but one suggestion is that the material is a type of mucin, functioning to separate and lubricate the region between the egg and the forthcoming capsule (Nalini 1940). Other suggestions include the secretion of the caudal tubules functions to bind the perimeter of egg jelly to the egg capsule (Knight *et al.* 1996; Hamlett *et al.* 1998). In *S. canicula*, papillary secretions contain carbohydrate and stain metachromatically (Threadgold 1957). Feng and Knight (1992) identified the material as a sulfated glycosaminoglycan because it stained AB+. Although we cannot assume that the material secreted in *C. milii* is the same as that of oviparous elasmobranchs, its functions of surrounding the egg and filling the egg capsule are thought to be similar.

The fundamental process of egg capsule production and assembly in elasmobranchs is a blown extrusion die mechanism involving secretion of a liquid crystalline polymer through baffle plates and luminal assembly into a multilaminate egg capsule (Knight *et al.* 1996). The egg case in *S. canicula* is constructed of collagen fibrils (Knight *et al.* 1996). The capsule is constructed from approximately 30 lamellae, each extruded from its own transverse groove within the lumen of the OG. Each transverse groove contains a row of spinnerets. Each spinneret is supplied by a short secretory duct that opens between two plate-like folds of epithelium termed baffle plates (Knight and Feng 1992). The spinnerets serve to orientate unaggregated molecules. Flow from adjacent baffle plates merges to form one layer of the capsule. Extrusion of collagen from the elasmobranch OG is analogous to the wet spinning of synthetic lyotropic liquid crystals (Lewis and Fellars 1988) in two respects: in both cases, a liquid crystalline solution is fed to spinnerets and, second, in the OG fibrillogenesis is initiated by an increase in pH from approximately 6.5 to 8.0 when the material reaches the main lumen of the gland (Feng and Knight 1994). This is analogous to extrusion into an alkaline bath in wet spinning technology. The steps of this process appear to be essentially the same in *C. milii* for the secretion of the PAS–, AB– capsule material. Based on similar histological organisation, it appears that the egg capsule material in *C. milii* is likewise a liquid crystal polymer, extruded by a blown extrusion die mechanism.

The last zone of the OG is the terminal zone, which is the actual site of formation of surface hairs that adorn the exterior of the capsule in species that have hairs and sperm storage. A feature of some oviparous OG is the secretion of hair filaments from the terminal zone. In *C. milii* and in *R. eglanteria* (Hamlett *et al.* 1999), the terminal zone differentiates into a region of hair production and a region of sperm storage. The hair region has mucoid tubules near the lumen and the base of the same tubules contains secretory cells that resemble baffle zone secretory cells. Initially, the basal portion of the tubule produces a secretion that extrudes up the tubule by secretion pressure from below. As the emerging hair filament passes the cells of the luminal mucoid region, it is coated with secretions. There are similar observations of two cell types in the same tubule in *Hydrolagus colliei* (Prasad 1945*a*; Stanley 1963). The dorsal surface of the OG in *C. milii* has abundant hairs that aid adhesion of sand to the egg case. The ventral surface is smooth with lateral flanges that are concave. This formation results in a suction cup arrangement that helps to attach the egg case to the muddy ocean floor. Hence, the egg case is attached to the mud and the sticky dorsal surface with hairs is rapidly coated with sand, thereby providing camouflage. Similar arrangements occur in skates, where the egg case is smooth but elongate and spiral tendrils extend from the lateral margin and ends of the egg case. These tendrils are sticky and serve the same purpose of attaching sand for camouflage. Additionally, the tendrils coil with seagrass to attach and support the egg case.

Metten (1939) described sperm storage in *S. canicula* as occurring in the shell-secreting tubules. This is contrary to the observations of Hamlett *et al.* (1998, 1999). Metten (1939) examined the OG of the oviparous shark *S. canicula* from animals in various stages of secretion of the tertiary egg envelope. He cited Hobson's (1930) work in the skate and stated that ova were found in the upper oviducts, between the ostium and OG, whereas egg capsules were three-quarters completed. In his own observations in *S. canicula*, Metten (1939) reported that in fish with ova in the coelom or upper oviduct, the egg capsule was half secreted or less. He believed that fertilisation and egg capsule secretion occur simultaneously and that the shell-secreting tubules provided some nutrient material in the capsule substance. He noted that some sperm were incorporated into the egg capsule substance and that it hardened immediately upon leaving the glands. He also claimed that sperm in the bottom of shell-secreting tubules actively secreting egg capsule material were in the process of 'turning around' to exit the glands along with the egg capsule material. In studies of the same animal, Knight *et al.* (1996) examined the structure of the shell-secreting tubules of the OG and concluded that fertilisation must occur in the upper oviduct or abdominal cavity. They noted sperm in the baffle zone tubules in animals secreting egg capsule but did not notice sperm in the caudal segment of the oviducal gland corresponding to the terminal zone. Knight *et al.* (1996) indicated that the tubules in this segment of the gland secreted sulfated and neutral mucopolysaccharides.

Metten (1939) did not recognise the terminal zone, but pictured a broad caudal region of the OG that he indicated had short mucous glands. This corresponds to the terminal zone. The histological organisation of the OG of two other oviparous chondrichthyans have been studied. In the skate *R. eglanteria* (Hamlett *et al.* 1999) and the chimaerid *C. milii* (present study), terminal zone tubules are short, broadly dispersed, and do not form lamellae. Their surface area is large owing to the width of the terminal zone, not the depth of each gland, as in viviparous species. In both *Raja* and *Callorhynchus*, sperm have been observed in the terminal zone and no sperm were seen in the baffle zone. The results of Metten (1939) show incidental sperm occurrence in baffle zone tubules and sperm being purged from the tubules with secretion of egg capsule. The refilling of gland tubules may be the result of repeated inseminations.

The organisation and distribution of terminal zone tubules in *R. eglanteria*, *R. erinacea* (Hamlett *et al.* 1998) and *S. canicula* (Knight *et al.* 1996) are all very similar. The terminal zone tubules in *C. milii* strongly resemble the tubules of these oviparous elasmobranchs and presumably perform similar functions.

The organisation and distribution of terminal zone tubules in placental sharks is in sharp contrast with terminal zone tubules in oviparous chondrichthyans (Hamlett *et al.* 2003). In *Mustelus canis* (Hamlett *et al.* 2002*a*), *Mustelus antarcticus* (Storrie *et al.* 2001), and *Iago omanensis* (Hamlett *et al.* 2002*b*), terminal zone tubules sweep laterally from the OG lumen and form laterally situated dilated recesses that harbor sperm year round. Recently, Conrath and Musick (2002) studied various aspects of the reproductive biology in *M. canis* and reported observations on sperm storage. She made transverse histological sections of the caudal one-third of the OG from samples collected throughout the year and consistently found sperm in the OG, specifically the terminal zone.

The fate of spermatozoa deposited within the female reproductive tract has been described in the smooth hound *M. canis* (Hamlett *et al.* 2002*b*, 2003). Evidence of sperm–uterine association is presented as well as documentation of sperm storage, specifically in the terminal zone of the OG. Immediately post partum, the placental–uterine attachment sites, now termed uterine or placental scars, begin to remodel to a mucous epithelium for the next gestation cycle. Sperm become embedded in the uterine epithelium adjacent to placental scars. Bundled sperm then occur throughout gestation in the terminal zone of the OG. Fertilisation is presumed to occur in the anterior oviduct above the OG. The physiological mechanisms that mediate sperm–uterus attachment, release, and storage in the terminal zone of the OG are currently under investigation.

Storrie *et al.* (2001) reported on sperm in the OG in *M. antarcticus* during different periods of gestation. Their evidence demonstrated sperm storage exclusively in the terminal zone, although transient occurrence of sperm was noted in other gland tubules in animals not actively secreting jelly or egg envelope. Of note is the fact that terminal zone sperm were found in both mature (pregnant, non-pregnant, and post partum) and immature (before first ovulation) animals throughout the year. Efforts are being directed at elucidating whether terminal zone sperm are being stored for prolonged periods from a single mating event with one or more males or result from multiple matings throughout the year. Feldheim *et al.* (2001) have recently applied genetic analysis using DNA microsatellite loci developed for lemon sharks (*Negaprion brevirostris*) to investigate the possibility of multiple paternity. Their results demonstrate that at least three males sired a single litter. In addition, using other molecular methods for genetic analysis, Saville *et al.* (2002) noted that at least four fathers contributed to a brood of 32 pups in the nurse shark *Ginglymostoma cirratum*. Histological examination of the OG from either species was not performed.

Various workers have commented on uterine sperm, generally immediately after insemination. Metten (1944) reported uterine digestion of sperm in *S. canicula*. The observations of Hamlett *et al.* (2002*a*) on *M. canis* do not confirm Metten's (1944) conclusions. Fishelson and Baranes (1998) described the folded endometrium of gravid placental *I. omanensis* as forming simple tubular glands at the bases of the folds. They saw aggregations of sperm in the tubules, but did not report sperm being embedded in the uterus.

In conclusion, we have determined that the overall morphology of the OG in the holocephalan *C. milii* closely resembles the OG of oviparous elasmobranchs. Zonation within the gland is virtually identical histologically, yet the shape of the egg capsule differs, reflecting the three-dimensional arrangement of secretory tubules. Both the club and papillary zones elaborate egg jelly and the baffle zone produces the bulk of the multilaminate egg capsule. The terminal zone forms hairs to decorate the exterior of the egg case and additional tubules store sperm. The similarity in OG structure and function between elasmobranchs and holocephalans reinforce their related evolutionary origin.

Acknowledgments

We are grateful to Professor Marilyn Renfree, Dr Rob Day, Amy Beck, Matt Reardon, Joan Clark, Bruce Abaloz and David Paul of The University of Melbourne (UM) and to Russell Hudson of Primary Industries Research Victoria (PIRVic) who assisted this research. Most of this work was accomplished while W. C. H. was on sabbatical leave at UM. The research was supported by an Enhancing International Science Networks Visiting Fellowship (PIRVic) and a Visiting Research Scholar Travel Grant, 2000 (UM). The research was undertaken in support of a project entitled 'Saw shark and elephant fish assessment and by-catch evaluation in the Southern Shark Fishery'. That project was undertaken by PIRVic and funded by the Australian Fisheries Research and Development Corporation (Project 1999/103).

References

Callard, I. P., and Koob, T. J. (1993). Endocrine regulation of the elasmobranch reproductive tract. *The Journal of Experimental Zoology* **266**, 368–377.

Conrath, C. L., and Musick, J. A. (2002). Reproductive biology of the smooth dogfish, *Mustelus canis*, in the northwest Atlantic Ocean. *Environmental Biology of Fishes* **64**, 367–377.

Cook, H. (1990). Carbohydrates. In 'The Theory and Practice of Histological Techniques'. 3rd edn. (Eds J. D. Bancroft and A. Stevens.) pp. 177–214. (Churchill Livingstone: London, UK.)

Dean, B. (1895). 'Fishes, Living and Fossil. Columbia University Series III.' (Columbia University: New York, USA.)

Dean, B. (1906). 'Chimaeroid Fishes and Their Development.' (Carnegie Institute: Washington, DC, USA.)

Dean, B. (1912). Orthogenesis in the egg capsules of *Chimaera*. *Bulletin of the American Museum of Natural History* **31**, 35–40.

Feldheim, K. A., Gruber, S. H., and Ashley, M. V. (2001). Multiple paternity of a lemon shark litter (Chondrichthyes; Carcharhinidae). *Copiea* **2001**, 781–786.

Feng, D., and Knight, D. P. (1992). Secretion and stabilization of the egg capsule of the dogfish *Scyliorhinus canicula*. *Tissue & Cell* **24**, 773–790.

Feng, D., and Knight, D. P. (1994). Structure and formation of the egg capsule tendrils in the dogfish *Scyliorhinus canicula*. *Philosophical Transactions of the Royal Society of London* **343B**, 285–302.

Fishelson, L., and Baranes, A. (1998). Observations on the Oman shark, *Iago omanensis* (Triakidae), with emphasis on the morphological and cytological changes of the oviduct and yolk sac during gestation. *Journal of Morphology* **236**, 151–165.

Hamlett, W. C., and Koob, T. J. (1999). Female reproductive system. In 'Sharks Skates and Rays: The Biology of Elasmobranch Fishes'. (Ed. W. C. Hamlett.) pp. 398–443. (The Johns Hopkins University Press: Baltimore, MD, USA.)

Hamlett, W. C., Knight, D. P., Koob, T. J., Jezior, M., Luong, T., Rozycki, T., Brunette, N., and Hysell, M. K. (1998). Survey of oviducal gland structure and function in elasmobranchs. *The Journal of Experimental Zoology* **282**, 399–420.

Hamlett, W. C., Hysell, M. K., Jezior, M., Rozycki, T., Brunette, N., and Tumilty, K. (1999). Fundamental zonation in elasmobranch oviducal glands. 'Proceedings of the 5th Indo-Pacific Fish Conference, Nouméa, 1997'. (Eds B. Seret and J.-Y. Sire.) pp. 271–280. (Society of French Ichthyology: Paris, France.)

Hamlett, W. C., Musick, J. A., Hysell, C. K., and Sever, D. M. (2002*a*). Uterine epithelial–sperm interaction, endometrial cycle and sperm storage in the terminal zone of the oviducal gland in the placental smoothhound, *Mustelus canis*. *The Journal of Experimental Zoology* **292**, 129–144.

Hamlett, W. C., Fishelson, L., Baranes, A., Hysell, C. K., and Sever, D. M. (2002*b*). Ultrastructural analysis of sperm storage and morphology of the oviducal gland in the Oman shark, *Iago omanensis* (Triakidae). *Marine and Freshwater Research* **53**, 601–613.

Hamlett, W. C., Greven, H., and Schindler, J. (2003). Sperm storage in the Class Chondrichthyes & Class Osteichthyes. In 'The New Panorama of Animal Evolution. Proceedings of the 18th International Congress of Zoology'. (Eds A. Legakis, S. Sfenthourakis, R. Polymeni and M. Thessalou-Legaki.) pp. 405–413. (Pensoft: Moscow, Russia.)

Hobson, A. D. (1930). A note on the formation of the egg case of the skate. *Journal of the Marine Biological Association of the UK* **16**, 577–581.

Humason, G. L. (1972). Staining connective tissue and muscle. In 'Animal Tissue Techniques'. 3rd edn. (Ed. G. L. Humason.) pp. 297–300. (WH Freeman and Co.: San Francisco, CA, USA.)

Knight, D. P., and Feng, D. (1992). Formation of the dogfish egg case, a coextruded, multilayer laminate. *Biomimetics* **1**, 151–175.

Knight, D. P., Feng, D., Stewart, M., and King, E. (1993). Changes in macromolecular organization in collagen assemblies during secretion in the nidamental gland and formation of the egg capsule wall in the dogfish *Scyliorhinus canicula. Philosophical Transactions of the Royal Society of London* **341B**, 419–436.

Knight, D. P., Feng, D., and Stewart, M. (1996). Structure and function of the selachian egg case. *Biological Reviews* **71**, 81–111.

Koob, T. J., and Straus, J. W. (1998). On the role of egg jelly in *Raja erinacea. Bulletin of the Mt. Desert Island Biological Laboratory* **37**, 117–119.

Lewis, D. N., and Fellars, J. F. (1988). Processing of polymer liquid crystals. In 'High Modulus Polymers: Approaches to Design and Development'. (Eds P. A. E. Zacharides, R. S. Porter, R. Polymeni and M. Thessalou-Legaki.) pp. 28–36. (Marcell Dekker: New York, USA.)

Lo Bianco, S. (1909). Notizie biologiche riguardanti specialmente il periodo di maturita sessuale degli animali del golfo di Napoli. *Mitteilungen an der zoolischen Station zu Neapel* **19**, 513–791.

Metten, H. (1939). Studies on the reproduction of the dogfish. *Philosophical Transactions of the Royal Society of London* **230B**, 217–238.

Metten, H. (1944). The fate of spermatozoa in the female dogfish (*Scyliorhinus canicula*). *The Quarterly Journal of Microscopical Science* **84**, 283–295.

Nalini, K. P. (1940). Structure and function of the nidamental gland of *Chiloscyllium griseum* (Mull. and Henle). *Proceedings of the Indian Academy of Science* **128**, 189–214.

Prasad, R. R. (1945*a*). The structure, phylogenetic significance, and function of the nidamental glands of some elasmobranchs of the Madras coast. *Proceedings of the National Institute of Sciences India* **11**, 282–303.

Prasad, R. R. (1945*b*). Further observations on the structure and function of the nidamental glands of a few elasmobranchs of the Madras coast. *Proceedings of the Indian Academy of Science* **22**, 368–373.

Prasad, R. R. (1948). Observations on the nidamental glands of *Hydrolagus colliei, Raja rhina* and *Platyrhinoids triseriatus. Copeia* **1948**, 54–57.

Pratt, H. L. Jr (1993). The storage of spermatozoa in the oviducal glands of western north Atlantic sharks. *Environmental Biology of Fishes* **38**, 139–149.

Rusaouën, M. (1976). The dogfish shell gland, a histochemical study. *Journal of Experimental Marine Biology and Ecology* **23**, 267–283.

Saville, K. J., Lindley, A. M., Maries, E. G., Carrier, J. C., and Pratt, H. L. Jr (2002). Multiple paternity in the nurse shark, *Ginglymostoma cirratum. Environmental Biology of Fishes* **63**, 347–351.

Stanley, H. P. (1963). Urogenital morphology in the chimaeroid fish *Hydrolagus colliei* (Lay and Bennett). *Journal of Morphology* **112**, 99–125.

Stevens, A. (1990). The haemotoxylins. In 'The Theory and Practice of Histological Techniques'. 3rd edn. (Eds J. D. Bancroft, A. Stevens, R. Polymeni and M. Thessalou-Legaki.) pp. 107–118. (Churchill Livingstone: London, UK.)

Storrie, M. T., Walker, T. I., Laurenson, L., and Hamlett, W. C. (2001). Aplacental yolk sac viviparity in the gummy shark, *Mustelus antarcticus*. 'Sixth Indo-Pacific Fish Conference Scientific Programme and Book of Abstracts, 20–25 May 2001, Durban, South Africa'. pp. 59. (Oceanographic Research Institute: Durban, South Africa.)

Threadgold, L. T. (1957). A histochemical study of the shell gland of *Scyliorhinus caniculus. The Journal of Histochemistry and Cytochemistry* **5**, 159–166.

Manuscript received 11 May 2001; revised and accepted 23 January 2004.

Appendix 4: Stock assessments

This appendix contains a report initially prepared for SharkRAG

CHAPTER 10 : INITIAL ASSESSMENTS OF SAWSHARK (*PRISTIOPHORUS CIRRATUS* AND *P. NUDIPINNIS*) AND ELEPHANT FISH (*CALLORHINCHUS MILII*)

André E. Punt[1], Terence I. Walker[2], and Anne S. Gason[2]
1 – CSIRO Marine Research, GPO Box 1538, Hobart, TAS 7001, Australia
2 - Marine and Freshwater Systems, Primary Industries Research Victoria, Box 114, Queenscliff, VIC 3225, Australia

10.1. Introduction

Three endemic species of sawshark whose distributions have not been precisely described and the elephant fish (*Callorhinchus milii*) occur in waters off southern Australia. Common sawshark (*Pristiophorus cirratus*) is reported to range from Jurien Bay in Western Australia to Eden in New South Wales, including Tasmania, to depths of 310 m. Southern sawshark (*P. nudipinnis*) is considered to range from the western region of the Great Australian Bight to eastern Gippsland in Victoria, including Tasmania, to depths of 70 m. Eastern sawshark (*Pristiophorus* sp. A) occurs from about Lakes Entrance in eastern Victoria to Coffs Harbour in New South Wales at depths of 100–630 m. The elephant fish is distributed from Esperence in Western Australia to Sydney in New South Wales, including Tasmania, at depths to at least 200 m (Last and Stevens, 1994). Elephant fish also occur in New Zealand, but the stocks there are assumed to be separate from those in southern Australia. The three sawshark species exhibit aplacental viviparity (live bearing of pups), whereas the elephant fish exhibits oviparity (egg laying).

For assessment purposes, all sawsharks south of the Victoria–NSW border are assumed to be common sawshark and southern sawshark whereas those north of this border are assumed to be eastern sawshark. Only common sawshark and southern sawshark are included in the present stock assessment; the eastern sawshark provides a very small component of the sawshark catch and is excluded from the assessment because there are no biological data available on this species.

These species, along with gummy and school shark, form the primary target and byproduct species of the directed shark fishery off southern Australia. In contrast to gummy and school shark, sawshark and elephant fish are 'data poor' with the only stock assessment-related information for these species being species-specific demographic and gillnet selectivity parameters, species-aggregated catch and effort data, and some limited information on the size- and age-structure of the historical catches. This lack of data severely restricts the type of analyses on which stock assessments can be based. This is because, for example, none of the data available for these species permit independent estimation of the rate of natural mortality, M (in contrast, it is possible, in principle at least, to estimate M for gummy and school shark from the results of tagging experiments).

This chapter first outlines the data available for sawshark and elephant fish and uses these data to develop time-series of catches, catch-rates and catch length-composition for use in population model-based stock assessments. Assessments of sawshark and elephant fish are then undertaken using a population dynamics model tailored to the peculiarities of shark life-history. The final section of this chapter outlines some caveats and identifies future work.

10.2. Data

The data available for assessments of target and byproduct shark species off southern Australia include: catches (in mass), catch-rate-based indices of relative abundance, length-frequency data, age-composition data, and the results of tagging studies. All five of these data-types are included in the assessments of gummy shark (Punt *et al.*, 2001; Pribac *et al.*, in press; Chapter 7) and school shark (Punt *et al.*, 2000a). In contrast to the situation for gummy and school shark, very little information on catch length-frequency is available for sawshark and elephant fish (Walker *et al.*, 1997). Few animals of these species have been aged, and the number of animals tagged is much too small to enable reliable estimation of mortality rates (31 common sawshark recaptured, 41 southern sawshark recaptured, and 24 elephant fish recaptured). Therefore, assessments of sawshark and elephant fish must rely primarily on the information from the time-series of catches and from the catch and effort information.

10.2.1 Catch data

Figures 10.1 and 10.2 show the time-trajectories of catch by the directed shark fisheries (by SharkFAG sub-region – see Fig. 7.1) during 1973–2002. The catches of sawshark in Bass Strait (sub-regions SAV-E, western Bass Strait and eastern Bass Strait) make up the vast bulk of the catch of these species by this fishery off southern Australia (90%; Figure 10.1). In contrast to the situation for sawshark, sizeable quantities of elephant fish have been caught off eastern Tasmania (Figure 10.2). Although the catches of elephant fish off eastern Tasmania constitute 24% of the total catch over 1973–2003, the assessments of this chapter are restricted to elephant fish in Bass Strait only. A key reason for this is that, although catch information is available for eastern Tasmania, there are no reliable estimates of effort for this area before 1995 and catch-effort standardizations are not currently based on the catch and effort data for this region (see Appendix 7.A). Sub-region NSW is excluded because the sawshark catch is from a separate species and the catch of elephant fish is negligible.

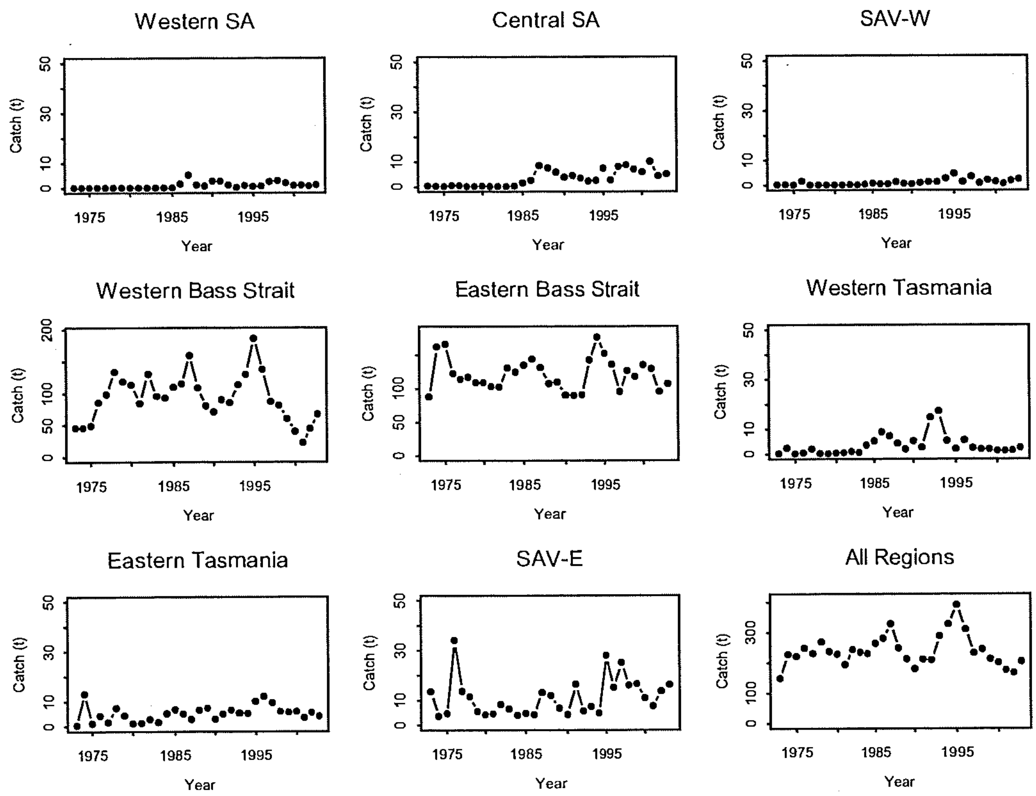


Figure 10.1 : Catches (carcass weight, t) of sawshark by the directed shark fisheries by SharkFAG region.

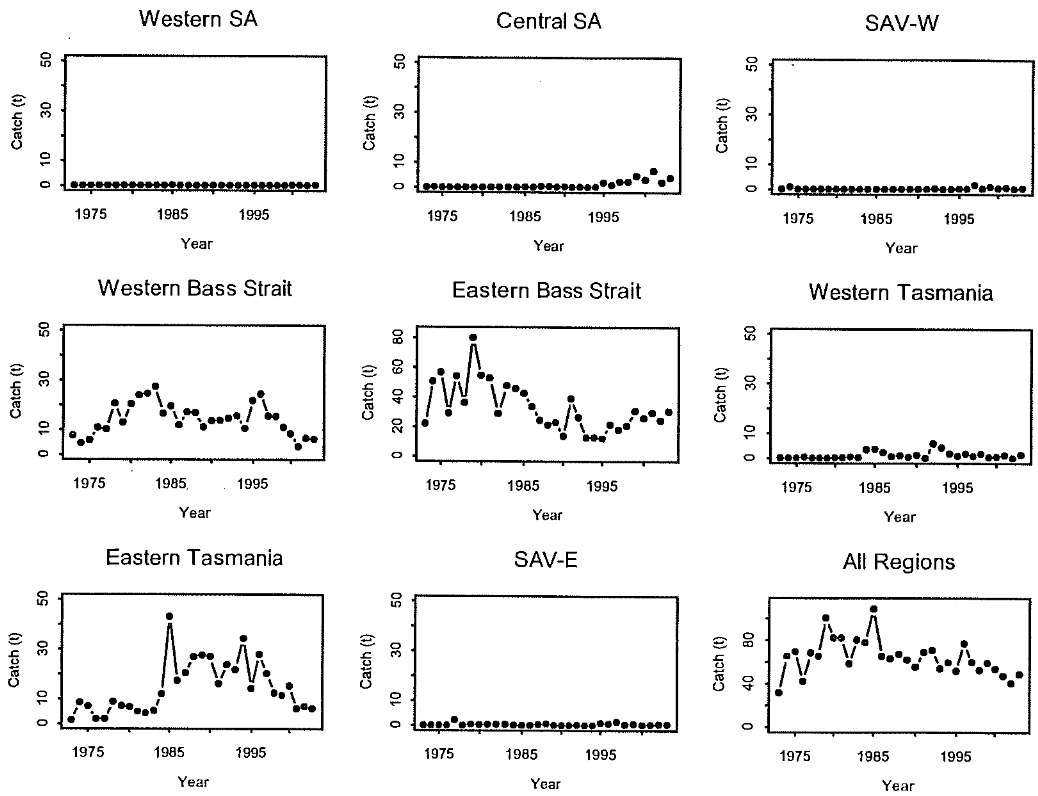


Figure 10.2 : Catches (carcass weight, t) of elephant fish by the directed shark fisheries by SharkFAG region.

Table 10.1 lists the annual catches by the directed shark fisheries by gear-type (longlines and four sizes of gill-net) for Bass Strait that were used in the analyses of this chapter. The bulk of the historical catches of sawshark and elephant fish (94%) has been taken by 6" gill-nets although fairly substantial catches were taken during 1973–76[1] using 7" gill-nets.

It is necessary to estimate the catches by the directed shark fisheries for the years prior to 1973 and to include catches by the trawl sector (otter trawlers and Danish seine vessels) for the assessments of sawshark and elephant fish. The remainder of this section therefore uses available information to make estimates of these catches. However, it needs to be recognized that even though the estimates are based on all of the available information, these estimates are still subject to considerable uncertainty.

Table 10.2 lists the catches by the South East Trawl Fishery (SETF) and the Great Australian Bight Trawl Fishery (GABTF) based on logbook data for years 1985–2003 (SETF) and 1988–2003 (GABTF)[2]. The catches of sawshark reported for NSW are excluded from the assessment because the animals landed from this sub-region are a separate species. Table 10.3 lists historical (1950–69) information on catches of sawshark

[1] Data for 1970–72 exist but, for consistency with how these data have been treated in assessments of gummy and school shark, they are not used in the analyses of this chapter.

[2] Data are presented separately for the SETF and GABTF for 2002 and 2003 even though these two fisheries are now combined for management and reporting purposes.

landed in Victoria, which covers the sub-regions of SAV-E, Western Bass Strait and Eastern Bass Strait. The algorithm to estimate catches for those years for which catches are missing using the information in Tables 10.1–10.3 is[3]:

a) The total catches by the directed shark fisheries (all gear-types and sub-regions combined) for years 1969–72 are computed using the formula:

$$C_y^{\text{SSF}} = C_{1973}^{\text{SSF}}\, C_y^{\text{Gummy}} \,/\, C_{1973}^{\text{Gummy}} \tag{10.1}$$

where C_y^{SSF} is the catch by the directed shark fisheries during year y, and

C_y^{Gummy} is the catch of gummy shark by the directed shark fisheries during year y.

