The biology of sharks of the family Carcharhinidae from the nearshore waters of Cleveland Bay, with particular reference to *Rhizoprionodon taylori*.

Thesis submitted by
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in April 1993

for the degree of Doctor of Philosophy in
the Department of Zoology at
James Cook University of North Queensland.

FRONT PIECE: ***Rhizoprionodon taylori***

"This is the most common of all the smaller galeids on our coast"

J. Douglas Ogilby. 1915
Mem. Qld. Mus., 3, p.132

DECLARATION

I declare that this thesis is my own work and has not been submitted in any other form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished works of others has been acknowledged in the text and a list of references is given.

29/4/93

Colin A. Simpfendorfer Date

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ABSTRACT

Sharks of the family Carcharhinidae occurring in the nearshore waters (<5m depth) of Cleveland Bay, northern Queensland, were studied between June 1987 and February 1990. Specimens were collected using gillnets at three sites in Cleveland Bay, as well as demersal otter trawls throughout Cleveland Bay. The aims of the study were to examine the biology of the species of carcharhinid sharks that occur in the nearshore waters of Cleveland Bay and to determine the importance of this area to populations of carcharhinid sharks.

Thirteen species of the family Carcharhinidae were examined. One species - *Rhizoprionodon taylori* - was particularly abundant and most of the research was directed at this species. Of the remaining twelve species all were normally caught as juveniles. At least six species, not including *R. taylori*, utilise Cleveland Bay as a communal nursery area. Two patterns of nursery area utilisation were identified. Juvenile *R. taylori* also occurred, but their distribution overlapped with that of the adults. The importance of nearshore waters to populations of carcharhinid sharks, and the role that the sharks have in these areas, are discussed.

The distribution of *R. taylori* was analysed using catch rates from gillnets. There was no significant difference in the catch rate of *R. taylori* between bottom or surface set nets, or between nets set at each of the three main sampling sites. The catch rate of *R. taylori* was significantly different between seasons and years of this study, and was influenced by temperature, salinity, tidal variation and the amount of teleost by-catch. Mechanisms for the possible action of these factors are discussed.

Age and growth of *R. taylori* were studied using the techniques of vertebral ageing, back calculation and length frequency analysis. Vertebrae contained circuli produced annually in January or February, possibly as a result of stress during mating. The oldest male examined was 5.7 years old, and the oldest female 6.9 years. The age

at maturity was one year. Von Bertalanffy growth parameters estimated from vertebral ageing data for males were t_0=0.41yr, K=1.337, L_∞= 652mm TL, and for females t_0=0.46yr, K=1.013, L_∞= 733mm TL. Growth parameters estimated by length frequency and back calculation techniques concurred with those from vertebral ageing.

Stomach content analysis was used to investigate the food and feeding habits of *R. taylori*. The most common identifiable prey were members of the demersal teleost families Leiognathidae, Clupeidae and Teraponidae, the prawn family Penaeidae, and the squid family Loliginidae. 59.4% of specimens had no food in their stomachs. The mean weight of prey in the stomachs containing food was 14.1g (1.1% body weight), and the mean weight of original prey items (prior to digestion) was estimated to be 29.5g (2.3% body weight). It was estimated that individual *R. taylori* feed, on average, every two days, normally on a single prey item.

Maturity in *R. taylori* occurs at 560mm TL in males and 575mm TL in females. Mating occurs in late January and early February. Mature females mate every year, producing litters of one to ten offspring. The gestation period of 11.5 months is divided into an initial seven month period of embryonic diapause, followed by a 4.5 month period of development. During the diapause period embryos remain at the blastoderm stage. Nutrition of the embryos is initially lecithotrophic, however, embryos over 30mm TL have structures to absorb secretions from the uterus and a yolk sac placenta forms during the third month of development. The young of *R. taylori* are born in mid-January of each year at sizes ranging from 220 to 260mm TL.

The results indicate that *R. taylori* has an atypical life history when compared to other members of the family Carcharhinidae. Factors that may cause intra- and inter-specific variations in life history traits are discussed. Finally, the results are discussed in relation to the management of shark stocks and nearshore waters.

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Acknowledgements

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Section I.

Introduction, study area and sampling

Chapter 1.

General Introduction

The family Carcharhinidae (whaler sharks) is a large group of common tropical and temperate sharks that includes many well known species (e.g. tiger, lemon, bull, blue and blacktip sharks). There are approximately 50 species in 12 genera recognised within the family (Springer 1964; Garrick 1982, 1985; Compagno 1984, 1988; Stevens and Wiley 1986; Lavery and Shaklee 1991). Compagno (1988) has suggested that there may also be a case for including the hammerhead sharks in the Carcharhinidae but for the purposes of this study the conventional view, that these are a separate family, was followed.

It is in tropical and sub-tropical inshore waters that the family Carcharhinidae is the most abundant and diverse (Garrick 1982; Compagno 1984). Some members of the family do occur in temperate areas, but the greatest diversity and abundance occurs between latitudes 30^{o} North and South (Garrick 1982). Only a few species are considered truly oceanic (e.g. *Carcharhinus falciformis*, *C. longimanus* and *Prionace glauca*), the remainder having primarily neritic distributions (i.e. over the continental shelves).

Species of the genus *Carcharhinus* are the dominant form of sharks in tropical inshore waters in terms of variety, abundance, and biomass (Garrick 1967). Other genera of the family (e.g. *Rhizoprionodon*, *Scoliodon*, and *Triaenodon*) are also very abundant in inshore waters where they occur (Springer 1964; Randall 1977; Compagno 1988). The abundance of members of the family, and their predatory feeding habits, make

them one of the major groups of high order predators in most tropical inshore marine ecosystems. Larger species (e.g. *Carcharhinus amboinensis*, *C. leucas*, *Galeocerdo cuvier*, and *Negaprion* spp.) are at the top of the trophic pyramid in tropical inshore regions (e.g. Springer 1967; Polovina 1984; Simpfendorfer 1992). Small and medium sized species are more abundant than the large species, but occupy lower trophic positions.

The abundance of carcharhinids in inshore tropical waters has resulted in their exploitation on both a subsistence level (Bonfil *et al.* 1990), and on a commercial basis (Anderson and Teshima 1990). Initial commercial exploitation of carcharhinid species was mostly as a source of vitamin A (Springer 1963), but this declined when synthetic vitamins became available. In more recent times shark flesh has gained wide acceptance as a source of protein, while their fins are used to make shark fin soup in south east Asia. Other commercial uses of shark products include the tanning of the skin for leather and the extraction of oils from the liver for a variety of purposes from lubricants to cosmetics.

Along with commercial exploitation has come research to provide a greater understanding of the biology of carcharhinid species and so enable effective management of stocks. This has been particularly important for sharks since their life history traits make them very susceptible to over-fishing (Holden 1974, 1977; Hoenig and Gruber 1990; Pratt and Casey 1990; Simpfendorfer 1991). Another factor that stimulated early research on sharks is their tendency to occasionally attack man (Gilbert *et al.* 1967). The region in which carcharhinid sharks have been studied in the most detail has been the western North Atlantic, especially off the east coast of the United States of America, and in the Gulf of Mexico (e.g. Bigelow and Schroeder 1948; Springer 1960, 1963; Clarke and von Schmidt 1965; Branstetter 1981; Snelson

and Williams 1981; Gruber 1982; Parsons 1983a; Branstetter and McEachran 1986; Hamlett 1986; Morrissey 1987; Gruber *et al.* 1988; Branstetter 1990; Hoff and Musick 1990). Other regions where research on carcharhinid sharks has been carried out include: the south western Indian Ocean (e.g. Wheeler 1959a,b, 1960, 1962; Bass *et al.* 1973, 1975; van der Elst 1979; Cliff *et al.* 1988; Cliff and Dudley 1991; Smale 1991; Walter and Ebert 1991), South East Asia (e.g. Mahadevan 1940; Setna and Sarangdhar 1948, 1949a,b; Taniuchi 1971; Nair 1976; Teshima *et al.* 1978; Devadoss 1979; Krishnamoorthi and Jagadis 1986), islands of the Pacific Ocean (e.g. Hobson 1963; Clarke 1971; Randall 1977; Johnson 1978; McKibben and Nelson 1986; Nelson *et al.* 1986), and the western South Atlantic Ocean (e.g. Ferreira 1988; Lessa 1988).

Research on sharks of the family Carcharhinidae has also been undertaken in Australian waters in response to increasing exploitation in northern Australia by both local and foreign fishermen. Fisheries related studies have been carried out in tropical Australia in recent years, primarily by the CSIRO, Northern Territory Fisheries, and the Australian Fisheries Service. These studies have concentrated on the region between the North West Shelf and far northern Queensland (Figure 1.1). Initially this work produced basic fisheries related information (e.g. Church 1981; Millington 1981; Okera *et al.* 1981; Church *et al.* 1982; Stevens *et al.* 1982; Lyle and Timms 1984a,b; Lyle *et al.* 1984; Stevens and Church 1984; Welsford *et al.* 1984; Lyle and Griffin 1987; McPherson 1988; Stevens 1989), but more recently biological information on all of the species that occur in the region has been published (e.g. Stevens and Wiley 1986; Lyle 1987a; Davenport and Stevens 1988; Lavery and Shaklee 1989, 1991; Stevens and McLoughlin 1991; Lavery 1992). There has also been some research on the ecological role of sharks in inshore, nearshore and estuarine areas as part of studies on the ecology of these habitats (e.g. Blaber 1980, 1986; Blaber *et al.* 1985,

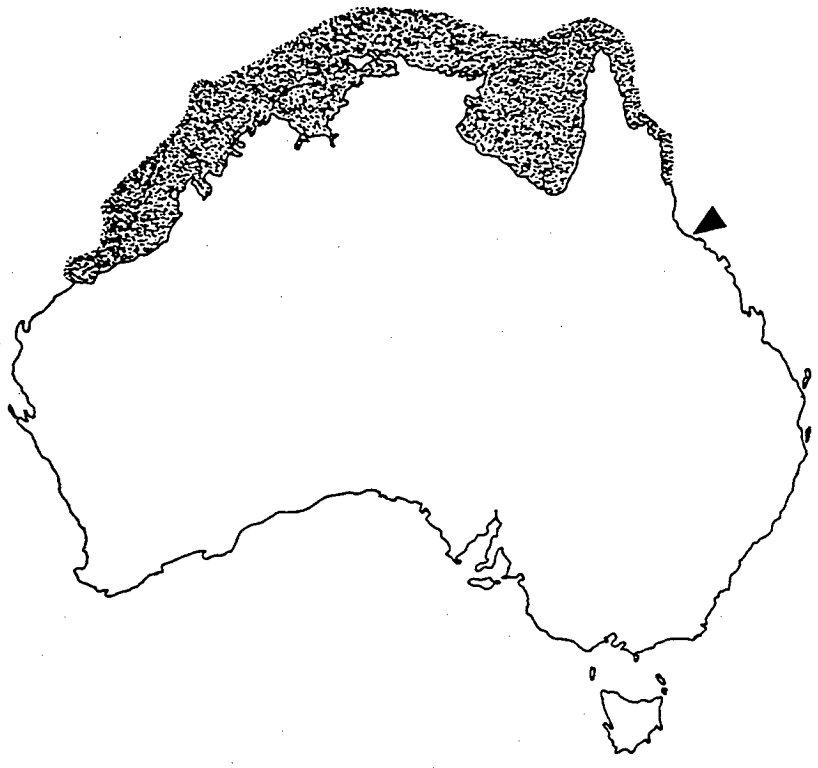


Figure 1.1: Region of shark fisheries related research previously carried out in northern Australia (stippled; from Fig. 1, Stevens and McLoughlin 1991) and location of the study area in Cleveland Bay, North Queensland (arrow).

1989, 1990a,b; Brewer *et al.* 1991; Salini *et al.* 1992). On the basis of both the ecological and fisheries studies, as well as taxonomic and identification studies (e.g. Garrick 1982, 1985; Compagno 1984, 1988) at least 29 species of the family Carcharhinidae are known to occur in the neritic and oceanic waters of northern Australia (Table 1.1).

Table 1.1: Species of shark from the family Carcharhinidae recorded from the waters of northern Australia. (Based on Garrick 1982 and Compagno 1984.)

Carcharhinus albimarginatus (Rüppell, 1837)
Carcharhinus altimus (Springer, 1950)
Carcharhinus amblyrhynchoides (Whitley, 1934)
Carcharhinus amblyrhynchos (Bleeker, 1856)
Carcharhinus amboinensis (Müller & Henle, 1839)
Carcharhinus brevipinna (Müller & Henle, 1839)
Carcharhinus cautus (Whitley, 1945)
Carcharhinus dussumieri (Valenciennes, 1839)
Carcharhinus falciformis (Bibron, 1839)
Carcharhinus fitzroyensis (Whitley, 1943)
Carcharhinus hemiodon (Valenciennes, 1839)
Carcharhinus leucas (Valenciennes, 1839)
Carcharhinus limbatus (Valenciennes, 1839)
Carcharhinus longimanus (Poey, 1861)
Carcharhinus macloti (Müller & Henle, 1839)
Carcharhinus melanopterus (Quoy & Gaimard, 1824)
Carcharhinus obscurus (LeSueur, 1818)
Carcharhinus plumbeus (Nardo, 1827)
Carcharhinus sealei (Pietschmann, 1916)
Carcharhinus sorrah (Valenciennes, 1839)
Carcharhinus tilstoni (Whitley, 1950)
Galeocerdo cuvier (Peron & LeSueur, 1822)
Loxodon macrorhinus Müller & Henle, 1839
Negaprion acutidens (Rüppell, 1837)
Prionace glauca (Linnaeus, 1758)
Rhizoprionodon acutus (Rüppell, 1837)
Rhizoprionodon oligolinx Springer 1964
Rhizoprionodon taylori (Ogilby, 1915)
Triaenodon obesus (Rüppell, 1837)

Although much work has been done off the northern coast of Australia, biological information specifically relating to carcharhinid species occurring off the east coast of Queensland is scarce. Some information is available from the work by CSIRO (e.g. Stevens and Wiley 1986; Stevens and McLoughlin 1991) but this was based on sampling north of Cairns (see Figure 1.1). Only Blaber (1980) and Simpfendorfer (1986, 1992) have provided any biological information on carcharhinid species from tropical waters off Cairns or further south. The family Carcharhinidae has received mention in the scientific literature for this region from time to time, but this has either been as part of taxonomic work (e.g. Whitley 1934, 1939, 1964), reports of occurrence (e.g. McKay and Beinssen 1988), or physiological work (e.g. Denton and Breck 1981; Baldwin and Wells 1990). These studies have provided very little general biological information.

The commercial catch of sharks in Queensland waters has increased enormously since the mid-seventies (Figure 1.2). This increase in the catch has resulted in the need for management of the stocks to be considered (Simpfendorfer 1991). Given the paucity of biological data on the east coast stocks, however, it has not been possible to make such considerations. This project was initiated in 1987 to provide biological information that would lead to an understanding of the populations of sharks in the coastal waters of the tropical east coast of Queensland, and thus provide a firmer basis on which some management options could be assessed. Research was focussed upon the family Carcharhinidae as it is the most commonly caught group in coastal waters, and represents the largest component of the commercial catch. The project was planned to build on the work of Simpfendorfer (1986) that had shown that Cleveland Bay, off Townsville ($19^{o}15'$S., $146^{o}55'$E.; see Figure 1.1), was used as a nursery area by several members of the family. During the initial stages of the

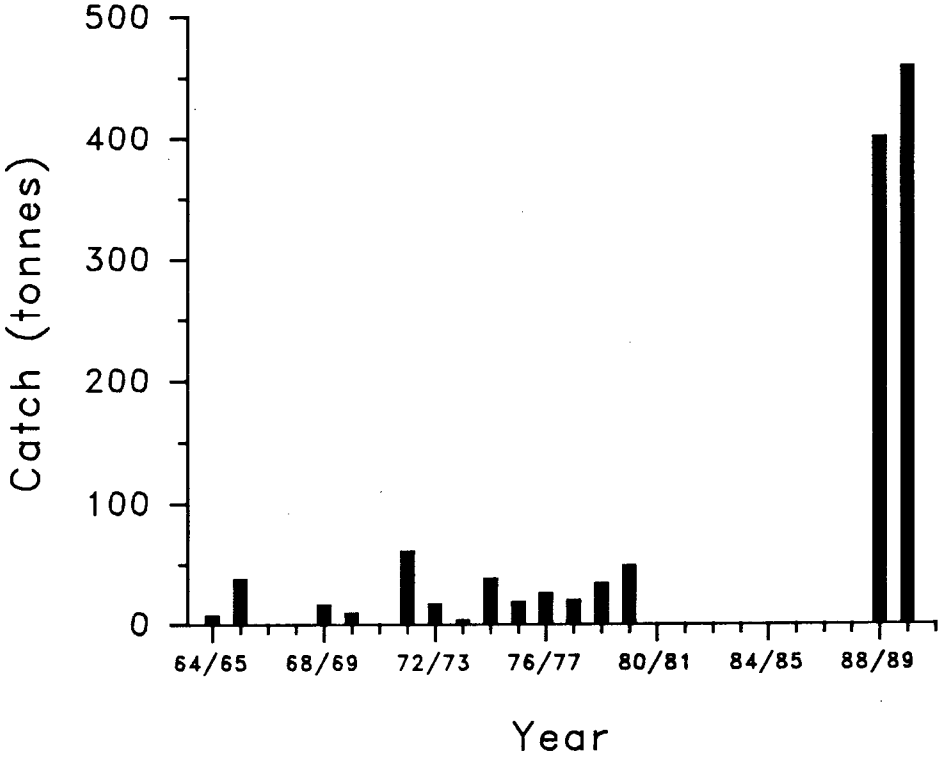


Figure 1.2: Catches of sharks reported by Queensland's commercial fishers between 1964/65 and 1989/90. Information from Australian Bureau of Statistics (1964/65 to 1979/80) and the QFMA/QDPI (1988/89 to 1989/90). No data are available for the period from 1980/81 to 1987/88.

project it was found that adult *Rhizoprionodon taylori* were also commonly caught. As a result the project was expanded to include a detailed investigation of the biology of *R. taylori*. The study was restricted to the nearshore waters of Cleveland Bay to allow adequate sampling within the resources available to the project. The term "nearshore" is defined in this study as those coastal waters that are less than five metres deep (below chart datum). Salini *et al.* (1992) used a similar definition in ecological studies in the Gulf of Carpentaria.