The rationale for this approach to estimating total catches (suggested at the 2 March 2004 meeting of SharkFAG) is that the effort directed at elephant fish and sawshark should follow that targeted towards gummy shark. Equation 10.1 is not used to estimate the total catch of sawshark for 1968 and 1969 because data from across sub-regions of SAV-E, Western Bass Strait and Eastern Bass Strait are available on these catches (Table 10.3)

b) The catches computed using Equation (10.1) are split to sub-region and gear-type based on the data for 1973. The rationale for this approach is that 1973 is the first year for which information on the split to sub-region is available and the data for 1973 should consequently provide the best information on the split for the years prior to 1973.

c) The total catch by trawl (all sub-regions) for each of the years 1950–69 for sawshark is set to the estimates in Table 10.3, whereas the catches by trawl of elephant fish prior to 1970 are assumed to be zero. There is no information on the catches from 1970–84 so the total catch by trawl for 1970–85 is determined by linear interpolation between the catches for 1969 and 1986[4].

d) The total catches by trawl are split to sub-region using the proportion of the catch taken in each sub-region during 1986–88 (the first years for which reliable logbook data are available). The selectivity pattern for the trawl catches is taken to be the same as that for the longline component of the fishery because longline catches the widest size range of fish.

Figure 10.3 shows the time-series of catches of sawshark and elephant fish on which the present assessment is based (i.e. the catches in Bass Strait) compared with the total (southern Australia-wide) catches. Figure 10.3 also shows the breakdown of the catches used in the assessment into those taken using trawl gear and those taken by the directed shark fisheries.

---

[3] This algorithm is applied separately to sawshark and elephant fish.

[4] This process therefore ignores the data from logbooks for 1985. These data are ignored because the otter trawl logbook data for 1985 are incomplete because the logbook programme only started near the end of 1985.

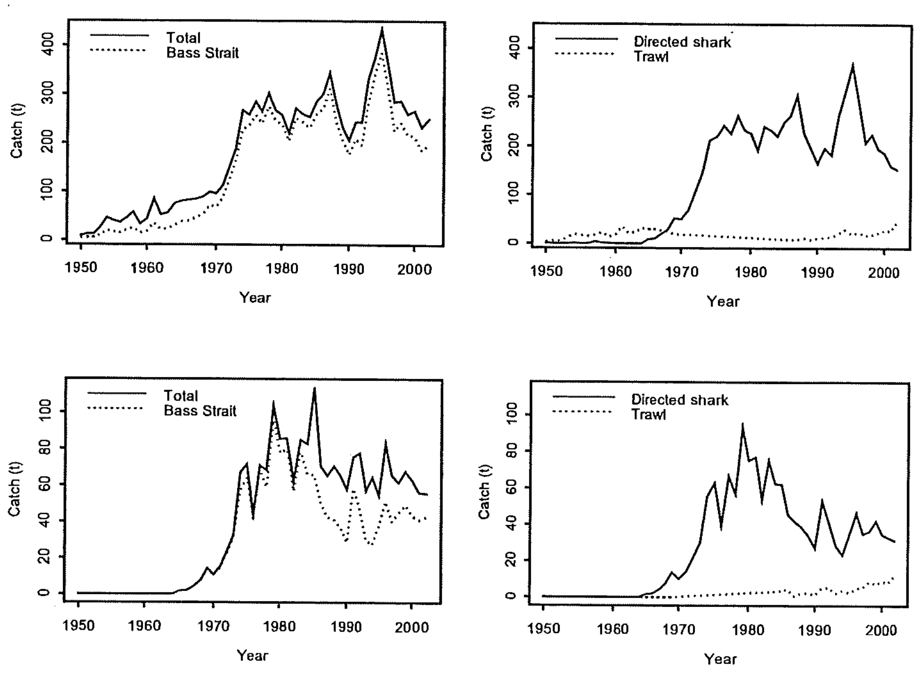


Figure 10.3 : Catch series for sawshark (upper panels) and elephant fish (lower panels). The left panels show the catches off southern Australia and those in Bass Strait while the right panels show the split of the catch in Bass Strait between the trawl fisheries and the directed shark fisheries.

It is known that some sawshark and elephant fish are discarded by the trawl sector and that most of the historical catches by otter trawl were recorded in the logbooks as "unspecified species". It is possible (in principle at least) to estimate the actual trawl catch of sawshark and elephant fish using the data collected by the Scientific Monitoring Programme (SMP) and the Integrated Scientific Monitoring Programme (ISMP) using the formula:

$$C_y^{\text{Trawl}} = \sum_r I_y^r E_y^r \qquad (10.2)$$

where I_y^r is the catch-rate (either catch-per-haul or catch-per-hour) in sub-region r during year y, and

E_y^r is the effort (either the number of hauls or the number of hours, depending on how I_y^r is defined) in sub-region r during year y.

Table 10.4 lists the estimated catches based on Equation 10.2 (with asymptotic coefficients of variation). The estimates of elephant fish catch are very imprecise as are the estimates of sawshark catch for the years prior to 1996. The estimates of the sawshark catches based on Equation (10.2) are generally much larger than those based on the trawl logbooks (compare Tables 10.2 and 10.4). The discrepancy between the catches of sawshark in Table 10.4 and those in Table 10.2 cannot be attributed to discarding (the discard rate for sawshark based on the ISMP data is only 1.7%). Instead, the discrepancy can best be explained by reporting of catches of sawshark as "unspecified species". A sensitivity test is therefore conducted for the sawshark assessment in which the trawl catches in the base-case analysis are increased by 150% to examine the implications of the catches of sawshark in Table 10.4 better representing the removals of sawshark by the trawl sector.

10.2.2 Catch-rate indices

In principle, the ideal way to develop catch-rate-based indices of relative abundance is to apply a method of catch-effort standardization (e.g. Gavaris, 1980; Kimura, 1981; Vignaux, 1994; Punt *et al.*, 2000b) to the data for sawshark and elephant fish. However, this approach cannot be applied at present because SharkFAG have yet to define 'indicative' fishers for sawshark and elephant fish. Instead, the effort estimated from the catch-effort standardization for gummy shark (See Appendix 7.A) estimated to targeted towards gummy shark in Bass Strait (Figure 10.4) is assumed to be an appropriate measure of the effort directed towards sawshark and elephant fish in Bass Strait. The catch-rate for sawshark for year y is therefore defined as the catch of sawshark during year y divided by the effort estimated to be directed toward gummy shark during year y (the ratio of the catch by 6" mesh gill-nets for year y to the standardized catch-rate for year y). Table 10.5 lists the catch-rate series considered in the analyses of this chapter.

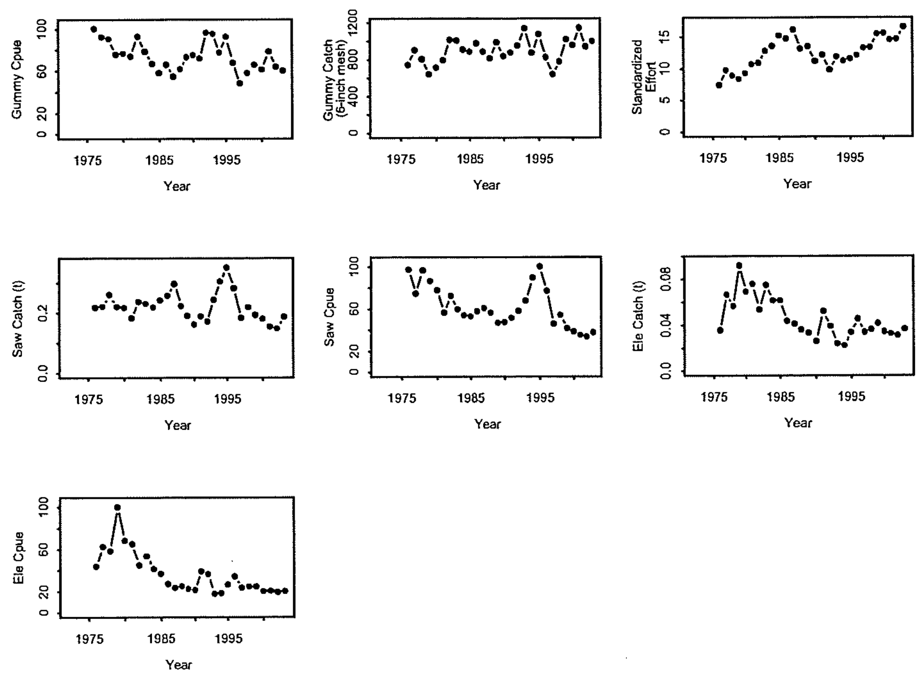


Figure 10.4 : Catch and catch-rate indices for sawshark and elephant fish in Bass Strait based on standardized effort for gummy shark.

10.2.3 Length-frequency information

Length-frequency and sex-composition data are available for gummy shark, school shark, sawshark, and elephant fish from commercial and research sampling. The sample sizes for sawshark and elephant fish are, however, very small (Table 10.6 lists the numbers of sharks measured annually by gear-type and sex). The analyses of this chapter are restricted to those combinations of gear-type and sex for which the sample sizes are "reasonable" – these combinations are highlighted in Table 10.6.

10.2.4 Biological parameters

The number of pups (actually embryos) per pregnant sawshark (or number of eggs laid per mature female elephant fish) of age a (total length $\ell_{1,a}$) is given by:

$$P'_a = \max(0, a' + b'\ell_{1,a}) \tag{10.3}$$

where a' and b' are the parameters that govern the relationship between total length and number of pups per pregnant female.

The proportion of female animals of age a (total length $\ell_{1,a}$) that are in maternal[5] (or egg laying) condition each year is given by:

$$P''_a = P''_{\max}\left(1+\exp(-\ell n(19)\frac{\ell_{1,a}-\ell''_{50}}{\ell''_{95}-\ell''_{50}})\right)^{-1} \tag{10.4}$$

where $P''_{\max}$ is the proportion of very large ($\ell_{1,a} \to L_{\infty,1}$) females that are in maternal (or egg laying) condition each year,

ℓ''_{50} is the length at which half of the maximum proportion of females are in maternal (or egg laying) condition each year, and

ℓ''_{95} is the length at which 95% of the maximum proportion of females are in maternal (or egg laying) condition each year.

The total length of a fish of age a and sex g at the start of the year, $\ell_{g,a}$, is described by the von Bertalanffy growth equation:

$$\ell_{g,a} = L_{\infty,g}(1-e^{-\kappa_g(a-t_{0,g})}) \tag{10.5}$$

and the live mass by the allometric equation:

$$w_{g,L} = a_g(\overline{L}_L)^{b_g} \tag{10.6}$$

where $\overline{L}_L$ is the mid-point of length-class L.

The values assumed for the parameters of Equations (10.3)–(10.6) are listed in Table 10.7.

The probability that a fish of age a and sex g lies in length-class L (length-class L is defined to be $[L-\Delta L, L+\Delta L]$) is given by:

[5] A female is classed as being in maternal condition if it will give birth to young before or soon after the following 1 January (i.e. it contributes to the following year's recruitment (Walker in press)).

$$\Phi(g,a,L) = \int_{L-\Delta L}^{L+\Delta L} \frac{1}{\sqrt{2\pi}\,\sigma_{g,a} l} e^{-\frac{(\ell n l - \ell n \ell_{g,a})^2}{2\sigma_{g,a}^2}} dl \tag{10.7}$$

where ΔL is half the width of a length-class (250 mm), and
$\sigma_{g,a}$ is (approximately) the coefficient of variation of the length of an animal of age a and sex g (assumed to be 0.1 for all ages).

10.2.5 Selectivity

Different selectivity patterns are assumed for the two major gear-types (hooks and gill-nets). By analogy with the assessments of gummy and school shark, the catch by hooks is assumed to be taken uniformly from the 2+ component of the population, i.e.:

$$S_{g,j,L} = \begin{cases} 0 & \bar{L}_L < \ell_{g,2} \\ 1 & \text{otherwise} \end{cases} \tag{10.8}$$

where $S_{g,j,L}$ is the selectivity of gear-type j on fish of sex g in length-class L.

The selectivity pattern for gill-nets is assumed to follow a gamma function (Kirkwood and Walker, 1986):

$$S_{g,j,L} = \left(\frac{\bar{L}_L}{\alpha_{g,j}\,\beta_{g,j}}\right)^{\alpha_{g,j}} e^{\alpha_{g,j} - \frac{\bar{L}_L}{\beta_{g,j}}} \tag{10.9}$$

where α, β are the parameters of the selectivity pattern, i.e.:

$$\alpha = \tfrac{1}{2}\left(\theta_1 m - \sqrt{(\theta_1 m) + 4\theta_2}\right) \qquad \alpha = \theta_1\, m / \beta \tag{10.10}$$

where θ_1, θ_2 are parameters (Table 10.7), and
m is the mesh size (in inches).

Figures 10.5–10.7 summarize the biological parameters for common sawshark, southern sawshark and elephant fish in terms of the relationships between length and age, mass and age, pup (or egg) production and age. Pup (or egg) production is the product of the number of pups per maternal female (or eggs per egg-laying female) and the proportion of females of each age that are in maternal (or egg-laying) condition. These relationships imply a particular level of natural mortality for pups (or eggs laid) in order for the population to remain in balance; Figures 10.5–10.7 therefore show natural mortality as a function of age when the natural mortality rate for animals 2 and older is 0.2yr^{-1}. These figures also show selectivity as a function of length (solid line – longlines; dotted lines – gillnets) and age. Finally, these figures include the distributions of length-at-age for ages 3, 8, 11, etc.

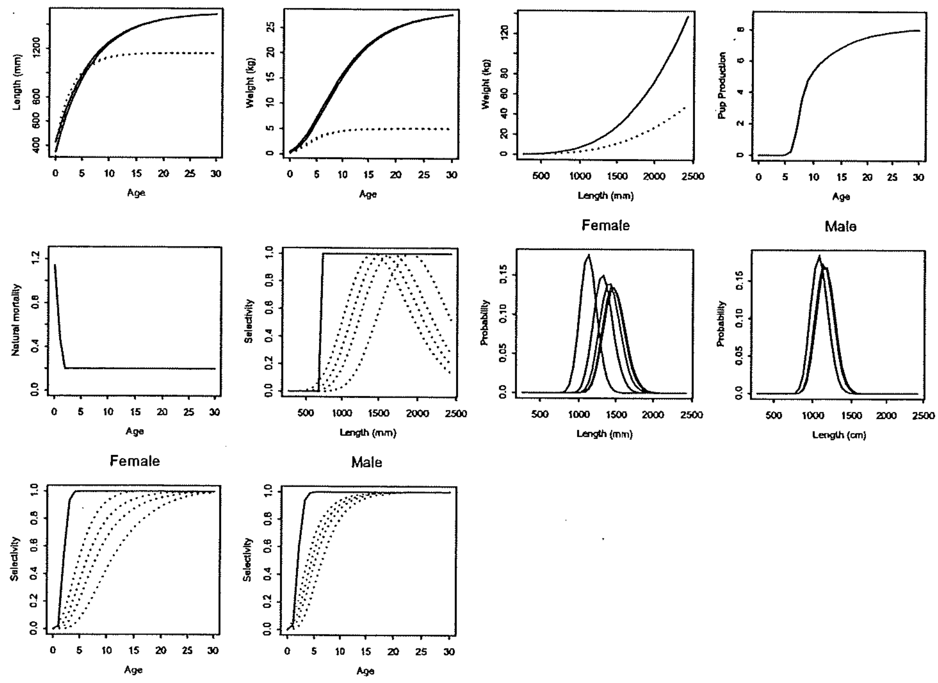


Figure 10.5 : Biological and technological parameters for common sawshark. The first two panels on the upper row of panels show length-at-age and mass-at-age at the start and in the middle of year (females and males solid and dotted lines respectively).

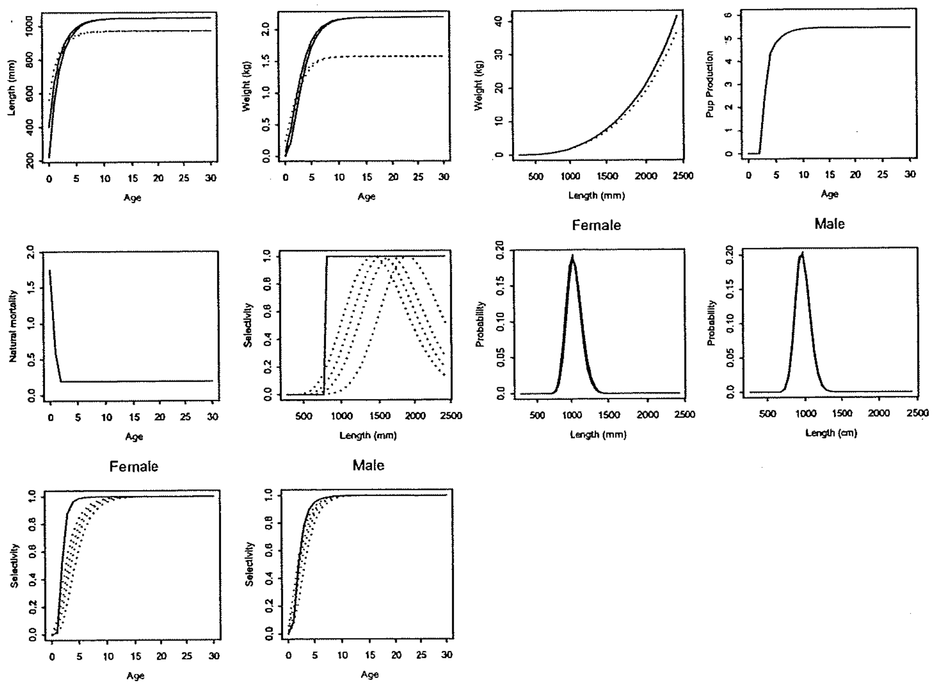


Figure 10.6 : Biological and technological parameters for southern sawshark. The first two panels on the upper row of panels show length-at-age and mass-at-age at the start and in the middle of year (females and males solid and dotted lines respectively).

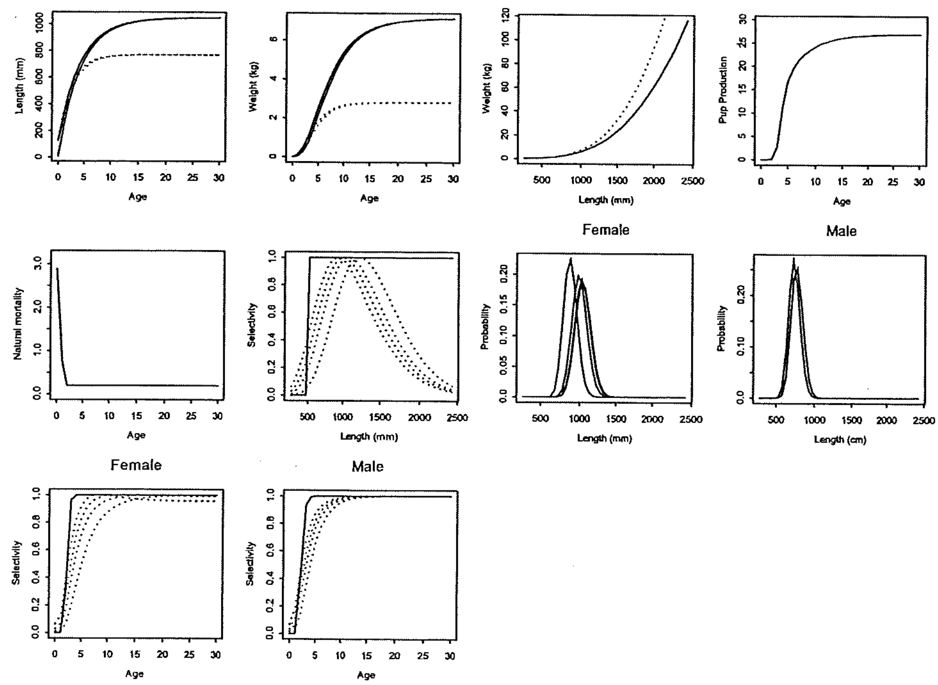


Figure 10.7 : Biological and technological parameters for elephant fish. The first two panels on the upper row of panels show length-at-age and mass-at-age at the start and in the middle of year (females and males solid and dotted lines respectively).

10.3. Analytical framework

The assessments of this chapter are based on the approach developed to assess gummy shark in Bass Strait and off South Australia (see Appendices 7.B and 7.C for details), with a few differences:

a) The relationship between length and number of pups (or eggs) per maternal (or egg-laying) female is assumed to be a linear rather than an exponential function of length.
b) No information is available on the age-structure of the historical catches.
c) Availability is assumed to be independent of length in the absence of data that can be used to determine the relationship between availability and length – this assumption should lead to somewhat more pessimistic results than had availability been assumed to be domed-shaped.
d) Natural mortality is pre-specified (rather than being estimated) in the absence of sufficient tagging, length-, and age-composition data. The base-case value for the natural mortality rate of animals aged 2 and older, M_{2+}, is 0.2yr^{-1}. Sensitivity to the value assumed for M_{2+} is examined in the tests of sensitivity.
e) Catch-rate is assumed to be related linearly to abundance. This assumption is necessitated because of the absence of tagging data and sufficient information on the length- and age-composition of the catches from which the relationship between catch-rate and abundance could potentially be estimated.
f) The population is assumed to be at its unfished equilibrium level at the start of 1950 (the first year for which catches are available – see Section 10.1).

g) Recruitment residuals are estimated for 1951–2000 (for those analyses that allow for changes over time in pup (or egg) survival).
h) The coefficient of variation of the catch-rate data is assumed to be 0.3 (rather than 0.15) to reflect less confidence in the catch-rate index as an index of abundance.
i) The effective sample size for the length-frequency data is set to 10 to reflect the fact that these data are based on very small sample sizes and few sample locations.

Parameter uncertainty is examined through the use of sensitivity tests and by applying the Markov Chain Monte Carlo (MCMC) algorithm (Hastings, 1970; Gelman *et al.*, 1995). Table 10.8 lists the specifications for the base-case analyses and the sensitivity tests. Two sets of analyses are conducted for sawshark, one set in which the values for the biological parameters are set to those estimated for common sawshark (Table 10.7; Figure 10.5) and another set in which the values for the biological parameters are set to those estimated for southern sawshark (Table 10.7; Figure 10.6). The sensitivity test in which allowance is made for gear-competition (sensitivity test 10) involves assuming that the relationship between exploitation rate and fishing effort is governed by Equation 7.C.2 where the value of the parameter γ_1 is set equal to 0.1075 – the base-case value for this parameter from the gummy shark assessment.

10.4 Results

The results of the assessments are summarized by the following five quantities:

a) the estimate of the Maximum Sustainable Yield rate (if the fishery operated uniformly on mature animals);
b) the value of the density-dependence parameter (V or Q_0, depending on whether density-dependence impacts natural mortality or the survival rate of pups);
c) the depletion of the pup production in 1973 (relative to that in 1950);
d) the depletion of the pup production in 2004 (relative to that in 1950); and
e) the value of negative log-likelihood function corresponding to the parameter values presented.

Table 10.9 lists the values for these five quantities (and their asymptotic standard errors) for the base-case analyses and the sensitivity tests. One of the sensitivity tests did not converge (the Hessian matrix was not positive definite – indicated in Table 10.8 by an asterisk). The results for this sensitivity tests are consequently omitted from Table 10.9.

10.4.1. The base-case analyses (sawshark)

The fits of the model to the fishing effort data for sawshark (Table 10.9, rows "Base-case"; Figures 10.8 and 10.9) are good. The analysis in which the biological parameters for southern sawshark are assumed fits the change in fishing effort from 1990–95 slightly better than when the biological parameters for common sawshark are assumed. The model is able to capture the central tendency of the length-frequency data. However, the model predicts that a wider range of size-classes should be caught using 6" mesh gill-nets than is actually the case. There are several possible reasons for the inability of the model to adequately capture the size-range of the catch: a) the samples on which the length-frequencies are based (which were collected during surveys) are unrepresentative of the length-frequency of the catch, b) the assumed selectivity curves (which were estimated using data pooled over both species) are in error, c) the values of the parameters of the growth curve (including the extent of variation about that curve) are in error, and d) larger and small animals are not available to the fishing gear. Unfortunately, in the absence of data, it is not possible to determine which of these reasons is most plausible. Clearly, collection of additional length-frequency data would help resolve this issue. Additional length-frequency data should be collected from the catch and an attempt should be made to assemble length-frequencies from the trawl catch. The long period of highly length-selective fishing mortality by gillnets in Bass Strait may have biased the von Bertalanffy growth parameters as has been demonstrated for gummy shark (Walker *et al.*, 1998), through the Phenomenon of Apparent Change of Growth Rate (Lee, 1912).

The productivity of the resource (as measured by *MSYR*) is low (28% or 17% depending on whether the biological parameters are set to those for common or southern sawshark; Table 10.9). These estimates of productivity are, however, very imprecise (standard deviations of 7–8%). The pup production in 2004 is assessed to be 32% (common sawshark parameters) or 26% (southern sawshark parameters) of its 1950 value. These estimates are also fairly imprecise (standard deviations of ~10%).

10.4.2 The base-case analysis (elephant fish)

The fit of the model to the catch-rate data is again adequate (Figure 10.10). However, the fit to the length-frequency data for females is very poor with the model predicting that there should be a large catch of small females. However, in contrast to the situation for males, few small females are caught. The productivity of elephant fish is estimated to be quite substantially lower than that of sawshark (5%). In common with sawshark, the elephant fish resource in Bass Strait is estimated to be depleted to below 40% of the pup production in 1950 (point estimate 20%). The base-case estimate of the pup production in 2004 of elephant fish is somewhat more precise than those of sawshark (the standard deviation of P_{2004} / P_{1950} is only 3%; a coefficient of variation nevertheless close to 25%).

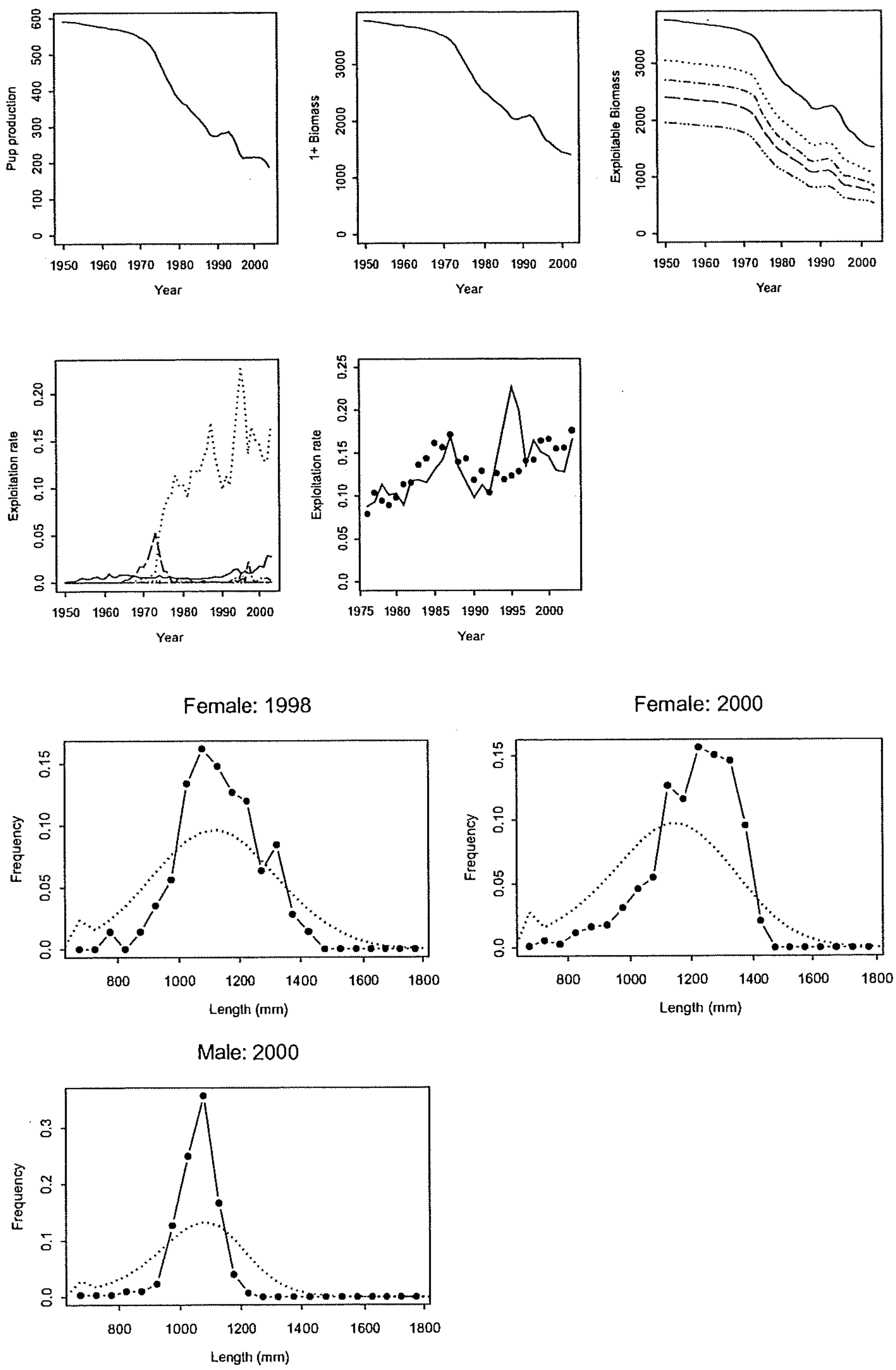


Figure 10.8 : Diagnostic statistics for the base-case assessment of sawshark (common sawshark biological parameters).