With the intention to examine the biology of carcharhinid sharks in nearshore waters two specific areas of investigation were undertaken. The aim of the first part was to examine the biology of *Rhizoprionodon taylori*. As this species was the most abundant in the study area most of the research was devoted to this component of work. This involved the collection, and analysis, of data on: spatial and temporal distribution (Chapter 3), age and growth (Chapter 4), food and feeding (Chapter 5), reproduction (Chapter 6), and embryonic development (Chapter 7). These data provided information on the utilisation of nearshore waters by *R. taylori*, its role in this habitat, and its life history, all of which are valuable when making management decisions. The aim of the second part was to investigate the biology of the other species of sharks from the family Carcharhinidae that occur in the nearshore waters of Cleveland Bay. This was mostly intended to examine the utilisation of nearshore waters as a nursery area and the role that the other species play in these ecosystems. The results of this component of work are presented in Chapter 8.

The thesis is split into four sections. The first section comprises the General Introduction (Chapter 1) and a Chapter describing the study area and sampling methods (Chapter 2). The second section includes the five Chapters describing the biology of *R. taylori*, and the third section the Chapter dealing with the other carcharhinid

species. The final section contains the General Conclusions. Due to the diverse nature of the subject matter investigated a review of previous literature is included in each Chapter as appropriate, rather than being entirely concentrated in the General Introduction.

Chapter 2.

Study area and sampling methods.

2.1 Study area

Cleveland Bay is a shallow embayment on the north-east coast of Queensland (19°15'S, 146°55'E; see Figure 1.1). It is approximately 27km wide and covers an area of 225km^2. The city of Townsville is located on its western shore and Magnetic Island is just north of the Bay on the western side (Figure 2.1). The principal habitat within the Bay is a soft mud substratum, together with areas of seagrasses (mostly *Cymodocea serrulata*, *Halophila* spp. and *Halodule uninervis*) and small coastal reefs. The southern shore of the Bay is lined with mangroves. Three substantial waterways enter the Bay: Ross River on the western side, and Crocodile and Alligator Creeks on the southern side. Tidal sections of these waterways also have extensive stands of mangroves. During the wet season (January to April) the outflow from these waterways, and the Burdekin River to the south, can cause large drops in the salinity of the Bay (Wolanski and Jones 1981; Walker 1981a). There have been a number of studies on the physical environment of Cleveland Bay, including information on temperature (Kenny 1974; Walker 1981b), salinity (Wolanski and Jones 1981; Walker 1981a), nutrients (Walker and O'Donnell 1981) and sediments (Belperio 1983). The maximum tidal variation recorded in Townsville is approximately 4.2m (Kenny 1979).

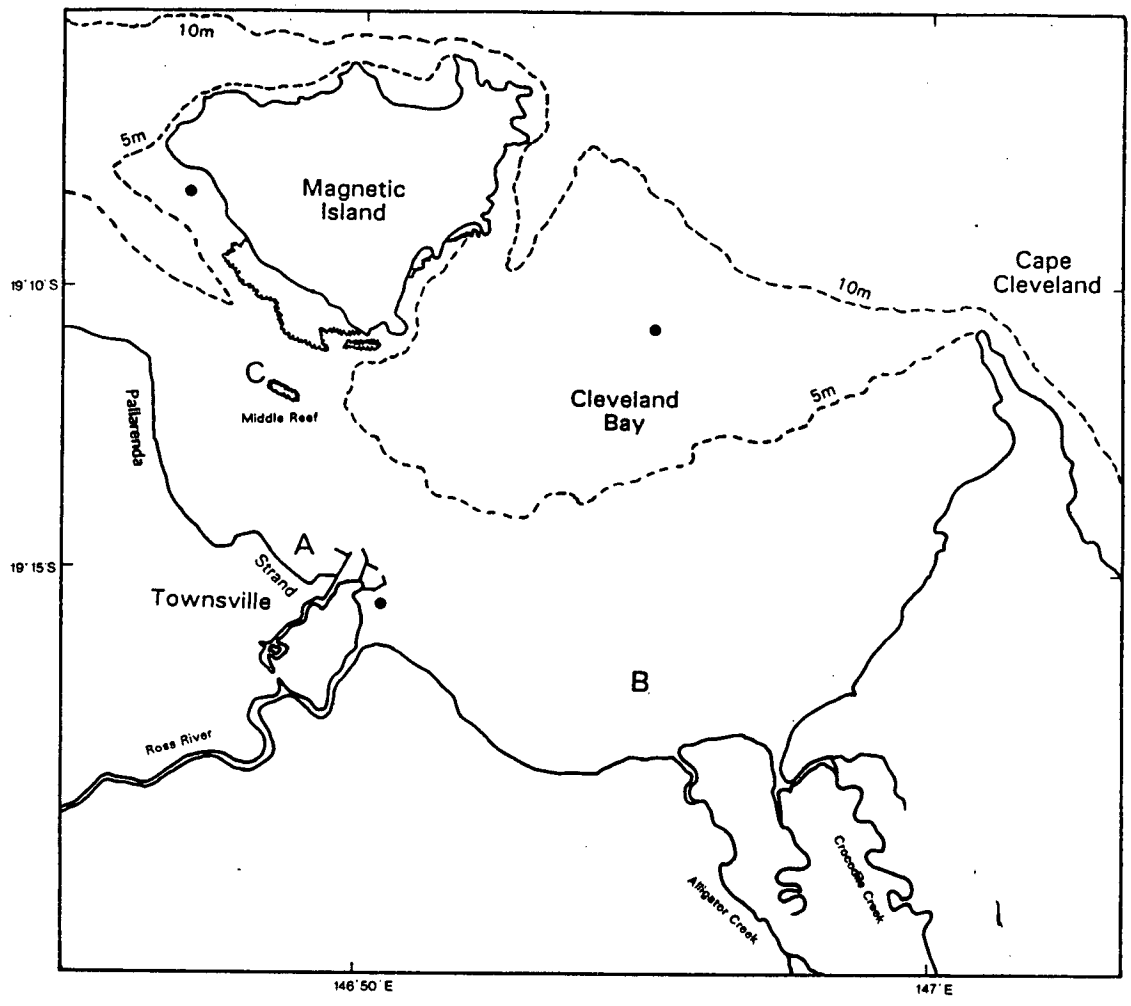


Figure 2.1: Details of the Cleveland Bay study area. The letters A-C signify the three main sampling sites: A, Strand; B, southern Bay; and C, Middle Reef. Dots represent other sampling sites. Isobaths are indicated by dashed lines.

2.1.1 Sampling sites

A number of sites were sampled within Cleveland Bay to obtain specimens. Three main sampling sites were used: 1-2km offshore from the Strand Beach, Townsville; adjacent to Middle Reef; and the southern area of Cleveland Bay off Alligator Creek (Figure 2.1). These sampling sites are referred to throughout this thesis as Strand, Middle Reef and southern Bay, respectively. The three sites were chosen as each encompassed a different habitat type within the Bay. Greater detail of these sampling sites is given below. Sampling was occasionally undertaken at other sites, but no specimens were obtained from them. These included Bolger Bay at Magnetic Island, a mid-Bay area, and the mouth of Ross River (Figure 2.1). Samples were also obtained from demersal otter trawls throughout Cleveland Bay and around Magnetic Island on an ad-hoc basis.

The Strand

Sampling occurred at the Strand on 40 occasions. The depth at this site varied from 3 to 4 metres, and the bottom was of sandy-mud with patches of rubble. The benthic assemblage includes patches of white gorgonian (*Solenocaulon grandis*), sponges, anemones and seagrass.

Middle Reef

Middle Reef is a small coral reef located between Pallarenda Beach and Magnetic Island (Figure 2.1). The reef lies in 4 metres of water, is approximately 1.5km long, and consists of several bommies. The main species of coral found at Middle Reef are of the genera *Acropora*, *Porites* and *Goniopora* (Dr. John Collins, Marine

Biology Department, James Cook University, pers. comm.). The substratum around the reef is mud, with patches of coral rubble. Sampling occurred at the western end of Middle Reef eight times and at the eastern end once.

Southern Bay

This site was situated in beds of seagrass (mostly *Cymodocea serrulata*) in the southern section of Cleveland Bay. Sampling occurred between 1 and 3 kilometres from shore in water 2 to 4m deep. The site was sampled 11 times.

2.2 Sampling methodology

2.2.1 Gillnetting

Specimens for this study were obtained primarily from sampling trips to the sites described above. Sampling trips occurred between May 1987 and February 1990. Vessels used for sampling were less than six metres in length and so the frequency and destination of these trips was dependent upon the prevailing sea conditions. Trips occurred mostly at night or in the late afternoon, although some were undertaken during the day to compare catches between day and night sampling.

Specimens were caught using gillnets constructed of monofilament nylon mesh hung on 6mm rope. Three mesh sizes were used: 5, 10 and 15cm. The 5cm net and one 10cm net were fished on the surface, and two 10cm nets and a 15cm net were fished on the bottom. All nets were 60m in length, except for the 5cm net which was 30m long. The hanging ratio of all nets was 0.6. The 5cm net was 1.6m deep, the 10cm nets 2.5m deep, and the 15cm net 3.2m deep.

Each sampling trip normally involved setting up to three 10cm nets, and from time-to-time 5cm and/or 15cm nets. The nets were set parallel to the prevailing wind. If there was no wind then they were set perpendicular to the shore or reef. Several metres of rope were left between each of the panels of net. Set times varied from 0.75 to 7.50 hours, with a mean set time of 2.82 hours. Set time was dependent mostly upon prevailing sea conditions, with sampling curtailed if conditions deteriorated. Nets were hauled over the stern and the sharks removed. Specimens caught were returned to the laboratory where identification, and detailed examination and dissection were undertaken. Details of other organisms caught in the nets were also recorded. Details of the gillnet sets are given in Appendix A.1.

Information from the night set 10cm gillnets was used to examine distributions. Sampling effort of these nets, measured in netting hours (one netting hour equivalent to one net set for one hour), varied considerably between seasons (summer, December to February; autumn, March to May; winter, June to August, and spring, September to November) and sampling sites (Figure 2.2). Sampling effort was pooled into seasons to reduce the number of categories for statistical analysis. Only the Strand was sampled in all 11 seasons during the study. The southern Bay site was sampled during six seasons, and Middle Reef during five seasons. The mean netting effort per season, for all locations combined, was 39.3 netting hours.

2.2.2 Demersal otter trawls

A small number of samples were obtained from otter trawls carried out by the R.V. "James Kirby" throughout Cleveland Bay and surrounding waters. These samples were obtained on an ad-hoc basis. The R.V. "James Kirby" operates paired demersal otter trawls, each with a mouth width of 12m. Details of trawls from which samples were obtained are given in Appendix A.2.

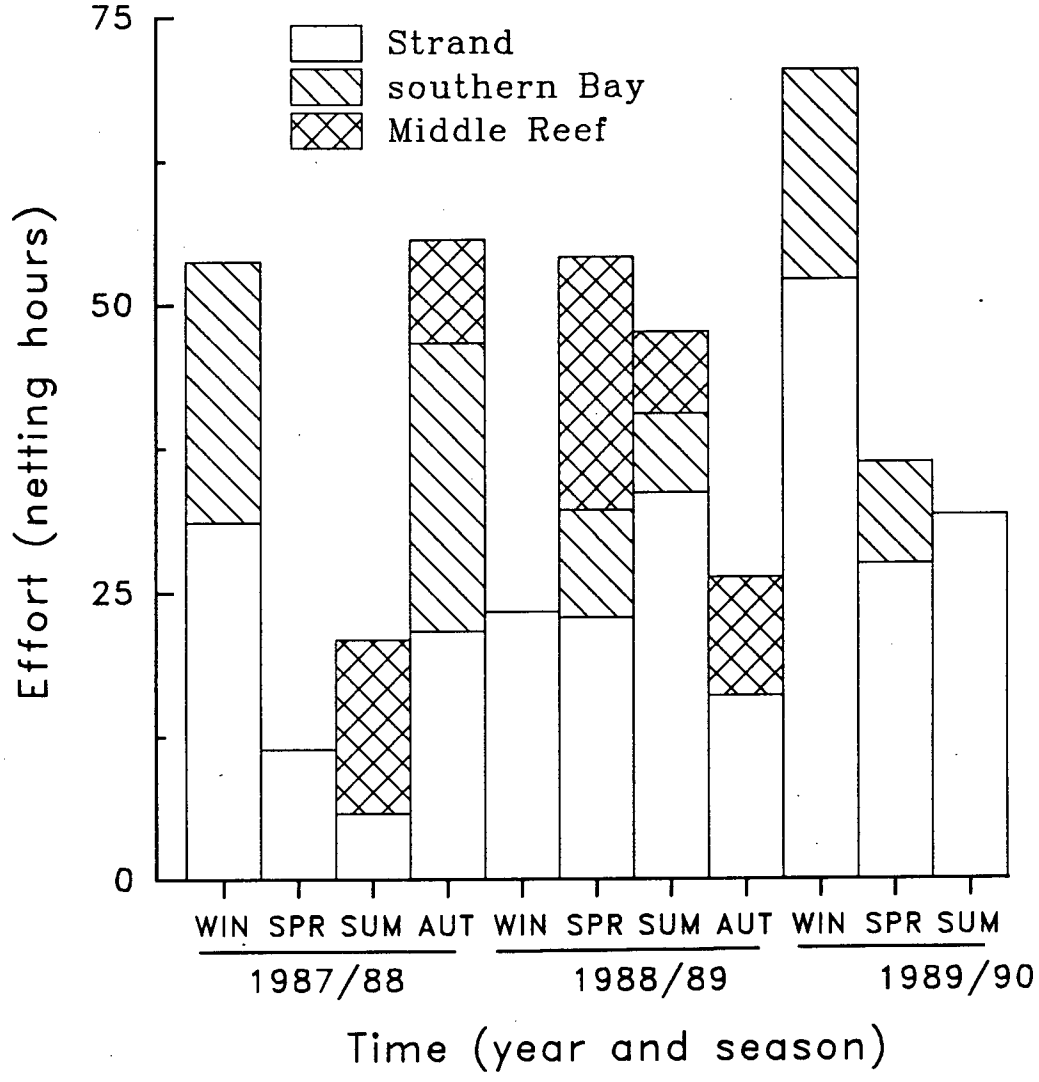


Figure 2.2: Total seasonal effort, in netting hours, of night set 10cm gillnets at three sites in Cleveland Bay between Winter 1987 and Summer 1990.

2.3 Data collected on sampling trips

2.3.1 Environmental parameters

Data relating to the physical, chemical and biological conditions were collected on each gillnet sampling trip. Seven environmental parameters were measured or recorded:

a. Sea surface temperature - measured to the nearest 0.1^{o}C using a mercury thermometer.

b. Sea surface salinity - a water sample was collected during each trip and the salinity determined in the laboratory using an inductive salinometer.

c. Depth - water depth was measured, either with a depth sounder or a weighted line, to the nearest 0.1m at the commencement of sampling.

d. Tidal variation - tidal variation was calculated as the difference between the water depth at the start of sampling and the end of sampling. When nets were fished over a changing tide any tidal variation in excess of that calculated from depth measurements was determined from tidal information for Townsville harbour.

e. Wind strength - obtained from meteorological data and observations during sampling trips.

f. Sea state - estimated based on meteorological data and observations during sampling trips.

g. Teleost by-catch - the by-catch of the nets was expressed as the number of teleosts caught in each net.

The values of environmental parameters from all gillnet sampling trips are given in Appendix A.3.

2.3.2 Identification of catch

The species of sharks caught were identified using keys in recent taxonomic works, including Compagno (1984, 1988), Garrick (1982, 1985) and Springer (1964). The only species that could not be identified using these keys were *Carcharhinus tilstoni* and *C. limbatus*, which have only recently been recognised as distinct species (Stevens and Wiley 1986; Lavery and Shaklee 1991). These two species were distinguished on the basis of differences given by Stevens and Wiley (1986) and Stevens (CSIRO Division of Fisheries, pers. comm.).