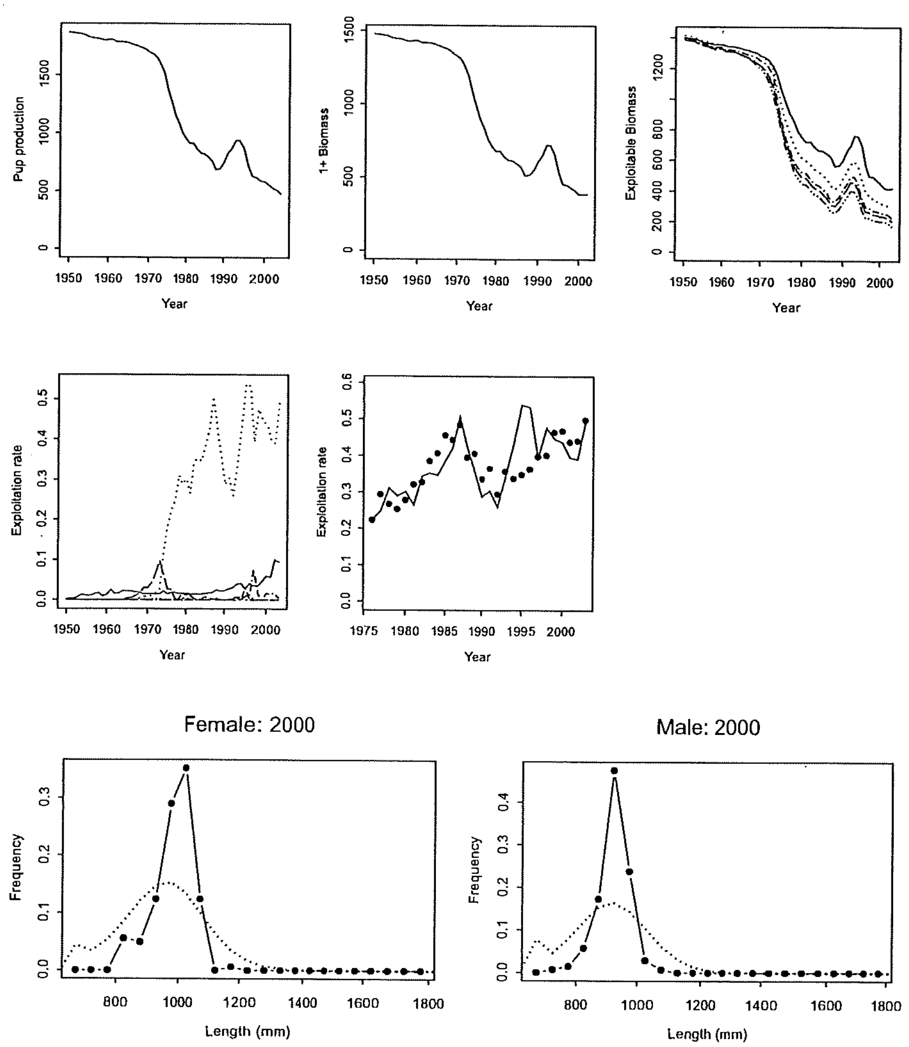


Figure 10.9 : Diagnostic statistics for the base-case assessment of sawshark (southern sawshark biological parameters).

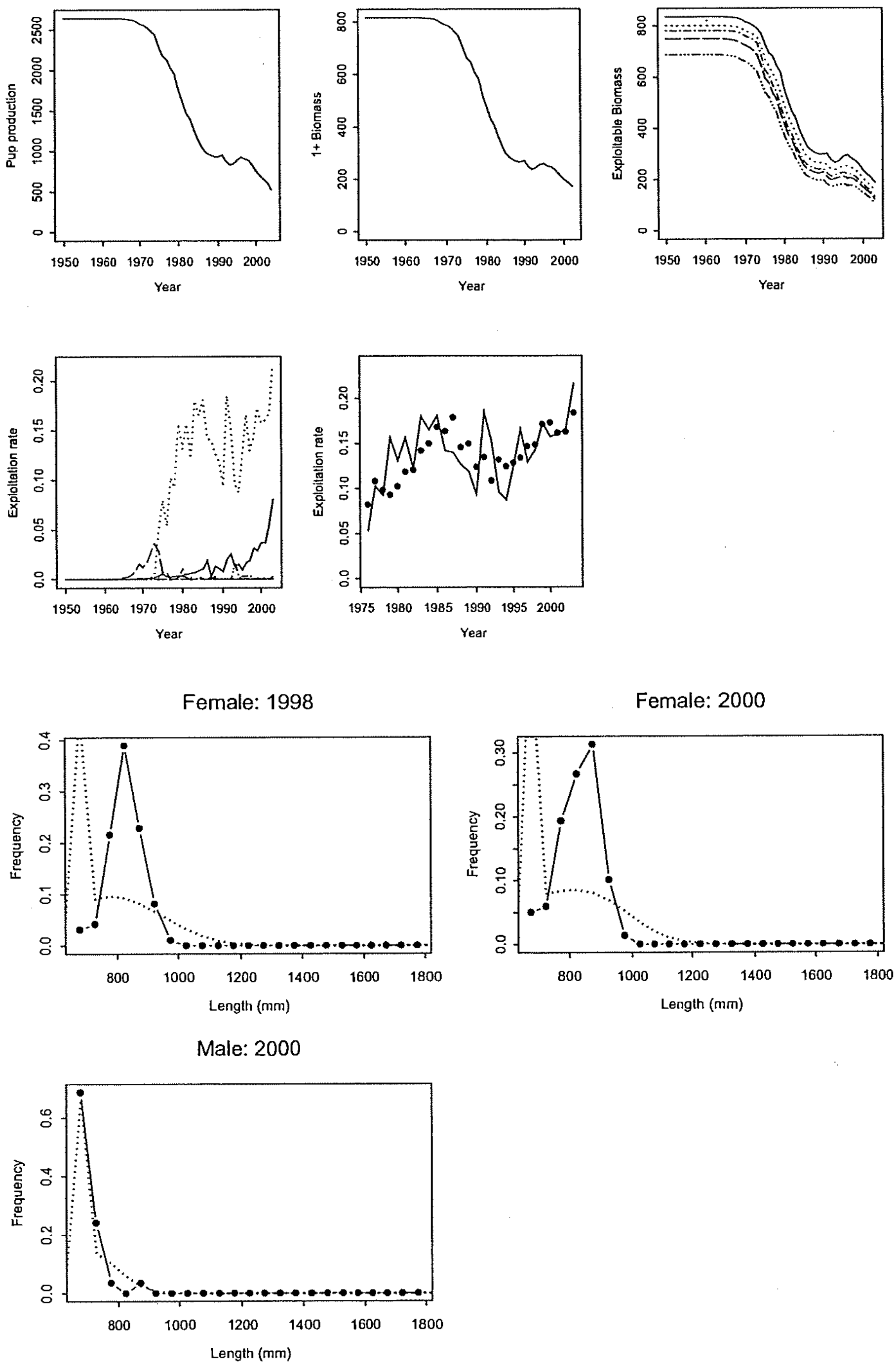


Figure 10.10 : Diagnostic statistics for the base-case assessment of elephant fish.

10.4.3 Sensitivity tests

The point estimate of the current depletion of sawshark ranges from 17% (sensitivity test 6) to 39% (sensitivity tests 3 and 10) while productivity (as measured by *MSYR*) ranges from 3% to 23%. The factors that influence the current depletion of sawshark to the greatest extent are: a) whether the biological parameters are set to those for common or southern sawshark, b) the weight assigned to the catch-rate data (increasing this weight leads to a more depleted resource), and c) allowing for some gear-competition. It is perhaps noteworthy that the best fit (of those analyses for which the values for the negative log-likelihood are comparable) is that for which allowance is made for gear competition – note, however, that none of the analyses can be distinguished statistically.

The range of depletion levels is less for elephant fish (14–22%) than for sawshark (17–39%) although the range of *MSYR* values (point estimates: 3–14%) remains very broad.

10.4.4 Bayesian analyses

Bayesian posterior distributions for the parameters and outputs of the model were developed using the Markov Chain Monte Carlo (MCMC) algorithm. The number of cycles was set to 5,000,000, the first 1,000,000 of which were ignored as a burn-in period and the chain was thinned by selecting every 5,000th parameter vector thereafter. Whether the MCMC algorithm had reached convergence was evaluated using standard diagnostic statistics and plots (see section 7.4.2).

The diagnostics (e.g. Figures 10.11a and 10.11b) suggest that convergence had failed to occur for sawshark. The results in Figures 10.11a and 10.11b indicate that this problem is such that convergence of the MCMC algorithm for sawshark cannot be expected unless: a) the model is reparameterized so that all high posterior correlations (e.g. Figure 10.12, a correlation of -0.91) are eliminated, or b) the number of cycles is increased very substantially (by at least an order of magnitude, prossibly by two orders of magnitude). The value of the parameter *V* has substantial posterior probability very near its maximum of 1 (Figure 10.12) – this will also tend to cause problems for the convergence of the MCMC algorithm unless a prior is selected for *V* which prevents this. Resolution of the problems with the convergence of the MCMC algorithm for sawshark is beyond the scope of the present preliminary assessment. Detailed Bayesian results are therefore not presented for sawshark in this chapter.

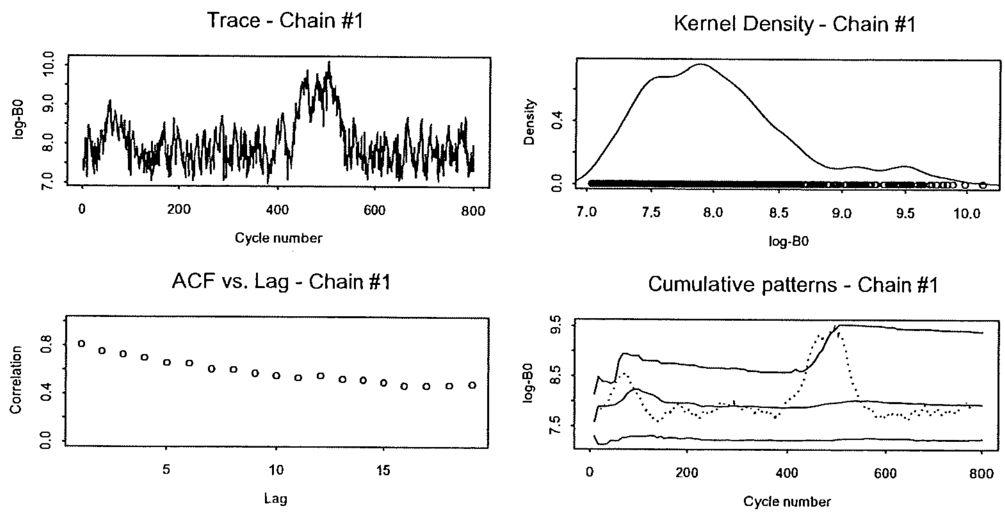


Figure 10.11(a) : Convergence statistics for the logarithm of the virgin recruitment for sawshark (common sawshark biological parameters).

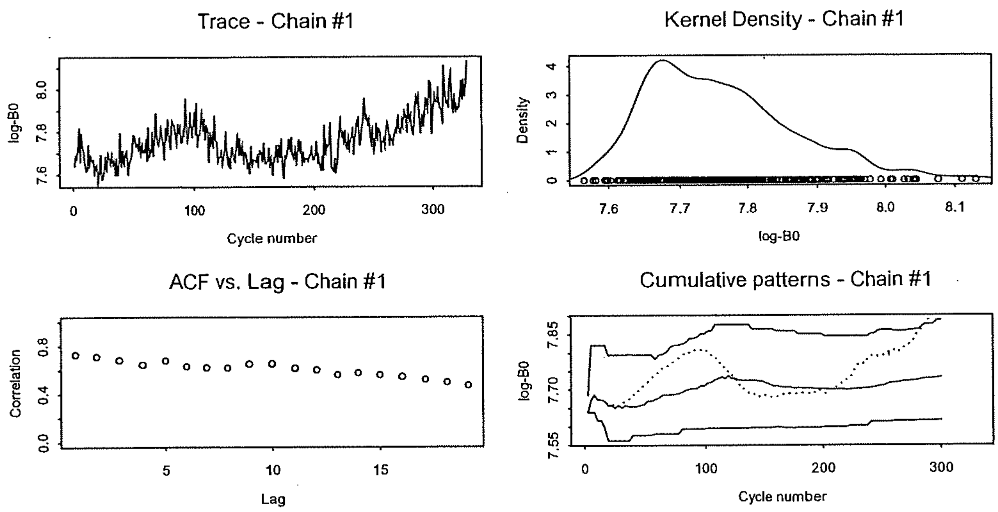


Figure 10.11(b) : Convergence statistics for the logarithm of the virgin recruitment for sawshark (southern sawshark biological parameters).

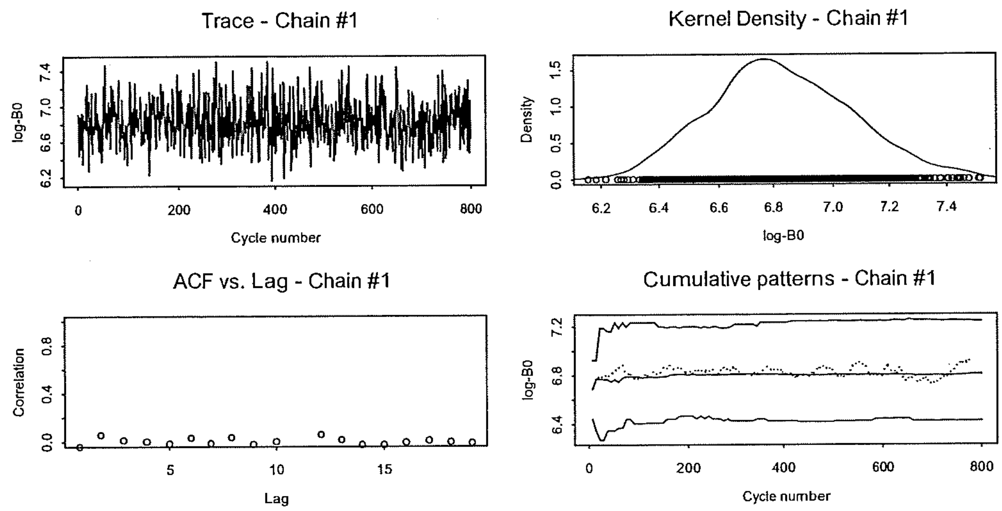


Figure 10.11(c) : Convergence statistics for the logarithm of the virgin recruitment for elephant fish.

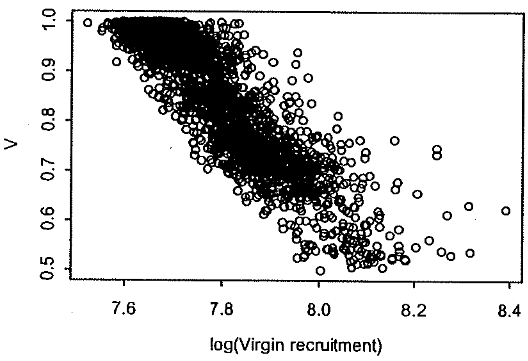


Figure 10.12 : Posterior correlation between the value of the density dependence parameter (V) and the logarithm of the virgin recruitment for sawshark (common sawshark biological parameters).

Some concerns with the Bayesian results for elephant fish remain although the diagnostics (e.g. Fig 10.11c) provide no evidence for a lack of convergence of the MCMC algorithm. For example, the posterior median for P_{2004}/P_{1950} (0.26) is notably larger than the posterior mode (0.2), although the large imprecision (95% posterior interval [0.13, 0.48]) implies that the posterior mode and median are actually not inconsistent. The bimodal distribution for *MSYR* (modes at 5 and 13%) (Figure 10.13) is also perhaps a cause for some concern.

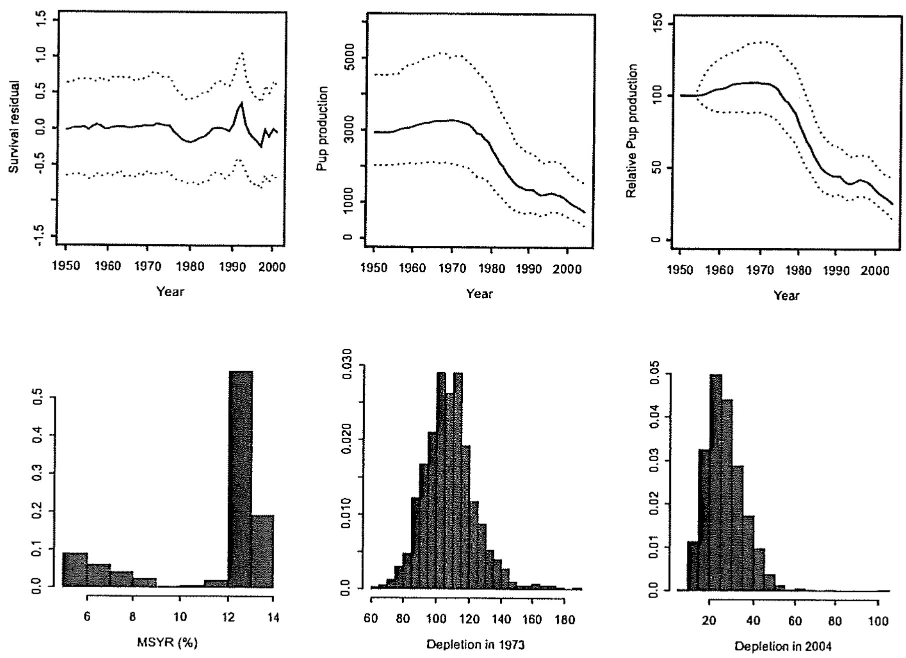


Figure 10.13 : Posterior distributions for the time-trajectories of the survival residuals and pup production for elephant fish (posterior medians and posterior 90% intervals) and posterior distributions for *MSYR*, P_{1973}/P_{1950} and P_{2004}/P_{1950}.

10.5. Discussion and further work

10.5.1 Stock status

The results of the assessment suggest that both sawshark and elephant fish are depleted to below 40% of the 1950 pup production (perhaps substantially so in the case of elephant fish). The results are, however, imprecise, particularly those for sawshark. For example, the point estimates of the current depletion of sawshark pup production range from 17–39% depending on the assumptions of the assessment.

The assessment of sawshark combines data for common and southern sawshark. These species differ quite markedly in terms of their biological parameters (Table 10.7; Figures 10.5 and 10.6). It is therefore quite likely that these two species also differ in terms of biological productivity but there are no data to examine this quantitatively. The impact of catches being aggregated across species cannot be assessed at present and the measures of uncertainty do not capture this source of uncertainty.

Changes in targeting practices have occurred over the history of the fishery. This assessment attempts to account for this by basing the effort used to construct the catch-rate-based indices of abundance on that for gummy shark. However, more subtle changes in targeting practices have occurred. In the absence of data to qualify these changes, however, their impacts on the results of the assessment remain unknown.

The fits to the catch-rate data appear good (Figures 10.8–10.10). This is, however, not surprising because these are only the data available to determine trends in population size and the extent of variability in pup survival. The results are therefore completely determined by the trend in historical catch-rates. It is well-known that catch-rates may not index abundance adequately, but the extent to which catch-rates may be inadequate indicators of abundance is unknown for sawshark and elephant fish. Cooke and Beddington (1984) and Cooke (1985) describe various scenarios in which catch rate is unlikely to be linearly related to abundance. Cooke and Beddington (1984) highlight the possibility that catch rates may decline more slowly than abundance ("hyperstability") and this expectation is supported by the meta-analysis conducted by Harley *et al.* (2001). However, the opposite problem ("hyperdepletion") can also occur (e.g. Prince and Hilborn, 1998).

Another consequence of an assessment that relies on a single data source is that additional data can lead to marked changes in impressions of stock status and productivity. For example, Punt and Walker (1998) using a model and data set similar to that considered in this chapter concluded that the mature biomass of the school shark resource off southern Australia lay between 13 and 45% of its 1927 level at the start of 1995. However, Punt *et al.* (2000a) estimated the pup production to be between 12 and 18% of its 1927 level at the start of 1997 based on a larger data set and a spatially-structured population dynamics model. The two sets of results are not inconsistent, but the additional data did not lead to a narrowing of the uncertainty towards the centre of the range considered initially to be plausible. There is no reason to believe that additional data could not impact the results of the assessments of this chapter in a similar (i.e. non-symmetric) manner.

10.5.2 Future work

The analyses of this chapter are clearly preliminary. There are several aspects of the analyses which could be improved.

a) The catch-rate series (Table 10.5) are based on the standardized effort data for gummy shark in Bass Strait. In principle, catch-rate indices could be developed specifically for sawshark (both species combined) and elephant fish based on data for 'indicative' fishers chosen by SharkFAG or using the data subsetting approach developed by Stephens and MacCall (In press). Once a data set has been chosen, the data could be analyzed using a variant of the delta-negative binomial approach used in Appendix 7.A.

b) The analyses are restricted to Bass Strait owing to a lack of data. However, catches of, for example, elephant fish are fairly substantial outside of Bass Strait (e.g. Figure 10.2). SharkFAG need to consider: i) whether consideration needs to be given to attempting to include other regions in future assessments, and ii) how the results for an assessment of a subset of the area fished can be used to provide management advice for the whole fishery.

c) The impact of combining the two sawshark species for assessment purposes should be examined by simulation. Alternatively (or in addition) an assessment framework should be developed that fits two population dynamics models (one for each of common and southern sawshark) simultaneously and that assesses the relative sizes of the two species using survey data such as that reported by Walker *et al.* (in press).

d) Availability is assumed to be independent of length in the absence of data. SharkFAG should consider whether sensitivity to the possibility that availability is domed-shaped should be examined. However, given the lack of data, hypotheses for how availability might change with length would need to be developed by SharkFAG based on *a priori* considerations.

e) Assessments of elephant fish have been conducted in New Zealand (e.g. McClatchie and Lester (1994)). The assumptions that underlie those assessments should be compared with those of the present assessments. Such a comparison could lead to a revision to some of the assumptions and / or additional sensitivity tests.

f) The values assumed for the biological parameters should be reviewed by SharkFAG. Specifically, the growth rate of southern sawshark is very rapid with maximum size attained in less than 10 years (Figure 10.6). The gill-net selectivity patterns for sawshark and elephant fish are also such that few animals are "fully selected" because the length-at-full-selection is greater than ℓ_∞ (Figures 10.5–10.7). One of the model inputs is the first size at which a female can be mature (which impacts how *MSYR* is defined). This quantity is assumed to be: 800 mm (common sawshark), 400 mm (southern sawshark), and 90 mm (elephant fish). SharkFAG needs to consider, and possibly revise, these values.

g) The base-case value for the natural mortality rate for animals aged 2 and older is $0.2yr^{-1}$. This value is based on the results of the assessment of gummy shark. The growth curves, particularly for southern sawshark, would suggest that this is likely to be an under-estimate.

h) Information of catch-rates of sawshark and elephant fish is available from the Integrated Scientific Monitoring Programme. Once analyzed, these data could be included as an alternative index of abundance.

i) Consideration should be given to reparameterizing the model in an attempt to reduce the extent of correlation among the parameters of the model.

j) Additional length-frequency data (from the catch of gillnetters and trawlers), as well as catch age-composition data, should be collected and included in future assessments.

10.6 References

Cooke, J.G. 1985. On the relationships between catch per unit effort and whale abundance. *Reports of the International Whaling Commission* 35: 511–519.

Cooke, J.G. and J.R. Beddington. 1984. The relationship between catch rates and abundance in fisheries. *IMA J. Mathematics Applied in Medicine and Biology* 1: 291–405.

Gavaris, S. 1980. Use of a multiplicative model to estimate catch rate and effort from commercial data. *Canadian Journal of Fisheries and Aquatic. Sciences* 37: 2272–2275.

Gelman, A, Carlin, J.B. Stern, H.S. and D.B. Rubin. 1995. Bayesian data analysis. Chapman and Hall. London.

Harley, S.J., Myers, R.A. and A. Dunn. 2001. Is catch-per-unit-effort proportional to abundance? *Canadian Journal of Fisheries and Aquatic Sciences* 58: 1760–1772.

Hastings, W.K. 1970. Monte Carlo sampling methods using Markov chains and their applications. *Biometrika* 57: 97–109.

Kimura, D.K. 1981. Standardized measures of relative abundance based on modelling log(c.p.u.e.), and the application to Pacific ocean perch (*Sebastes alutus*). *Journal de Consiel int. Exploration Mer.* 39: 211–218.

Kirkwood, G.P. and T.I. Walker. 1986. Gill net mesh selectivities for gummy shark, *Mustelus antarcticus* Günther, taken in south-eastern Australian waters. *Australian Journal of Marine and Freshwater Research* 37: 689–697.

Last, P.R. and J.D. Stevens. 1994. Sharks and rays of Australia. CSIRO, Melbourne.

Lee, R.M. 1912. An investigation into the methods of growth determination in fishes. *Publications de Circonstance Conseil Permanent International Pour l'Exploration de la Mer* 63: 1–34.

McClatchie, S. and P. Lester. 1994. Stock assessment of the elephant fish (*Callorhinchus milii*). *NZ Fisheries Assessment Research Document* 94/6.

Pribac, F., Punt, A.E., Walker, T.I. and B.L. Taylor. In press. The value of length, age and tagging data in a stock assessment of a length selective fishery for gummy shark *(Mustelus antarcticus). Journal of Northwest Atlantic Fisheries Science* 00: 00–00.

Prince, J.D. and R. Hilborn, 1998. Concentration profiles and invertebrate fisheries management. p. 187–196 *in* G.S. Jamieson and A. Campbell [Ed.] Proceedings of the North Pacific Symposium on Invertebrate Stock Assessment and Management. *Canadian Journal of Fisheries and Aquatic Sciences* 125.

Punt, A.E. and T.I. Walker. 1998. Stock assessment and risk analysis for the school shark (*Galeorhinus galeus*) off southern Australia. *Marine and Freshwater Research* 49: 719–731.

Punt, A.E., Pribac, F., Walker, T.I., Taylor, B.L. and J.D. Prince. 2000a. Stock assessment of school shark *Galeorhinus galeus* based on a spatially-explicit population dynamics model. *Marine and Freshwater Research* 51: 205–220.

Punt, A.E., Walker, T.I., Taylor, B.L. and F. Pribac. 2000b. Standardization of catch and effort data in a spatially-structured shark fishery. *Fisheries Research* 45: 129–145.

Punt, A.E., Pribac, F., Walker, T.I. and B.L. Taylor. 2001. Population modelling and harvest strategy evaluation for school and gummy shark. Report of FRDC 99/102. CSIRO Marine Research, Hobart (136pp).

Stephens, A. and A. MacCall. In press. A multispecies approach to subsetting logbook data for purposes of estimating CPUE. Fisheries Research 00: 00–00.

Vignaux, M. 1994. Catch per unit effort (CPUE) analysis of west coast South Island Cook Strait spawning hoki fisheries, 1987-93. *NZ Fisheries Assessment Research Document* 94/11.

Walker, T.I. In press. Chapter 4 Reproduction in fisheries science. p. 00–00 *in* W.C. Hamlett (Series Ed) and B.G.M. Jamieson [Ed.] Reproductive Biology and Phylogeny of Chondrichthyes: Sharks, Rays and Chimaeras. Science Publishers, Inc., Enfield, New Hampshire 03478, USA.

Walker, T.I., Hudson, R.J., and A.S. Gason. In press. Catch evaluation of target, byproduct, and bycatch species taken by gillnets and longlines in the shark fishery of south-eastern Australia. *Journal of Northwest Atlantic Fisheries Science* 00: 00–00.

Walker, T.I., Taylor, B.L., Hudson, R.J., Brown, L.P. and N.F. Bridge. 1997. Monitoring data sets held by MAFRI on the Southern Shark Fishery. SharkFAG Document SS/97/D18/3 (18pp).

Walker, T.I., Taylor, B.L., Hudson, R.J. and J.P. Cottier. 1998. The phenomenon of apparent change of growth rate in gummy shark (*Mustelus antarcticus*) harvested off southern Australia. *Fisheries Research* 39: 139–163.

Table 10.1. Time-series of historical catches (1973–2002) of sawshark and elephant fish in Bass Strait by the directed shark fisheries.

(a) Sawshark

Year	Gear-Type				
	Longline	6" Mesh	6.5" Mesh	7" Mesh	8" Mesh
1973	1.71	30.69	0.03	111.27	3.82
1974	10.73	139.71	0.00	61.02	0.00
1975	1.81	194.77	0.00	23.01	0.00
1976	3.33	218.49	0.00	19.91	0.20
1977	3.87	220.12	0.00	2.16	0.16
1978	0.58	259.78	0.42	0.91	0.02
1979	0.24	220.52	2.77	9.01	0.00
1980	0.11	217.57	2.45	6.18	0.00
1981	0.10	183.13	1.26	7.40	0.00
1982	0.12	237.76	0.03	2.33	0.00
1983	0.35	231.60	0.29	0.16	0.00
1984	0.26	219.99	0.52	0.49	0.00
1985	2.52	243.01	3.29	0.07	0.00
1986	3.39	257.31	1.44	0.02	0.00
1987	3.84	296.68	1.12	0.95	0.83
1988	2.80	223.66	0.54	0.75	0.02
1989	4.30	191.00	0.49	0.39	0.04
1990	3.09	161.99	0.00	0.00	0.00
1991	5.99	188.82	0.90	0.02	0.00
1992	8.39	172.09	0.25	1.34	0.00
1993	11.41	243.81	0.00	3.35	4.44
1994	3.28	305.19	0.00	0.34	1.85
1995	1.21	351.32	10.83	3.03	0.00
1996	1.06	282.79	1.06	3.56	0.00
1997	0.57	183.22	23.55	0.00	0.00
1998	0.31	219.19	4.27	0.00	0.00
1999	0.39	193.58	0.20	0.00	0.00
2000	0.35	180.86	4.43	0.00	0.00
2001	0.28	155.34	3.62	0.00	0.00
2002	0.06	148.60	4.34	0.00	0.00
2003	0.15	187.84	1.15	0.03	0.00

(Table 10.1 Continued)

(b) Elephant fish

Year	Gear-Type				
	Longline	**6" Mesh**	**6.5" Mesh**	**7" Mesh**	**8" Mesh**
1973	0.43	2.21	0.00	25.54	1.78
1974	1.65	35.26	0.00	18.70	0.00
1975	2.92	55.59	0.00	4.15	0.00
1976	0.50	35.58	0.00	3.80	0.00
1977	0.00	66.50	0.00	0.01	0.00
1978	0.00	56.68	0.18	0.01	0.00
1979	0.00	91.96	0.36	1.00	0.00
1980	0.00	69.08	4.79	1.37	0.00
1981	0.00	75.81	0.47	0.74	0.00
1982	0.06	53.62	0.00	0.03	0.00
1983	0.02	74.94	0.03	0.08	0.00
1984	0.24	61.56	0.77	0.00	0.00
1985	0.42	61.53	0.03	0.01	0.00
1986	1.92	43.62	0.12	0.00	0.00
1987	0.07	41.34	0.00	0.00	0.37
1988	1.91	36.14	0.00	0.69	0.00
1989	0.79	33.15	0.00	0.07	0.00
1990	1.14	26.13	0.00	0.00	0.00
1991	1.05	51.89	0.00	0.00	0.00
1992	1.79	39.08	0.08	0.09	0.00
1993	1.35	23.88	0.00	0.00	2.97
1994	0.50	22.30	0.00	0.00	0.52
1995	0.29	33.70	0.75	0.00	0.00
1996	0.28	45.27	0.62	0.18	0.00
1997	0.06	33.85	0.90	0.00	0.00
1998	0.14	36.04	0.04	0.00	0.00
1999	0.35	41.38	0.19	0.00	0.00
2000	0.02	34.31	0.17	0.00	0.00
2001	0.05	32.56	0.23	0.00	0.00
2002	0.11	31.10	0.09	0.00	0.00
2003	0.01	36.62	0.32	0.00	0.00

Table 10.2 : Reported catches (kg) by SETF and GABTF trawlers of sawshark and elephant fish by year and sub-region.

(a) Sawshark

Year	Sub-region									Total
	WSA	CSA	SAV-W	SAV-E	WBas	EBas	WTas	ETas	NSW	
South East Trawl Fishery										
1985	0	0	365	81	374	2343	0	349	588	4100
1986	0	0	1257	225	412	7114	150	10	10333	19501
1987	0	0	762	330	36	7804	0	110	7390	16432
1988	0	0	790	60	1325	10043	30	135	18068	30451
1989	0	0	615	0	150	7982	0	25	9408	18180
1990	0	0	1735	1760	595	8383	120	105	4995	17693
1991	0	0	1664	496	70	11661	140	260	9574	23865
1992	0	0	2895	1585	637	11537	0	145	8735	25534
1993	0	0	1795	540	1966	17023	0	247	10120	31691
1994	0	0	2180	770	551	27374	0	267	11999	43141
1995	0	0	7107	1615	835	16432	30	147	6710	32876
1996	0	0	8602	4607	1435	16841	30	217	7234	38966
1997	0	35	11275	4450	1349	11611	0	231	8233	37184
1998	0	99	6695	2955	1020	13981	0	91	5116	29957
1999	0	10	8020	6330	1956	14051	0	36	5771	36174
2000	0	0	20305	5015	3163	19462	91	362	6813	55211
2001	0	40	10155	5710	1556	17978	172	1259	5865	42735
2002	10	55	13138	5682	1628	36460	276	1825	12095	71168
2003	10	43	8517	7398	1721	32702	674	2150	26312	79526
Great Australian Bight Trawl Fishery										
1988	0	120	0	0	0	0	0	0	0	120
1989	700	79	0	0	0	0	0	0	0	779
1990	8346	0	0	0	0	0	0	0	0	8346
1991	7217	0	0	0	0	0	0	0	0	7217
1992	8765	0	0	0	0	0	0	0	0	8765
1993	10505	0	0	0	0	0	0	0	0	10505
1994	5322	43	0	0	0	0	0	0	0	5365
1995	9366	50	0	0	0	0	0	0	0	9416
1996	9803	92	0	0	0	0	0	0	0	9895
1997	13695	221	0	0	0	0	0	0	0	13916
1998	11821	156	0	0	0	0	0	0	0	11977
1999	9639	292	0	0	0	0	0	0	0	9931
2000	9520	89	0	0	0	0	0	0	0	9609
2001	14221	169	0	0	0	0	0	0	0	14390
2002	11170	50	0	0	0	0	0	0	0	11220
2003	25395	154	0	0	0	0	0	0	0	25548

(Table 10.2 Continued)

(b) Elephant fish

Year	Sub-region									Total
	WSA	CSA	SAV-W	SAV-E	WBas	EBas	WTas	ETas	NSW	
South East Trawl Fishery										
1985	0	0	0	0	124	244	0	330	210	908
1986	0	0	100	0	3549	851	40	455	154	5149
1987	0	0	0	0	200	449	30	55	1056	1790
1988	0	0	90	0	190	2016	140	170	492	3098
1989	0	0	0	0	1110	1398	260	45	94	2907
1990	0	0	0	0	305	895	270	0	373	1843
1991	0	0	0	0	1140	4016	437	255	462	6310
1992	0	0	32	32	2813	2481	555	100	377	6390
1993	0	0	0	0	605	1432	0	467	273	2777
1994	0	0	0	0	1350	2360	62	110	105	3987
1995	0	0	0	0	1140	1325	210	109	75	2859
1996	0	0	15	0	2590	2181	275	177	172	5410
1997	0	0	30	0	2622	2664	170	67	75	5628
1998	0	0	0	0	4747	3755	0	168	15	8685
1999	0	0	60	0	3815	3290	75	175	35	7450
2000	0	0	80	0	3824	4961	20	18	0	8903
2001	0	0	70	30	3314	4751	49	167	93	8474
2002	0	0	1035	119	4384	7040	1238	797	252	14865
2003	0	154	763	291	6135	9245	1538	1388	1134	20648
Great Australian Bight Trawl Fishery										
1988	0	100	0	0	0	0	0	0	0	100
1989	125	14	0	0	0	0	0	0	0	139
1990	45	0	0	0	0	0	0	0	0	45
1991	32	0	0	0	0	0	0	0	0	32
1992	30	0	0	0	0	0	0	0	0	30
1993	0	0	0	0	0	0	0	0	0	0
1994	705	0	0	0	0	0	0	0	0	705
1995	0	0	0	0	0	0	0	0	0	0
1996	220	0	0	0	0	0	0	0	0	220
1997	0	0	0	0	0	0	0	0	0	0
1998	30	0	0	0	0	0	0	0	0	30
1999	965	0	0	0	0	0	0	0	0	965
2000	0	0	0	0	0	0	0	0	0	0
2001	21	0	0	0	0	0	0	0	0	21
2002	127	0	0	0	0	0	0	0	0	127
2003	1384	30	0	0	0	0	0	0	0	1414

Table 10.3 : Historical (1950–69) catches of sawshark in Bass Strait .

Year	Total	Longline	Danish seine	Trawl
1950	8.14	0.70	7.44	7.44
1951	12.48	0.13	12.34	12.34
1952	12.22	0.14	12.08	12.08
1953	26.81	0.08	26.73	26.73
1954	46.48	0.56	45.91	45.91
1955	40.06	0.04	40.02	40.02
1956	36.06	0.06	36.01	36.01
1957	45.68	3.94	41.75	41.75
1958	57.37	1.52	55.84	55.84
1959	33.50	0.56	32.94	32.94
1960	43.43	0.08	43.34	43.34
1961	85.06	0.17	84.89	84.89
1962	52.05	0.09	51.96	51.96
1963	55.92	0.23	55.69	55.69
1964	75.69	0*		75.69
1965	80.89	0*		72.66
1966	82.23	0*		72.39
1967	84.07	0*		64.74
1968	89.19	0.08	37	61.11
1969	98.88	0.11	42	46.77

* assumed value

Table 10.4 : Estimates of trawl catches (otter trawl and Danish seine) based on the data collected by the SMP and the ISMP.

Year	Sawshark				Elephant fish			
	Haul-based		Hour-based		Haul-based		Hour-based	
	Estimate	CV	Estimate	CV	Estimate	CV	Estimate	CV
1992					11337	0.877	8130	0.721
1993	16239	0.448	19900	0.610	437	0.526	380	0.430
1994	7835	0.359	11488	0.373	18393	0.449	33111	0.537
1995	53413	0.169	63971	0.162	4092	0.244	5536	0.354
1996	40365	0.21	37193	0.393	766	0.617	686	0.542
1997	80332	0.171	68081	0.154	72	0.705	66	0.634
1998	146180	0.172	208190	0.197	5729	0.454	6779	0.427
1999	184399	0.125	176540	0.103	1762	0.481	1369	0.335
2000	231278	0.138	260989	0.147	5942	0.316	7243	0.320
2001	134469	0.184	170217	0.275	346556	0.425	671445	0.462
2002	145765	0.155	164301	0.145	1778	0.452	1978	0.435
2003	178815	0.114	168558	0.145	11331	0.248	8317	0.277

Table 10.5 : Catch-rate series for sawshark and elephant fish.

Year	Sawshark	Elephant fish
1976	97.17	43.92
1977	74.33	62.32
1978	96.44	58.40
1979	86.40	100.00
1980	77.60	68.38
1981	56.51	64.92
1982	72.10	45.12
1983	59.65	53.57
1984	53.67	41.68
1985	52.81	37.11
1986	57.58	27.09
1987	60.69	23.47
1988	56.18	25.19
1989	46.72	22.51
1990	47.85	21.42
1991	51.27	39.10
1992	57.84	36.46
1993	67.56	18.37
1994	89.69	18.19
1995	100.00	26.62
1996	77.18	34.29
1997	45.66	23.41
1998	54.08	24.67
1999	41.35	24.54
2000	38.27	20.15
2001	35.22	20.48
2002	33.46	19.43
2003	37.41	20.24

Table 10.6 : Length-frequency sample sizes for sawshark and elephant fish. The combinations of year, sex, and mesh-size indicated in bold underline are included in the analyses of this chapter.

		Year											
Sex	Mesh	1973	1974	1975	1976	1986	1987	1994	1995	1998	1999	2000	2001
Elephant fish													
F	6	16	12	23	3	4	0	0	0	**380**	62	**217**	3
F	6.5	0	0	0	0	0	0	0	0	0	1	0	8
F	7	17	5	21	0	9	0	0	0	0	0	0	0
F	8	0	0	2	0	5	0	0	0	0	0	0	0
M	6	8	12	9	5	45	0	0	0	4	40	**83**	0
M	6.5	0	0	0	0	0	0	0	0	1	9	0	2
M	7	5	11	3	2	27	0	0	0	0	0	0	0
M	8	0	1	0	0	17	0	0	0	0	0	0	0
Common sawshark													
F	6	13	6	0	2	39	37	0	0	**142**	23	**672**	96
F	6.5	0	0	0	0	0	0	0	0	2	21	0	0
F	7	9	31	2	1	29	27	16	10	0	0	0	0
F	8	0	1	0	0	20	18	0	0	0	0	0	0
M	6	29	18	3	6	50	26	0	0	94	24	**300**	33
M	6.5	0	0	0	0	0	0	0	0	1	8	0	0
M	7	11	12	1	1	12	12	2	3	0	0	0	0
M	8	1	1	0	0	10	10	0	0	0	0	0	0
Southern sawshark													
F	6	0	0	1	6	13	7	0	0	39	36	**162**	52
F	6.5	0	0	0	0	0	0	0	0	11	3	0	0
F	7	0	0	0	2	8	6	0	0	0	0	0	0
F	8	0	0	0	0	8	3	0	0	0	0	0	0
M	6	0	1	4	3	18	5	0	0	17	15	**139**	48
M	6.5	0	0	0	0	0	0	0	0	2	2	0	0
M	7	0	0	0	1	11	5	0	0	0	0	0	0
M	8	0	0	1	0	6	2	0	0	0	0	0	0

Table 10.7 : Values for the biological parameters (source: PIRVic, unpublished data).

Quantity	Common sawshark		Southern sawshark		Elephant fish	
	Female	Male	Female	Male	Female	Male
L_∞ (mm)	1502	1165	1047	971	1049	770
κ (yr^{-1})	0.149	0.309	0.488	0.575	0.238	0.400
t_0 (yr)	-1.76	-1.00	-0.49	-1.00	-0.05	-0.04
a ($x10^{-9}$)[A]	0.990	1.520	0.060	0.078	0.591	0.063
b	3.292	3.015	3.498	3.450	3.337	3.688
a' (yr)	-14.52		-8.36		-2.37	
b' (yr^{-1})	0.0205		0.0184		0.0279	
P''_{max}	0.5 [B]		0.5 [B]		1.0 [C]	
ℓ''_{50} (mm)	1109		841		602	
ℓ''_{95} (mm)	1199		893		746	
θ_1	237.91		237.91		154.23	
θ_2	185075		185075		185097	

A – Non-pregnant
B – Maternity
C – Maturity

Table 10.8 : The specifications for the base-case analyses and the sensitivity tests.

(a) Sawshark

Abbreviation	Biological parameters	M_{2+}	Recruitment residuals	Density-dependence	CPUE σ	Trawl Catches	Length data effective sample size	Gear Competition
Base-A	Common	0.2yr^{-1}	1951–2002	*M*; ages 0-30	0.3	Base	10	No
Base-B	Southern	0.2yr^{-1}	1951–2002	*M*; ages 0-30	0.3	Base	10	No
Sen-1A	Common	0.15yr^{-1}	1951–2002	*M*; ages 0-30	0.3	Base	10	No
Sen-1B	Southern	0.15yr^{-1}	1951–2002	*M*; ages 0-30	0.3	Base	10	No
Sen-2A	Common	0.25yr^{-1}	1951–2002	*M*; ages 0-30	0.3	Base	10	No
Sen-2B	Southern	0.25yr^{-1}	1951–2002	*M*; ages 0-30	0.3	Base	10	No
Sen-3A	Common	0.2yr^{-1}	None	*M*; ages 0-30	0.3	Base	10	No
Sen-3B	Southern	0.2yr^{-1}	None	*M*; ages 0-30	0.3	Base	10	No
Sen-4A	Common	0.2yr^{-1}	1951–2002	*M*; ages 0-5	0.3	Base	10	No
Sen-4B	Southern	0.2yr^{-1}	1951–2002	*M*; ages 0-5	0.3	Base	10	No
Sen-5A	Common	0.2yr^{-1}	1951–2002	Pup survival	0.3	Base	10	No
Sen-5B	Southern	0.2yr^{-1}	1951–2002	Pup survival	0.3	Base	10	No
Sen-6A	Common	0.2yr^{-1}	1951–2002	*M*; ages 0-30	0.15	Base	10	No
Sen-6B	Southern	0.2yr^{-1}	1951–2002	*M*; ages 0-30	0.15	Base	10	No
Sen-7A	Common	0.2yr^{-1}	1951–2002	*M*; ages 0-30	0.3	x 2.5	10	No
Sen-7B	Southern	0.2yr^{-1}	1951–2002	*M*; ages 0-30	0.3	x 2.5	10	No
Sen-8A	Common	0.2yr^{-1}	1951–2002	*M*; ages 0-30	0.3	Base	0	No
Sen-8B	Southern	0.2yr^{-1}	1951–2002	*M*; ages 0-30	0.3	Base	0	No
Sen-9A	Common	0.2yr^{-1}	1951–2002	*M*; ages 0-30	0.3	Base	20	No
Sen-9B	Southern	0.2yr^{-1}	1951–2002	*M*; ages 0-30	0.3	Base	20	No
Sen-10A	Common	0.2yr^{-1}	1951–2002	*M*; ages 0-30	0.3	Base	10	Yes
Sen-10B	Southern	0.2yr^{-1}	1951–2002	*M*; ages 0-30	0.3	Base	10	Yes

(Table 10.8 Continued)

(b) Elephant fish

Abbreviation	M_{2+}	Recruitment residuals	Density-dependence	CPUE σ	Length data effective sample size	Gear Competition
Base	0.2yr^{-1}	1951–2002	*M*; ages 0-30	0.3	10	No
Sen-1	0.15yr^{-1}	1951–2002	*M*; ages 0-30	0.3	10	No
Sen-2	0.25yr^{-1}	1951–2002	*M*; ages 0-30	0.3	10	No
Sen-3*	0.2yr^{-1}	None	*M*; ages 0-30	0.3	10	No
Sen-4	0.2yr^{-1}	1951–2002	*M*; ages 0-5	0.3	10	No
Sen-5	0.2yr^{-1}	1951–2002	Pup survival	0.3	10	No
Sen-6	0.2yr^{-1}	1951–2002	*M*; ages 0-30	0.15	10	No
Sen-8	0.2yr^{-1}	1951–2002	*M*; ages 0-30	0.3	0	No
Sen-9	0.2yr^{-1}	1951–2002	*M*; ages 0-30	0.3	20	No
Sen-10	0.2yr^{-1}	1951–2002	*M*; ages 0-30	0.3	10	Yes

* Did not converge

Table 10.9 : Estimates of the output quantities (estimates with asymptotic standard deviations in parenthesis).

(a) Sawshark

Scenario	Common sawshark parameters					Southern sawshark parameters				
	MSYR	V/Q_0	P_{1973}/P_{1950} (%)	P_{2004}/P_{1950} (%)	$-\ell nL$	*MSYR*	V/Q_0	P_{1973}/P_{1950} (%)	P_{2004}/P_{1950} (%)	$-\ell nL$
Base-case	0.08 (0.08)	0.479 (0.456)	88 (7)	32 (11)	18.01	0.17 (0.07)	0.758 (0.279)	86 (7)	26 (10)	15.32
Sen-1	0.12 (0.08)	0.679 (0.342)	87 (5)	28 (10)	17.47	0.18 (0.07)	0.825 (0.236)	85 (6)	25 (9)	15.06
Sen-2	0.17 (0.11)	0.312 (0.647)	90 (9)	35 (14)	18.71	0.16 (0.07)	0.692 (0.321)	86 (7)	26 (10)	15.57
Sen-3	0.15 (0.07)	0.277 (0.461)	91 (4)	39 (11)	19.17	0.12 (0.08)	0.535 (0.325)	88 (3)	37 (9)	17.77
Sen-4	0.17 (0.01)	0.447 (0.486)	89 (9)	33 (12)	18.09	0.16 (0.07)	0.750 (0.273)	85 (8)	25 (10)	15.22
Sen-5	0.03 (0.06)	1.712 (1.785)	91 (11)	35 (13)	18.37	0.11 (0.05)	3.901 (2.123)	88 (8)	27 (10)	15.97
Sen-6	0.18 (0.00)	1.000 (0.001)	84 (4)	17 (4)	33.32	0.23 (0.00)	1.000 (0.001)	83 (6)	20 (6)	23.11
Sen-7	0.09 (0.08)	0.490 (0.452)	84 (7)	29 (11)	17.94	0.16 (0.07)	0.709 (0.286)	82 (7)	25 (9)	15.45
Sen-8	0.18 (0.00)	1.000 (0.002)	84 (4)	23 (9)	7.27	0.23 (0.00)	1.000 (0.001)	84 (6)	21 (9)	5.06
Sen-9	0.08 (0.06)	0.463 (0.360)	88 (6)	32 (10)	27.66	0.16 (0.06)	0.695 (0.248)	86 (7)	26 (9)	24.70
Sen-10	0.08 (0.09)	0.448 (0.509)	89 (7)	39 (13)	16.92	0.16 (0.08)	0.723 (0.306)	86 (7)	31 (11)	14.92

(b) Elephant fish

Scenario	*MSYR*	V/Q_0	P_{1973}/P_{1950} (%)	P_{2004}/P_{1950} (%)	$-\ell nL$
Base-case	0.05 (0.03)	0.195 (0.112)	94 (10)	20 (6)	27.27
Sen-1	0.07 (0.02)	0.269 (0.097)	93 (8)	19 (6)	26.36
Sen-2	0.14 (0.01)	0.127 (0.121)	96 (13)	21 (7)	28.35
Sen-4	0.13 (0.00)	0.197 (0.105)	95 (11)	21 (6)	27.13
Sen-5	0.03 (0.02)	1.540 (0.572)	97 (13)	22 (7)	28.10
Sen-6	0.09 (0.03)	0.340 (0.103)	89 (8)	19 (4)	47.65
Sen-7	0.05 (0.03)	0.195 (0.112)	94 (10)	20 (6)	27.27
Sen-8	0.14 (0.10)	0.510 (0.350)	89 (8)	14 (9)	7.44
Sen-9	0.12 (0.01)	0.160 (0.098)	95 (11)	20 (6)	44.25
Sen-10	0.05 (0.03)	0.185 (0.113)	94 (10)	22 (7)	27.11

Appendix 5a: Catch evaluation

This appendix contains a manuscript in press, which evaluates catches of target, byproduct and bycatch species taken by gillnets and longlines in the shark fishing component of the Gillnet Hook and Trap Fishery.

J. Northw. Atl. Fish. Sci., Vol. 35: 505–530

Catch Evaluation of Target, By-product and By-catch Species Taken by Gillnets and Longlines in the Shark Fishery of South-eastern Australia

Terence I. Walker, Russell J. Hudson, and Anne S. Gason
Primary Industries Research Victoria, P. O. Box 114
Queenscliff, Victoria 3225, Australia

Abstract

Experimental demersal gillnets and demersal longlines were deployed from research vessels on grounds of *Mustelus antarcticus* during 1973–76. Gillnet mesh-size had major effects on catch composition and catch rate, whereas gillnet hanging ratio, hook-size, hook shank-length and hook-spacing had minor effects. The gillnets and longlines were much more effective at catching chondrichthyans than teleosts, and catches of species of cephalopoda, bivalvia, gastropoda, mammalia, aves and reptilia were negligible. Any reduction in the present legal minimum mesh-size of 6 inch the shark fishery would markedly increase by-catch. In gillnets monitored by scientific observers aboard commercial vessels during 1998–01, the ratio of the number of chondrichthyan to teleost animals was ~24:1 in Bass Strait and ~5:1 in South Australia. In Bass Strait between 1973–76 and 1998–2001, the catch rate by 6 inch mesh gillnets of chondrichthyans declined by one-third, whereas a change in the catch rate of teleosts was not statistically detectable. Most of this decline is explained by reductions of 54% by *Cephaloscyllium laticeps* and of 87% by *Galeorhinus galeus*. The retained commercial catch was 74% of the chondrichthyan animals and 58% of the teleosts caught; only 3% of the chondrichthyans and 2% of the teleosts were discarded dead. There are occasional interactions with protected species (marine mammals and *Carcharodon carcharias*).

Key words: Australia, by-catch, catch rates, gillnet, longline, observers, shark fishery

Introduction

The International Plan of Action for the Conservation and Management of Sharks (IPOA-Sharks) recognises that the life history characteristics of chondrichthyan species can lead to low 'biological productivity' making these animals more prone to overexploitation from fishing than most teleost and invertebrate species. The IPOA-Sharks, developed by the Food and Agriculture Organization of the United Nations, also recognizes that these species require special management, research, and monitoring if they are to be harvested sustainably (Anon., 2000). Globally, the catches of chondrichthyans are often under-reported and it is likely to go unrecognized that many species, particularly those taken as by-catch, are at high risk (Walker, 1998). 'Critical by-catches' pertains to species or populations that are in danger of extinction, and 'unsustainable by-catches' are by-catches of species or populations that are not currently at risk but will decline at current levels of by-catch (Hall, 1996).

In Australia, several initiatives in recent years have created legislative requirements to evaluate catch composition and catch rates of all species of fish in Australian fisheries. The requirements apply to both targeted and non-targeted species. Non-targeted species comprise by-product (species where the catch is mostly retained) and by-catch (species where the catch is mostly discarded). In response to legislative obligations, the Commonwealth Government has recently developed by-catch action plans for major Australian fisheries. The Government has also responded to the legislative requirement for "strategic assessment" of certain fisheries for ecological impacts on a) target and by-product species, b) by-catch species, c) threatened, endangered and protected species, d) marine habitats, and e) marine food chains. The process requires collection of appropriate data, risk assessment, and appropriate management responses. Also as a signatory nation to the IPOA-Sharks, Australia has developed a National Plan of Action for the Conservation and Management of Sharks (NPOA-Sharks), which identifies catch evaluation and risk assessment of chondrichthyan species as high priority needs.

The present study is designed to evaluate the catch composition and catch rates in the shark fishery of southern Australia. The catch of each species was evaluated in terms of whether the animals were landed on board 'live' or 'dead' and whether they were 'retained' or 'discarded'. The study addresses catches taken both by demersal monofilament gillnets and demersal longlines from data available for the two periods of 1973–76 and 1998–2001.

Materials and Methods

Data utilized in the present study were collected opportunistically during three separate investigations. Data from the first of these investigations were collected on two research vessels during 1973–76, where the biology of gummy shark (*Mustelus antarcticus*) and the length selective characteristics of fishing gear were investigated (Walker, 1983). Data from the second of these investigations were collected on two commercial fishing vessels during 1998 as part a pilot fixed-station fishery-independent survey designed to determine survey intensity for monitoring abundance of harvested species (Punt *et al.*, 2002). Data from the third investigation were collected on eight fishing vessels during 1999–2001 as part of a study of common sawshark (*Pristiophorus cirratus*), southern sawshark (*P. nudipinnis*), and elephant fish (*Callorhinchus milii*).

During 1973–76, most of the research sampling was undertaken in Bass Strait, with a small amount of sampling undertaken in waters off the east and south coasts of Tasmania and in waters off South Australia. Five separate experiments were undertaken to test for the effects of gillnet mesh size, gillnet hanging ratio, hook size, hook shank length and hook spacing on catch rate. During 1998–2001, sampling was undertaken during normal commercial fishing operations in Bass Strait and South Australia. For Bass Strait, comparisons of catch rates from gillnet with 6 inch mesh were made between 1973–76 and 1998–2001. Other than recording mesh size of gillnets, it was not possible to control the design of the fishing gear or undertake experiments during the second period. Catch rates for gillnet 7 inch mesh size and longlines with Mustad 11/O long-shank hooks during 1973–76 are also presented for Bass Strait, because these gears were used extensively by the fishing industry during that period. For Tasmania, similar data are presented for 1973–76, but there are no data for 1998–2001. For South Australia, there are insufficient data for 1973–76, but gillnet 6 inch mesh and 6½ inch mesh size data are presented for 1998–2001. During 1998–2001, most of the fishing gear deployed in South Australia and Tasmania was 6½ inch mesh size and most of the fishing gear deployed in Bass Strait was with 6 inch mesh.

Field sampling 1973–76

During June 1973 to November 1976, catch composition and catch rates were examined at 162 fishing sites during 155 fishing days on the FV *Moondara* and FRV *Sarda*, at depths of 9–79 m on the continental shelf between Streaky Bay, South Australia; Gabo Island, Victoria; and Hobart, Tasmania. Most fishing sites were in Bass Strait (126 sites), but some were off eastern Tasmania, south of latitude 41° South (20 sites), and off South Australia (16 sites) (Fig. 1a).

Longlines used consisted of 400 hooks attached to two separate lines. The hooks (2/O–10/O Mustad short-shank and 11/O Mustad long-shank) were clipped 5, 7.5, 10, or 20 m apart to a sinking super saran rope main line. Each hook was connected to one end of a 1 m long snood, constructed of 6 mm diameter braided polypropylene, by a 10 cm long monel wire trace. The other end of the snood was attached to the main line by way of a snap-clip. Each of 12 gillnets was 250 m long and ~1.7 m deep. Eight had a hanging ratio of 0.60 and mesh sizes ranging 2–9 inch mesh (51–229 mm), in steps of 1 inch mesh (25 mm). Two had a hanging ratio of 0.53 and mesh sizes of 6 inch mesh (152 mm) and 7 inch mesh (178 mm), and two had a hanging ratio of 0.67 and mesh sizes of 6 and 7 inch mesh.

The monofilament polyamide webbing used to construct the nets was green, double knotted, double selvedge, and of neutral buoyancy. The bridle and headline were made of 10 mm (diameter) polypropylene rope. The headline with attached webbing was 250 m long. Vinyl floats ('3TV-5' each with 128 g wt upthrust) were attached to the headline at 5 m intervals. The leadline was made of 6 mm diameter polyethylene rope, with eight 57 g lead weights per 5 m. The leadline was made 5% longer than the headline to reduce the incidence of tangling when setting of the nets. The number of meshes deep, the thickness of the filaments of the webbing (0.47–1.05 mm), and the breaking strain of the filaments varied with mesh size (101–467 Newton) (Table 1).

The gillnets and longlines were set on the seabed mainly between the times of 0400 hr and 0600 hr; the nets were set first, followed by the longlines. Set as groups of two or three nets, the ends of the headlines of adjoining nets were connected and separated by 100 m lengths of 10 mm diameter polypropylene rope. Two lead anchor weights (each 12.5 kg) were attached to the bridles at the two ends of each net. Two buoy lines, with buoys, were attached to the headlines of the nets at the two free ends of each group of nets. Similar configurations of buoy lines, buoys, and anchor weights were adopted for each longline. The groups of nets and the two longlines were usually set in a line 100–300 m apart.