2.3.3 Measurement of the catch

The total length (TL) and fork length (FL) of specimens (Figure 2.3) were measured to the nearest millimetre. The relationships between fork length and total length for the commonly caught species are given in Table 2.1. These relationships were used to determine the total lengths of specimens with damaged caudal fins, or those for which only fork length was recorded. The weight of specimens less than 5kg was determined using an electronic balance accurate to the nearest gram, while a 200kg scale accurate to 0.5kg was used for larger specimens

Table 2.1: Fork length - total length relationships for commonly caught species of carcharhinid shark from Cleveland Bay based on data collected in the present study.

Species	n	relationship	r^2
Carcharhinus tilstoni	132	TL = 1.14(FL) + 48.90	0.991
Rhizoprionodon acutus	125	TL = 1.22(FL) + 6.57	0.984
R. taylori	448	TL = 1.15(FL) + 23.43	0.981

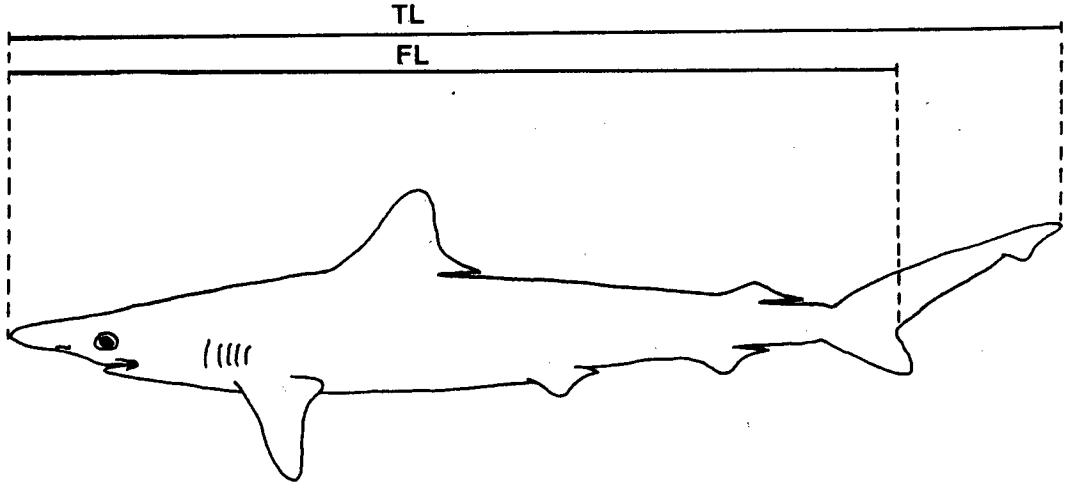


Figure 2.3: Length measurements taken from specimens caught in Cleveland Bay. TL, total length; FL, fork length.

Section II.

Biology of *Rhizoprionodon taylori*

Chapter 3.

Catch statistics and distribution of *Rhizoprionodon taylori*

3.1 Introduction

Rhizoprionodon taylori (Ogilby, 1915) is a small species of carcharhinid shark that is endemic to the continental shelf of Australia north of 30°S (Compagno 1984). Stevens and McLoughlin (1991) gave its range as the North West Shelf in Western Australia east to Brisbane, Queensland, at depths of 9 to 111 metres. Few other studies in northern Australia have specifically identified this species. Blaber (1980) recorded juveniles and adults as abundant in intertidal and subtidal areas of Trinity Bay, Cairns. Lyle and Griffin (1987) reported that *R. taylori* was more commonly caught on bottom, rather than surface, longlines in northern Australia. Lyle (1987b) noted that *R. taylori* occurred across northern Australia, and was particularly abundant in the eastern Gulf of Carpentaria. Blaber *et al.* (1990b) recorded *R. taylori* from Albatross Bay, in the eastern Gulf of Carpentaria, at a depth of 11-20m, but because of mis-identification probably under-estimated its abundance (Dr. John Salini, CSIRO Marine Laboratories, Cleveland, Queensland, pers. comm.).

The aims of this section of the study were to provide data on the effectiveness and selectivity of the gear used in catching *R. taylori*; to examine the distribution of *R. taylori*, both spatially and temporally, within Cleveland Bay based on catch rate data; and to investigate the influence that environmental factors may have on the catch rate of *R. taylori*.

3.2 Materials and methods

Details of the study area, sampling sites, sampling methods, and measurement of the catch, are given in Chapter 2. Length data were used to examine the size selectivity of the different sampling methods. A power curve (Equation 3.1) was fitted to the length (L) and weight (W) data to produce a length-weight relationship.

$$W = aL^b \qquad 3.1$$

where a is the intercept, and b is the slope, of the log-log transformed data.

The numbers of *R. taylori* caught in gillnets were converted to catch rates (sharks $net^{-1}hr^{-1}$) to allow for comparisons between nets. These comparisons were restricted to nets of the same mesh size due to differences in the materials used in their construction (e.g. line thickness) and their dimensions (e.g. drop) which would have affected their catching rates.

Analysis of the distribution of *Rhizoprionodon taylori*

Analysis of the spatial and temporal distribution patterns of *R. taylori* in Cleveland Bay was based on data from night set 10cm gillnets. The data were analysed using analysis of variance (ANOVA) utilising the statistical program "SAS" and based on SAS type III sums of squares (SAS Institute 1988). Factors that were not significant at any level of a model were removed and the analysis re-run until a final result was obtained. The improvement in the normality of the catch rate data when transformed using a logarithmic function was judged using the Shapiro-Francia statistic calculated by the "Statistix" computer program (Analytical Software [no

date]). If the Shapiro-Francia statistic increased substantially then the transformed data were used for the ANOVA and other statistical tests. In all tests the critical value of probability (α) used was 0.05.

Catch rates were compared between the three main sites using an ANOVA with two factors: site (Strand, southern Bay, or Middle Reef) and net type (surface or bottom). Site and net type were fixed effects.

Sampling effort was inconsistent at the southern Bay and Middle Reef sites (see Figure 2.2) and so temporal distribution patterns were only analysed for the Strand site. The initial ANOVA model specified for the temporal distribution analysis included three factors: year (June 1987 to May 1988, June 1988 to May 1989, and June 1989 to May 1990), season (see section 2.2), and net type (surface or bottom). Years were taken as June to May, and seasons were used instead of months, to reduce the possible combinations of factors and so increase the robustness of the model. Net type was a fixed effect, and year and season random effects.

The variance to mean ratio (VMR) of catch rates from 10cm gillnets was calculated by dividing the mean catch rate by the variance of the catch rate data. A VMR equal to one indicated a uniform distribution, a VMR less than one a random distribution, and a VMR greater than one an aggregated distribution.

Analysis of the influence of environmental factors on catch rates of *Rhizoprionodon taylori* in 10cm gillnets.

Multiple regression analysis was used to model the influence of environmental parameters on the catch rates of 10cm gillnets set at night in Cleveland Bay. The analysis was undertaken using the maximum R-square improvement technique in

the statistical program "SAS" (SAS Institute 1988). The final model selected had to fit several criteria: (1) it had to be statistically significant, (2) its value of Mallow's C_p had to be less than the number of independent variables (Analytical Software [no date]), and (3) it had to explain a reasonable proportion of the variation.

The environmental parameters included in the analysis were: surface temperature, surface salinity, depth, tidal variation and wind strength. Sea state was not included in the analysis as it was closely correlated with wind strength. Wind strength was used in the model instead of sea state because it could be more accurately determined. Also included in the analysis was the number of teleosts caught in the net as their presence may have attracted sharks. Data were pooled for surface and bottom nets, and for the three sampling sites, to provide data from 142 nets for analysis.

3.3 Results

A total of 454 specimens of *Rhizoprionodon taylori* were collected by all sampling methods between May 1987 and February 1990. Thirty six of these were caught in 5cm gillnets; 414 in 10cm gillnets; none in the 15cm gillnets; and four in demersal otter trawls.

The specimens caught ranged in size from 232mm TL to 784mm TL. The smallest specimens were caught in otter trawls off the north side of Magnetic Island in January 1989. The specimens caught in the 10cm gillnets ranged in size from 451 to 784mm TL, with the majority between 580 and 720mm TL (Figure 3.1). The mean size of *R. taylori* specimens caught in 10cm gillnets was 635mm TL. The mean size of specimens caught in the 5cm gillnet was 616mm TL, and ranged from 426 to 784mm TL.

The weight of specimens ranged from 57 to 2898g, with a mean of 1236g. There was a significant relationship between total length and total weight ($r^2=0.9438$, d.f.=445, $p<0.0001$). This relationship was:

$$WT = (2.00 \times 10^{-6}) TL^{3.1286} \qquad 3.2$$

3.3.1 Catch rates of 10cm gillnets

The catch rates of 10cm gillnets set during the day were lower than nets set at night. The mean catch rate for 16 day set 10cm nets was 0.076 sharks $net^{-1}hr^{-1}$, and at night the mean catch rate from 150 nets was 0.89 sharks $net^{-1}hr^{-1}$.

There was a significant correlation between the number of *R. taylori* caught in a net and the length of time the net was set (Figure 3.2a; $r^2=0.0758$, d.f.=162, p=0.0004). However, there was no significant correlation between the time a net was set and its catch rate (Figure 3.2b; $r^2=0.0203$, d.f.=162, p=0.0689).

The variance to mean ratio of the catch rates from night set nets was 3.51, indicating an aggregated distribution. Eighty one of the 150 sets of the 10cm gillnets that were analysed did not catch any *R. taylori*. Of the remainder most had catch rates less than 2 sharks $net^{-1}hr^{-1}$ (Figure 3.3), but reached as high as 11.53 sharks $net^{-1}hr^{-1}$.

The catch rate data were transformed for subsequent analyses as the Shapiro-Francia statistic increased from 0.548 to 0.762 with the logarithmic transformation.

Distribution of *Rhizoprionodon taylori*

The ANOVA model comparing the catch rates at the three main sampling sites included only one factor - location - as net type was not significant at any level (net type, d.f.=1, F=0.23, p=0.634; net type*site, d.f.=2, F=0.06, p=0.946). There were no significant differences in the mean catch rates between sites suggesting an homogeneous distribution of *R. taylori* in Cleveland Bay (Figure 3.4; ANOVA, d.f.=2, F=1.94, p=0.1474).

Net type was also excluded from the ANOVA model examining temporal distribution of *R. taylori* at the Strand site (net type, d.f.=1, F=0.19, p=0.660; net type*year, d.f.=2, F=0.01, p=0.988; net type*season, d.f.=3, F=0.61, p=0.610; net type*year*season, d.f.=5, F=0.44, p=0.818). The results of the final model, given in Table 3.1 show that seasons, years, and interaction terms (season*year)

all had significant effects on the catch rates of *R. taylori* at the Strand. The mean annual catch rate was highest in 1987/88, decreasing in 1988/89 and again in 1989/90 (Figure 3.5a). The mean seasonal catch rates, for all years combined, varied between 0.80 and 1.25 sharks $net^{-1}hr^{-1}$, with the highest catch rates in spring and summer (Figure 3.5b). Mean seasonal catch rates for all seasons in 1987/88 were above 1.50 sharks $net^{-1}hr^{-1}$, however, in 1988/89 and 1989/90 they were not above 1.00 sharks $net^{-1}hr^{-1}$ (Figure 3.6). The highest mean seasonal catch rates occurred in winter and spring of 1987/88 (Figure 3.6).

Table 3.1: Final results of ANOVA for the catch rates of *Rhizoprionodon taylori* in night set 10cm gillnets set at the Strand site between May 1987 and February 1990.

Factor(s)	d.f.	F	p
year	2	26.6	0.0001
season	3	3.92	0.0111
year*season	5	2.72	0.0247

Influence of environmental parameters on the catch rate of *R. taylori*

Mean monthly temperature and salinity values for Cleveland Bay varied through the study period (Figure 3.7). Temperature varied seasonally, with the highest values in the study recorded each year during January and February, and the lowest during June and July. Salinity did not show a distinct seasonal variation, remaining above 35 parts per thousand except for a period from January 1989 to August 1989.

The best results from the multiple regression analysis of environmental parameters were obtained when the absolute value of tidal variation was used (i.e. the amount

of tidal movement irrespective of whether it was ebb or flood). The model which provided the best prediction of catch rate included the parameters tidal variation (absolute value), salinity, temperature and by-catch (Table 3.2). This relationship was significant ($F=8.50$, d.f. $=4$, $p<0.0001$); Mallow's C_p value was 3.782, the only model to have a C_p value less than the number of independent variables; and the r^2 value was 0.2012. Tidal variation, salinity and temperature had positive effects, while by-catch was negative.

Table 3.2: Results of multiple regression model that provided the best prediction of the catch rate of *Rhizoprionodon taylori* in 10cm gillnets in Cleveland Bay.

Variable	Estimate	F	p
Intercept	-2.546	6.43	0.0124
Tidal variation	0.399	22.57	0.0001
Salinity	0.062	4.77	0.0308
Temperature	0.022	2.42	0.1224
By-catch	-0.041	4.61	0.0336

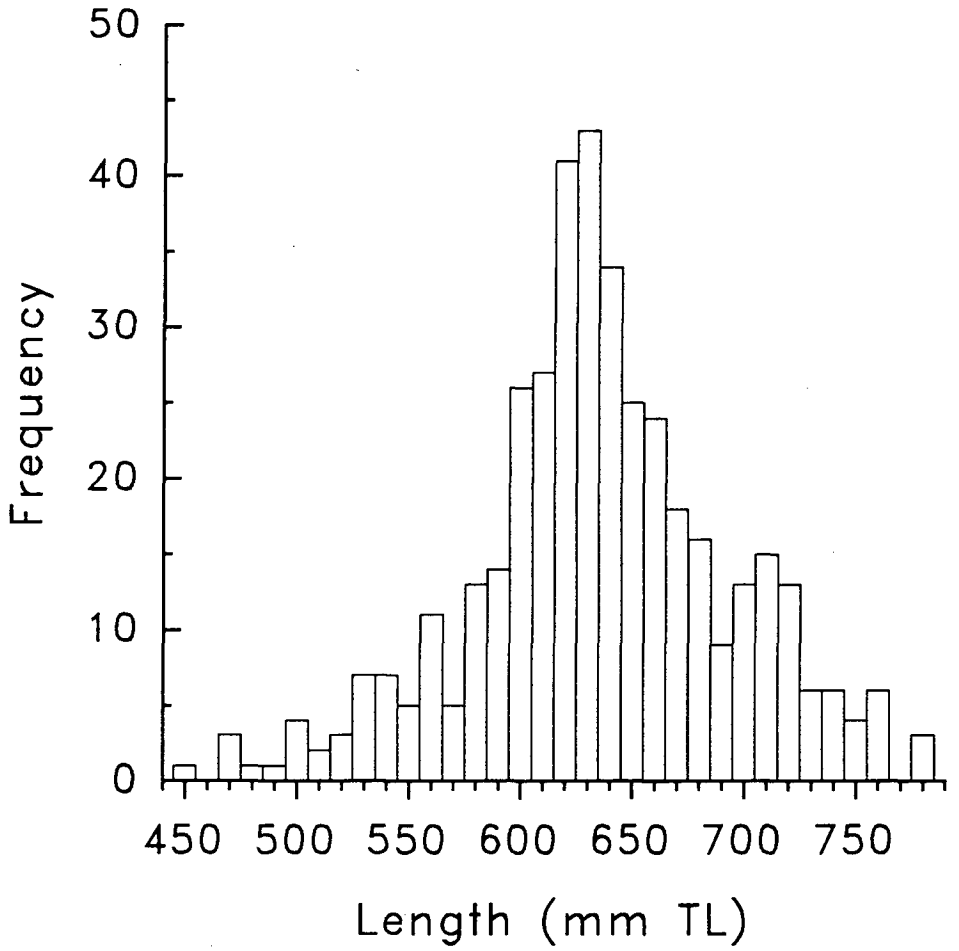


Figure 3.1: Length frequency distribution of *Rhizoprionodon taylori* specimens caught in 10cm monofilament gillnets set in Cleveland Bay.