Five separate experiments were undertaken during 1973–76 using various combinations of this fishing gear

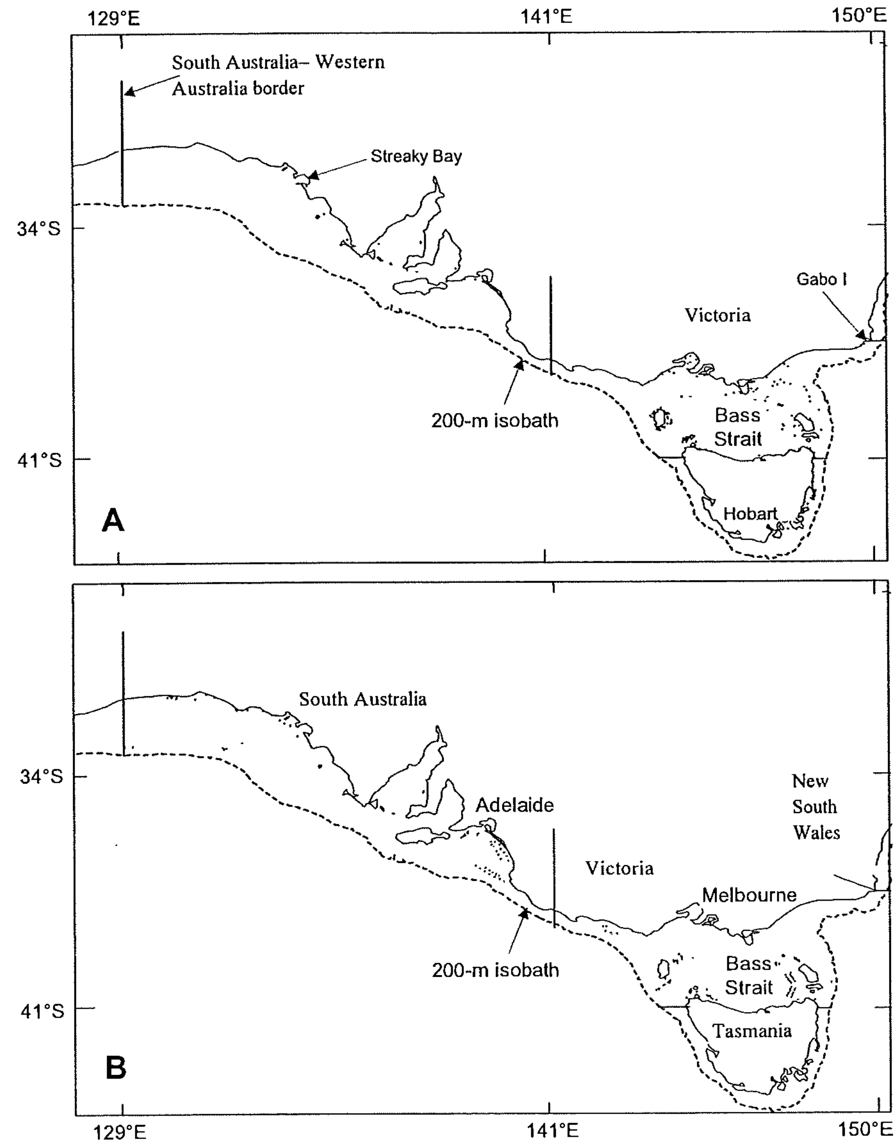


Fig. 1. (A) Fishing sites during 1973–76 and (B) fishing sites during 1998–2001.

to determine the effects on the catch rate for each species. Three experiments tested the effects of mesh size of gillnets (2–9 inch mesh), hanging ratio of gillnets (0.53, 0.60, and 0.67), and hook size (short-shank Mustad 2/O–10/O), respectively. Two experiments tested the effects of hook size (short-shank Mustad 5/O and 10/O), hook shank length (Mustad short-shank 10/O and long-shank 11/O), and hook spacing (Mustad long-shank 11/O 5, 10 and 20 m). Mean fishing times for the gears were 6.1 hr for Experiment 1, 6.3 hr for Experiment 2, 4.3 hr for Experiment 3, 4.3 hr for Experiment 4, and 3.2 hr for Experiment 5 (Table 2).

Field sampling 1998–2001

During November 1998 to February 2001, nine different commercial vessels were used during 10 separate fishing trips for sampling at 153 fishing sites (91 sites in Bass Strait and 62 sites off South Australia) (Fig. 1b). The vessels operated under normal commercial fishing conditions, where the fishing gear consisted of 6 inch or 6½ inch mesh size gillnets. The vessels were all licensed to deploy gillnets up to a maximum of 4 200 m long and 20 meshes deep; each gillnet was ~2.4 m deep with a hanging ratio of ~0.60. The thickness and breaking strain

TABLE 1. Variable characteristics of the eight experimental gillnets used for Experiments 1 and 2.

Mesh size (inch)	Number of meshes deep (mm)	Filament thickness (N)	Breaking strain
2	42	0.47	101
3	28	0.57	146
4	21	0.66	193
5	17	0.74	240
6	14	0.81	285
7	12	0.87	326
8	10	0.90	348
9	9	1.05	467

TABLE 2. Summary of fishing gear specifications and variables for each of five experiments and for between period and between gear statistical tests.

Experiment/ test	Period	Fishing gear	Fishing gear specifications	Explanatory variables	Gear units	Times set
Expt 1	1973–76	Gillnet	8 mesh-sizes[1] × 1 hanging-ratio	Mesh-size	8 × 250 m	73
Expt 2	1973–76	Gillnet	2 mesh-sizes[2] × 3 hanging-ratios[3]	Hanging-ratio	6 × 250 m	32
Expt 3	1973–76	Longline	8 hook-sizes[4] × 1 shank-length[5] × 1 space[6]	Hook-size	8 × 50 hks	39
Expt 4	1973–76	Longline	3 hook-sizes[7] 2 shank-lengths[8] 2 spaces[9]	Hook-size, shank-length, hook-spacing	4 × 50 hks	41
Expt 5	1973–76	Longline	3 hook-sizes[7] 2 shank-lengths[8] 2 spaces[10]	Hook-size, shank-length, hook-spacing	4 × 50 hks	22
Between periods	1973–76 1998–01	Gillnet	1 mesh-size × 2 periods[11] (Bass Strait only)	Period	172 × 250 m 91 × 4 200 m	172 91

[1] Mesh-sizes 2-, 3-, 4-, 5-, 6-, 7-, 8- and 9-inch of hanging-ratio 0.60
[2] Mesh-sizes 6- and 7-inch
[3] Gillnet hanging ratios of 0.53, 0.60 and 0.67
[4] Hook-sizes Mustad 2/O, 3/O, 4/O, 5/O, 7/O, 8/O, 9/O and 10/O
[5] Short-shank
[6] 7.5 m hook-space
[7] Hook-sizes Mustad 5/O, 10/O and 11/O
[8] Short-shank and long-shank
[9] 10- and 20 m hook-spaces
[10] 5- and 10 m hook-spaces
[11] Periods 1973–76 and 1998–2001

of the filaments of the gillnet webbing were ~0.90 mm and ~359 Newton, respectively. The gear was set on the seabed, mostly twice a day. Those set between the times of 2100 hr and 0500 hr were mostly hauled after sunrise, whereas those set between the times of 0800 hr and 2000 hr were mostly hauled after sunset. Mean fishing time for the gear was 8.2 hr. Depths at the fishing sites ranged 17–130 m; there were only 10 sites >79 m (all in South Australia), the maximum depth fished during 1973–76. The full length of gillnet was deployed at most fishing sites (4 200 m at 128 sites) or a little less was deployed when the gear was damaged (4 000 m at 21 sites). Half the available gillnets were set when searching for target species or when avoiding strong tidal flow or damage to the catch from predation (2 100 m at 2 sites, and 2 000 m at 2 sites).

Data collection

When hauling the fishing gear, the catch was sorted for up to 22 sampling units of fishing gear. All chondrichthyes, teleostei, cephalopoda, mammalia, aves, and reptilia, and selected (large sized) species of crustacea, bivalvia, gastropoda, were identified and counted. No information was recorded for other invertebrate and chordate taxonomic groups. Common, scientific, and family name for each animal identified was assigned according to the Codes for Australian Aquatic Biota (CAAB) maintained by CSIRO Division of Marine Research as of June 2002. In addition, during 1998–2001, where practical, each animal caught was classed as 'live', 'dead', or 'unknown' when removed from the water, and classed as 'retained' or 'discarded'. Because 'sea lice' (isopods and copepods) and leatherjackets (family *Monocanthidae*), can cause damage and loss of a portion of the catch, the proportion of each retained animal was recorded.

Data analysis

The data were managed and analysed using the statistical package SAS (Ver. 8.1, SAS Institute, North Carolina, USA). Catch rates were statistically tested for each of the five experiments separately and for each of three regions adopted for comparisons of the fishing gears used most widely in the shark fishery during 1973–76 and 1998–2001. For each experiment, the data were pooled over all fishing sites, whereas, for inter-period and commercial gear comparisons, the data were separated into the three regions Bass Strait, Tasmania, and South Australia. For the purpose of the present study, a one way analysis of variance was applied to test for the effect of each of several explanatory (independent) variables separately for each species and each major taxonomic group. For each analysis separately, the variance was tested for homogeneity and, where this was true, the following model was applied.

$$\textit{Catch rate} = \textit{Explanatory variable(s)} + \tilde{\varepsilon}$$

In the model, ε is the error term and catch rate is the number of animals caught divided by the fishing effort, where fishing effort was applied separately in the model for each of several alternative units. For gillnets, the unit of fishing effort applied was 'metre-lift-hours', and, for longlines, the unit of fishing effort applied was 'hook-lifts' (number of hooks). The explanatory variable in the model varied depending on experiment or on region for the inter-period or gear comparisons. The explanatory variable was mesh size for Experiment 1, hanging ratio for Experiment 2, and hook size for Experiment 3, and the three explanatory variables were hook size, hook shank-length, and hook-space for each of Experiments 4 and 5. For inter-period comparisons, the explanatory variable was sampling period for gillnet 6 inch mesh size in Bass Strait and, for commercial gear comparisons, the explanatory variable was mesh size for gillnet 6 inch and 6½ inch mesh size in South Australia during 1998–2001. No statistical test was applied to the data presented for Tasmania during 1973–76.

Results

During 1973–76 and 1998–2001 combined, a much higher number of animals and a higher number of species were caught by gillnets (22 918 animals, 124 species) than by longlines (4 006 animals, 54 species). The wider range of gillnet mesh sizes and longline hook sizes deployed caught both a higher number of animals and higher number of species during 1973–76 (16 657 animals, 112 species) than during 1998–2001 (10 267 animals, 65 species), despite a much lower fishing effort during 1973–76. Some of the differences in numbers of animals and numbers of species caught between the two periods can be explained by longlines being used only during 1973–76 (4 006 animals, 54 species). However, most of the differences in the numbers caught is explained by eight mesh sizes (2–9 inches) used during 1973–76 (12 651 animals, 104 species) and only two mesh sizes (6 and 6½ inches) during 1998–2001 (10 267 animals, 65 species).

The catch comprised mostly chondrichthyes (21 633 animals, 33 species) and teleosts (5 118 animals, 87 species), with small quantities of cephalopoda (26 animals, 4 species), bivalvia (14 animals, 1 species), gastropoda (9 animals, 1 species), crustacea (121 animals, 3 species), and mammalia (3 animals, 2 species) (Table 3).

A breakdown of the number of different chondrichthyan and teleost species caught and number of animals caught by species for each of the five experiments undertaken during 1973–76 is presented in Table 4. Catch rates are presented separately where explanatory variables were

TABLE 3. Number of animals and number of species caught by gillnet and longline during 1973–76 and 1998–2001.

Taxonomic group	No. of animals 1973–76 Longline	Gillnet	Total	1998–2001 Gillnet	Total	No. of species 1973–76 Longline	Gillnet	Total	1998–2001 Gillnet	Total
Chondrichthyes	3 093	9 104	12 197	9 436	21 633	23	27	31	22	33
Teleostei	905	3 501	4 406	712	5 118	28	70	74	35	87
Cephalopoda	8	14	22	4	26	3	4	4	2	4
Bivalvia	–	14	14	–	14	–	1	1	–	1
Gastropoda	–	–	–	9	9	–	–	–	1	1
Crustacea	–	18	18	103	121	–	2	2	3	3
Mammalia	–	–	–	3	3	–	–	–	2	2
Aves	–	–	–	–	–	–	–	–	–	–
Reptilia	–	–	–	–	–	–	–	–	–	–
Total	4 006	12 651	16 657	10 267	26 924	54	104	112	65	131

TABLE 4. Summary of results from five experiments.

Expt	Fishing gear	No. species caught Chondricthyans	Teleosts	No. animals caught Chondricthyans	Teleosts	No. species sig.[1] Chondricthyans	Teleosts
1	Gillnet	25	63	5 038	2 284	8	13
2	Gillnet	14	16	1 117	148	–	–
3	Longline	18	16	1 291	561	1	–
4	Longline	25	63	827	109	–	1
5	Longline	11	5	366	80	–	–

[1] Statistically significant

statistically significant for several species (Experiment 1) or where the data are of special interest (Experiment 3). Separate tables are also presented of catch rates for comparison between the 1973–76 and 1998–2001 sampling periods in Bass Strait, and of available data for 1973–76 in Tasmania and for 1998–2001 in South Australia. In each table, the catch rates are presented by species categorised as chondrichthyes, teleostei, cephalopoda, and other. The category "Other" includes bivalvia, gastropoda, crustacea and mammalia. Within each taxonomic category, the species are ordered from the highest to lowest on the basis of the number of animals caught. The probability values for the effects of various variables tested by 'one way analysis of variance' are presented where the condition of homogeneity of variance is met.

Experiment 1: Effect of gillnet mesh size on catch rates

Results from Experiment 1 (Table 5) indicate that the effect of gillnet mesh size on catch rate was statistically highly significant for many of the species caught. Overall the gillnets had much higher catch rates of chondrichthyans than of teleosts for all mesh sizes 3–9 inches, but the 2 inch mesh had a higher catch rate of teleosts than of chondrichthyans. There is a roughly linear relationship between the ratio of the number of chondrichthyans divided by the number of teleosts against mesh size where the ratio increases with increasing mesh size (Fig. 2).

Of the total catch of 7 356 animals across all species and mesh sizes, more than two thirds were chondrichthyans (5 038 animals, 68%) and most of the rest were teleosts (2 284 animals, 31%). Together, cephalopoda (9 animals), bivalvia (14), and crustacea (11) made up <1% of the catch. No gastropoda, mammalia, aves or reptilia were caught. There were 25 species of chondrichthyes, 62 species of teleostei plus *Monacanthidae* (covering unidentified animals in this family), 3 species of cephalopoda, 1 species of bivalvia, and 1 species of crustacea.

The highest catch rates of chondrichthyans were taken in larger mesh sizes than the highest catch rates of teleosts. The highest catch rate of chondrichthyans was in the 4 inch mesh (25%), followed by 3 inch mesh (20%), 5 inch mesh (15%), 2 inch mesh (11%), 6 inch mesh (10%), 7 inch mesh (10%), 8 inch mesh (5%), and 9 inch

TABLE 5. Experiment 1: Effect of gillnet mesh-size on number of animals caught off south-eastern Australia during 1973–76. Eight fishing gear sampling units of gillnet, each 250 m long, and of 8 mesh-sizes (2–9 inch) were set at each of 73 sites; s.e., standard error; P, probability value for an effect of mesh-size; $P \geq 0.05$, * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

Common name or effort	Scientific name	Mean (s.e.) No. of animals caught per 1 000 km-hours								Animals caught		
		2-inch	3-inch	4-inch	5-inch	6-inch	7-inch	8-inch	9-inch	Number	%	P
Fishing effort (km-hours)		113	112	109	110	114	110	110	111			
Number of fishing gear sampling units		73	73	73	73	73	73	73	73			
Chondrichthyes												
Piked spurdog	*Squalus megalops*	3 524(1363)	5 915(2368)	5 904(1959)	1 181(445)	320(140)	66(45)	62(52)	88(62)	1850	36.7	.0000***
Gummy shark	*Mustelus antarcticus*	221(102)	518(158)	1 669(398)	1 965(385)	1 390(259)	832(207)	428(97)	214(77)	850	16.9	.0000***
Port Jackson shark	*Heterodontus portusjacksoni*	125(77)	141(60)	398(154)	404(112)	641(171)	797(163)	465(122)	519(142)	367	7.3	.0035**
School shark	*Galeorhinus galeus*	427(206)	559(235)	871(455)	723(385)	187(78)	463(143)	301(81)	278(76)	352	7.0	.5207
Elephant fish	*Callorhinchus milii*	69(58)	334(165)	582(261)	996(503)	666(355)	704(481)	217(154)	245(142)	351	7.0	.4117
White-spotted spurdog	*Squalus acanthias*	233(172)	139(129)	922(491)	517(359)	432(357)	205(175)	1 62(130)	50(50)	302	6.0	.3724
Common sawshark	*Pristiophorus cirratus*	335(127)	510(169)	469(136)	774(291)	332(105)	105(39)	76(42)	–	295	5.9	.0017**
Draughtboard shark	*Cephaloscyllium laticeps*	131(117)	113(56)	161(73)	179(52)	332(104)	1 151(373)	308(94)	106(50)	294	5.8	.0000***
Southern sawshark	*Pristiophorus nudipinnis*	269(72)	574(259)	448(178)	354(148)	99(45)	32(19)	34(17)	6(6)	185	3.7	.0054**
Gulf catshark	*Asymbolus vincenti*	157(99)	182(85)	78(78)	–	–	–	–	–	49	1.0	.0434*
Rusty catshark	*Parascyllium ferrugineum*	28(21)	166(68)	87(44)	45(45)	24(24)	9(9)	–	–	39	0.8	.0103*
Southern eagle ray	*Myliobatis australis*	14(14)	–	–	7(7)	–	9(9)	19(14)	232(198)	25	0.5	.2560
Broadnose sevengill shark	*Notorynchus cepedianus*	20(14)	–	36(29)	50(32)	14(12)	52(26)	79(38)	–	24	0.5	.1851
Varied catshark	*Parascyllium variolatum*	26(18)	66(61)	5(5)	10(10)	–	–	–	–	15	0.3	.4224
Australian angel shark	*Squatina australis*	42(42)	–	–	10(10)	–	35(35)	10(10)	54(24)	13	0.3	.4346
Bronze whaler	*Carcharhinus brachyurus*	–	–	–	33(33)	–	35(27)	–	32(32)	8	0.2	.5552
Longnose skate	*Raja* sp A	–	–	–	8(8)	15(11)	–	17(12)	–	5	0.1	.2546
Southern dogfish	*Centrophorus uyato*	–	–	–	57(57)	–	–	–	–	4	0.1	
Sparsely-spotted stingaree	*Urolophus paucimaculatus*	–	–	–	9(9)	–	–	7(7)	14(14)	3	0.1	.6337
Whiskery shark	*Furgaleus macki*	–	–	–	–	–	13(13)	10(10)	–	2	0.0	.5377
Shortfin mako	*Isurus oxyrinchus*	–	–	–	–	–	–	14(14)	–	1	0.0	
Thresher shark	*Alopias vulpinus*	–	–	–	–	–	–	–	11(11)	i	0.0	
Ornate wobbegong	*Orectolobus ornatus*	–	–	–	8(8)	–	–	–	–	1	0.0	
Smooth hammerhead	*Sphyrna zygaena*	–	–	–	–	–	–	–	18(18)	1	0.0	
Melbourne skate	*Raja whitleyi*	–	9(9)	–	–	–	–	–	–	1	0.0	
Sub-total	*Chondrichthyes*	5621(1475)	9 227(2516)	11 628(2632)	7 329(1361)	4 451(770)	4 507(790)	2 211(370)	1 867(358)	5 038	100.0	.0000***
Teleostei												
Sand flathead	*Platycephalus bassensis*	7 433(4106)	2 173(488)	368(101)	94(54)	135(113)	–	–	–	770	33.7	.0029**
Yellowtail scad	*Trachurus novaezelandiae*	2 316(1774)	12(9)	9(9)	–	–	–	–	–	223	9.8	.1066
Ruddy gurnard perch	*Neosebastes scorpaenoides*	315(134)	495(146)	741(268)	39(23)	35(26)	10(10)	–	39(28)	159	7.0	.0000***
Butterfly perch	*Caesioperca lepidoptera*	1 420(749)	–	6(6)	–	–	–	–	–	151	6.6	.0008***
Silverbelly	*Parequula melbournensis*	637(419)	259(238)	6(6)	–	–	–	–	–	108	4.7	.0795
Goldspot flathead	*Neoplatycephalus aurimaculatus*	524(307)	360(191)	91(32)	26(15)	–	–	–	–	92	4.0	.0160*
Unspecified leatherjacket	*Family Monacanthidae*	15(15)	626(378)	–	25(25)	8(8)	–	–	–	64	2.8	.0100*
Long-finned pike	*Dinolestes lewini*	366(298)	11(11)	–	13(13)	–	–	–	–	50	2.2	.1723
Barracouta	*Thyrsites atun*	7(7)	69(50)	359(235)	5(5)	–	–	–	–	45	2.0	.0349*
Jackass morwong	*Nemadactylus macropterus*	–	161(83)	131(101)	62(44)	–	–	–	–	42	1.8	.0717
Senator fish	*Pictilabrus laticlavius*	464(464)	122(122)	–	–	–	–	–	–	42	1.8	.4829
Tiger flathead	*Neoplatycephalus richardsoni*	458(293)	137(80)	25(18)	41(32)	–	–	–	–	33	1.4	.0361*
Bastard trumpeter	*Latridopsis forsteri*	–	8(8)	156(148)	112(70)	–	–	11(11)	11(11)	33	1.4	.3604
Queen snapper	*Nemadactylus valenciennesi*	–	13(13)	109(76)	54(46)	36(28)	54(38)	34(29)	–	29	1.3	.4696
Southern goatfish	*Upeneichthys vlamingii*	65(29)	199(79)	11(11)	–	–	–	–	–	28	1.2	.0000***

TABLE 5. (Cont'd). Experiment 1: Effect of gillnet mesh-size on number of animals caught off south-eastern Australia during 1973–76. Eight fishing gear sampling units of gillnet, each 250 m long, and of 8 mesh-sizes (2–9 inch) were set at each of 73 sites; s.e., standard error; P, probability value for an effect of mesh-size; $P \geq 0.05$, * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

Common name or effort	Scientific name	Mean (s.e.) No. of animals caught per 1 000 km-hr								Animals caught		
		2-inch	3-inch	4-inch	5-inch	6-inch	7-inch	8-inch	9-inch	Number	%	P
Barber perch	*Caesioperca rasor*	308(221)	44(38)	–	–	–	–	–	–	27	1.2	.0762
White trevally	*Pseudocaranx dentex*	231(198)	35(17)	25(18)	9(9)	–	24(24)	–	–	27	1.2	.2938
Bearded rock cod	*Pseudophycis barbata*	59(29)	101(58)	167(108)	–	9(9)	–	–	–	25	1.1	.0579
Yank flathead	*Platycephalus speculator*	1 155(1143)	131(87)	40(31)	8(8)	–	15(15)	–	–	25	1.1	.4466
Saddled wrasse	*Notolabrus fucicola*	28(28)	61(33)	123(78)	13(13)	–	11(11)	–	–	24	1.1	.0859
Herring cale	*Odax cyanomelas*	70(63)	61(61)	7(7)	–	–	–	–	–	20	0.9	.4849
Globefish	*Diodon nicthemerus*	143(132)	–	13(13)	–	–	–	–	–	20	0.9	.3311
Red gurnard	*Chelidonichthys kumu*	41(27)	24(17)	114(62)	–	–	–	–	–	19	0.8	.0107*
Velvet leatherjacket	*Meuschenia scaber*	22(16)	213(170)	–	–	–	–	–	–	19	0.8	.1542
Long-snouted boarfish	*Pentaceropsis recurvirostris*	–	–	–	75(32)	17(12)	35(22)	42(21)	19(19)	18	0.8	.0291*
Magpie perch	*Cheilodactylus nigripes*	10(10)	8(8)	8(8)	76(38)	30(21)	45(37)	12(12)	–	17	0.7	.1836
Chinaman-leatherjacket	*Nelusetta ayraudi*	–	190(183)	9(9)	17(17)	–	–	–	–	16	0.7	.4076
Brown-spotted wrasse	*Notolabrus parilus*	–	61(61)	26(26)	5(5)	–	–	–	–	14	0.6	.5320
Brown-striped leatherjacket	*Meuschenia australis*	–	94(71)	–	10(10)	–	–	–	–	13	0.6	.1086
Butterfly gurnard	*Lepidotrigla vanessa*	–	69(37)	41(24)	–	–	–	–	–	12	0.5	.0065**
Blue-throated wrasse	*Notolabrus tetricus*	14(14)	–	10(10)	57(29)	6(6)	6(6)	–	–	12	0.5	.0207*
Blue warehou	*Seriolella brama*	11(11)	21(21)	21(15)	38(32)	–	–	–	–	11	0.5	.5099
Rough gurnard perch	*Neosebastes pandus*	24(24)	58(58)	–	–	–	–	–	–	10	0.4	.5064
Sergeant baker	*Aulopus purpurissatus*	–	27(16)	47(26)	–	–	–	–	–	8	0.4	.0071**
Redfish	*Centroberyx affinis*	–	85(85)	–	–	–	10(10)	–	–	7	0.3	.4537
Bight redfish	*Centroberyx gerrardi*	–	–	–	8(8)	–	55(55)	–	–	7	0.3	.4606
Sandpaper fish	*Paratrachichthys sp 1*	75(75)	–	–	–	–	–	–	–	6	0.3	.4301
Rosy wrasse	*Pseudolabrus psittaculus*	43(24)	–	–	–	–	–	–	–	6	0.3	
Jewfish	*Argyrosomus japonicus*	–	–	–	8(8)	–	50(41)	–	–	5	0.2	.2119
Rock ling	*Genypterus tigerinus*	11(11)	22(16)	–	10(10)	–	–	–	–	4	0.2	.3290
Silver dory	*Cyttus australis*	–	–	14(14)	–	16(11)	–	–	–	4	0.2	.3074
Eastern school whiting	*Sillago flindersi*	23(16)	5(5)	–	–	–	–	–	–	4	0.2	.0815
Marblefish	*Aplodactylus arctidens*	–	–	7(7)	19(19)	–	–	–	–	4	0.2	.4963
King George whiting	*Sillaginodes punctata*	32(22)	18(18)	–	–	–	–	–	–	3	0.1	.1881
Common bullseye	*Pempheris multiradiatus*	19(13)	10(10)	–	–	–	–	–	–	3	0.1	.1886
Toothbrush leatherjacket	*Acanthaluteres vittiger*	–	31(18)	–	–	–	–	–	–	3	0.1	
Snapper	*Pagrus auratus*	–	–	–	–	–	18(18)	–	8(8)	2	0.1	.5116
Sea sweep	*Scorpis aequipinnis*	–	–	–	16(16)	–	–	–	–	2	0.1	
Striped trumpeter	*Latris lineata*	–	–	–	–	–	8(8)	9(9)	–	2	0.1	.5394
Horse-shoe leatherjacket	*Meuschenia hippocrepis*	–	–	17(17)	–	–	–	–	–	2	0.1	
Ornate cowfish	*Aracana ornata*	11(11)	–	9(9)	–	–	–	–	–	2	0.1	.5391
John dory	*Zeus faber*	12(12)	–	–	–	–	–	–	–	1	0.0	
Harlequin fish	*Othos dentex*	–	–	–	8(8)	–	–	–	–	1	0.0	
Blue devil	*Paraplesiops meleagris*	–	17(17)	–	–	–	–	–	–	1	0.0	
Southern cardinalfish	*Vincentia conspersa*	15(15)	–	–	–	–	–	–	–	1	0.0	
Tailor	*Pomatomus saltatrix*	11(11)	–	–	–	–	–	–	–	1	0.0	
Eastern Australian salmon	*Arripis trutta*	–	–	–	15(15)	–	–	–	–	1	0.0	
Zebra fish	*Girella zebra*	–	–	14(14)	–	–	–	–	–	1	0.0	
Old wife	*Enoplosus armatus*	–	–	10(10)	–	–	–	–	–	1	0.0	
Dusky morwong	*Dactylophora nigricans*	–	–	10(10)	–	–	–	–	–	1	0.0	
Western blue groper	*Achoerodus gouldii*	–	–	–	17(17)	–	–	–	–	1	0.0	

TABLE 5. (Cont'd). Experiment 1: Effect of gillnet mesh-size on number of animals caught off south-eastern Australia during 1973–76. Eight fishing gear sampling units of gillnet, each 250 m long, and of 8 mesh-sizes (2–9 inch) were set at each of 73 sites; s.e., standard error; *P*, probability value for an effect of mesh-size; $P \geq 0.05$, * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

		Mean (s.e.) No. of animals caught per 1 000 km-hr								Animals caught		
Common name or effort	Scientific name	2-inch	3-inch	4-inch	5-inch	6-inch	7-inch	8-inch	9-inch	Number	%	*P*
Speckled stargazer	*Kathetostoma canaster*	9(9)	–	–	–	–	–	–	–	1	0.0	
Six-spined leatherjacket	*Meuschenia freycineti*	7(7)	–	–	–	–	–	–	–	1	0.0	
Sub-total	*Teleostei*	16 401(5423)	6 031(1020)	2 746(585)	881(185)	293(123)	340(105)	108(38)	77(36)	2284	100.0	.0000***
Cephalopoda												
Gould's squid	*Nototodarus gouldi*	15(15)	35(18)	–	–	–	–	–	–	6	66.7	.0254*
Giant cuttlefish	*Sepia apama*	24(18)	–	–	–	–	–	–	–	2	22.2	
Octopus	*Octopus pallidus*	–	–	–	10(10)	–	–	–	–	1	11.1	
Sub-total	*Cephalopoda*	40(24)	35(18)	–	10(10)	–	–	–	–	9	100.0	.0257*
Other												
Commercial scallop	*Pecten fumatus*	–	–	–	–	136(136)	–	–	–	14		.4301
Swollen spider crab	*Leptomithrax gaimardii*	–	–	–	–	–	–	54(54)	129(129)	11		.5078

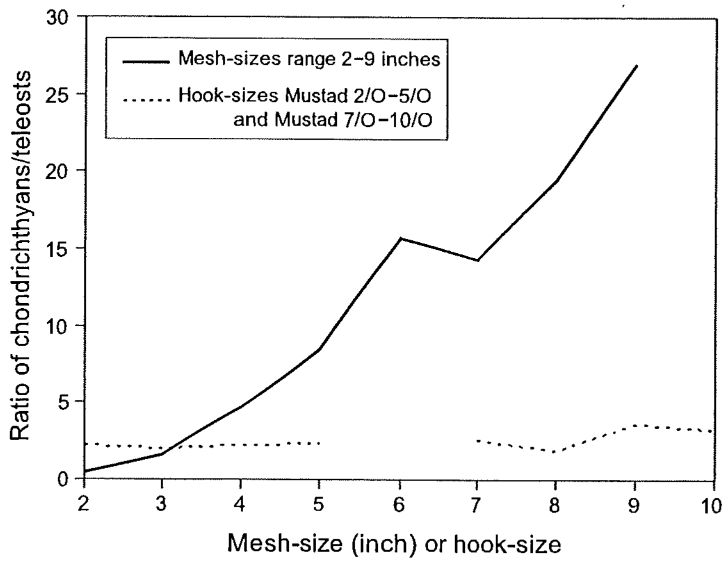


Fig. 2. Ratio of number of animals of chondrichthyes/number of animals of teleostei against gillnet mesh-size or hook-size. Mesh-sizes range 2–9 inches and hook-sizes Mustad 2/O–5/O and Mustad 7/O–10/O.

mesh (4%). The highest catch rate of teleosts was in the 2 inch mesh (54%), followed by 3 inch mesh (27%), 4 inch mesh (12%), 5 inch mesh (2%), 6 inch mesh (1%), 7 inch mesh (1%), 8 inch mesh (1%), and 9 inch mesh (0%).

Over all eight mesh sizes observed, the catch rates of chondrichthyan species varied widely, with two species, *Squalus megalops* (37%) and *Mustelus antarcticus* (17%), accounting for more than half these animals. Seven other species had similar catch rates and accounted for most of the rest of the animals captured. The catch rates of teleost fishes also varied widely between species, where *Platycephalus bassensis* (34%), constituted more than one-third of these animals. This species along with nine other species provided three-fourths of the teleost animals. The remaining 25% of animals caught comprised 52 species and unidentified fishes of the family *Monocanthidae* (Table 5).

Most chondrichthyan and teleost species exhibit a pattern of a highest catch (mode) for a particular mesh size, the catch falling progressively with both decreasing and increasing mesh size. The modal catch corresponded to 3 inch mesh for *Pristiophorus nudipinnis*, *Asymbolis vincenti*, and *Parascyllium ferrugineum*; 4 inch mesh for *Squalus megalops*, *Galeorhinus galeus*, and *Squalus acanthias*; 5 inch mesh for *Mustelus antarcticus*, *Callorhinchus milii*, and *Pristiophorus cirratus*; 7 inch mesh for *Heterodontus portusjacksoni*, and *Cephaloscyllium laticeps*; and 9 inch mesh for *Myliobatis australis*. Most of the *Platycephalus bassensis* catch, expressed as a percentage of the total number of teleost fishes caught, were taken by the 2 inch (21%), 3 inch (10%) and 4 inch (2%) mesh. Other teleost species taken predominantly by the 2 inch mesh size include *Trachurus novaezelandiae* (10%), *Caesioperca lepidoptera* (7%), *Parequula melbournensis* (3%), *Neoplatycephalus aurimaculatus* (2%), and *Dinolestes lewini* (2%). *Nemadactylus macropterus* was mainly taken by 3 inch mesh size (1%) and *Neosebastes scorpaenoides* by 4 inch mesh size (3%). The 6 and 7 inch meshes, used commercially in the fishery, each took 10% of the chondricthyan animals and 1% of the teleost animals (Table 5).

Experiment 2: Effect of gillnet hanging ratio on catch rates

For Experiment 2, there were sufficient data to test 9 chonchrichthyan species and 11 teleosts for the effect of gillnet hanging ratio for the 6 inch and 7 inch mesh sizes on catch rate. The effect of hanging ratio was statistically not significant for any of these species.

Experiment 3: Effect of hook size on catch rates

Results from Experiment 3 indicate that the effect of hook size for the eight short-shank Mustad 2/O, 3/O, 4/O, 5/O, 7/O, 8/O, 9/O, and 10/O hooks with a 7.5 m hook space on catch rate was statistically significant for only one of the 18 chondrichthyan species (*Heterodontus* *portusjacksoni*) and none of the 16 teleost species caught (Table 6). The results were pooled over all hook sizes because of the lack of statistical significance of hook size. Similarly, the effect of hook size was not statistically significant for either the 18 chondrichthyan species pooled or the 16 teleost species pooled. Of the total catch of 1 856 animals, across all species and hook sizes, more than two-thirds were chondrichthyans (1 291 animals, 70%) and most of the rest were teleosts (561 animals, 30%). There was a small catch of three species of cephalopoda (4 animals, <1%), and zero catches of animals of bivalvia, gastropoda, crustacea, mammalia, aves, and reptilia. For the chondrichthyans, the catch rates were similar for the three top species: *Squalus megalops* (27%), *Mustelus antarcticus* (24%), and *Cephaloscyllium laticeps* (22%). For the teleosts, the catch was dominated by two species: *Platycephalus bassensis* (47%) and *Neosebastes scorpaenoides* (37%) (Table 6).

Experiments 4 and 5: Effects of hook size, shank length and hook space on catch rates

As expected, the catch rates for the top four or five chondrichthyan species and top two teleost species caught during Experiments 4 and 5 were similar to those caught during Experiment 3. Across these two experiments, the effects of hook size, shank length, and hook space on catch rates were not statististically significant, with one exception. Shank length of hook for the teleost *Neosebastes scorpaenoides* was statistically significant ($P < 0.01$) in Experiment 4; a higher catch rate was obtained with short shank hooks than long shank hooks.

Effects of sampling period and commercial fishing gears on catch rates

Catch rates for commercial fishing gears were available from fishing aboard research vessels during 1973–76 and from commercial shark fishing vessels during 1998–2001. In Bass Strait, direct comparisons in catch rate between 1973–76 and 1998–2001 can only be made for gillnet 6 inch mesh (Table 7a). These data indicate a statistically significant decrease in the catch rate for all chondrichthyan fishes, and no significant difference in the catch rate for all teleost fishes. Among the chondrichthyan species, *Cephaloscyllium laticeps* exhibits a statistically significant decrease of 54% and *Galeorhinus galeus* exhibits a statistically highly significant decrease of 87% between the two periods. One species, *Notorynchus cepedianus*, taken in low numbers during 1973–76 exhibits a statistically highly significant increase in catch. In addition, 10 chondrichthyan species and 17 teleost species exhibit zero catch rates during 1973–76 and low catch rates during 1998–2001, whereas, conversely, 3 chondrichthyan species and 5 teleost species had low catch rates during 1973–76 and zero catch rates during 1998–2001. These differences are interpreted as an artifact of the data where the probability of catching low numbers of animals of species that are either rare or of low catchability in the depth range 0–79 m was higher during 1998–01 than during 1973–76. This is because the total fishing effort was 12.2 times higher during 1998–01 than during 1973–76. For these reasons, the effect of sampling period was not tested for any species where the catch rate was zero during either 1973–76 or 1998–2001 (Table 7a).

In Tasmania, there were too few data to properly characterise catch composition and catch rates. The data suggest that catch rates of *Squalus acanthias* in Tasmania were higher than in Bass Strait and South Australia (Table 7b).

In South Australia, the catch rate by gillnet was statistically significantly higher in 6 inch mesh than in 6½ inch mesh size for all chondrichthyans combined, but the effect of mesh size was not significant for teleosts. Most of the higher catch rate by the 6 inch mesh size for *Mustelus antarcticus* and *Notorynchus cepedianus*. As in Bass Strait and Tasmania, catch rates of teleosts was low compared with chondrichthyan species in South Australia (Table 7c).

There were some minor differences in catch rates between Bass Strait, Tasmania, and South Australia. Among the chondrichthyan species, the data suggest that the catch rates of *Cephaloscyllium laticeps*, *Pristiophorus cirratus*, *P. nudipinnis*, and *Callorhinchus milii* were higher in Bass Strait than in South Australia. Several minor species, *Myliobatis australis*, *Carcharhinus brachyurus*, and *Alopias vulpinis*, were more common in South Australia than in Bass Strait. Among the teleosts, several species appeared in the catch off South Australia that were absent or provided very low catch rates in Bass Strait and Tasmania. These species include *Centroberyx gerrardi*, *Kyphosus gibsoni*, and *Nemadactylus valenciennesi*. One species, *Platycephalus bassensis*, appears to be less common in South Australia than in Bass Strait and Tasmania.

Breakdown of total catch as retained and discarded, and live and dead

Percentages of the commercial catch taken as retained and discarded animals, broken down as live and dead, for 1998–2001 are presented for Bass Strait (8 198 animals) and South Australia (2 069 animals) separately. The catches were taken by 6 inch mesh in Bass Strait and a combination of 6 inch and 6½ inch mesh in South Australia. The catch rate of chondrichthyans in Bass

TABLE 6. Experiment 3: Effect of hook-size on the number of animals caught off south-eastern Australia during 1973–76. Eight fishing gear sampling units of 50 hooks for each of 8 Mustad hook-sizes, with short-shank and 7.5-m hook-space, were set at each of 39 sites; s.e., standard error; P, probability value for an effect of hook-size; * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

Common name or effort	Scientific name	Mean (s.e.) number of animals caught per 100 000 hook-lifts	Animals caught Number	%	P
Fishing effort (100 hook-lifts)		156			
Number of fishing gear sampling units		312			
Chondrichthyes					
Piked spurdog	*Squalus megalops*	2 205 (373)	344	26.6	.3951
Gummy shark	*Mustelus antarcticus*	1 974 (196)	308	23.9	.7553
Draughtboard shark	*Cephaloscyllium laticeps*	1 859 (195)	290	22.5	.9746
School shark	*Galeorhinus galeus*	923 (131)	144	11.2	.5478
Gulf catshark	*Asymbolus vincenti*	314 (65)	49	3.8	.1319
Rusty catshark	*Parascyllium ferrugineum*	192 (69)	30	2.3	.5755
Port Jackson shark	*Heterodontus portusjacksoni*	147 (34)	23	1.8	.0486*
Melbourne skate	*Raja whitleyi*	135 (49)	21	1.6	.2843
Southern fiddler ray	*Trygonorrhina fasciata*	128 (49)	20	1.5	.9245
Common sawshark	*Pristiophorus cirratus*	122 (30)	19	1.5	.8066
White-spotted spurdog	*Squalus acanthias*	90 (39)	14	1.1	.6211
Broadnose sevengill shark	*Notorynchus cepedianus*	58 (31)	9	0.7	.5479
Longnose skate	*Raja* sp *A*	51 (22)	8	0.6	.2234
Elephant fish	*Callorhinchus milii*	32 (17)	5	0.4	.7109
Southern sawshark	*Pristiophorus nudipinnis*	19 (19)	3	0.2	.4312
Shortfin mako	*Isurus oxyrinchus*	13 (9)	2	0.2	.5406
Smooth stingray	*Dasyatis brevicaudata*	6 (6)	1	0.1	
Sandyback stingaree	*Urolophus bucculentus*	6 (6)	1	0.1	
Sub-total	*Chondrichthyes*	8276 (500)	1291	100.0	.2268
Teleostei					
Sand flathead	*Platycephalus bassensis*	1 705 (212)	266	47.4	.1282
Ruddy gurnard perch	*Neosebastes scorpaenoides*	1 327 (220)	207	36.9	.8344
Bearded rock cod	*Pseudophycis barbata*	154 (39)	24	4.3	.8460
Jackass morwong	*Nemadactylus macropterus*	71 (33)	11	2.0	.5612
Red rock cod	*Scorpaena papillosa*	64 (33)	10	1.8	.7442
Red gurnard	*Chelidonichthys kumu*	64 (31)	10	1.8	.1398
Tiger flathead	*Neoplatycephalus richardsoni*	58 (25)	9	1.6	.7380
Yank flathead	*Platycephalus speculator*	32 (17)	5	0.9	.2310
Blue-throated wrasse	*Notolabrus tetricus*	32 (17)	5	0.9	.7444
Silverbelly	*Parequula melbournensis*	26 (20)	4	0.7	.1033
Goldspot flathead	*Neoplatycephalus aurimaculatus*	19 (11)	3	0.5	.5916
Sergeant baker	*Aulopus purpurissatus*	13 (9)	2	0.4	.5407
Butterfly gurnard	*Lepidotrigla vanessa*	13 (9)	2	0.4	
Senator fish	*Pictilabrus laticlavius*	6 (6)	1	0.2	
Rosy wrasse	*Pseudolabrus psittaculus*	6 (6)	1	0.2	
Velvet leatherjacket	*Meuschenia scaber*	6 (6)	1	0.2	
Sub-total	*Teleostei*	3 596 (340)	561	100.0	.5775
Cephalopoda		2 205 (373)			
Giant cuttlefish	*Sepia apama*	13 (9)	2	50.0	.5399
Gould's squid	*Nototodarus gouldi*	6 (6)	1	25.0	
Octopus	*Octopus pallidus*	6 (6)	1	25.0	
Sub-total	*Cephalopoda*	26 (13)	4	100.0	.7534

TABLE 7A. Comparison of number of animals caught by various fishing gears in Bass Strait between 1973–76 and 1998–2001. s.e., standard error; *P*, probability value for a difference in catch between 1973–76 and 1998–2001 for 6-inch mesh-size; $P \geq 0.05$, * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

		Mean (s.e.) number of animals caught per 100,000 hook-lifts or 1000 km-hr					
		1973–76			1998–2001	Number	
Common name or effort	Scientific name	Hooks	7-inch	6-inch	6-inch	caught	*P*
Fishing effort (100 hook-lifts or gillnet km-hr)		126	220	271	3 317		
Number of fishing gear sampling units		148	139	172	91		
Chondrichthyes							
Gummy shark	*Mustelus antarcticus*	4 055 (430)	1 105 (153)	1457 (197)	1 220 (118)	4 797	.4067
Draughtboard shark	*Cephaloscyllium laticeps*	1 141 (175)	1 063 (217)	660 (112)	305 (53)	1609	.0265*
Common sawshark	*Pristiophorus cirratus*	33 (13)	171 (34)	381 (76)	292 (35)	1145	.4112
Elephant fish	*Callorhinchus milii*	20 (14)	515 (261)	340 (154)	229 (57)	910	.6068
Port Jackson shark	*Heterodontus portusjacksoni*	255 (62)	684 (154	269 (63)	160 (41)	794	.2354
School shark	*Galeorhinus galeus*	2 041 (360)	360 (78)	246 (47)	32 (8)	425	.0012**
Piked spurdog	*Squalus megalops*	314 (119)	46 (26)	115 (53)	71 (26)	326	.5576
Southern sawshark	*Pristiophorus nudipinnis*	247 (74)	57 (21)	151 (42)	68 (11)	299	.1561
Broadnose sevengill shark	*Notorynchus cepedianus*	91 (38)	15 (11)	1 (1)	17 (6)	71	.0009***
White-spotted spurdog	*Squalus acanthias*	313 (95)	61 (52)	46 (26)	–	65	
Southern eagle ray	*Myliobatis australis*	14 (14)	11 (8)	7 (7)	11 (3)	45	.6839
Sparsely-spotted stingaree	*Urolophus paucimaculatus*	–	–	7 (5)	12 (4)	43	.4962
Australian angel shark	*Squatina australis*	–	23 (19)	4 (4)	8 (2)	31	.5289
Melbourne skate	*Raja whitleyi*	64 (33)	6 (6)	–	>0 (>0)	9	
Gulf catshark	*Asymbolus vincenti*	18(8)	–	–	–	5	
Western shovelnose ray	*Aptychotrema vincentiana*	–	–	–	1 (1)	5	
Shortfin mako	*Isurus oxyrinchus*	–	–	–	1 (1)	4	
Rusty catshark	*Parascyllium ferrugineum*	14 (14)	5 (5)	10 (10)	–	4	
Varied catshark	*Parascyllium variolatum*	7(7)	–	–	–	3	
Longnose skate	*Raja sp A*	3(3)	–	–	>0 (>0)	2	
Smooth stingray	*Dasyatis brevicaudata*	7(7)	–	–	–	2	
Thresher shark	*Alopias vulpinus*	–	–	–	>0 (>0)	1	
Bronze whaler	*Carcharhinus brachyurus*	–	–	4 (4)	–	1	
Thornback skate	*Raja lempriert*	3(3)	–	–	–	1	
Skates (unspecified)	*Raja* spp	–	–	–	>0 (>0)	1	
Sub-total	*Chondrichthyes*	8 640 (630)	4121 (467)	3 699 (349)	2 429 (144)	10 598	.0104*
Teleostei							
Sand flathead	*Platycephalus bassensis*	642 (142)	40 (24)	86(70)	3 (1)	126	.3907
Long-snouted boarfish	*Pentaceropsis recurvirostris*	2 (2)	42 (17)	62(19)	17 (3)	73	.0868
Blue warehou	*Seriolella brama*	–	30 (30)	–	15 (5)	60	
Jack mackerel	*Trachurus declivis*	–	–	–	16 (12)	53	
White trevally	*Pseudocaranx dentex*	–	12 (12)	–	11 (8)	46	
Latchet	*Pterygotrigla polyommata*	–	–	–	7 (5)	23	
Blue-throated wrasse	*Notolabrus tetricus*	86 (38)	8 (6)	8 (5)	4 (2)	22	.4774
Ruddy gurnard perch	*Neosebastes scorpaenoides*	186 (58)	4 (4)	15 (11)	–	20	
Magpie perch	*Cheilodactylus nigripes*	–	5 (5)	8 (6)	4 (2)	17	.6495
Goldspot flathead	*Neoplatycephalus aurimaculatus*	–	–	47 (47)	–	14	

TABLE 7A. (Cont'd). Comparison of number of animals caught by various fishing gears in Bass Strait between 1973–76 and 1998–2001. s.e., standard error; *P*, probability value for a difference in catch between 1973–76 and 1998–2001 for 6-inch mesh-size; $P \geq 0.05$, * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

Common name or effort	Scientific name	Hooks	Mean (s.e.) number of animals caught per 100,000 hook-lifts or 1000 km-hr 1973–76 7-inch	1973–76 6-inch	1998–2001 6-inch	Number caught	P
Queen snapper	*Nemadactylus valenciennesi*	–	19 (19)	17 (17)	1 (1)	14	.4997
Bight redfish	*Centroberyx gerrardi*	– –	–	4 (2)	12		
Red gurnard	*Chelidonichthys kumu*	2 (2)	–	3 (3)	2 (1)	10	.8514
Bearded rock cod	*Pseudophycis barbata*	12 (10)	–	11 (8)	1 (1)	12	.3704
Barracouta	*Thyrsites atun*	–	–	–	3 (2)	8	
Deepsea trevalla	*Hyperoglyphe antarctica*	–	–	–	2 (2)	6	
Swallow-tail	*Centroberyx lineatus*	–	–	–	1 (1)	5	
Sergeant baker	*Aulopus purpurissatus*	3 (3)	–	–	1 (1)	4	
Silver dory	*Cyttus australis*	–	–	13 (7)	–	4	
Giant boarfish	*Paristiopterus labiosus*	–	–	–	2 (1)	4	
Knifejaw	*Oplegnathus woodwardi*	–	–	–	1 (1)	4	
Pink ling	*Genypterus blacodes*	–	–	–	1 (1)	3	
Tiger flathead	*Neoplatycephalus richardsoni*	3 (3)	–	–	1 (>0)	3	
Snapper	*Family Pagrus auratus*	14 (14)	–	4 (4)	>0 (>0)	3	.5025
Jackass morwong	*Family Nemadactylus macropterus*	–	19 (11)	–	1 (1)	6	
Stargazer	*Family Uranoscopidae*	2 (2)	–	–	1 (>0)	3	
Leatherjacket	*Family Monacanthidae*	–	–	3 (3)	1 (1)	3	
Bastard trumpeter	*Latridopsis forsteri*	–	–	10 (7)	–	2	
Greenback flounder	*Rhombosolea tapirina*	–	–	–	1 (>0)	2	
Red cod	*Pseudophycis bachus*	2 (2)	–	–	–	1	
Redfish	*Centroberyx affinis*	–	5 (5)	–	–	1	
Tailor	*Pomatomus saltatrix*	–	4 (4)	–	–	1	
Marblefish	*Aplodactylus arctidens*	–	–	3 (3)	–	1	
Western blue groper	*Achoerodus gouldii*	–	–	–	>0 (>0)	1	
Saddled wrasse	*Notolabrus fucicola*	–	6 (6)	–	–	1	
Common stinkfish	*Synchiropus calauropomus*	–	–	4 (4)	–	1	
Sub-total	*Teleostei*	955 (167)	195 (49)	294 (130)	102 (18)	569	.2827
Cephalopoda							
Octopus	*Octopus pallidus*	–	–	–	1 (1)	3	
Giant cuttlefish	*Sepia apama*	17 (14)	–	–	–	2	
Gould's squid	*Nototodarus gouldi*	–	–	–	>0 (>0)	1	
Sub-total	*Cephalopoda*	17 (14)	–	–	1 (1)	6	
Other							
Swollen spider crab	*Leptomithrax gaimardii*	–	–	–	24 (13)	83	
Southern rock lobster	*Jasus edwardsii*	–	–	–	1 (1)	2	
False bailer shell	*Livonia mammilla*	–	–	–	3 (1)	9	
Australian fur seal	*Arctocephalus pusillus dorifer*	–	–	–	1 (>0)	2	

TABLE 7B. Comparison of number of animals caught by various fishing gears in Tasmania during 1973–76. (s.e. is standard error).

Common name or effort	Scientific name	Mean (s.e.) number of animals caught per 100 000 hook-lifts or 1000 km-hr Hooks	7-inch	6-inch	Number caught
Fishing effort (100 hook-lifts or gillnet km-hr)		8	35	36	
Number of fishing gear sampling units		4	23	23	
Chondrichthyes					
Gummy shark	*Mustelus antarcticus*	2 000 (736)	846 (349)	1 962 (486)	117
White-spotted spurdog	*Squalus acanthias*	125 (125)	689 (556)	1 288 (1 124)	78
Elephant fish	*Callorhinchus milii*	–	480 (224)	911 (332)	50
Piked spurdog	*Squalus megalops*	750 (250)	19 (19)	759 (280)	36
Draughtboard shark	*Cephaloscyllium laticeps*	375 (125)	220 (92)	214 (122)	19
School shark	*Galeorhinus galeus*	1 000 (0)	59 (44)	18 (18)	11
Southern sawshark	*Pristiophorus nudipinnis*	–	79 (47)	197 (89)	10
Port Jackson shark	*Heterodontus portusjacksoni*	–	135 (80)	83 (83)	6
Common sawshark	*Pristiophorus cirratus*	–	–	120 (56)	4
Broadnose sevengill shark	*Notorynchus cepedianus*	250 (250)	–	37 (37)	3
Longnose skate	*Raja sp A*	–	–	48 (34)	2
Sub-total	*Chondrichthyes*	4 500 (1021)	2 527 (716)	5 637 (1534)	336
Teleostei					
Jackass morwong	*Nemadactylus macropterus*	–	–	107 (76)	5
Striped trumpeter	*Latris lineata*	–	73 (40)	22 (22)	4
Sand flathead	*Platycephalus bassensis*	125 (125)	–	37 (37)	2
Bearded rock cod	*Pseudophycis barbata*	125 (125)	–	–	1
Red rock cod	*Scorpaena papillosa*	125 (125)	–	–	1
Tiger flathead	*Neoplatycephalus richardsoni*	125 (125)	–	–	1
Sub-total	*Teleostei*	500 (204)	73 (40)	166 (100)	14
Other					
Commercial scallop	*Pecten fumatus*	–	–	432 (432)	14

Strait was ~2.5 times higher than that in South Australia, whereas the catch rate of teleosts in Bass Strait was about half that in South Australia (Tables 8a, b).

Chondrichthyan fishes provided a higher proportion of the commercial catch in Bass Strait (95%) than in South Australia (82%), whereas teleost fishes provided a higher proportion of the catch in South Australia (18%) than in Bass Strait (4%). In Bass Strait, of the chondrichthyan fishes (7 761 animals), 74% (38% live and 36% dead) were retained and 26% (24% live and 2% dead) were discarded, and of the teleost fishes (337 animals), 54% were retained (40% live and 14% dead) and 46% were discarded (18% live and 28% dead). In South Australia, of the chondrichthyan fishes (1 675 animals), 72% (42% live and 30% dead) were retained and 28% (25% live and 3% dead) were discarded, and, of the teleost fishes (375 animals), 91% were retained (91% live and 0% dead) and 9% were discarded (7% live and 2% dead).

In Bass Strait, 48% the catch of chondrichthyan animals was the target species *Mustelus antarcticus*, 28% comprised by-product species (*Pristiophorus cirratus*, *Callorhinchus milii*, *P. nudipinnis*, *Galeorhinus galeus*, and *Notorynchus cepedianus*), and 24% comprised 10 by-catch species. The 3 principal chondrichthyan by-catch species, *Cephaloscyllium laticeps* (13%), *Heterodontus portusjacksoni* (7%), and *Squalus megalops* (3%), were discarded live, except for 6% of *Squalus megalops*, which was discarded dead. In South Australia, 55% of the catch of chondrichthyan fishes was *Mustelus antarcticus*, 19% comprised by-product species (*Pristiophorus cirratus*, *Callorhinchus milii*, *P. nudipinnis*, *Galeorhinus galeus*, *Sphyrna zygaena*, *Notorynchus cepedianus*, and *Furgaleus macki*), and 26% comprised 9 by-catch species. The three most caught by-catch species, *Heterodontus portusjacksoni* (15%), *Squalus megalops* (4%), and *Myliobatis australis* (3%), were discarded live, except for 9% of *Myliobatis australis* discarded dead.

In Bass Strait, none of the 26 teleost species caught provide high catches; 54% of the animals were retained. Most of the catch of the top 4 species (*Seriolella brama*, *Pentaceropsis recurvirostris*, *Trachurus declivis*, and

TABLE 7C. Comparison of number of animals caught by various fishing gears in South Australia during 1998–2001. s.e., standard error; *P*, probability value for a difference in catch between 6 and 6½-inch mesh-size during 1998–2001; $P \geq 0.05$, *$P<0.05$, ** $P<0.01$, *** $P<0.001$.

Common name or effort	Scientific name	Mean (s.e.) number of animals caught per 100 000 hook-lifts or 1 000 km-hr: 6-inch	6½-inch	No. caught	*P*
Fishing effort (100 hook-lifts or gillnet km-hr)		531	1 335		
Number of fishing gear sampling units		14	48		
Chondrichthyes					
Gummy shark	*Mustelus antarcticus*	1 150 (202)	253 (44)	939	.0000***
Port Jackson shark	*Heterodontus portusjacksoni*	61 (15)	141 (53)	315	.4232
School shark	*Galeorhinus galeus*	–	94 (50)	139	
Smooth hammerhead	*Sphyrna zygaena*	2 (2)	75 (30)	77	.2011
Piked spurdog	*Squalus megalops*	4 (3)	62 (37)	71	.3962
Southern eagle ray	*Myliobatis australis*	62 (19)	20 (7)	53	.0145*
Bronze whaler	*Carcharhinus brachyurus*	2 (2)	45 (19)	45	.2152
Common sawshark	*Pristiophorus cirratus*	2 (2)	40 (13)	43	.1190
Southern sawshark	*Pristiophorus nudipinnis*	18 (7)	14 (7)	29	.7411
Broadnose sevengill shark	*Notorynchus cepedianus*	37 (13)	2 (1)	27	.0000***
Elephant fish	*Callorhinchus milii*	16 (5)	9 (4)	23	.3896
Thresher shark	*Alopias vulpinus*	25 (13)	10 (7)	19	.2930
Australian angel shark	*Squatina australis*	9 (4)	16 (10)	19	.7146
Spotted wobbegong	*Orectolobus maculatus*	–	4 (2)	4	
Whiskery shark	*Furgaleus macki*	–	1 (1)	2	
Skates (unspecified)	*Raja spp*	–	2 (1)	2	
Sparsely-spotted stingaree	*Urolophus paucimaculatus*	3 (3)	–	2	
White shark	*Carcharodon carcharias*	–	–	1	
Draughtboard shark	*Cephaloscyllium laticeps*	–	–	1	
White-spotted spurdog	*Squalus acanthias*	2 (2)	–	1	
Western shovelnose ray	*Aptychotrema vincentiana*	–	1 (1)	1	
Sub-total	*Chondrichthyes*	1 394 (208)	788 (110)	1 813	.0116*
Teleostei					
Bight redfish	*Centroberyx gerrardi*	10(10)	43 (20)	64	.3789
Southern drummer	*Kyphosus gibsoni*	–	36 (26)	62	.4496
Long-snouted boarfish	*Pentaceropsis recurvirostris*	36(13)	29 (11)	61	.7483
Queen snapper	*Nemadactylus valenciennesi*	12(8)	31 (14)	48	.4888
Snapper	*Pagrus auratus*	42(32)	12 (7)	36	.1772
Dusky morwong	*Dactylophora nigricans*	–	17 (9)	24	
Western blue groper	*Achoerodus gouldii*	6(4)	14 (7)	21	.5701
Red gurnard	*Chelidonichthys kumu*	33 (10)	–	18	
Jewfish	*Argyrosomus japonicus*	10 (7)	3 (2)	14	.1943
Magpie perch	*Cheilodactylus nigripes*	8 (7)	5 (3)	13	.5919
Yellow-spotted boarfish	*Paristiopterus gallipavo*	–	3 (2)	6	
Leatherjacket	*Family Monacanthidae*	–	6 (4)	6	
Latchet	*Pterygotrigla polyommata*	–	4 (2)	5	
Sand flathead	*Platycephalus bassensis*	8 (3)	1 (1)	5	.0378*
Tiger flathead	*Neoplatycephalus richardsoni*	5 (4)	–	3	
Sergeant baker	*Aulopus purpurissatus*	–	1 (1)	2	
Blue-throated wrasse	*Notolabrus tetricus*	4 (4)	–	2	
Pink ling	*Genypterus blacodes*	2 (2)	–	1	
Mirror dory	*Zenopsis nebulosus*	–	>0 (>0)	1	
Ruddy gurnard perch	*Neosebastes scorpaenoides*	–	–	1	
Jack mackerel	*Trachurus declivis*	–	1 (1)	1	
Samsonfish	*Seriola hippos*	–	1 (1)	1	

TABLE 7C. (Cont'd). Comparison of number of animals caught by various fishing gears in South Australia during 1998–2001. s.e., standard error; *P*, probability value for a difference in catch between 6 and 6½-inch mesh-size during 1998–2001; $P \geq 0.05$, * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

Common name or effort	Scientific name	Mean (s.e.) number of animals caught per 100 000 hook-lifts or 1 000 km-hr		No. caught	*P*
		6-inch	6½-inch		
Bumpnose trevally	*Carangoides hedlandensis*	–	2 (2)	1	
Sweep	*Scorpis lineolatus*	–	1 (1)	1	
Old wife	*Enoplosus armatus*	–	1 (1)	1	
Wrasse	*Labridae* spp	–	1 (1)	1	
Greenback flounder	*Rhombosolea tapirina*	2 (2)	–	1	
Toadfish	*Tetraodon erythrotaenia*	–	1 (1)	1	
Sub-total	*Teleostei*	179 (58)	212 (67)	401	.7952
Other					
Swollen spider crab	*Leptomithrax gaimardii*	7 (7)	14 (11)	13	.7404
Southern rock lobster	*Jasus edwardsii*	–	3 (2)	4	
Southern bay lobster	*Ibacus peronii*	2 (2)	–	1	
Common dolphin	*Delphinus delphis*	2 (2)	–	1	

Pseudocaranx dentex), together providing 61% of the catch of teleost fishes, were retained, except for *T. declivis* which was discarded (68% live and 32% dead). In South Australia, most of the catch of 27 teleost species were retained (91%). The top 4 species (*Kyphosus gibsoni, Centroberyx gerrardi, Pentaceropsis recurvirostris,* and *Nemadactylus valenciennesi*) provided 58% of the catch.