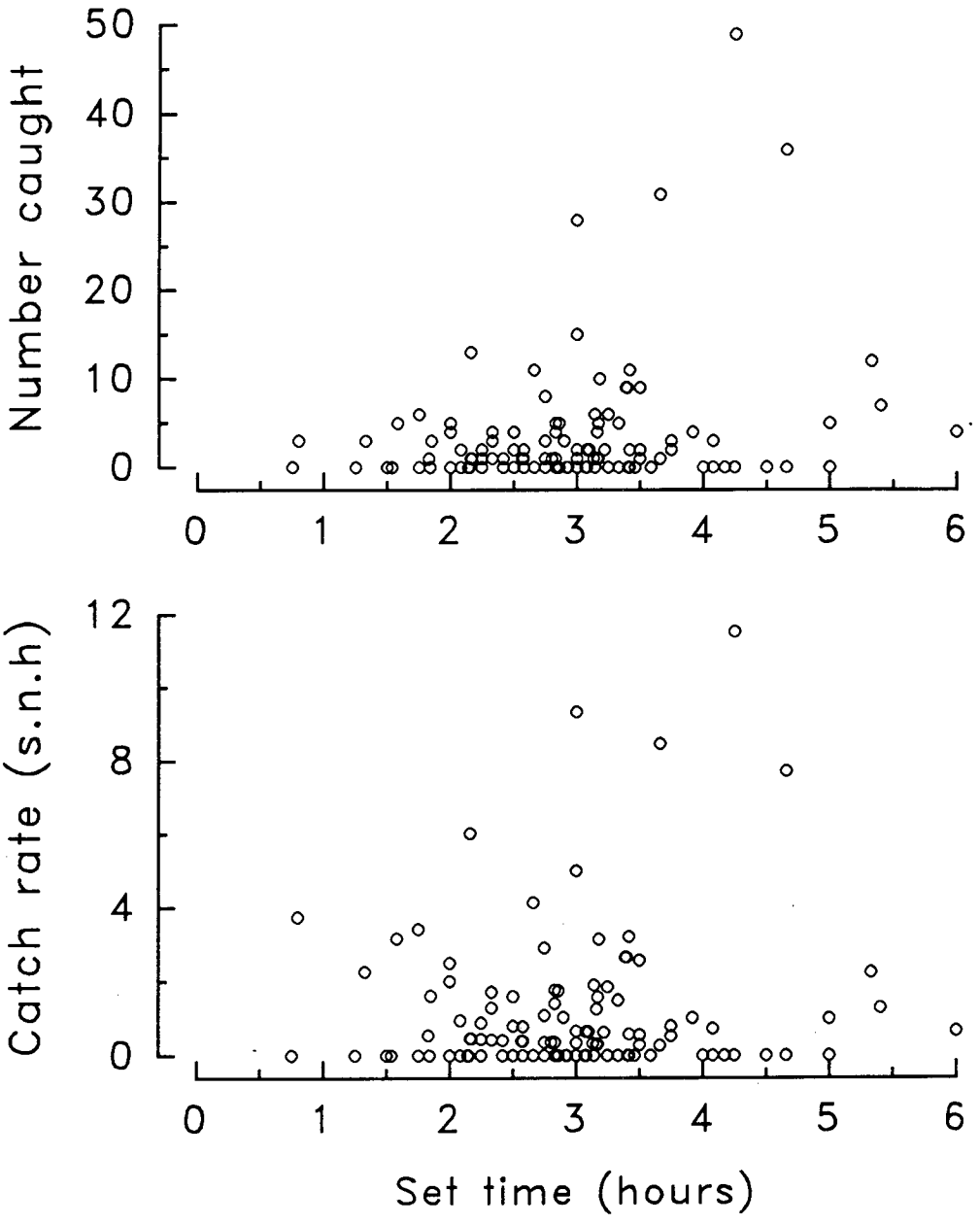


Figure 3.2: The effect of set time on (a) the number, and (b) catch rate, of *Rhizoprionodon taylori* caught in 10cm gillnets set in Cleveland Bay. s.n.h, sharks $net^{-1}hr^{-1}$.

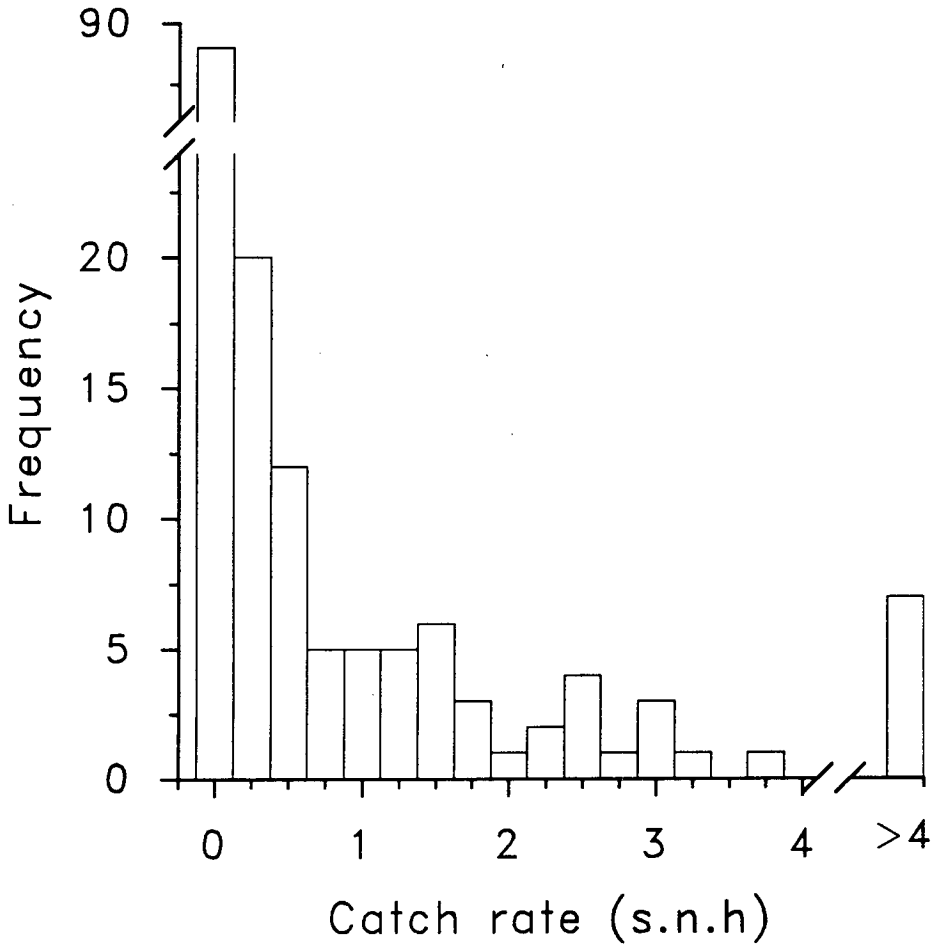


Figure 3.3: Frequency distribution of catch rates of *Rhizoprionodon taylori* caught in 10cm gillnets set in Cleveland Bay. s.n.h, sharks $net^{-1}hr^{-1}$.

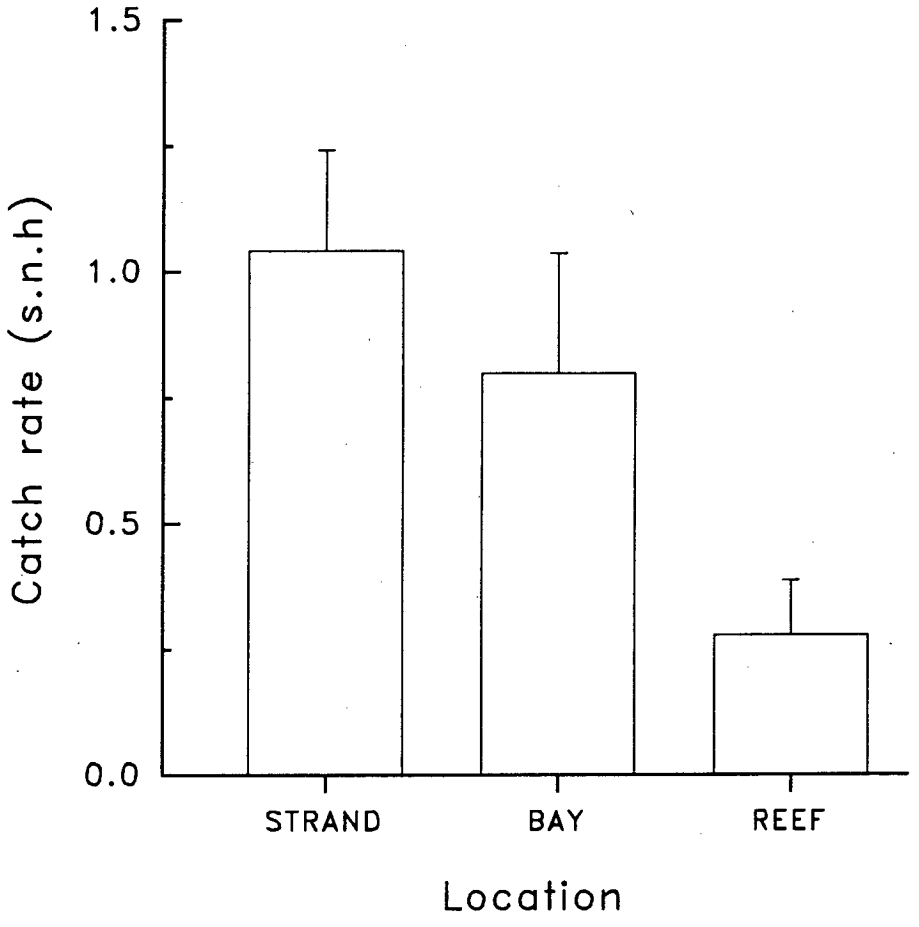


Figure 3.4: Mean catch rates of *Rhizoprionodon taylori* caught in night set 10cm gillnets at three sampling sites in Cleveland Bay. Error bars plus one standard error; s.n.h, sharks $net^{-1}hr^{-1}$.

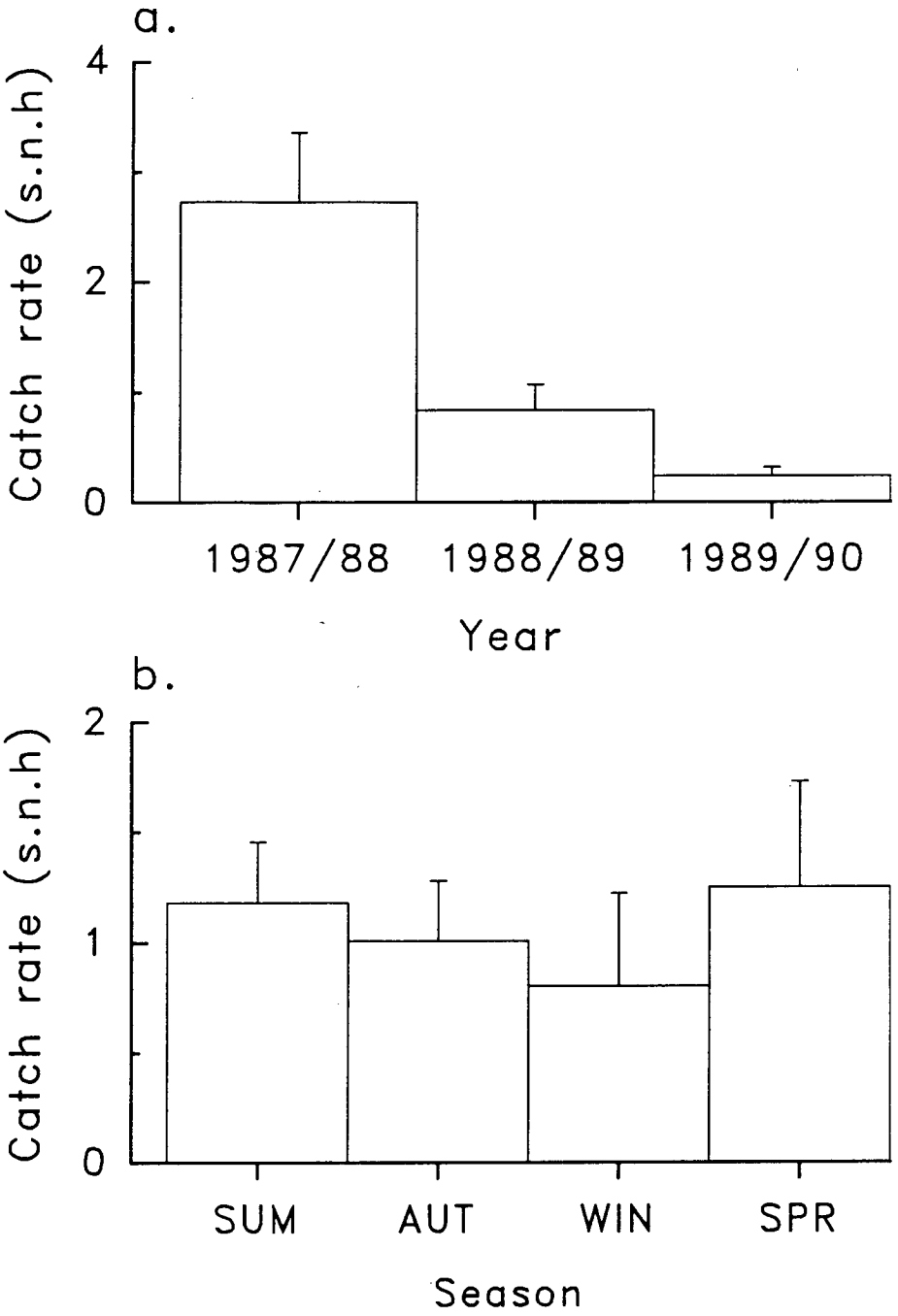


Figure 3.5: Mean annual (a), and seasonal (b), catch rates of *Rhizoprionodon taylori* in night set 10cm gillnets at The Strand, Cleveland Bay, between Winter 1987 and Summer 1990. Error bars plus one standard error; s.n.h, sharks $net^{-1}hr^{-1}$.

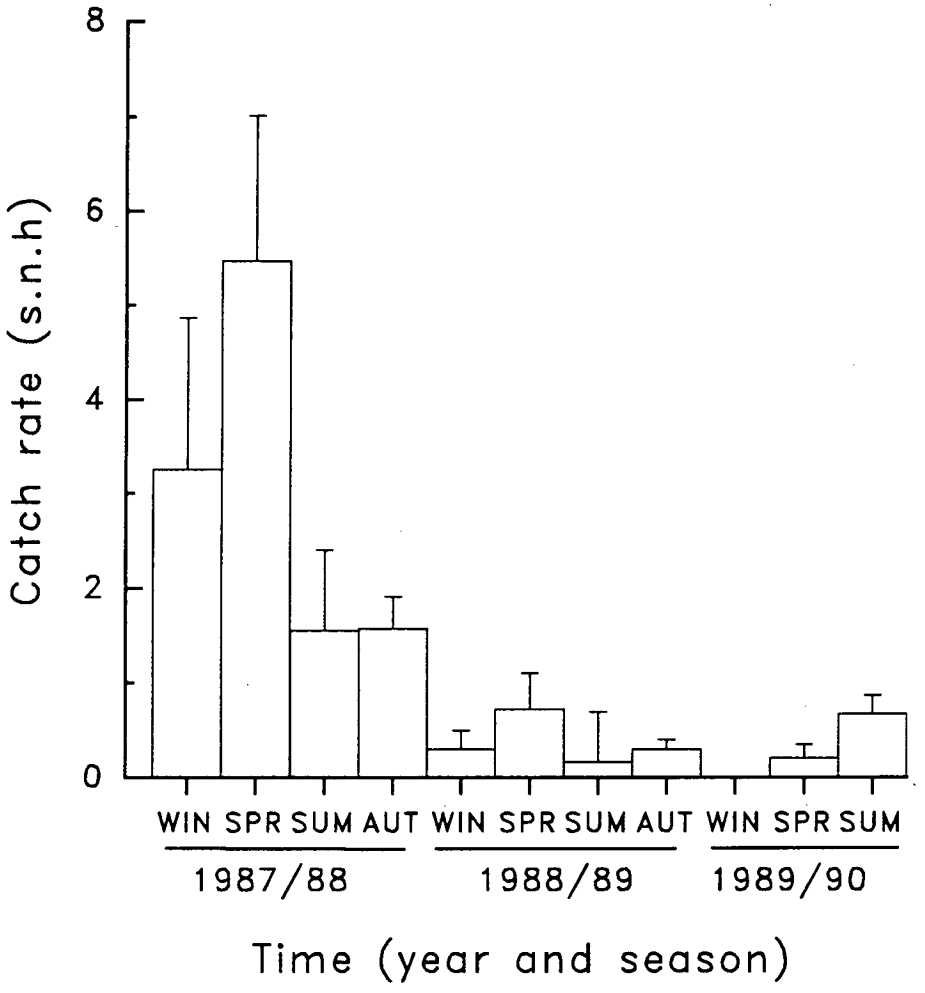


Figure 3.6: Mean seasonal catch rates of *Rhizoprionodon taylori* in Cleveland Bay for all years of this study. Error bars plus one standard error; s.n.h, sharks $net^{-1}hr^{-1}$.