Three wildlife interactions occurred during 1998–2001 as part of the present study. Two Australian fur seals (*Arctocephalus pusillus dorfer*) were discarded dead in Bass Strait and one common dolphin (*Delphinus delphis*) was discarded dead in South Australia.

Discussion

From the mid-1920s when the fishery began until the early-1970s, *Galeorhinus galeus* was the principal target species taken by baited hooks on longlines. Since the early-1970s, most of the catch was taken by gillnets and targeting switched early and rapidly from *G. galeus* to *Mustelus antarcticus* in Bass Strait. However, in South Australia and Tasmania, as the abundance of *G. galeus* continually declined, the species switch was more gradual (Walker, 1999). Since 2001, a total allowable catch applies to each species. Today, most fishing effort in the fishery targets *M. antarcticus*, which is the more biologically productive species (Walker, 1998; Pribac *et al.*, 2004); the earlier practice of targeting *Galeorhinus galeus* has ceased almost completely. Common sawshark (*Pristiophorus cirratus*), southern sawshark (*P. nudipinnis*), elephant fish (*Callorhinchus milii*), and broadnose sevengill shark (*Notorynchus cepedianus*) are taken as by-product, although not all fishers retained these species earlier in the history of the fishery.

Of the total catch of *M. antarcticus* produced from the fishery during 2000 (1 651 tons, carcass weight), 91% was taken by demersal monofilament gillnet and 9% was taken by demersal longline (Walker *et al.*, 2003). The fishing effort was distributed in Bass Strait (55% of gillnet effort, 30% of longline effort), South Australia (40% of gillnet effort, 64% of longline effort), and Tasmania (5% of gillnet effort, 6% of longline effort). Most of the gillnet effort deployed in Bass Strait and Tasmania was 6 inch mesh size, whereas most deployed in South Australia was 6½ inch mesh size. Baited Mustad 11/O long-shank hooks were mostly used on the longlines.

Ten important conclusions are drawn from the present study about the catch rates of gillnets and longlines deployed in the fishery on the continental shelf in the depth range 9–130 m:

1. Both gillnets and longlines are much more effective at catching chondrichthyan species than teleost species, and catches of species of cephalopoda, bivalvia, gastropoda, mammalia, aves and reptilia are negligible.

2. The effect of gillnet mesh size on catch rates is strong, whereas the effects of gillnet hanging ratio, hook size, hook shank length, and hook space are weak.

TABLE 8A. Breakdown of total catch by gill-net 6-inch mesh size as retained, discarded, live, and dead animals for each species in Bass Strait during 1998–2001. Catch-per-unit effort (CPUE) is measured as number of animals per 1000 km-hr.

			Total catch (%)								
			Retained		Discarded		Total catch (%)		Total catch (%)		No.
Common name or effort	Scientific name	CPUE	Live	Dead	Live	Dead	Live	Dead	Retained	Discarded	caught
Fishing effort (km-hr)		3 317									
Chondrichthyes											
Gummy shark	*Mustelus antarcticus*	1 114	40	59	–	1	40	60	99	1	3 697
Draughtboard shark	*Cephaloscyllium laticeps*	312	–	–	100	–	100	–	–	100	1 034
Common sawshark	*Pristiophorus cirratus*	304	77	22	–	1	77	23	99	1	1 008
Elephant fish	*Callorhinchus milii*	223	70	28	1	1	71	29	98	2	741
Port Jackson shark	*Heterodontus portusjacksoni*	157	–	–	100	–	100	–	–	100	521
Piked spurdog	*Squalus megalops*	77	–	–	94	6	94	6	–	100	254
Southern sawshark	*Pristiophorus nudipinnis*	67	78	20	–	2	78	22	98	2	223
School shark	*Galeorhinus galeus*	32	29	68	1	2	30	70	97	3	105
Broadnose sevengill shark	*Notorynchus cepedianus*	18	17	83	–	–	17	83	100	–	59
Sparsely-spotted stingaree	*Urolophus paucimaculatus*	12	–	–	77	23	77	23	–	100	41
Southern eagle ray	*Myliobatis australis*	12	–	–	79	21	79	21	–	100	41
Australian angel shark	*Squatina australis*	7	–	17	67	16	67	33	17	83	24
Western shovelnose ray	*Aptychotrema vincentiana*	2	–	–	80	20	80	20	–	100	5
Shortfin mako	*Isurus oxyrinchus*	1	–	25	25	50	25	75	25	75	4
Thresher shark	*Alopias vulpinus*	–	100	–	–	–	100	–	100	–	1
Longnose skate	*Raja sp A*	–	–	–	100	–	100	–	–	100	1
Melbourne skate	*Raja whitleyi*	–	–	–	100	–	100	–	–	100	1
Skates (unspecified)	*Raja spp*	–	–	–	100	–	100	–	–	100	1
Sub-total	*Chondrichthyes*	2 339	38	36	24	2	62	38	74	26	7 761
Teleostei											
Blue warehou	*Seriolella brama*	17	25	33	–	42	25	75	58	42	55
Long-snouted boarfish	*Pentaceropsis recurvirostris*	16	92	6	–	2	92	8	98	2	54
Jack mackerel	*Trachurus declivis*	16	–	–	68	32	68	32	–	100	53
White trevally	*Pseudocaranx dentex*	13	43	57	–	–	43	57	100	–	44
Latchet	*Pterygotrigla polyommata*	7	–	–	–	100	–	100	–	100	23
Magpie perch	*Cheilodactylus nigripes*	4	–	–	64	36	64	36	–	100	14
Bight redfish	*Centroberyx gerrardi*	4	67	25	–	8	67	33	92	8	12
Sand flathead	*Platycephalus bassensis*	3	9	–	36	55	45	55	9	91	11
Blue-throated wrasse	*Notolabrus tetricus*	3	33	23	11	33	44	56	56	44	9
Red gurnard	*Chelidonichthys kumu*	2	25	–	–	75	25	75	25	75	8

TABLE 8A. (Cont'd). Breakdown of total catch by gillnet 6-inch mesh size as retained, discarded, live, and dead animals for each species in Bass Strait during 1998–2001. Catch-per-unit effort (CPUE) is measured as number of animals per 1000 km-hr.

			Total catch (%)								
			Retained		Discarded		Total catch (%)		Total catch (%)		No.
Common name or effort	Scientific name	CPUE	Live	Dead	Live	Dead	Live	Dead	Retained	Discarded	caught
Barracouta	*Thyrsites atun*	2	–	–	–	100	–	100	–	100	8
Deepsea trevalla	*Hyperoglyphe antarctica*	2	–	100	–	–	–	100	100	–	6
Swallow-tail	*Centroberyx lineatus*	2	100	–	–	–	100	–	100	–	5
Bearded rock cod	*Pseudophycis barbata*	1	–	–	–	100	–	100	–	100	4
Giant boarfish	*Paristiopterus labiosus*	1	100	–	–	–	100	–	100	–	4
Knifejaw	*Oplegnathus woodwardi*	1	100	–	–	–	100	–	100	–	4
Queen snapper	*Nemadactylus valenciennesi*	1	50	50	–	–	50	50	100	–	4
Sergeant baker	*Aulopus purpurissatus*	1	–	–	–	100	–	100	–	100	3
Pink ling	*Genypterus blacodes*	1	33	67	–	–	33	67	100	–	3
Jackass morwong	Family *Nemadactylus macropterus*	1	33	–	–	67	33	67	33	67	3
Tiger flathead	Family *Neoplatycephalus richardsoni*	1	50	50	–	–	50	50	100	–	2
Stargazer	Family *Uranoscopidae*	1	–	–	100	–	100	–	–	100	2
Greenback flounder	*Rhombosolea tapirina*	1	100	–	–	–	100	–	100	–	2
Leatherjacket	*Family Monacanthidae*	1	–	–	50	50	50	50	–	100	2
Snapper	*Pagrus auratus*	–	100	–	–	–	100	–	100	–	1
Western blue groper	*Achoerodus gouldii*	–	–	–	100	–	100	–	–	100	1
Sub-total	*Teleostei*	102	40	14	18	28	58	42	54	46	337
Cephaplopoda											
Octopus	*Octopus pallidus*	1	33	–	67	–	100	–	33	67	3
Gould's squid	*Nototodarus gouldi*	–	100	–	–	–	100	–	100	–	1
Sub-total	*Cephalopoda*	1	50	–	50	–	100	–	50	50	4
Other											
Swollen spider crab	*Leptomithrax gaimardii*	25	–	–	70	30	70	30	–	100	83
Southern rock lobster	*Jasus edwardsii*	1	100	–	–	–	100	–	100	–	2
False bailer shell	*Livonia mammilla*	3	67	–	33	–	100	–	67	33	9
Australian fur seal	*Arctocephalus pusillus dorifer*	1	–	–	–	100	–	100	–	100	2

TABLE 8B. Breakdown of total catch by gillnet 6 inch and 6 1/2 inch mesh size as retained, discarded, live, and dead animals for each species in South Australia during 1998–2001. Catch-per-unit effort (CPUE) is measured as number of animals per 1 000 km-hr.

Common name or effort	Scientiifc name	CPUE	Total catch (%) Retained Live	Retained Dead	Discarded Live	Discarded Dead	Total catch (%) Live	Dead	Total catch (%) Retained	Discarded	No. caught
Fishing effort (km-hr)		1 865									
Chondrichthyes											
Gummy shark	*Mustelus antarcticus*	497	47	52	–	1	47	53	99	1	928
Port Jackson shark	*Heterodontus portusjacksoni*	138	–	–	100	–	100	–	–	100	257
School shark	*Galeorhinus galeus*	44	94	2	4	–	98	2	96	4	82
Smooth hammerhead	*Sphyrna zygaena*	41	97	2	–	1	97	3	99	1	77
Piked spurdog	*Squalus megalops*	38	–	–	100	–	100	–	–	100	71
Southern eagle ray	*Myliobatis australis*	28	2	–	89	9	91	9	2	98	53
Common sawshark	*Pristiophorus cirratus*	23	91	7	2	–	93	7	98	2	43
Bronze whaler	*Carcharhinus brachyurus*	23	100	–	–	–	100	–	100	0	42
Southern sawshark	*Pristiophorus nudipinnis*	14	63	30	4	3	67	33	93	7	27
Broadnose sevengill shark	*Notorynchus cepedianus*	13	21	–	–	79	21	79	21	79	24
Elephant fish	*Callorhinchus milii*	12	68	14	9	9	77	23	82	18	22
Thresher shark	*Alopias vulpinus*	10	5	–	32	63	37	63	5	95	19
Australian angel shark	*Squatina australis*	10	21	–	68	11	89	11	21	79	19
Spotted wobbegong	*Orectolobus maculatus*	2	100	–	–	–	100	–	100	–	4
Skates (unspecified)	*Raja spp*	1	–	–	100	–	100	–	–	100	2
Sparsely-spotted stingaree	*Urolophus paucimaculatus*	1	–	–	50	50	50	50	–	100	2
Whiskery shark	*Furgaleus macki*	1	100	–	–	–	100	–	100	–	1
White-spotted spurdog	*Squalus acanthias*	1	–	–	100	–	100	–	–	100	1
Western shovelnose ray	*Aptychotrema vincentiana*	1	–	–	100	–	100	–	–	100	1
Sub-total	*Chondrichthyes*	898	42	30	25	3	67	33	72	28	1 675
Teleostei											
Southern drummer	*Kyphosus gibsoni*	33	100	–	–	–	100	–	100	–	62
Bight redfish	*Centroberyx gerrardi*	31	98	–	2	–	100	–	98	2	58
Long-snouted boarfish	*Pentaceropsis recurvirostris*	30	98	–	–	2	98	2	98	2	56
Queen snapper	*Nemadactylus valenciennesi*	23	98	–	2	–	100	–	98	2	42
Snapper	*Pagrus auratus*	19	100	–	–	–	100	–	100	–	35
Dusky morwong	*Dactylophora nigricans*	13	58	–	42	–	100	–	58	42	24
Western blue groper	*Achoerodus gouldii*	11	100	–	–	–	100	–	100	–	21
Red gurnard	*Chelidonichthys kumu*	10	56	–	22	22	78	22	56	44	18
Jewfish	*Argyrosomus japonicus*	5	90	–	–	10	90	10	90	10	10

TABLE 8B. (Cont'd). Breakdown of total catch by gillnet 6 inch and 6 1/2 inch mesh size as retained, discarded, live, and dead animals for each species in South Australia during 1998–2001.Catch-per-unit effort (CPUE) is measured as number of animals per 1 000 km-hr.

			Total catch (%)								
			Retained		Discarded		Total catch (%)		Total catch (%)		Number
Common name or effort	Scientiifc name	CPUE	Live	Dead	Live	Dead	Live	Dead	Retained	Discarded	caught
Magpie perch	*Cheilodactylus nigripes*	5	100	–	–	–	100	–	100	–	10
Yellow-spotted boarfish	*Paristiopterus gallipavo*	3	100	–	–	–	100	–	100	–	6
Leatherjacket	*Family Monacanthidae*	3	100	–	–	–	100	–	100	–	6
Latchet	*Pterygotrigla polyommata*	3	40	–	60	–	100	–	40	60	5
Sand flathead	*Platycephalus bassensis*	3	60	–	20	20	80	20	60	40	5
Tiger flathead	*Neoplatycephalus richardsoni*	2	100	–	–	–	100	–	100	–	3
Sergeant baker	*Aulopus purpurissatus*	1	–	–	100	–	100	–	–	100	2
Blue-throated wrasse	*Notolabrus tetricus*	1	100	–	–	–	100	–	100	–	2
Pink ling	*Genypterus blacodes*	1	100	–	–	–	100	–	100	–	1
Mirror dory	*Zenopsis nebulosus*	1	100	–	–	–	100	–	100	–	1
Jack mackerel	*Trachurus declivis*	1	100	–	–	–	100	–	100	–	1
Samsonfish	*Seriola hippos*	1	100	–	–	–	100	–	100	–	1
Bumpnose trevally	*Carangoides hedlandensis*	1	–	–	100	–	100	–	–	100	1
Sweep	*Family Scorpis lineolatus*	1	100	–	–	–	100	–	100	–	1
Old wife	*Family Enoplosus armatus*	1	–	–	100	–	100	–	–	100	1
Wrasse	*Family Labridae*	1	100	–	–	–	100	–	100	–	1
Greenback flounder	*Family Rhombosolea tapirina*	1	100	–	–	–	100	–	100	–	1
Toadfish	*Tetraodon erythrotaenia*	1	–	–	100	–	100	–	–	100	1
Sub-total	*Teleostei*	201	91	–	7	2	98	2	91	9	375
Other											
Swollen spider crab	*Leptomithrax gaimardii*	7	–	–	69	31	69	31	–	100	13
Southern rock lobster	*Jasus edwardsii*	2	100	–	–	–	100	–	100	–	4
Southern bay lobster	*Ibacus peronii*	1	–	–	100	–	100	–	–	100	1
Common dolphin	*Delphinus delphis*	1	–	–	–	100	–	100	–	100	1

3. Overall catch rates of chondrichthyan and teleost fishes by mesh size are very different. For chondrichthyans, the modal catch rate is by 4 inch mesh size with decreasing catch rates for both increasing and decreasing mesh size, whereas for teleosts the modal catch rate is by 2 inch mesh size with decreasing catch rates as mesh size increases.

4. For gillnets, there is linear increase in the ratio of the number of chondrichthyan fishes divided by the number of teleost fishes with increasing mesh size, whereas for hooks the ratio is approximately constant with increasing hook size.

5. For chondrichthyes, the top four species taken by gillnet across 8 mesh sizes (Experiment 1), *Squalus megalops*, *Mustelus antarcticus*, *Heterodontus portusjacksoni*, and *Galeorhinus galeus*, are similar to the top four species taken by longline across 8 hook sizes (Experiment 3), *Squalus megalops*, *M. antarcticus*, *Cephaloscyllium laticeps*, and *G. galeus*. The only difference is that *H. portusjacksoni* is more prevalent than *C. laticeps* in the gillnet catch, whereas the converse occurs for the longline catch.

6. For teleostei, *Platycephalus bassensis* is the most prevalent species caught by both gillnets across 8 mesh sizes (Experiment 1) and longlines across 8 hook sizes (Experiment 3). *Neosebastes scorpaenoides* is the second most prevalent species caught by longline and the third most prevalent species caught by gillnet. The second most prevalent species taken by gillnet, *Trachurus novaezelandiae*, is not caught by longline.

7. For chondrichthyes in Bass Strait, there has been about a one-third overall reduction in abundance across all species combined between 1973–76 and 1998–2001. About half of this reduction is attributable to an 87% reduction in the catch-per-unit effort (CPUE) of *Galeorhinus galeus* and a 54% reduction in the CPUE of *Cephaloscyllium laticeps*.

8. Only small proportions of the commercial catch of chondrichthyan (3%) and teleost (2%) animals taken by demersal gillnets of 6 inch and 6½ inch mesh size coming aboard dead are discarded. The discarded animals are mostly *Cephaloscyllium laticeps*, *Heterodontus portusjacksoni*, *Squalus megalops*, and *Myliobatis australis*, which come aboard live.

9. Fishery-wildlife interactions occur occasionally with Australian fur seals (*Arctocephalus pusillus dorfer*) and common dolphin (*Delphinus delphis*).

10. Of ten chondrichthyan species on the continental shelf and continental slope identified by the IUCN Shark Specialist Group as threatened, two are identified by the present study as caught by the fishery. White shark (*Carcharodon carcharius*) are taken occasionally and *Galeorhinus galeus*, once the primary target species, is presently taken as significant by-product (253 tons during 2000) (Walker *et al.*, 2003).

In summary, most of the by-catch from the shark fishery of southern Australia consists of four chondrichthyan species that are discarded live. Only small quantities of teleost species are taken and these are mostly retained and marketed, and, with the exception of *Galeorhinus galeus*, interactions with protected or threatened species are minimal. The main challenge for management of the fishery is to allow sustainable use of the highly productive resource of *Mustelus antarcticus*, while rebuilding the depleted stocks of *G. galeus*. There is little spatial overlap between the shark fishery and other fisheries.

The 87% reduction in CPUE of *G. galeus*, detected by the present study in Bass Strait between 1973–76 and 1998–2001, is consistent with the reduction in CPUE reported by commercial fishers (Walker *et al.*, 2003). The magnitude of the reduction is also consistent with the results of stock assessment for the species using independent data (Punt and Walker, 1998; Punt *et al.*, 2000).

The 54% reduction in the catch of *Cephaloscyllium laticeps* is more difficult to explain. Fishing mortality of these animals is not expected to be high, because they are highly robust animals; they are mostly alive when removed from gillnets. Part of the explanation for this observed reduction is that commercial fishers tend to avoid fishing grounds where these animals are known to be abundant. In addition, fishers often move away from fishing grounds where catch numbers of this species are high to avoid untangling large numbers of these animals from the gillnets. Some fishers claim that *M. antarcticus* tend not to aggregate in regions inhabited by large numbers of *C. laticeps*. In Bass Strait, no attempt was made to avoid *C. laticeps* during 1973–76 (172 fishing sites) or during the pilot fixed station fishery-independent survey in 1998 (24 fishing sites). However, some of the fishers operating under normal commercial conditions might have avoided such regions during 1999–2001 (67 fishing sites).

Ten chondrichthyan species occurring in the region of the shark fishery are listed as threatened by the IUCN Shark Specialist Group. The grey nurse shark (*Carcharias taurus*), Harrison's dogfish (*Centrophorus harrisoni*), and southern dogfish (*C. uyato*) are listed as critically endangered. Greeneye spurdog (*Squalus mitsukurii*) and endeavour dogfish (*C. moluccensis*) are listed as endangered. *Carcharodon carcharias*, *G. galeus*, Herbst's nurse shark (*Odontaspis ferox*), eastern angel shark (*Squatina* sp A), and Maugean skate (*Raja* sp L) are listed as vulnerable (Cavanagh *et al.*, 2003).

On the upper continental slope of southern Australia, several species of dogfish (*Squalidae*) and holocephalans (*Holocephali*), taken as by-product by demersal trawl, gillnet or longline, have been identified as severely depleted and requiring special management. Upper slope dogfish species are more vulnerable to capture than mid slope species, because they are targeted throughout their vertical distribution and most of their geographic distribution. Demersal trawl surveys off central and southern New South Wales during 1977 and 1997 indicate a reduction in catch rates of *Centrophorus* spp of 98.4–99.7% (Andrew *et al.*, 1997; Graham *et al.*, 2001). The shark fishery now only occasionally operates outside depths of 100 m, and therefore does not impact the severely depleted populations of *Centrophorus* spp or holocepahalans on the continental slope, which occur mainly in depths >200 m.

Reports by fishers indicate that a small by-catch of *Carcharodon carcharias* occurs, which is consistent with one animal caught by longline during 1973–76 as part of the present study (Experiment 4). The species is now totally protected in all Australian waters and the unintentional fishing mortality of the species is being reduced as various waters are closed to shark fishing. All Victorian waters (coastal waters out to 3 nm and all enclosed bays and inlets) have been closed to shark fishing since 1988. Area closures are presently under consideration in South Australia and Tasmania.

There are no reported catches of *Carcharias taurus* from the shark fishery of southern Australia. Although the distribution of *C. taurus* is reported to include Victoria, South Australia, and Tasmania (Last and Stevens, 1994), the species is extremely rare in these waters. The species occurs mainly in New South Wales and Western Australia (Pollard, 1996). Similarly there are no reported catches of *Odontapsis ferox*, *Squatina* sp A or *Raja* sp L from the shark fishery. *Odontapsis ferox* is distributed off New South Wales and *Squatina* sp A is distributed mainly in the coastal waters of New South Wales and Queensland (Last and Stevens, 1994) outside the range of the shark fishery. However, *Raja* sp L occurs inshore off southern Tasmania (Last and Stevens, 1994) where it can potentially interact with the shark fishery.

The small catch of marine mammals by gillnets during 1998–2001, two Australian fur seals (*Arctocephalus pusillus dorfer*) and one common dolphin (*Delphinus delphis*), is consistent with the anecdotal information of a small by-catch for these species. Several other species of seals (families *Otariidae* and *Phocidae*) and dolphins (family *Delphinidae*) that occur within the range of the fishery (Menkhorst, 1995) may be caught on rare occasions. The Victorian closure to shark fishing is likely to have reduced the unintentional fishing mortality of *Arctocephalus pusillus dorfer* within at least 3 naut. miles around four major seal breeding colonies (Lady Julia Percy Island, Seal Rock, Kanowa Island and The Skerries) and other haul out sites. Closure of other important seal habitat is under consideration in other States.

At a world level, based on limited data, 27 million tons of material are estimated to be discarded annually. Most of this is from industrial rather than artisanal fisheries. The highest number of records of discards is from trawl fisheries (966 records), followed by drift net and gillnet fisheries (232), line fisheries (150), pot fisheries (83), and purse seine fisheries (82) (Alverson *et al.*, 1994). Management of fishery-wildlife interactions, particularly with mammals, seabirds, and turtles, have become the key factors in the management strategies of some fisheries (Jennings *et al.*, 2001).

Most of the world's catch of chondrichthyan species is captured by demersal trawl, demersal gillnet, and pelagic and demersal longlines (Bonfil, 1994; Walker, 1998). Various studies have evaluated catches from demersal trawl (Van Der Molen *et al.*, 1998; Stobutzki *et al.*, 2001; Anderson and Clark, 2003) and longline fisheries (Bailey *et al.*, 1996; Marín *et al.*, 1998; Williams, 1999), but there has been little attempt to comprehensively evaluate catches in demersal gillnet fisheries.

The effects of mesh size in trawl codends on catch has been investigated extensively for prawns and teleosts (Sparr and Venema, 1992; Millar and Fryer, 1999; D'Onghia *et al.*, 2003), but not for chondrichthyans. Square mesh panels in demersal trawl codends has been shown to facilitate escapement of small teleost fish (Broadhurst *et al.*, 1997; Graham *et al.*, 2003), but not yet for small chondrichthyan animals. Another approach is to fit a rigid grid in front of the codend to deflect large animals such as turtles, mammals and sharks through an escape panel; this by-catch reduction device (BRD) is often referred to as a turtle exclusion device or trawl efficiency device (TED) (Anon., 2000; Jennings *et al.*, 2001).

As demonstrated for sharks (Kirkwood and Walker, 1986; McLoughlin and Stevens, 1994; Simpfendorfer and Unsworth, 1998; Carlson and Cortés, 2003) and teleosts

(Millar and Fryer, 1999; Holgård and Lassen, 2002), the present study confirms that gillnets are highly length selective and mesh size markedly affects species composition of the catch and the length frequency composition of each species in the catch. The relative abundances of the various species taken in the 2–9 inch mesh sizes adopted were very different and there are distinct trends with mesh size. This means mesh size can be regulated to provide for the efficient catch of target species with escapement of pre-recruit and large breeding animals (Walker, 1998) and escapement of certain by-catch species (present study). In some fisheries, regulation of filament thickness has been suggested to facilitate escapement of particular by-catch species by allowing the filaments of gillnet webbing to break (Anon., 2000).

The effects of hook size on catch can be detected for some teleost species (Sparr and Venema, 1992; Sousa *et al.*, 1999; Holgård and Lassen, 2002) and hook type, hook shape, and bait can also have length selective effects on the catch (Woll *et al.*, 2001). Although not extensively investigated, it appears the effects of hook size are weak for demersal chondrichthyan species (present study). Increasing the distance for setting hooks above the seabed can markedly reduce the by-catch of deep water sharks (Coelho *et al.*, 2003). Anecdotal reports from observers on board vessels operating in the tropical and subtropical tuna longline fisheries indicate increasing the distance of hooks below the sea surface can reduce the by-catch of pelagic sharks. Also, preventing use of wire traces between the snoods and hooks can facilitate escapement of chondrichthyan species, particularly large sharks, by allowing snoods to be broken or bitten through (Anon., 2000).

Changes in the structure of demersal fish communities have been detected by studies with trawl gear, which is less size selective than gillnets. For example annual trawl surveys during 1970–2000, a time scale similar to the present study, demonstrated a change in community composition in an area following its closure in 1987 on the continental shelf of Nova Scotia, Canada. Fish from a total of 74 species were caught in either the area closed in 1987 (60 species) or the nearby Brown's Bank area (62 species). The change was demonstrated by multivariate analysis and a randomised pertubation test (Fischer and Frank, 2002). Another study, trawling regularly at 14 sites during 1970–75 and 1990/91 in Port Phillip Bay, Victoria, Australia, also provides evidence of detectable changes in the demersal fish communities (Hobday *et al.*, 1999). The present study shows that a gillnet fishery based on the narrow mesh size range of 6–7 inch mesh size can cause detectable changes in the relative abundance of particular species, providing evidence of a detectable change in demersal fish community composition. The observation from the present study of a linear increase in the ratio of the number of chondrichthyan fishes to the number of teleost fishes with increasing mesh size is consistent with the tendency for chondrichthyan animals to attain larger body size than teleost animals (Freedman and Noakes, 2002). The observation is also consistent with the tendency for teleost animals to be more abundant than chondricthyan animals in coastal demersal fish communities.

Acknowledgements

The authors thank all former technical staff of Primary Industries Research Victoria who participated in the field sampling during 1973–1976 and Lauren P. Brown of that institute and Jeremy D. Prince of Biospherics Pty. Ltd. who participated in field sampling during 1998. The many shark fishers who kindly provided accommodation, assistance, and hospitality aboard their fishing vessels are also thanked. Collection of data during 1973–76 was funded from the former Australian Fishing Industry Research Trust Account and the former Victorian Fisheries and Wildlife Division. Collection of data during 1998 was funded by the Australian Fisheries Management Authority as part of the Pilot Fixed Site Stations Survey. Collection of data during 1999–2001 and analysis of the data was funded by the Australian Fisheries Research and Development Corporation as part of FRDC Project 1999/103. Species names are based on the Codes for Australian Aquatic Biota, accessed at the internet address www.marine.csiro.au/caab/caabsearch. Alexander K. Morison and Sonia G. Talman of Primary Industries Research Victoria and an anonymous reviewer are acknowledged for their helpful suggestions for improvement of the manuscript.

References

ALVERSON, D. L., M. H. FREEBERG, J. G. POPE, and S. A. MURAWSKI. 1994. A global assessment of fisheries bycatch and discards. *FAO Fish. Tech. Pap.*, **339**: 233 p.

ANDERSON, O. F., and M. R. CLARK. 2003. Analysis of bycatch in the fishery for orange roughy, *Hoplostethus atlanticus*, on the South Tasman Rise. *Mar. Freshw. Res.*, **54**: 643–652.

ANDREW, N. L., K. J. GRAHAM, K. E. HODGSON, and G. N. G. GORDON. 1997. Changes after twenty years in relative abundance and size composition of commercial fishes caught during fishery independent surveys on SEF trawl grounds. FRDC Project No. 96/139. NSW Fisheries Final Report Series No. 1. NSW Fisheries Research Institute, Cronulla, New South Wales, Australia, 109 p.

ANON. 2000. Fisheries management 1. Conservation and management of sharks. *In*: FAO Technical Guidelines for Responsible Fisheries. Food and Agriculture Organization of the United Nations, Rome. Vol. 4, Supplement 1, 37 p.

BAILEY, K., P. G. WILLIAMS, and D. ITANO. 1996. Bycatch and discards in Western Pacific Tuna Fisheries: a review of SPC data holdings and literature. South Pacific Commission, Noumea, New Caledonia.

BONFIL, R. 1994. Overview of world elasmobranch fisheries. *FAO Fish. Tech. Pap.*, **341**: 1–119.

BROADHURST, M. K., S. J. KENNELLY, and D. T. BARKER. 1997. Simulated escapement of juvenile sand whiting (*Sillago ciliata*, Cuvier) through square-meshes: effects on scale-loss and survival. *Fish. Res.*, **31**: 51–60.

CARLSON, J. K., and E. CORTÉS. 2003. Gillnet selectivity of small coastal sharks off the southeastern United States. *Fish. Res.*, **60**: 405–414.

CAVANAGH, R. D., P. M. KYNE, S. L. FOWLER, J. A. MUSICK, and M. B. BENNETT (eds). 2003. The Conservation Status of Australasian Chondrichthyans: Report of the IUCN Shark Specialist Group Australia and Oceanaria Regional Red List Workshop. Queensland, Australia, 7–9 March 2003. The University of Queensland, School of Biomedical Sciences, Brisbane, Australia, x + 170 p.

COELHO, R., L. BENTES, J. M. S. GONÇALVES, P. LINO, J. RIBEIRO, and K. ERZINI. 2003. Reduction of elasmobranch by-catch in the hake semipelagic near-bottom longline fishery in the Algarve, Southern Portugal. *Fish. Sci.*, **69**: 293–299.

D'ONGHIA, G., R. CARLUCCI, P. MAIORANO, and M. PANZA. 2003. Discards from deep-water bottom trawling in the eastern-central Mediterranean Sea and effects of mesh size changes. *J. Northw. Atl. Fish. Sci.*, **31**: 245–261.

FISCHER, J. A. D., and K. T. FRANK. 2002. Changes in finfish community structure associated with an offshore fishery closed area on the Scotian shelf. *Mar. Ecol. Prog. Ser.*, **240**: 249–265.

FREEDMAN, J. A., and D. L. G. NOAKES. 2002. Why are there no really big bony fishes? A point-of-view on a maximum body size in teleosts and elasmobranchs. *Rev. Fish Biol. Fish.*, **12**: 403–416.

GRAHAM, K. J., N. L. ANDREW, and K. E. HODGSON. 2001. Changes in the relative abundances of sharks and rays on Australian South East Fishery Trawl grounds after twenty years of fishing. *Mar. Freshw. Res.*, **52**: 549–561.

GRAHAM, N., R. J. KYNOCH, and R. J. FRYER. 2003. Square mesh panels in demersal trawls: further data relating haddock and whiting selectivity to panel position. *Fish. Res.*, **62**: 361–375.

HALL, M. A. 1996. On bycatches. *Rev. Fish Biol. Fish.*, **6**: 319–352.

HOBDAY, D. K., R. A. OFFICER, and G. D. PARRY. 1999. Changes to demersal fish communities in Port Phillip Bay, Australia, over two decades, 1970–91. *Mar. Freshw. Res.*, **50**: 397–407.

HOLGÅRD, H., and H. LASSEN. 2002. Manual on estimation of selectivity for gillnet and longline gears in abundance surveys. *FAO Fish. Tech. Pap.*, **397**: 1–84.

JENNINGS, S., M. J. KAISER, and J. D. REYNOLDS. 2001. *Marine fisheries ecology*, Blackwell Science Ltd, Melbourne, 417 p.

KIRKWOOD, G. P., and T. I. WALKER. 1986. Gill net mesh selectivities for gummy shark, *Mustelus antarcticus* Günther, taken in south-eastern Australian waters. *Australian J. Mar. Freshw. Res.*, **37**: 689–697.

LAST, P. R., and J. D. STEVENS. 1994. Sharks and rays of Australia, CSIRO Australia, Melbourne, 513 p.

MARÍN, Y., F. BRUM, L. C. BAREA, and J. F. CHOCCA. 1998. Incidental catch associated with swordfish longline fisheries in the south-west Atlantic Ocean. *Mar. Freshw. Res.*, **49**: 633–639.

MCLOUGHLIN, K. L., and J. D. STEVENS. 1994. Gill-net mesh selectivities for two species of commercial carcharhinid shark taken in northern Australia. *Australian J. Mar. Freshw. Res.*, **45**: 521–534.

MENKHORST, P. W. (ed.). 1995. Mammals of Victoria distribution, ecology and conservation. Oxford University Press: Oxford, 360 p.

MILLAR, R. B., and R. J. FRYER. 1999. Estimating the size-selection curves of towed gears, traps, nets and hooks. *Rev. Fish Biol. Fish.*, **9**: 89–116.

POLLARD, D. A. 1996. The biology and conservation status of the grey nurse shark (*Carcharias taurus* Rafinesque 1810) in New South Wales, Australia. *Aquat. Conserv.: Mar. Freshw. Ecosystems*, **6**: 1–20.

PRIBAC, F., A. E. PUNT, T. I. WALKER, and B. L. TAYLOR. 2004. The value of length, age and tagging data in a stock assessment of a length selective fishery for gummy shark. *J. Northw. Atl. Fish. Sci.*, **35**: 267–290 (this volume).

PUNT, A. E., F. PRIBAC, T. I. WALKER, B. L. TAYLOR, and J. D. PRINCE. 2000. Stock assessment of school shark *Galeorhinus galeus* based on a spatially-explicit population dynamics model. *Mar. Freshw. Res.*, **51**: 205–220.

PUNT, A. E., and T. I. WALKER. 1998. Stock assessment and risk analysis for the school shark (*Galeorhinus galeus*) off southern Australia. *Mar. Freshw. Res.*, **49**: 719–731.

PUNT, A. E., T. I. WALKER, and J. D. PRINCE. 2002. Assessing the management-related benefits of fixed-station fishery-independent surveys in Australia's southern shark fishery. *Fish. Res.*, **55**: 281–295.

SIMPFENDORFER, C. A., and P. UNSWORTH. 1998. Gill-net selectivity of dusky sharks (*Carcharhinus obscurus*) and whiskery sharks (*Furgaleus macki*) from south-western Australia. *Mar. Freshw. Res.*, **49**: 713–718.

SPARR, P., and S. C. VENEMA. 1992. Introduction to tropical fish stock assessment Part I – Manual. *FAO Fish. Tech. Pap.*, **306/1**: 1–376.

STOBUTZKI, I.C., M. J. MILLER, P. JONES, and J. P. SALINI. 2001. Bycatch diversity and variation in a tropical Australian penaeid fishery; the implications for monitoring. *Fish. Res.*, **53**: 283–301.

SOUSA, F., E. ISIDRO, and K. ERZINI. 1999. Semi pelagic longline selectivity for two demersal species from the Azores: the black sea bream (*Pagellus bogaraveo*) and the bluemouth rockfish (*Helicolenus dactylopterus dactylopterus*). *Fish. Res.*, **41**: 25–35.

VAN DER MOLEN, S., G. CAILLE, and R. GONZÁLEZ. 1998. By-catch of sharks in Patagonian coastal trawl fisheries. *Mar. Freshw. Res.*, **49**: 641–644.

WALKER, T. I. 1983. Investigations of the gummy shark, *Mustelus antarcticus* Günther, from south-eastern Australian waters. Report to Fishing Industry Research Committee. June 1983. *In*: Proceedings of the Shark Assessment Workshop, South East Fisheries Committee Shark Research Group, 7–10 March 1983, Melbourne. A. E. Caton (ed.). Department of Primary Industry, Canberra, ACT, Australia, p. 1–94.

WALKER, T. I. 1998. Can shark resources be harvested sustainably? A question revisited with a review of shark fisheries. *Mar. Freshw. Res.*, **49**: 553–572.

WALKER, T. I. 1999. Southern Australian shark fishery management. *In*: Case studies of management of elasmobranch fisheries. *FAO Fish. Tech. Pap.*, **378**(2): 480–514.

WALKER, T. I., B. L. TAYLOR, and R. J. HUDSON. 2003. Southern shark catch and effort 1970–2002 report to Australian Fisheries Management Authority, June 2003. Marine and Freshwater Resources Institute, Queenscliff, Victoria, Australia, 41 p.

WILLIAMS, P. G. 1999. Shark and related species catch in tuna fisheries of the Tropical Western and Central Pacific Ocean. *In*: Case studies of management of elasmobranch fisheries. *FAO Fish. Tech. Pap.*, **378**(2): 860–879.

WOLL, A. K., J. BOJE, R. HOLST, and A. C. GUNDERSEN. 2001. Catch rates and hook and bait selectivity in longline fishery for Greenland halibut (*Reinhardtius hippoglossoides*, Walbaum) at East Greenland. *Fish. Res.*, **51**: 327–246.

Appendix 5b: Damaged catch evaluation

This appendix contains a report prepared for SharkRAG, which determines damage levels caused by the effects of predation from invertebrates (sea-lice), other fish and mammals on target and byproduct shark species taken by gillnets in the Gillnet Hook and Trap Fishery.

SharkRAG/2005/10 28–29 July 2005

Predation Damage Rates to Shark in the Gillnet Hook and Trap Fishery

Terence I. Walker and Russell J. Hudson

Primary Industries Research Victoria, PO Box 114, Queenscliff, Vic. 3225

Abstract

Damage from predation by invertebrates, fish and mammals to sharks landed on-board from gillnets of 6-inch or 6½-inch mesh-size was investigated on board nine vessels operating under normal commercial fishing conditions. The work was undertaken during November 1998–February 2001 at 153 fishing sites (91 sites in Bass Strait and 62 sites off South Australia). Of 3187 gummy sharks (*Mustelus antarcticus*), 145 school sharks (*Galeorhinus galeus*), 1099 common sawshark (*Pristiophorus cirratus*), 315 southern sawshark (*P. nudipinnis*), and 916 elephant fish (*Callorhinchus milii*) examined for carcass damage, 42% of the animals were landed on the deck alive without damage. Part of the catch of animals landed on board dead had damage to carcasses resulting in 'lost carcass mass' and 'devalued retained carcass mass'. 'Lost carcass mass' from predation for gummy shark and school shark combined is estimated at 4.9% (4.7% for gummy shark and 6.9% for school shark); it is slightly higher in South Australia (5.3%) than in Bass Strait (4.7%). 'Lost carcass mass' for common sawshark and southern sawshark combined is estimated at 2.3% (2.1% for common sawshark and 3.5% for southern sawshark) and for elephant fish is estimated at 3.4%. 'Devalued retained carcass mass' from major damage is estimated at 9.2% for gummy shark and school shark combined (9.0% for gummy shark and 12.8% for school shark), 4.2% for common sawshark and southern sawshark combined (4.0% for common sawshark and 5.5% for southern sawshark), and 6.1% for elephant fish.

Introduction

Sharks captured by various fishing gears are often damaged before the gear is hauled. Sharks can be partly or totally eaten by invertebrates ('sea lice'), teleosts, other sharks, or mammals while captive in the fishing gear. Sharks come aboard in the full range of damage from very slight damage, causing minor devaluation of the carcass, to extensive damage, causing major or total devaluation of the carcass. Most damage begins as damage to gills, cloaca or viscera from sea lice and then extends to the axial muscle tissue of the carcass. Occasionally in tact carcasses greened by the effects of gummy shark eating predominantly crustaceans are devalued Depending on the extent of the damage, shark fishers decide on whether or not to market 'damaged carcasses' (or 'shark pieces'). This has raised management concerns that shark damage might lead to 'high grading' under the management scheme of individual transferable quotas (ITQs) adopted for gummy shark, school shark, sawshark and elephant fish in the Gillnet Hook and Trap Fishery (GHATF).

Several terms and concepts are defined for the purpose of this report. 'Shark carcass' refers to a beheaded and eviscerated shark with the tail, all fins, and, for males, the claspers, attached, and a 'damaged carcass' consists of two portions: the 'lost damaged portion' and the 'retained damaged portion'. The two damaged portions contribute to loss of income to the fishing industry in two ways. There is loss of income through the 'lost damaged portion' being unavailable for marketing, and there is loss of income through the 'retained damaged portion' being devalued on the market through a reduced price per kilogram. Hence, for the purpose of the present report, there is an 'undamaged catch' (sum of all the sharks with zero damage to the carcasses), a 'lost catch' (sum of all the 'lost damaged portions' from the 'damaged shark carcasses'), and 'devalued catch' (sum of all the 'retained damaged portions').

Two other types of catch—'drop-out catch' and 'annihilated catch'—also occur during fishing operations. These are important because they contribute to 'unaccounted fishing mortality' ('cryptic catch'), but they fall outside the scope of the present report because it is never observed and appropriate data cannot be readily collected by on-board scientific observers. 'Drop-out mortality' ('drop-out catch') includes sharks encountering the gear that either escape from or dropout of the gear and die. 'Predation mortality' ('annihilated catch') includes sharks totally consumed by 'sea-lice' or other animals before they can be landed on the vessel and observed.

Methods

During November 1998–February 2001, nine commercial vessels were used during 10 separate fishing trips for sampling for damage from predation at 153 fishing sites (91 sites in Bass Strait and 62 sites off South Australia). The vessels operated under normal commercial fishing conditions, where the fishing gear consisted of 6-inch or 6½-inch mesh-size gillnets. The vessels were all licensed to deploy gillnets up to a maximum of 4200 m long and 20 meshes deep; each gillnet was ~2.4 m deep with a hanging ratio of ~0.60. The gear was set on the seabed, mostly twice a day. Those set between the times of 2100 h and 0500 h were mostly hauled after sunrise, whereas those set between the times of 0800 h and 2000 h were mostly hauled after sunset. Mean fishing time for the gear was 8.2 h. Depths at the fishing sites ranged 17–130 m. The full length of gillnet was deployed at most fishing sites (4200 m at 128 sites) or a little less was deployed when the gear was damaged (4000 m at 21 sites). Half the available gillnets were set when searching for target species or when avoiding strong tidal flow or damage to the catch from predation (2100 m at 2 sites, and 2000 m at 2 sites) (Walker et al in press).

The species, sex, total length, 'condition of shark', 'extent of damage', and 'percentage mass loss' was recorded for each gummy shark, school shark, common sawshark, southern sawshark and elephant fish. Total length (TL) of shark was measured to the nearest millimetre; the tail of the shark was first allowed to take a natural position and the top caudal lobe was then placed parallel to the body axis. In addition, percentage of mass lost of the shark carcass ('%-loss') was estimated by eye and indices of 'condition of shark' were recorded as 1 (alive and strong), 2 (alive and weak), and 3 (dead). An index of 'carcass damage' was computed as 0, nil damage; 1, negligible damage (1–2% loss of carcass mass); 2, minor damage (3–9% loss of carcass mass); and 3, extensive damage (10–100% loss of carcass mass).

Several steps were required to calculate 'lost carcass mass' and 'devalued retained carcass mass'. The 'total mass' and 'carcass mass' (i.e. expected mass assuming no damage) were estimated for each shark from TL and the masses of the 'lost damaged portion' and the 'retained damaged portion' were estimated for each damaged shark from its TL and %-loss value. For each sex of each species separately, where sex or TL of a shark was not available because of extensive damage, sex was assumed to be female and TL was assumed to be equal to the mean TL of all sharks of known TL.

For gummy shark, the 'carcass mass', CW, was determined for each shark from the equation between carcass weight, total weight (TW) and TL (Walker 1983) as

$$CW = TW \times (0.540 + 1.28 \times 10^{-4} \times TL)$$

where, in turn, TW is determined from respective equations for males and females (Walker in prep)

$$TW = 4.210 \times 10^{-9}\, TL^{\,2.976},$$

and

$$TW = 0.990 \times 10^{-9}\, TL^{\,3.199}.$$

For school shark, CW was determined for each shark of both sexes from the equation (Walker 1986)

$$CW = 1.77 \times 10^{-10}\, TL^{\,3.41}.$$

For common sawshark, CW was determined for each shark from the equation between carcass weight and TL (unpublished data)

$$CW = 0.1366 \times 10^{-9}\, TL^{\,3.315},$$

and TW was determined from respective equations for males and females (unpublished data)

$$TW = 1.520 \times 10^{-9}\, TL^{\,3.015},$$

and

$$TW = 0.238 \times 10^{-9}\, TL^{\,3.292}.$$

For southern sawshark, CW was determined for each shark from the equation between carcass weight and TL (unpublished data)

$$CW = 0.0590 \times 10^{-9}\, TL^{\,3.426},$$

and TW was determined from respective equations for males and females (unpublished data)

$$TW = 0.0781 \times 10^{-9}\, TL^{\,3.450},$$

and

$$TW = 0.0485 \times 10^{-9}\ TL^{3.535}\ .$$

For elephant fish, ***CW*** was determined for each shark from the equation between carcass weight, total weight (TW) and TL (unpublished data)

$$CW = TW \times (0.661 - 0.0203 \times 10^{-4} \times TL)$$

where, in turn, ***TW*** is determined from respective equations for males and females (Walker in prep)

$$TW = 0.063 \times 10^{-9}\ TL^{3.688}\ ,$$

and

$$TW = 0.754 \times 10^{-9}\ TL^{3.301}\ .$$

For each species separately, 'lost carcass mass' of each animal was estimated by calculating the mass of the 'lost damaged portion' as ***CW*** x '%-loss/100'. Similarly, 'devalued retained carcass mass' of each animal was estimated by calculating the 'retained damaged portion' as ***CW*** x (1 – '%-loss'/100). The purpose of dividing by 100, in each case, was to convert percentage to a proportion. Then total 'lost carcass mass' was determined by summing 'lost carcass mass' over all individual animals in the catch and total 'devalued retained carcass mass' was determined by summing 'devalued retained carcass mass' over all individual animals in the catch

Results

Condition, expressed as alive and strong, alive and weak, or dead when landed on the deck of the vessel during normal commercial fishing operations, was recorded for 5816 animals (4314 gummy sharks, 111 school sharks, 778 common sawsharks, 217 southern sawsharks, and 396 elephant fish). Overall 42% of the animals were alive (36% for gummy shark, 29% for school shark, 67% for common sawshark, 71% for southern sawshark, and 41% for elephant fish) (Table 1). Only dead sharks were damaged; all sharks landed on the deck alive showed no sign of damage.

Extent of damage was recorded for 3187 gummy sharks (2969 in Bass Strait and 848 in South Australia), 145 school sharks (86 in Bass Strait and 59 in South Australia). Damage was also recorded for 1099 common sawshark, 315 southern sawshark and 914 elephant fish from throughout southern Australia. Damage prevented recording sex for 79 gummy sharks, 19 school sharks, 12 common sawshark, 1 southern sawshark, and 8 elephant fish, and because more than half the sexed animals in the catch were female, the unsexed animals were assumed to be female for the purpose of the analysis. Damage also prevented recording TL for 99 gummy sharks, 24 school sharks, 13 common sawshark, 2 southern sawshark, and 47 elephant fish. Mean TL values of 1021 mm for gummy shark, 1085 mm for school shark, 1139 mm for common sawshark, 976 mm for southern sawshark, and 742 mm for elephant fish, calculated as the mean for sharks of known TL, were assumed for sharks with missing TL.

Sample sizes and estimates of mean total mass, mean carcass mass, and mean carcass mass lost from damage are presented in Table 2. The overall 'lost carcass mass' for gummy shark and school shark combined was 4.9% (4.7% for gummy shark and 6.9% for school shark) and was slightly higher in SA (5.3%) than in BS (4.7%) (Table 3a). The overall 'lost carcass mass' for common sawshark and southern sawshark combined was 2.3% (2.1% for common sawshark and 3.5% for southern sawshark) and for elephant fish was 3.4% (Table 3b).

Of the retained catch, estimates of catch devalued were made from sharks classed 3 for damage (10–100% loss of carcass mass); it was assumed that sharks classed 0, 1 or 2 were not markedly devalued. This provided an estimate of 9.2% for gummy shark and school shark combined (9.0% for gummy shark and 12.8% for school shark), 8.5% from BS and 11.1% from SA (Table 3a). Estimates of catch devalued were 4.2% for common sawshark and southern sawshark combined (4.0% for common sawshark and 5.5% for southern sawshark) and 6.1% for elephant fish (Table 3b).

Discussion

Differences in sample size between the species results from differences in the number of animals caught between the species. In particular, low sample size for school shark reflects the low catches of school shark taken by shark fishermen mainly targeting gummy shark. The estimates of lost damaged catch and devalued retained damaged catch are imprecise for this species because of low sample size. This is indicated by the comparatively high standard error values for school shark estimates of lost carcass mass.

Differences in the levels of damaged catch between species captured in gillnets depends on the period of survival after being enmeshed. School shark has the highest levels of lost damaged catch (6.9%) and devalued retained damaged catch (12.8%). This species dies quickly in gillnets, because it lacks spiracles and needs to swim for ram-jet ventilation of the gills. Elephant fish lack spiracles and have intermediate levels of lost damaged catch (3.4%) and devalued retained damaged catch (6.1%). Gummy shark possess spiracles that improve survival, but these enable the species to struggle, which leads to either escape or the animals becoming tightly enmeshed around the gills after capture and eventually causing death. This species has intermediate levels of lost damaged catch (4.7%) and devalued retained damaged catch (9.0%). Common sawshark and southern sawshark have the lowest levels of lost damaged catch (2.3% for species combined) and devalued retained damaged catch (4.2% for species combined). These species are likely to be able to ventilate their gills and survive longer after capture than most species because they not only have spiracles, but tend to be tangled in gillnets by their blade-like rostrum with teeth rather than being enmeshed around the gills.

References

Brown, L. P. and Walker, T. I. (1999). Pilot fishery-independent fixed-station survey for Southern Shark Stocks: Report on the Furneaux Group cruise. 7 pp. (SharkFAG/99/D5).

Prince, J. D. (1998). Pilot fishery-independent fixed-station survey for Southern Shark Stocks: Report on the Robe cruise. 21pp. (SharkFAG/98/D6).

Walker, T. I. (1983). 'Investigations of the gummy shark, *Mustelus antarcticus* Günther, from south-eastern Australian waters'. Report to Fishing Industry Research Committee. June 1983. In 'Proceedings of the Shark Assessment Workshop, South East Fisheries Committee Shark Research Group, Melbourne.' 7–10 March 1983. 94pp. (Department of Primary Industry: Canberra).

Walker, T. I. (1986). Southern Shark Assessment Project. Second Review. October 1986. *Marine Science Laboratories Progress Review* No. 66. 48pp. (Marine Science Laboratories: Queenscliff).

Walker, T. I. (in prep). Spatial and temporal variation in the reproductive biology of gummy shark (*Mustelus antarcticus*) harvested off southern Australia. Prepared for *Marine and Freshwater Research* **00**, 00–00.

Walker, T. I., Hudson, R. J., and Gason, A. S. (2005). Catch evaluation of target, byproduct, and bycatch species taken by gillnets and longlines in the shark fishery of south-eastern Australia. In 'Proceedings of North Atlantic Fisheries Organisation Symposium. Elasmobranch Fisheries: Managing for Sustainable Use and Biodiversity Conservation'. 11–13 September 2002. Santiago de Compostela, Spain. *Journal of Northwest Atlantic Fishery Science* **35**, 505–530.

Acknowledgments

Professional shark fishers Ron Anthony, Ron Atterton, Steve Brockwell, Rod Casement, Mick Cook, Harry Ferrier, Jon Gazam, Mark Goulden, Neil Hosking, Adrian Rodgers, Arthur Sifford, and Robert White are thanked for accommodating scientific observers on their vessels. Lauren P Brown and Jeremy D. Prince are acknowledged for serving as scientific observers on the vessels for part of the study. The study was undertaken as part of FRDC Project 1999/103 and the AFMA funded Pilot Fishery-Independent Fixed-Station Survey for the Gillnet Hook and Trap Fishery.

Table 1. Condition of animals when landed on the deck of vessel

Species	Number of animals				Percentage of animals			
	Alive		Dead	Total	Alive		Dead	Total
	Strong	Weak			Strong	Weak		
Gummy shark	753	796	2,765	4,314	18	18	64	100
School shark	15	17	79	111	14	15	71	100
Common sawshark	251	268	259	778	32	35	33	100
Southern sawshark	98	57	62	217	45	26	29	100
Elephant fish	21	143	232	396	5	36	59	100
Total	1,138	1,281	3,397	5,816	20	22	58	100

Table 2. Mean total mass and carcass mass estimated from total length and mean carcass mass lost from damage

s.e., standard error; s.d., standard deviation.

Species	Region	Sample size	Total mass (kg)			Carcass mass (kg)			Carcass mass lost (kg)			Carcass mass lost (%)		
			Mean	s.e.	s.d.	Mean	s.e.	s.d.	Mean	s.e.	s.d.	Mean	s.e.	s.d.
Gummy shark	Bass Strait	2969	4.111	0.035	1.899	2.786	0.026	1.401	0.134	0.008	0.452	4.817	0.275	14.990
	South Australia	848	4.519	0.078	2.271	3.080	0.058	1.701	0.140	0.016	0.453	5.080	0.541	15.749
	Total	3817	4.202	0.032	1.994	2.851	0.024	1.478	0.135	0.007	0.452	4.876	0.245	15.161
School shark	Bass Strait	86	6.438	0.517	4.797	4.436	0.379	3.511	0.122	0.052	0.487	2.907	1.276	11.837
	South Australia	59	7.151	0.449	3.452	4.769	0.307	2.362	0.594	0.155	1.188	13.644	3.308	25.408
	Total	145	6.728	0.357	4.302	4.571	0.257	3.090	0.314	0.073	0.873	7.276	1.599	19.254
Gummy & school shark	Bass Strait	3055	4.177	0.037	2.072	2.832	0.028	1.525	0.133	0.008	0.453	4.764	0.270	14.912
	South Australia	907	4.690	0.081	2.451	3.190	0.060	1.799	0.169	0.018	0.543	5.637	0.553	16.664
	Total	3962	4.294	0.035	2.175	2.914	0.025	1.598	0.142	0.008	0.475	4.964	0.244	15.333
Common sawshark	Total	1099	2.866	0.036	1.180	1.966	0.024	0.785	0.041	0.007	0.222	2.169	0.333	11.042
Southern sawshark	Total	315	1.771	0.030	0.524	1.055	0.015	0.273	0.039	0.009	0.165	3.498	0.815	14.457
Total sawshark	Total	1414	2.622	0.031	1.162	1.763	0.021	0.799	0.041	0.006	0.211	2.465	0.316	11.895
Elephant fish	Total	916	2.537	0.038	1.149	1.635	0.024	0.737	0.056	0.008	0.246	3.044	0.436	13.198

Table 3a. Estimates of retained and lost catch of gummy shark and school shark from predation

0, nil damage; 1, negligible damage (1–2% loss of carcass with most damage to gills, cloaca or viscera); 2, minor damage (3–9% loss of carcass with most damage to gills, cloaca or viscera); 3, extensive damage (10–100% loss of carcass mass).

Region	Damage	Gummy shark					School shark					Gimmy & school shark combined				
	index	Sample	Carcass mass (kg)				Sample	Carcass mass (kg)				Sample	Carcass mass (kg)			
		size	Retained	Lost	Total	%	size	Retained	Lost	Total	%	size	Retained	Lost	Total	%
Amount of retained and lost catch and percentage of total catch																
Bass Strait	0	2220	6194	0	6194	75	73	323	0	323	85	2293	6517	0	6517	75
	1	32	93	1	94	1	2	10	0	10	3	34	102	1	103	1
	2	333	908	40	948	11	4	12	1	12	3	337	920	41	961	11
	3	384	679	356	1035	13	7	26	10	36	9	391	705	366	1071	12
	Total	2969	7874	398	8272	100	86	371	10	381	100	3055	8245	406	8652	100
	%		95.2	4.8	100.0			97.2	2.8	100.0			95.3	4.7	100.0	
South Australia	0	696	2184	0	2184	84	37	191	0	191	68	733	2375	0	2375	82
	1	2	5	0	5	0	0	0	0	0	0	2	5	0	5	0
	2	21	54	3	56	2	1	2	0	2	1	22	56	3	59	2
	3	129	251	116	367	14	21	53	35	88	31	150	304	151	455	16
	Total	848	2493	119	2612	100	59	246	35	281	100	907	2740	153	2893	100
	%		95.5	4.5	100.0			87.5	12.5	100.0			94.7	5.3	100.0	
Total	0	2916	8378	0	8378	77	110	514	0	514	78	3026	8893	0	8893	77
	1	34	97	1	98	1	2	10	0	10	1	36	107	1	108	1
	2	354	962	43	1005	9	5	14	1	15	2	359	976	44	1020	9
	3	513	930	472	1402	13	28	79	45	124	19	541	1009	517	1526	13
	Total	3817	10367	515	10882	100	145	617	46	663	100	3962	10983	563	11545	100
	%		95.3	4.7	100.0			93.1	6.9	100.0			95.1	4.9	100.0	
Percentage of retained catch devalued from major damage																
Bass Strait			8.6					7.0					8.5			
South Australia			10.1					21.6					11.1			
Total			9.0					12.8					9.2			

Table 3b. Estimates of retained and lost catch of common sawshark and southern sawshark from predation

0, nil damage; 1, negligible damage (1–2% loss of carcass with most damage to gills, cloaca or viscera); 2, minor damage (3–9% loss of carcass with most damage to gills, cloaca or viscera); 3, extensive damage (10–100% loss of carcass mass).

Region	Damage	Common sawshark					Southern sawshark					Sawshark combined					Elephant fish				
	index	Sample	Carcass mass (kg)				Sample	Carcass mass (kg)				Sample	Carcass mass (kg)				Sample	Carcass mass (kg)			
		size	Retained	Lost	Total	%	size	Retained	Lost	Total	%	size	Retained	Lost	Total	%	size	Retained	Lost	Total	%
Amount of retained and lost catch and percentage of total catch																					
Southern	0	1020	2010	0	2010	93	285	299	0	299	90	1305	2309	0	2309	93	839	1351	0	1351	90
Australia	1	4	9	0	9	0		0	0	0	0	4	9	0	9	0	0	0	0	0	0
	2	7	12	1	13	1	3	3	0	3	1	10	16	1	16	1	4	7	0	8	1
	3	68	84	45	129	6	27	18	12	30	9	95	102	57	159	6	73	88	51	139	9
	Total	1099	2116	45	2161	100	315	320	12	332	100	1414	2435	58	2493	100	916	1446	51	1498	100
	%		97.9	2.1	100.0			96.3	3.7	100.0			97.7	2.3	100.0			96.6	3.4	100.0	
Percentage of retained catch devalued																					
			4.0					5.5					4.2					6.1			