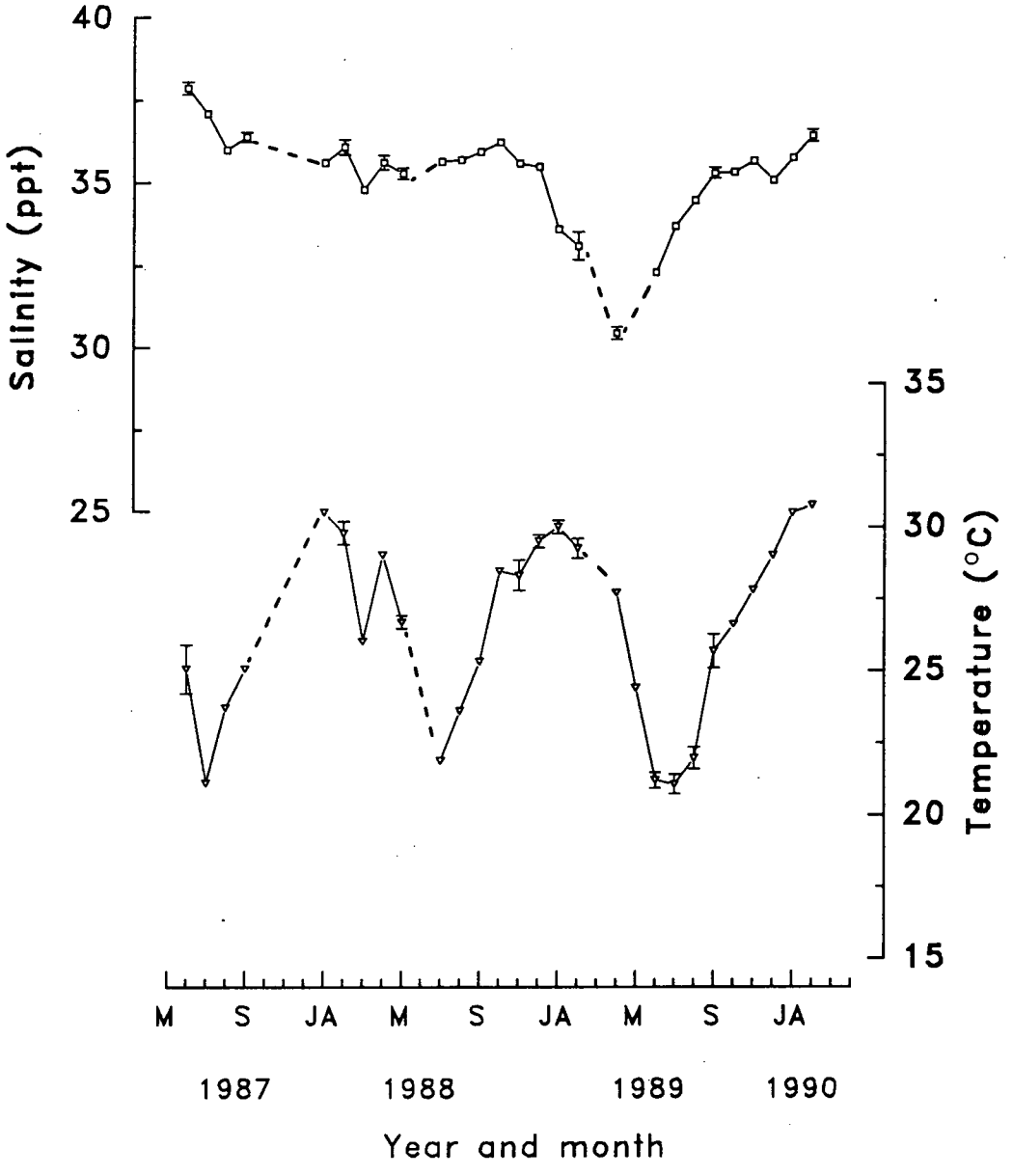


Figure 3.7: Mean monthly salinity and temperatures for Cleveland Bay from data collected during the sampling program. Error bars plus and minus one standard error

3.4 Discussion

Compagno (1984) gave the maximum size of *R. taylori* as 670mm TL, presumably based on a specimen examined by Springer (1964) from Salamander Rocks off Townsville. More recently, in work off the north coast of Australia, Stevens and McLoughlin (1991) recorded a maximum size of 660mm TL. Whitley (1940) described a 33 inch (838mm TL) specimen of *Protozygaena taylori* (=*R. taylori*) from Lindeman Island (149°E, 20°30'S) that contained two embryos approximately 215mm in length. Springer (1964), however, examined the embryo from this specimen and identified it as *R. acutus*. The maximum size of *R. taylori* in the present study (784mm TL) is therefore the largest recorded for this species. The larger size of *R. taylori* in Cleveland Bay suggests there may be growth differences between northern and eastern sections of the population.

The specimens of *R. taylori* obtained from Cleveland Bay covered the whole of the size range of the species. However, specimens in the size range from 230-580mm TL were poorly represented in the samples. This is probably a result of the selectivity of the 10cm gillnets which mostly caught specimens between 580 and 720mm TL, which corresponds to the size range of the adults (see Chapters 4 and 6). As a result few juveniles were caught. It was anticipated that the use of the 5cm gillnet would improve the representation of smaller specimens in the samples. However, the 5cm nets caught a similar size range of specimens to the 10cm net. This indicates that either smaller specimens were not present in the areas where the 5cm nets were used, or that the net had a similar selectivity to the 10cm nets. The latter of these is most likely since some smaller specimens were obtained. A consequence of the selectivity of the gear was that the analysis of catch rate data applied primarily to adults. Further resolution of the distribution and abundance of the smaller size

classes of *R. taylori* in Cleveland Bay will require sampling using gear capable of catching specimens less than 580mm TL (e.g. demersal otter trawls and small mesh gillnets).

Wallace (1972) reported that *Carcharhinus leucas* and *Carcharias taurus* were able to visually avoid multifilament gillnets during daylight. The low catch rates of *R. taylori* in nets set during the day are probably attributable to visual avoidance of monofilament netting. At night, however, it was assumed that sharks would not be able to see nets, thus explaining the higher catch rates. (However, there was also some evidence from this study for sharks (including *R. taylori*) being able to detect nets at night when sea conditions were calm and the nets produced a bioluminescent plume. Catches at such times were very low.) Another factor that may have increased the catch rate of *R. taylori* at night was increased activity. No information on the activity patterns of *R. taylori* is available, but tracking studies on other species of carcharhinid sharks have demonstrated that swimming speeds at night are higher than those during the day (e.g. McKibben and Nelson 1986; Gruber *et al.* 1988).

Lyle and Griffin (1987) suggested that *R. taylori* had a demersal habit. However, in Cleveland Bay adult *R. taylori* appear to utilise the whole water column at night in nearshore waters. Such behaviour was expected given the shallowness of the water at the sampling sites (most less than 4m deep). There were insufficient data to determine if they display similar behaviour during the day.

Environmental factors (tidal variation, salinity, temperature, and by-catch) were found to account for approximately 20% of the variation in the catch rate of *R. taylori* in Cleveland Bay. The factors that accounted for the remaining 80% of the

variation were not identified. Other factors responsible for variation in the catch rates may include behavioural traits, predator-prey interactions (e.g. Tricas 1979) and stochastic factors. The aggregated distribution of *R. taylori*, suggested by the VMR of the catch rates, may be one such behavioural factor. If as these data suggest *R. taylori* within Cleveland Bay are forming aggregations, then catch rates will largely be dependent upon one or more of these groups encountering the nets, and also the number of individuals in the groups. Aggregating behaviour has been well documented in the hammerhead shark *Sphyrna lewini* (Klimley and Nelson 1981; Klimley 1987; Klimley *et al.* 1988; Holland *et al.* 1992), but has been poorly documented in other species. The nature of the aggregations in *R. taylori*, if they exist, is unknown. It is also possible that the high VMR value was due to *R. taylori* being attracted to the nets by the presence of potential prey, but this was not supported by the results of the multiple regression analysis on the influences on catch rates which demonstrated an inverse relationship between by-catch and catch rate. The lack of a positive relationship between by-catch and *R. taylori* catch rate was probably due to the nets catching larger teleosts (e.g. mackerel and trevally) which do not represent potential prey for such a small species of shark.

The results indicate that the spatial distribution of adult *R. taylori* in Cleveland Bay is relatively homogeneous. Given the swimming ability of carcharhinids it is likely that *R. taylori* moves throughout Cleveland Bay and adjacent waters. A small tagging program (n=10) carried out on sharks in Cleveland Bay resulted in a single recapture of an *R. taylori* specimen. This specimen was tagged at the Strand and recaptured a month later in the southern part of Cleveland Bay (Simpfendorfer pers. data) showing that this species is capable at least of moving throughout the Bay. There was some evidence that non-adult *R. taylori* occurred more frequently at the

southern Bay site (see section 6.3.1), but there were insufficient data to test this hypothesis using catch records. It was also not possible to test for interactions between spatial and temporal factors given the inconsistent sampling at the southern Bay and Middle Reef sites.

The seasonal and yearly variations in catch rate at the Strand site may be attributable to several factors. Firstly, the decrease in mean annual catch rate may be largely due to decreases in population density caused by commercial fishing. Commercial gillnet fishermen in Cleveland Bay at times catch up to several hundred *R. taylori* in a day, although normal catches are much lower (Simpfendorfer pers. data). It is unlikely that the sampling for the present study was responsible for the decrease in catch rates given the size of commercial catches. Secondly, yearly and seasonal variation in catch rates may be the result of variations in environmental factors that influence catch rate (e.g. temperature and salinity). In particular the data suggest that the high catch rates obtained in Winter and Spring of 1987/88 may have been the result of high salinity affecting distribution, while seasonal variations in temperature may account for at least part of the seasonal variation in abundance of *R. taylori*. The influence of salinity on the distribution of *R. taylori* is of particular interest to management of stocks. If *R. taylori* is more abundant in Cleveland Bay when salinity is high then it will be more common in the Bay in years after poor wet seasons. The link between catch rate and salinity, and how distribution is affected need to be examined further. Seasonal variation in catch rates at the Strand site may also have resulted from seasonal movements of *R. taylori* within Cleveland Bay and/or adjacent waters. No data are currently available to determine whether this is the case.

Chapter 4.

Age and growth of *Rhizoprionodon taylori*

4.1 Introduction

There has been no previous research on the age or growth of *Rhizoprionodon taylori*. The aims of this section of the study were to develop a reliable method for determining the age of *R. taylori*; to use the estimates of age to determine the growth pattern of *R. taylori*; and to compare the age and growth of this species to other species of carcharhinid sharks. The techniques used to provide information on the age and growth of sharks are reviewed below, along with a summary of the growth patterns of other species of carcharhinid sharks.

4.1.1 A review of shark age and growth, with particular reference to the family Carcharhinidae

Age and growth studies on sharks have revealed that they are typically slow growing, long lived, and late maturing animals (Pratt and Casey 1990). Such a pattern has important implications for the dynamics of shark populations (Holden 1974). Thus the determination of age and growth parameters is essential to understanding shark life histories and the dynamics of populations (Cailliet and Tanaka 1990). The first section of this review covers the techniques used to determine age and growth parameters in sharks. The second section examines the age and growth parameters recorded for species of the family Carcharhinidae.

Methodology

Early work on the determination of growth parameters was based on techniques such as tagging (e.g. Kato and Carvallo 1967; Wass 1973) and size frequency analysis (e.g. Olsen 1954; Aasen 1966 in Stevens 1975). The effort required to obtain data for these techniques was arduous and so they were only obtained for a few species. Length frequency analysis is also limited by the slow growth of sharks which causes the overlap of year classes (Nair 1976; Ferreira and Vooren 1991). Length frequency analysis, however, is sometimes utilised to provide comparison with other techniques (Cailliet 1990).

More recent work on growth in sharks has centred around length at age techniques. These techniques require the age of specimens to be determined. Ageing techniques have been developed that utilise calcification patterns in the hard parts of sharks. The hard parts examined have normally been the vertebral centra (e.g. Stevens 1975; Branstetter *et al.* 1986), but fin spines have also been used for some species (e.g. Nammack *et al.* 1985; McFarlane and Beamish 1987). This review will deal only with ageing using vertebral centra since carcharhinid species do not have fin spines. However, similar techniques have been applied to ageing using fin spines.

The calcification of the vertebral centra results in a pattern of concentric bands (alternate opaque and translucent areas) and/or circuli (thin marks on the vertebrae) in the soft inner intermedialia and the hard outer corpus calcareum (Figure 4.1). The translucent and opaque bands correspond to periods of high and low calcification, respectively (Ferreira and Vooren 1991), while the circuli may be the result of stress causing disruption to the calcification process (e.g. Casey *et al.* 1985). The presence of marks on the vertebrae due to the pattern of calcification has been known for many years (e.g. Ridewood 1921). Haskell (1949 in Cailliet

et al. 1983) suggested that the bands could be used to age sharks. Early attempts to examine the patterns were experimental and examined the calcification patterns and how to observe them (e.g. LaMarca 1966; Applegate 1967). However, more recently these techniques have been applied to the determination of age.

A number of techniques have been developed to enhance the vertebral bands by staining either the heavily calcified areas (e.g. Alizaran red, silver nitrate, cobalt nitrate and crystal violet), or the areas between these which are high in protein (e.g. ninhydrin, mercurochrome) (Stevens 1975; Cailliet *et al.* 1983). Electron microprobe (e.g. Jones and Geen 1977; Cailliet and Radtke 1987) and x-radiograph (e.g. Martin and Cailliet 1988) techniques have also been applied to determine calcification patterns. The differences in the calcification pattern across the centrum are often reflected in the surface topography of the centrum face, a characteristic which Parsons (1983b) utilised by shading with a pencil to determine age. These techniques listed above have been applied to both whole centra, or thin sections of them. The use of these enhancement techniques has been discussed by Schwartz (1983), Cailliet *et al.* (1983), and Cailliet *et al.* (1986).

For the vertebral bands or circuli to be a useful ageing tool the periodicity with which they are laid down must be determined (Beamish and McFarlane 1983). In many species of elasmobranchs they have been shown to be laid down annually (e.g. Casey *et al.* 1985; Branstetter 1987b) and so the age is easily determined. However, in some species the bands are not produced annually (e.g. Pratt and Casey 1983; Brown and Gruber 1988; Natanson and Cailliet 1990). In reviewing ageing techniques Cailliet (1990) outlined six methods of verifying band periodicity: centrum edge analysis, comparison of results to parameters determined by size frequency analysis, comparison of results to back calculated growth parameters,

radiometric dating, tetracycline marking, and growth studies (field and laboratory). More details on age verification can be found in Beamish and McFarlane (1983) and Cailliet (1990).

Once age data have been collected and verified, growth parameters are normally obtained by fitting the von Bertalanffy growth curve (equation 4.1) to the length at age data. The parameters estimated with this growth function are: the asymptotic length (L_∞), a constant predicting the rate at which the asymptotic length is approached (K), the length at time t (l_t), and the age at length zero (t_0).

$$l_t = L_\infty(1 - e^{-K(t-t_0)}) \qquad 4.1$$

The ability of the von Bertalanffy growth curve to accurately model growth has been questioned by a number of authors. Knight (1968) argued against the biological significance of L_∞ when data from only part of the age range is available, or when the growth does not appear asymptotic. Roff (1980) contended that since the physiological basis for the von Bertalanffy curve had been disproved it should not be used and suggested that the parabolic function provided similar answers. Although the parabolic function does fit the data Roff noted that the parabolic function fitted best when the first six years of growth were discounted, a factor which counts against it when fitting a curve to short-lived species. Pratt and Casey (1990) have also suggest that a linear function may better describe growth in slow growing elasmobranch species. Despite these attempts to reduce the use of the von Bertalanffy function it provides a good fit in most situations and remains widely used in studies of elasmobranch growth.

Prior to the use of length at age techniques becoming widespread the von Bertalanffy parameter K was estimated using a method described by Holden (1974) (e.g. Francis

1981). Holden's method involved determining K from the gestation period and the size at birth. This method allowed the rapid estimation of K, but was reliant upon the embryonic growth rate being the same as post-partum growth. Pratt and Casey (1990) showed this assumption to be incorrect for most species of sharks.

Patterns in age and growth of carcharhinid species

Previous studies on the age and growth of sharks from the family Carcharhinidae have provided data for a number of species (Table 4.1). Most have relied upon length at age techniques, but some have used length frequency and growth to provide information. The value L_∞ represents an estimate of the maximum size, but in some species under-estimates the observed values (e.g. *Galeocerdo cuvier*). Values of K are typically low, normally ranging from 0.05 to 0.25. Higher values of K are normally obtained from small species. The absolute value of t_0 represents an approximation of the gestation period (Pratt and Casey 1990), but in general over-estimates the value indicating that embryonic growth is more rapid than post-partum growth. The ages at maturity of carcharhinid species are lowest in small species and higher in large species (Table 4.1). The maximum age of species varies but few carcharhinid sharks older than 25 years have been reported (Hoenig and Gruber 1990).

Many of the previous investigations of growth of carcharhinids have combined data from males and females. However, females are typically observed to grow larger than males (Springer 1967), and this is reflected in the growth parameters when the sexes are analysed separately. Females also normally have higher values of L_∞ and lower values of K than males (Table 4.1). There are also differences in the growth parameters within species between geographical areas as well as between techniques

Table 4.1: Von Bertalanffy growth parameters estimated for species of the family Carcharhinidae. Tech., technique (A - age at length; G - growth studies; LF - length frequency); t_{mat}, age at maturity.

Species	Sex	Tech.	L_∞ (cm)	K	t_0 (yr)	t_{mat} (yr)	Source
C. brachyurus	-	A	385	0.038	-3.48	13-20	Walter & Ebert 1991
C. brevipinna	-	A	214	0.212	-1.94	6-8	Branstetter 1987c
C. falciformis	-	A	291	0.153	-2.2	6-9	Branstetter 1987a
C. leucas	-	A	285	0.076	-3.0	14-18	Branstetter & Stiles 1987
C. limbatus	-	A	176	0.274	-1.20	4-8	Branstetter 1987c
	-	A	166	0.276	-1.15	4-5	Killam & Parsons 1989
	-	A	195	0.197	-0.88	6-7	
C. plumbeus	M	A	257	0.050	-4.5	13	Casey et al. 1985
	F	A	299	0.040	-4.9	12	
	M	G	181	0.663	?	-	Wass 1973
	F	G	195	0.684	?	-	
C. sorrah	M	A	98.4	1.17	-0.6	2-3	Davenport & Stevens 1988
	F		124	0.34	-1.9		
C. tilstoni	M	A	165	0.19	-2.6	3-4	Davenport & Stevens 1988
	F	A	194	0.14	-2.8		
Galeocerdo cuvier	-	A	388	0.184	-1.13	6-7	Branstetter *et al.* 1987
	-		440	0.107	-2.35		
Negaprion brevirostris	-	A	318	0.057	-2.30	11-13	Brown & Gruber 1988
Prionace glauca	M	A	295	0.175	-1.11		Cailliet & Bedford 1983
	F	A	242	0.251	-0.79		
Rhizoprionodon acutus	-	LF	100	0.2	-1.78	-	Krishnamoorthi & Jagadis 1986
R. terraenovae	-	A	108	0.359	-0.98	3-4	Branstetter 1987b
	-	A	92	0.45	-2.01	2-3	Parsons 1985
Scoliodon laticaudus	-	LF	76	0.273	-0.57	-	Nair 1976

for their estimation (Table 4.1). However, there has been only a very limited number of studies that have formally compared age and growth between geographic regions (e.g. Parsons 1987; Tanaka *et al.* 1990).

Conclusions

The methodologies for examining age and growth in sharks have been developed to the point where accurate estimates are possible for most species. As a result more and more information is being gathered, especially in response to the data requirements for exploited populations. What has not kept pace with these developments has been research on the calcification process in sharks, and how this related to the ageing techniques used. Studies such as Clement (1992) have gone part of the way to fulfilling this need, but more are required.

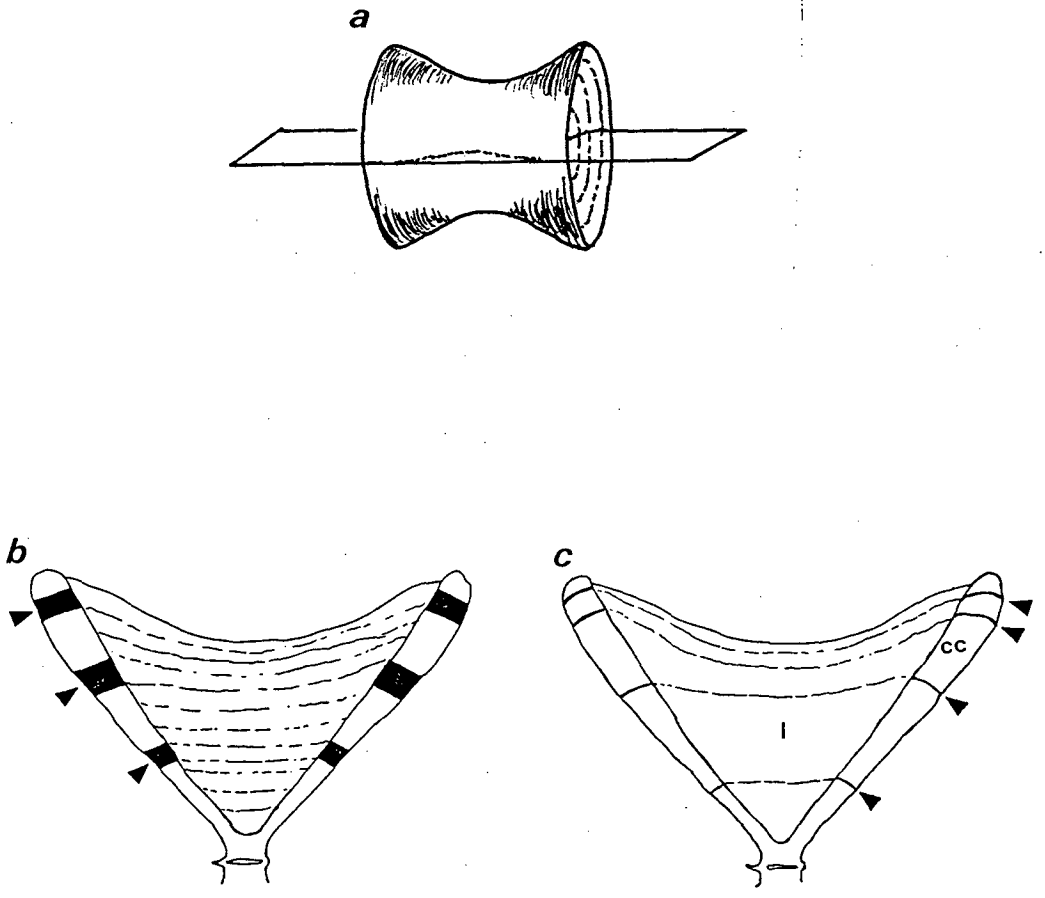


Figure 4.1: Diagrammatic longitudinal sections of vertebrae showing features referred to in the text. (a) whole vertebra showing plane of section, (b) vertebra with opaque (indicated by arrows) and translucent bands, and (c) vertebra with circuli (arrows). CC, corpus calcareum; and I, intermedialia.

4.2 Materials and Methods

Data were collected from 454 *Rhizoprionodon taylori* specimens for the determination of age and growth information. The collection of total length (TL) and fork length (FL) data were described in Chapter 2, along with the relationship between total length and fork length.

Several vertebrae anterior to the first dorsal fin were removed from a sub-sample of 138 specimens. This sub-sample was selected to represent the full size range of this species (230mm - 780mm). The vertebrae were frozen until prepared for ageing. Vertebrae were prepared by separating them, removing excess tissue and soaking in 5% sodium hypochlorite for 30 minutes. Following their immersion in sodium hypochlorite the vertebrae were thoroughly washed and then allowed to dry. Dry vertebrae were mounted on glass microscope slides and ground down, using 120-360 grit wet and dry abrasive paper, to just above the centre of the centrum. They were then remounted on the flat surface and the other side ground down to produce a thin (200-400 micrometres) horizontal section.

Narrow translucent circuli were observed in the corpus calcarea of sectioned vertebrae (Plate 4.1). The narrow width of the circuli suggested that they were check marks, rather than growth bands have been observed by many other authors (for examples see review by Cailliet *et al.* 1986). The circuli were counted in two vertebrae from each specimen. Counts were made without knowledge of the size or sex of the specimen. If the counts varied between the two vertebra a third was sectioned and circuli counted. If the count for the third vertebra matched either of the previous two it was taken as the count. If there was no agreement between any of the three samples then that specimen was excluded from the analysis. This

procedure was repeated a second time for the vertebrae of specimens not excluded at the first counting. The differences between the readings from the first and second counts were compared.

The error associated with the counting of the circuli in the vertebrae were estimated using the Index of Average Percentage Error (*IAPE*) described by Beamish and Fournier (1981):

$$IAPE = \frac{1}{N}\sum_{j=1}^{N}\left(\frac{1}{R}\sum_{i=1}^{R}\frac{|X_{ij}-X_j|}{X_j}\right)\times 100 \qquad 4.2$$

where N is the number of fish aged, R is the number of readings done, X_{ij} the age of the *jth* fish at the *ith* reading, and X_j the mean age of the *jth* fish. This method was used to calculate the error associated with each of the counts conducted, as well as the error between the two counts.

The radius of each centra, and the distance from the edge of the centra to each of the circuli, was measured using an optical micrometer on a high power microscope. Marginal increments were calculated as the distance between the last circulus and the edge of the centrum. The periodicity of the formation of circuli was determined by examining the change in the size of marginal increments through time.

Back calculation analysis was undertaken using a method described by Francis (1990). The length of a specimen when the ith band was laid down (L_i) was calculated using the equation:

$$L_i = (S_i/S_c)^v L_c \qquad 4.3$$

where S_i is the radius of the *ith* band, S_C is the radius of the vertebra at capture, L_C the length of the specimen at capture, and v is a constant derived from the power equation:

$$L = uS^v \qquad 4.4$$

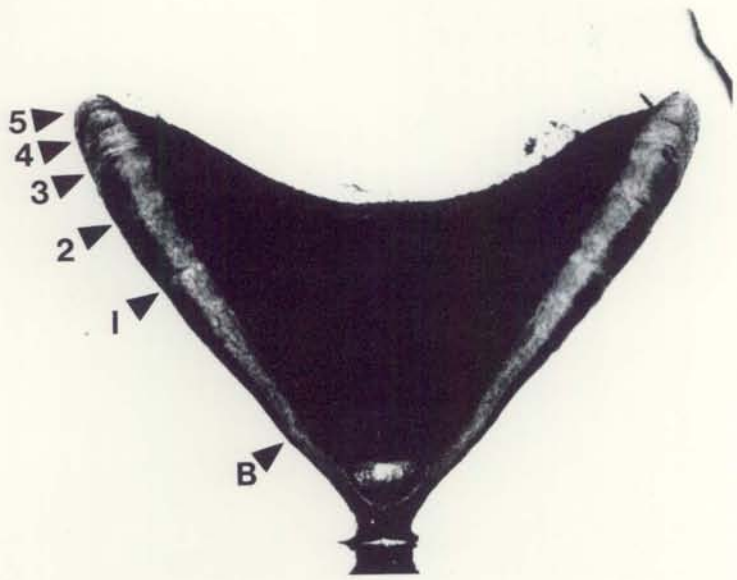
Equation 4.4 describes the relationship between the radius of the vertebra (S) and the length (L), with u being the intercept with the y-axis. When the relationship between L and S is isometric, v equals one, and equation 4.3 reverts to the Dahl-Lea equation (Francis 1990).

The value of v was calculated for *R. taylori* and used in equation 4.3 to determine the length when each circulus was produced. The von Bertalanffy growth function was then fitted to this back calculated data to provide parameter estimates for comparison to the length at age analysis.

Length frequency data were analysed using the ELEFAN I program (Pauly 1987). Data for males and females were analysed separately, except for juveniles which were combined because of small sample sizes. Length data from juvenile specimens caught by Simpfendorfer (1986) in Cleveland Bay were pooled with the data from the present study to provide sufficient data for analysis. Data from specimens caught within months were pooled, as were months between years.

Reproductive data for the determination of age at maturity were taken from data collected in Chapter 5. Juveniles caught just after birth were distinguished by the presence of a fresh umbilical scar between the pectoral fins.

Plate 4.1: Section of a centrum from a 738mm TL female caught in January and estimated to be 5+ years. Arrows indicate circuli; B, circulus formed at birth; 1-5, circuli formed after 1-5 years.


5
4
3
2
1
B

4.3 Results

4.3.1 Centrum morphology

Centrum growth was not significantly different between males and females (t-test, $p>0.05$) and was allometric (Figure 4.2, $v=0.707$, 95% C.I. $=0.038$). The relationship, however, did not adequately describe the growth of centra early in life, passing above the points of the youngest individuals caught. The allometric growth of centra necessitated the use of the non-linear back calculation method given in equation 4.3.

All specimens were found to have at least one circulus, which was assumed to be formed at birth. This birth circulus was associated in many specimens with a change in angle of the corpus calcareum, a characteristic of marks formed at birth in other species (e.g. Casey *et al.* 1985; Branstetter 1987a). The mean back calculated size at which this first mark was formed was 291mm TL.

The circuli on older specimens were close together and marginal increments were calculated only for specimens that had four or less circuli (including the circulus formed at birth). The smallest marginal increments occurred during February, while the largest increments were present in specimens from November to January (Figure 4.3). Circuli therefore apparently form annually in February, or possibly January.

4.3.2 Length at age

As the pupping season of *R. taylori* in Cleveland Bay lasts only three weeks (see Chapter 6) the age of individuals could be calculated from the number ofdays since birth and the number of circuli. A common birth date of January 15th was used,

which corresponds to the middle of the pupping season. The youngest individuals examined were estimated to be 9 days old and measured from 230-250mm TL. The oldest male specimen examined was estimated to be 5.7 years old, and the oldest female 6.9 years. The oldest individuals were not the largest specimens examined. The largest male examined was 691mm TL and largest female 784mm TL.

More than 66% of circulus counts from individual specimens at each reading matched, and over 93% were within one circulus (Table 4.2). Agreement between readings was higher, with over 85% matching and over 99% within one circulus. The error associated with each reading was similar, while the between readings error was lower than error for individual readings (Table 4.2).

Table 4.2: Differences in the circulus counts of vertebrae from *Rhizoprionodon taylori* and errors (IAPE) for readings and between readings.

Difference in circulus counts	1st reading (%)	2nd reading (%)	Between readings (%)
0	66.7	71.2	85.9
1	30.8	22.6	13.3
2	2.5	4.8	0.8
3	0	1.4	0
IAPE	17.3	14.9	8.09

Von Bertalanffy growth curves were fitted to length at age data for males and females separately (Figures 4.4 a and b respectively) because of the large difference in the maximum sizes. Initial growth was very rapid with both males and females growing approximately 315mm in their first year. Subsequently males on average grew 80mm in their second year and 20mm in their third. Females on average grew

125mm in their second year and 50mm in their third. Little growth occurred after three years in males or females. The variability of growth was large, with individuals of a similar age covering a wide range of sizes.

Back calculated sizes at which circulus formation occurred were also variable (Table 4.3). Estimates of von Bertalanffy growth parameters based on these back calculated data were similar to those from the length at age data (Table 4.3).

Table 4.3: Back calculated lengths at circulus formation, and von Bertalanffy growth parameters calculated from these data, for *Rhizoprionodon taylori* from Cleveland Bay. Lengths are in millimetres and are shown as range and mean (in parentheses).

Circulus	Male	Female
B (birth)	247-334(291)	232-333(291)
1	483-580(530)	490-692(544)
2	546-657(594)	584-721(640)
3	572-644(617)	619-738(685)
4	590-660(631)	636-738(704)
5	606-679(653)	716-748(726)
L_∞	630	715
t_0	-0.519	-0.586
K	1.20	0.894

4.3.3 Length frequency

The results of length frequency analysis using ELEFAN I are shown in Figure 4.5. The 0+ individuals show a distinct modal progression with very rapid growth from birth until May. However, modes were much less apparent in later age groups. The estimates of L_∞ and K which gave the best fit to the data are given in Table

4.4. ELEFAN I does not estimate values of t_0 but the growth curves produced indicate that they are between -0.3 and -0.4 years for males and females. The pooling of length data from juvenile males and females should not affect the results as vertebral ageing suggested that they grow at similar rates.

Table 4.4: Estimates of von Bertalanffy growth parameters for male and female *Rhizoprionodon taylori* from ELEFAN I.

	Parameter	
Sex	L_∞ (mm)	K
Male	685	1.3
Female	775	1.0

4.3.4 Size and age at maturity

Based on length at age data male and female *R. taylori* matured after a single years growth, at a size of approximately 550mm TL.

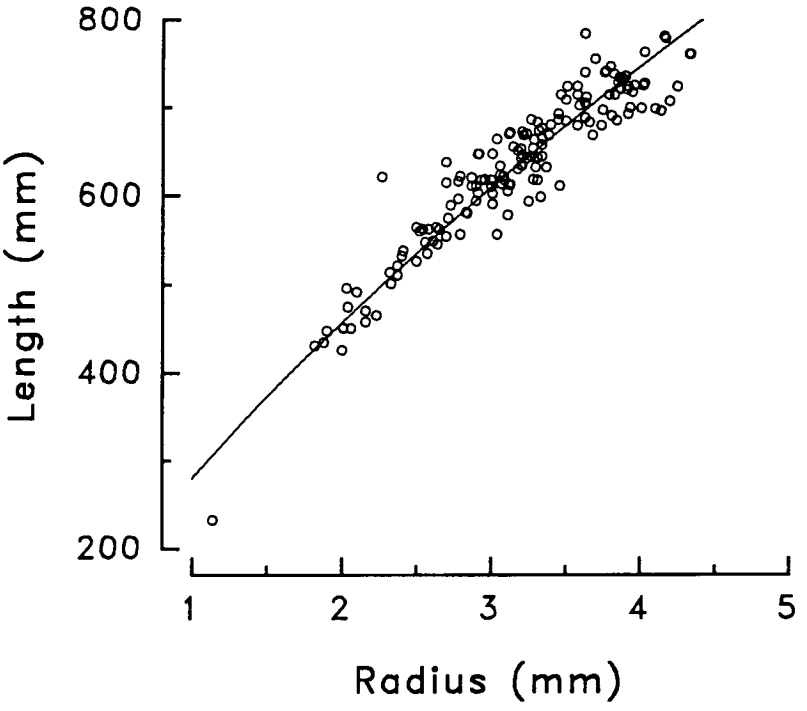


Figure 4.2: Relationship between centrum radius and total length for 151 *Rhizoprionodon taylori* caught in Cleveland Bay.

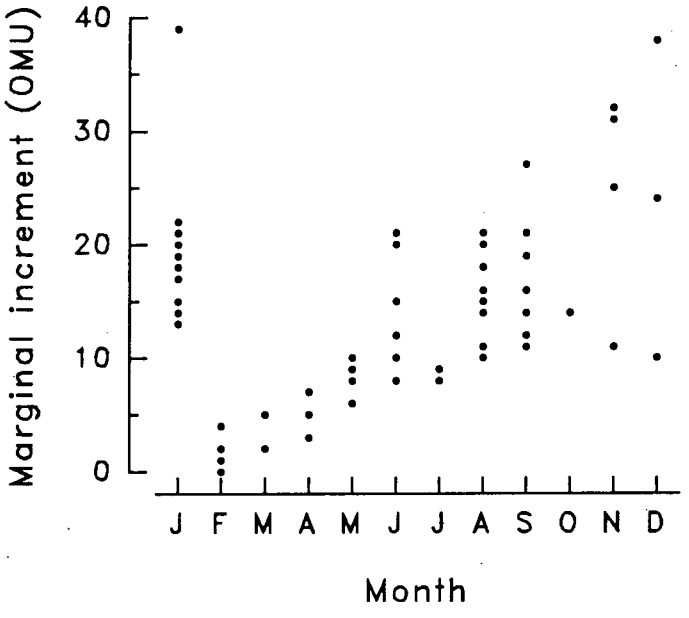


Figure 4.3: Marginal increments for *Rhizoprionodon taylori* from Cleveland Bay based on individuals with 4, or less, circuli (including the circulus formed at birth). 1 OMU = 0.027mm.

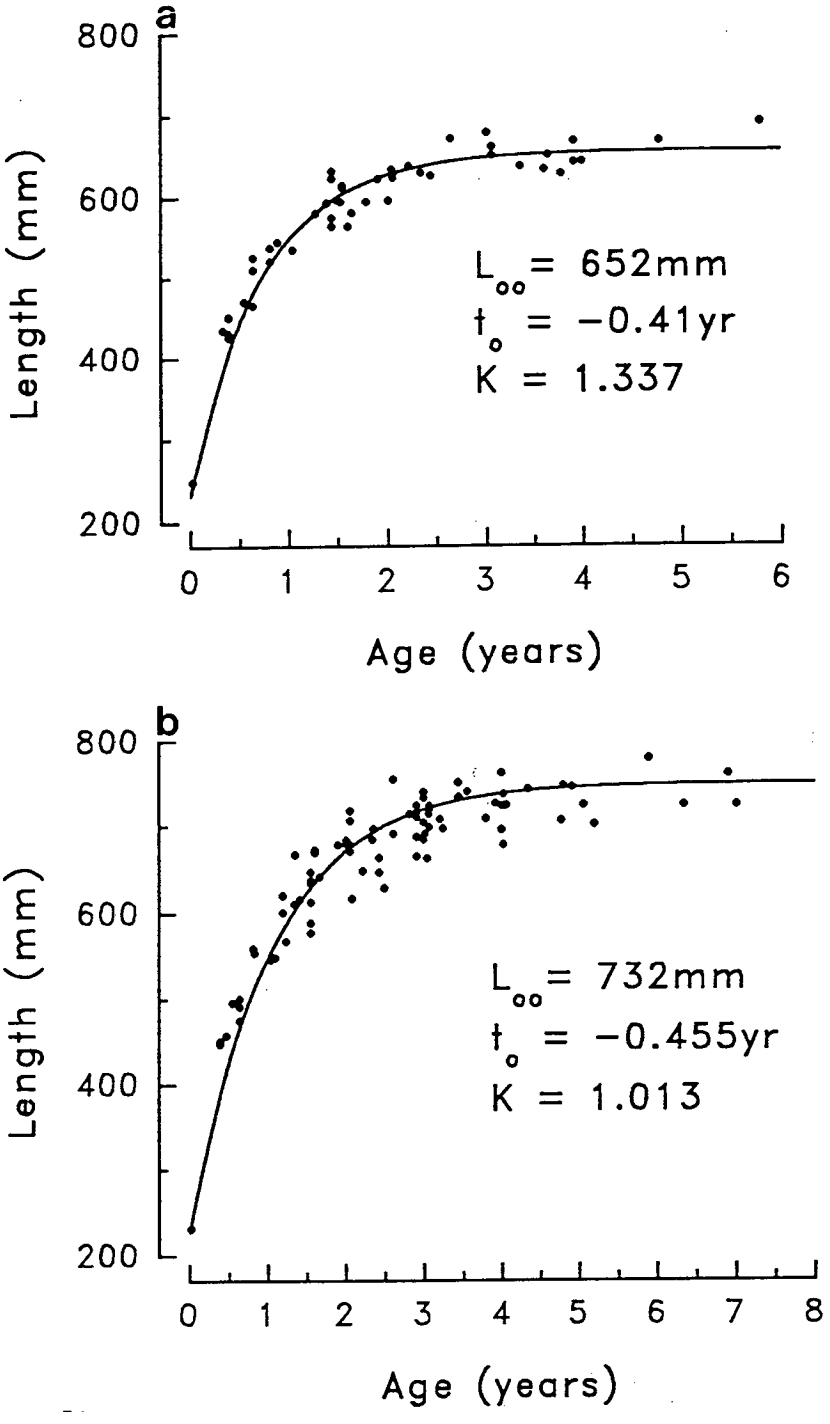


Figure 4.4: Von Bertalanffy growth curve fitted to length at age data for (a) 52 male and (b) 85 female *Rhizoprionodon taylori* caught in Cleveland Bay.

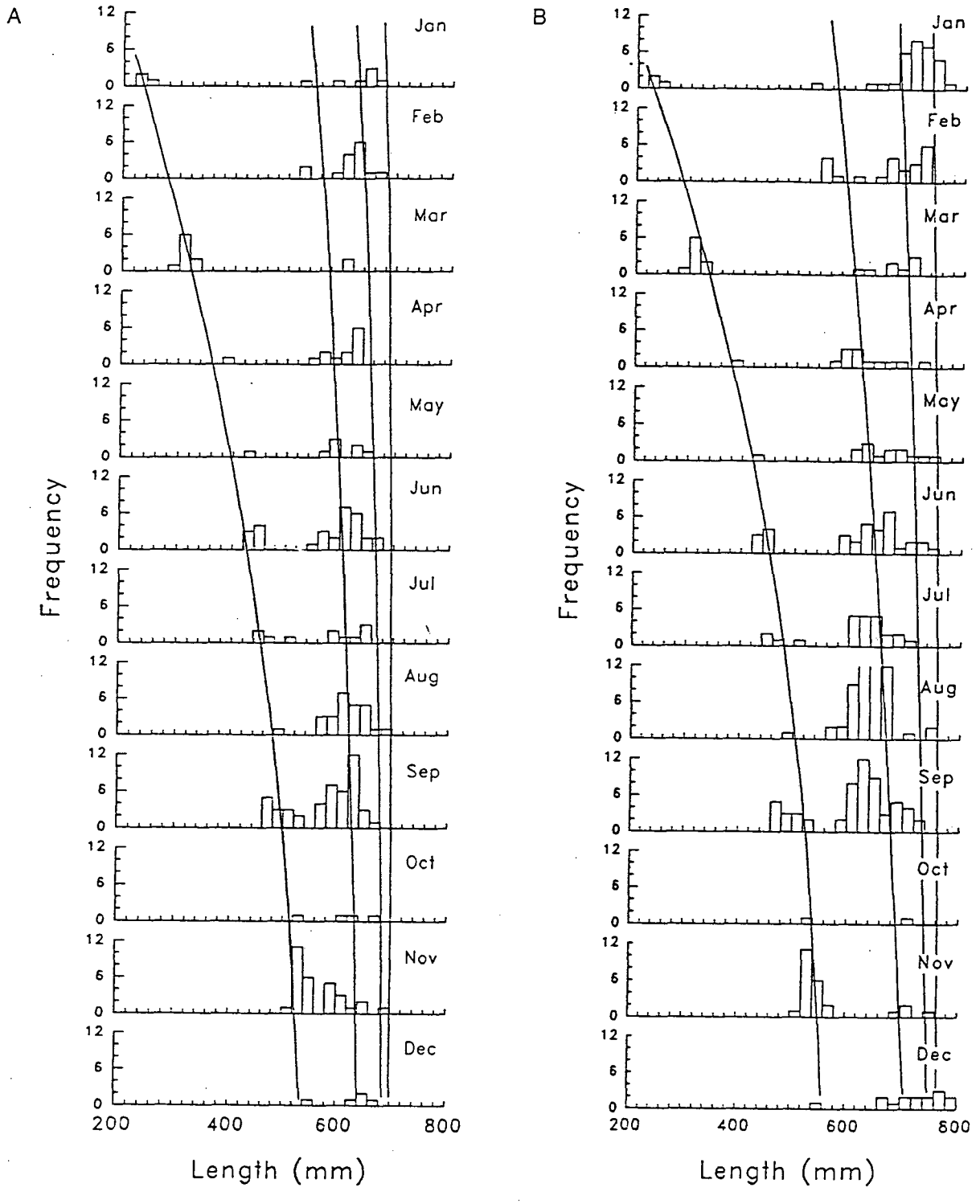


Figure 4.5: Length frequency data for (a) male and (b) female *Rhizoprionodon taylori* caught in Cleveland Bay. The von Bertalanffy growth curves calculated using ELEFAN I for the first four years are shown. Data for juvenile males and females (<580mm TL) are combined.

4.4 Discussion

Beamish and McFarlane (1983) pointed out that for length at age data to be meaningful the periodicity with which the bands or circuli within the centrum are produced needs to be verified. The results of the marginal increment analysis, as well as the similarity of the von Bertalanffy parameters derived from length at age, back calculation, and length frequency techniques, indicate that the periodicity of the circuli in *R. taylori* in Cleveland Bay is annual. Annual production of bands or circuli have been reported in a number of carcharhinid species (Cailliet 1990).

The production of circuli in January or February coincides with the mating season and may result from stress during this period. During mating the hepatosomatic index and condition factor of both male and female *R. taylori* are low (see Chapter 5). The stress that results from the poor body condition may cause the production of the circuli. Previous studies on fish and other aquatic animals have identified stress as a common cause of marks on the vertebrae (e.g. Beamish and McFarlane 1983; Campana 1983; Campana and Neilson 1985).

The accuracy of the von Bertalanffy growth parameters determined for *R. taylori* from the vertebral ageing technique is corroborated by the similarity of parameters determined from back calculation and length frequency data. Although useful as a check for vertebral ageing techniques the back calculation and length frequency techniques had limitations. As Cailliet (1990) observed, 'When .. back-calculated curves are compared with those from other methods, they provide only a check on the growth model used, and are actually more useful for providing data on sizes of missing age classes' (p.159). The use of length frequency analysis in *R. taylori* was limited because of the rapid decline in annual growth increments and variability in growth. Analysis was only possible due to the distinct 0+ age class. Analysis

of the data without the 0+ age class failed to yield comparable results as later age classes were not distinct. Length frequency techniques were also limited because of the need to pool samples within months and between years.

Although more than 93% of circulus counts matched, or were within one of each other, the values of IAPE were higher than previously reported for sharks. Brown and Gruber (1988), for example, reported an IAPE value of 3.4% for *Negaprion brevirostris*. High values of IAPE were obtained for *R. taylori* because its short life-span meant that even small discrepancies in counts produced a sizeable error relative to the life-span.

The size at birth estimated by back calculation is larger than that observed from neonates and full-term embryos (see Chapter 6). This over-estimation probably resulted from the vertebral radius-total length relationship not adequately describing growth of centra in young individuals. Additionally if the birth mark is produced after birth then the rapid growth of the juveniles could result in it producing the over-estimate. The over-estimation of the size at birth by back calculation probably resulted in the slightly lower value of K determined by this technique as compared to vertebral ageing or length frequency techniques.

The growth parameters of *R. taylori*, and the age at which they attain maturity, is not typical of the family Carcharhinidae. Most carcharhinid species studied to date have been found to have slow growth rates and mature after several years. Normal growth of carcharhinid species during the first year is 20-60% of the birth length (BL), with the highest values previously recorded in *Rhizoprionodon terraenovae* (69% BL), *Prionace glauca* (90% BL) and *Galeocerdo cuvier* (100% BL)

(Branstetter 1990). By comparison *R. taylori* grows 140% BL in its first year. Annual growth increments also decrease more rapidly in *R. taylori* than in other species.

Ages at maturity determined for other species of carcharhinid sharks vary from 2 years in *Scoliodon laticaudus* (Pratt and Casey 1990) to 18 years in *Carcharhinus leucas* (Branstetter and Stiles 1987). Maximum ages determined by vertebral analysis for carcharhinid species range from 8 years in *Rhizoprionodon terraenovae* (Parsons 1983) to 30 years in *Carcharhinus obscurus* (Hoenig and Gruber 1990). The age at maturity in long lived species is normally higher for females than males (see Table 4.1). In *R. taylori*, however, the short life-span and rapid growth probably result in the ages at maturity being the same for males and females.

The evolution of rapid early growth, early maturity, and short life-span, in *R. taylori* is possibly due to the risk of high levels of predation. Branstetter (1990) suggested that predation on sharks less than 100cm TL was normally high since they are less proficient swimmers and a more edible size. Accepting that this is the case then selection pressure will favour individuals that grow rapidly after birth and reproduce at an early age.

This section of work has produced the first information on the age and growth of *R. taylori*. It is clear that its pattern of growth differs from that normally observed in other members of the family Carcharhinidae. This has important implications for the life history and population dynamics of this species, and these are discussed further in Chapter 9.

Chapter 5.

Food and feeding of *Rhizoprionodon taylori*.

5.1 Introduction

Previous studies of *R. taylori* have produced only basic dietary information. Simpfendorfer (1986) presented preliminary data on the diet of *R. taylori* from Cleveland Bay, and Stevens and McLoughlin (1991) reported dietary information from 68 specimens from northern Australia. The aims of the research reported upon in the present chapter were to: determine the diet of *R. taylori* in Cleveland Bay based on examination of stomach contents, investigate the influence of sample size on the accuracy of dietary analysis, determine the trophic position of *R. taylori* in Cleveland Bay, examine the feeding habits of *R. taylori*, and determine if, and when, *R. taylori* is dependent upon stored energy reserves.

5.1.1 Food and feeding of carcharhinid sharks

Much has been written regarding feeding in sharks. Most of our current knowledge for the majority of species comes from reports of diets based on stomach content analysis. For only a few species do we have other kinds of information. The review presented below specifically addresses food and feeding as it relates to the family Carcharhinidae. For some aspects, however, it was necessary to draw on information from other groups of sharks as none was available for carcharhinid species.

Feeding mechanisms and behaviours

All species of the family Carcharhinidae are predatory, utilising good swimming ability and powerful jaws to capture and consume prey. Moss (1972) studied the feeding mechanism of members of the family and concluded that they are able to consume a variety of prey. He noted that the upper teeth are normally broad and capable of cutting or gouging large prey, while the lower teeth are more pointed and probably used for grasping prey that are eaten whole. Differentiation in the form of the teeth in the upper and lower jaws of some species, however, is minimal. For example, species of the genus *Rhizoprionodon* have blade-like teeth in both jaws (Springer 1964). The implications of such a dentition pattern for the types of prey consumed has not been investigated.

Observations of the feeding behaviour of carcharhinid species under natural conditions are rare. Normally feeding behaviour has been observed in specimens attracted by baits, or other stimuli, producing unnatural conditions for the observation of behaviour (Nelson 1977). One study that has reported natural feeding behaviour is that of Tricas (1979) who observed that *Prionace glauca* when feeding on anchovies approached from the rear, grasped the prey with a single bite, and swallowed the fish whole. He also described the behaviour of this species during feeding on large aggregations of squid noting different behaviours dependent upon the nature of the squid aggregations and their response to the presence of the shark. One type of feeding behaviour that has been noted in at least three carcharhinid species (*Carcharhinus amblyrhynchos*, *C. melanopterus* and *C. longimanus*) is the herding of schools of fish and squid (Strasburg 1958; Hobson 1963). Hobson (1963) failed to observe any natural feeding events during his study of reef associated species (*C. amblyrhynchos*, *C. melanopterus* and *Triaenodon obesus*) noting that

disinterest in potential prey. He supposed that such behaviour would enable these species to take prey by surprise when they wished. The validity of such an explanation is tenuous considering Hobson (1963) made no observations at night when at least *T. obesus* is known to normally feed (Randall 1977; Myrburg and Nelson 1990). There has been a variety of suggestions as to when during the day other carcharhinid species feed. Medved *et al.* (1985) and Cortes and Gruber (1990) reported that *C. plumbeus* and *N. brevirostris*, respectively, fed uniformly throughout the day and night. There have also been reports of crepuscular feeding in some species (Wetherbee *et al.* 1990).

Diet

Reports of the diets of individual carcharhinid species abound (e.g. Gudger 1948, 1949; Springer 1960; Hobson 1963; Stevens 1973; Bass *et al.* 1973, 1975; Randall 1977; Branstetter 1981; Snelson and Williams 1981; Stevens 1984a,b; Snelson *et al.* 1984; Blaber 1986; Stevens and Wiley 1986; Lyle 1987a; Stevens and McLoughlin 1991), but are not discussed in detail in this review as they are of little comparative value and have already been summarised by Compagno (1984). The summarised dietary information given by Compagno (1984) indicates that in general carcharhinid species have a diet consisting mostly of teleosts, as well as smaller amounts of crustaceans and cephalopods. Reports of crustaceans or cephalopods dominating the diet of a species exist (e.g. Stevens 1973; Medved and Marshall 1981), but are the exception rather than the rule. In large species (e.g. *Carcharhinus leucas*, *C. longimanus*, *Galeocerdo cuvier* and *Negaprion* spp.) other types of prey (e.g. elasmobranchs, marine reptiles, marine mammals, and birds) have been reported in the diet (Gudger 1948, 1949; Backus *et al.* 1956; Bass *et al.* 1973, 1975; Compagno 1984; Witzell 1987; Simpfendorfer 1992).

The relative importance of the main types of prey in the diet of individual species, and within species, can vary considerably. Inter-species variations have been attributed to differences in habits (e.g. Compagno 1984; Wetherbee 1990) and habitat preferences (e.g. Randall 1977; Wetherbee 1990) of shark species, while intra-species variation can result from changes in local prey abundance (e.g. Bigelow and Schroeder 1948; Tricas 1979; Stevens and Wiley 1986; Wetherbee *et al.* 1990; Salini *et al.* 1992) and ontogenic changes in feeding habits (e.g. Medved and Marshall 1981; Simpfendorfer 1986; Stevens and Wiley 1986; Wetherbee *et al.*1990; Simpfendorfer 1992).

Trophic position

The diets of carcharhinid sharks indicate that they are tertiary consumers, occurring at, or near, the top of the food chain. Medium and large sized species are apex predators in tropical and sub-tropical waters (Hiatt and Strasburg 1960; Springer 1967; Polovina 1984; Simpfendorfer 1992), while small species, and juveniles of medium and large species, normally occupy the penultimate trophic position (Hiatt and Strasburg 1960; Springer 1967; Longhurst and Pauly 1987). The abundance of members of the family makes them probably the most important group of predators in the higher trophic levels of tropical inshore ecosystems (Garrick 1967; Compagno 1984; Wetherbee *et al.* 1990). Although their trophic position is understood the importance of predation by carcharhinid sharks has been only rarely considered in community studies (e.g. Hiatt and Strasburg 1960), especially at the quantitative level (e.g. Polovina 1984). The lack of inclusion in community studies can probably be attributed to the difficulties in catching or observing them using traditional methods, and because of their transitory nature in most systems.

Feeding habits

A feature of many feeding studies of carcharhinid sharks is that large proportions of specimens have empty stomachs (e.g. Backus *et al.* 1956; Clark and von Schmidt 1965; Bass *et al.* 1973; Randall 1977; van der Elst 1979; Stevens 1984b; Lyle 1987a; Wetherbee *et al.* 1990). Some of these studies have found more than half of the specimens caught had empty stomachs. The high incidence of empty stomachs may be the result of one or both of two factors. Firstly, it may be the result of methodological problems, and, secondly, a consequence of the feeding habits of the species concerned.

A number of methodological problems may account for the observation of larger proportions of empty stomachs. The most commonly cited of these is that studies utilising baited lines have a greater chance of catching specimens with empty stomachs, as hungry sharks (and so those with empty stomachs) are more likely to take a bait (e.g. Medved *et al.* 1985; Wetherbee *et al.* 1990). Although gillnets may reduce this problem some studies using these techniques still produce large numbers of specimens with empty stomachs (e.g. Simpfendorfer 1986; Wetherbee *et al.* 1990), perhaps because hungry sharks are more active and so prone to capture. Another methodological problem may lie in the time and location of sampling. For example, if a species feeds at a particular time of the day then samples collected soon after the feeding period would be more likely to have smaller proportions of empty stomachs than samples collected just prior to the feeding period. A third methodological problem may lie in the occurrence of regurgitation of food. Although regurgitation *per se* is not a problem it is the ability to account for it in the analysis that is. Very few studies on shark feeding have considered the occurrence or degree of regurgitation. One that did was Medved *et al.* (1985), who found that in juvenile

C. plumbeus regurgitation was of minimal importance. Information on other species is not available presumably because of the difficulty of reliably identifying specimens which have regurgitated. Specimens with an everted stomach are easily accounted for, however, specimens having the stomach withdrawn back into the body cavity are difficult to recognise. Insufficient work has been carried out to date on this problem to provide an adequate method for consistently measuring the frequency of regurgitation.

The second factor that may explain the high incidence of empty stomachs is that of the feeding habits of sharks. To examine this factor it is first necessary to have a knowledge of the feeding habits and physiology of sharks (e.g. how often do sharks feed, how long does it take to digest food, etc.). The results of studies on the feeding habits of carcharhinid species of sharks are outlined below.

Only two studies have looked in detail at feeding in species of the family Carcharhinidae. One on juvenile *Carcharhinus plumbeus* was carried on in Chinoteague Bay, Virginia (Medved 1985; Medved *et al.* 1985, 1988), and the other was carried out on juvenile *Negaprion brevirostris* at Bimini (Wetherbee *et al.* 1987; Cortes and Gruber 1990; Wetherbee and Gruber 1990). Similar studies on adults remain to be undertaken, but are difficult because of the size and habits of the adults of many species. The two studies noted indicate that both species actively feed for a short period of time, often only consuming a single item, and then refrain from feeding until digestion of the meal is complete (Medved *et al.* 1985; Cortes and Gruber 1990; Wetherbee *et al.* 1990). Estimates of the length of time that species spend locating and capturing food (feeding duration) were 7-9 hours in *Carcharhinus plumbeus* (Medved *et al.* 1988) and 10-11 hours in *Negaprion brevirostris* (Cortes and Gruber 1990). The frequency of feeding (i.e. the time

between periods of active feeding) in *N. brevirostris* is approximately 32 hours, and in *C. plumbeus* is 95 hours. Data on the feeding duration and feeding frequency are not available for other species, but are likely to vary between species, types of prey consumed, and temperature regimes. For example, Medved *et al.* (1985) found that blue crabs were digested more rapidly than menhaden (a teleost).

To apply this information on feeding habits to the high incidence of empty stomachs it is necessary to assume that other species within the family have similar feeding habits to *C. plumbeus* and *N. brevirostris*. This feeding pattern involves a cycle between feeding and digestion. The only time during such a cycle that the stomach can be empty is between digestion and feeding. Large proportions of empty stomachs would therefore suggest that a species has a long period between the end of digestion and the consuming of food. This may be as a result of delaying feeding even after the stomach is emptied, or that it takes a long time (relative to the time it takes to digest food) to find and consume the next prey item or items. Most models of fish feeding allow for continuous consumption of food, however, Diana (1979) described a method which can be applied to these reported cyclic feeding habits of carcharhinid species (Cortes and Gruber 1990). This model works on the assumption that, if other possible factors are excluded, the proportion of specimens with empty stomachs will be equivalent to the proportion of time the stomachs are empty. The period between digestion and feeding represents an average, rather than a set period, since the exact timing of feeding in an individual will be the function of factors such as prey availability and feeding success. Thus it is possible to calculate the period of time between digestion and feeding if the proportion of empty stomachs and the time taken for digestion is known. The application of the Diana Method to the feeding data for *N. brevirostris* given by Wetherbee *et al.* (1990) (a digestive period

of 28-41 hours for natural prey, and 26% of specimens with empty stomachs) yields a period between digestion and feeding of 7-11 hours. This estimate agrees closely with the feeding duration given by Cortes and Gruber (1990) of 10-11 hours. Similar calculations for data from *C. plumbeus* provide an estimate slightly above that given by Medved *et al.* 1988 (9-12 hours versus 7-9 hours). Thus there is some evidence that feeding habits account for the occurrence of empty stomachs in some studies. However, it is likely that the relative importance of methodological problems and feeding habits will vary between species and so need to be examined on a species by species basis.

The studies discussed above on the feeding habits of carcharhinid sharks have also provided estimates of the rate of food consumption. Medved *et al.* (1988) calculated that in juvenile *C. plumbeus* consumption is approximately 1% body weight per day, and Cortes and Gruber (1990) calculated it to be 1.5-2.0% body weight per day in juvenile *N. brevirostris*. Clark (1960) suggested that juveniles have higher rates of food consumption than adults due to higher metabolic rates. However, this remains to be confirmed by experimentation.

Use of stored energy reserves

During periods when sharks do not consume enough food to meet their energy requirements they can rely on energy reserves stored in the liver (Springer 1967). The reliance on the liver can last several months, especially in sedentary benthic species (Clark 1960). Little is known, however, on how long active carcharhinid species can survive solely on stored reserves. Johnson (1978) suggested that active carcharhinid species could survive about 6 weeks in this way, but provided no data to support this suggestion.

The utilisation of energy reserves stored mostly in the liver mean that it may be possible to use the size, or chemical composition, of this organ as an indicator of the feeding status of a shark. However, changes in the liver size and composition have also been attributed to the processes of vitellogenesis and embryogenesis. There has been no specific study on carcharhinid species in this respect, but Craik (1978) demonstrated that in *Scyliorhinus canicula* yolk precursors are stored in the liver, and when vitellogenesis begins they are moved rapidly to the ovary. Rossouw (1987) calculated that in the shovelnose ray, *Rhinobatos annulatus*, vitellogenesis was not sufficient to account for all the variation observed in the liver weight. Thus the use of liver size as an indicator of feeding status is possible, but adequate provision for vitellogenesis in females must be taken into account. The use of liver data from male specimens only is one method to overcome the problems associated with vitellogenesis.

Ecology of feeding

One aspect of shark feeding on which there is little information is that of the ecology of shark feeding (Wetherbee *et al.* 1990). One of the few studies that has specifically addressed the ecology of shark feeding in the family Carcharhinidae is work carried out by CSIRO in the Gulf of Carpentaria (Salini *et al.* 1992). This research has shown that in inshore tropical waters feeding by a variety of carcharhinid species occurred in a non-selective, or opportunistic, manner (i.e. the proportion of a prey group in the diet reflects its abundance in the environment). In a review of shark feeding Wetherbee *et al.* (1990) noted that there is evidence that many species of sharks are non-selective feeders, based mostly on changes in diets with increasing size, changes in habitat, and seasonal variations in prey abundance. Although such changes supply evidence for non-selective feeding implicit evidence can only be

provided by studies such as Salini *et al.* (1992) that compare abundances of prey in stomach contents and the environment in which the sharks fed. Not all sharks, however, are non-selective feeders. For example, *Hemigaleus microstoma* a member of the family Hemigaleidae (a family closely related to the Carcharhinidae) almost exclusively consumes cephalopods (Stevens and Cuthbert 1983; Salini *et al.* 1992). Salini *et al.* (1992) also suggested that there is some partitioning of food resources, but that there is large amounts of overlap in the diets of sympatric species. They also concluded that predation by carcharhinid species has a substantial effect on populations of prawns. More studies such as Salini *et al.* (1992) are important if we are to understand the trophic significance of shark predation in marine ecosystems.

Conclusions

There remain large gaps in our knowledge of feeding in sharks of the family Carcharhinidae. In particular detailed study has been undertaken on juveniles of only a few species. More study on feeding in a range of species, and comparisons between adults and juveniles are required. The importance of shark feeding within marine communities will also only be fully understood with further ecological work.

5.2 Materials and Methods

5.2.1 Data collection

Information pertaining to the diet and feeding habits of *R. taylori* caught in Cleveland Bay was obtained by examination of stomach contents of the specimens caught in gillnets and trawls. Details of the methods of capture and measurement of specimens examined have been given in Chapter 2.

The contents of each stomach were identified to the lowest possible taxa and weighed to the nearest 0.1g using an electronic balance. The advanced state of digestion of many food items limited the accuracy of identification. As a result the taxonomic level used in the analysis of stomach content data was normally family. An exception to this procedure were the stomatopodans, which were grouped together for analysis. Specimens in which the stomach was everted, or in which the stomach was extremely flaccid but empty, were adjudged to have regurgitated during capture and handling, and were excluded from the analysis of stomach contents.

An estimate of the digestive state of stomach contents was made by assigning a index value (1-5) to each item. The state of digestion index (SDI) was based on the percentage of flesh remaining, compared with that expected in undigested prey items. Values of SDI, and the percentage of digestion that they represent are shown in Table 5.1. The original weight of stomach contents was then calculated by multiplying their weight by a correction factor. The correction factor for each SDI value was based on the mid-point of the range of percentage flesh remaining associated with each index value. Correction factors are given in Table 5.1.

Table 5.1: State of digestion index (SDI) values and correction factors assigned to each to enable the estimation of original prey weight.

SDI	% flesh remaining	Correction factor
1	100-81	1.11
2	80-61	1.43
3	60-41	2.00
4	40-21	3.33
5	20-1	10.0

Liver weights, measured to the nearest 0.1g using an electronic balance, were obtained from a sub-sample of specimens. Liver weights were used to calculate the hepatosomatic index (*HSI*) using the formula:

$$HSI = \frac{liver\ \ weight}{cleaned\ \ weight} \cdot 100 \qquad 5.1$$

where cleaned weight was the total body weight less the weights of the liver, stomach and pups.

Body condition (*K*) of specimens was calculated based on the cleaned weight (w, in grams) and total length (l, in millimetres) using the formula:

$$K = \frac{w}{(l^3)} \cdot 100000 \qquad 5.2$$

5.2.2 Analysis

Stomach contents

Three analyses were used to describe the stomach content data of *R. taylori*. The first of these was the Occurrence Method (Hynes 1950; Hyslop 1980). This method calculates the percentage occurrence of each food item (O_i) from the number of stomachs in which it occurs:

$$O_i = \frac{n_i}{N} \cdot 100 \qquad 5.3$$

where n_i is the number of occurrences of the *ith* food item in all stomachs examined that contained food, and N is the total number of stomachs examined that contained food. Since stomachs often contain more than one food item the sum of all O_i's will normally be greater than 100.

The second analysis used was the Gravimetric Method (Pillay 1952; Hyslop 1980). Percentage weight of prey groups was calculated using a similar formula to that used for determining percentage occurrence, but with the weight of the individual prey groups divided by total weight of stomach contents.

The third analysis of stomach contents combined aspects of both occurrence and weight. The method used is a modified form of the Weighted Resultant Index (R_W) described by Mohan and Sankaran (1988). Instead of using volume, as suggested by Mohan and Sankaran (1988), weight was used. R_W is a geometrically based index that takes account of the relative significance of both the occurrence and bulk of each prey group, a factor that other similar indices do not (Mohan and Sankaran 1988). Details of the calculation of R_W are included in Appendix B.

Sample size

The accuracy of stomach content analysis depends on sufficient stomachs being examined. One technique to determine if the sample size is sufficient is to calculate the diversity of the stomach contents for increasing numbers of stomachs. When sufficient stomachs have been examined diversity will not increase further. Simple measures of diversity (e.g. Shannon Index and Brillouin's Index) have been used, however, Hoffman (1979) points out that both of these indices may be inappropriate as, "the prey are not normally distributed among the fish and contents of individual stomachs do not represent the diet of the population" (p. 56). As an alternative Hoffman (1979) suggested that a method described by Pielou (1966), and based on Brillouin's Index, would be more appropriate:

$$H_k = \left(\frac{1}{N_k}\right) \cdot \log\left(\frac{N_k!}{\prod N_{ki}!}\right) \qquad 5.4$$

where H_k is the diversity of k pooled stomachs, N_k is the total number of individual prey groups in k pooled stomachs, and N_{ki} is the number of individuals of the *ith* prey group in k pooled stomachs. Values of H_k were plotted against k. The value of k at which H_k reached a stable value is the minimum sample size required to adequately describe the diet.

Comparisons of SDI distributions

Differences in the distribution of SDI values were tested between sexes, maturity states, years, season and sampling location, using Chi square contingency tables. Results were considered tentative if more than 20% of the expected values were less than five (Zar 1984).

Variations in hepatosomatic index and body condition

Monthly variations in HSI and body condition were analysed separately for males and females. Data between years were pooled and analysed on a monthly basis. The data sets were tested for equality of variances between months using Bartlett's Test. If the variances were equal the data were analysed using single factor analysis of variance (ANOVA) with HSI or body condition as the variate and months as the factor. If the variances were significantly different between months then the non-parametric Kruskal-Wallis test was used.

5.3 Results

5.3.1 Diet

Stomachs from 453 *R. taylori* specimens were examined. Twenty three specimens (5.3%) were adjudged to have regurgitated and were excluded from further analyses. Of the remaining 430 stomachs a further 242 (56.3%) did not contain food. Only 18.4% of the stomachs examined contained food items that were identifiable to the family level. Of the stomachs that contained food 84.3% contained one food item, 13.8% contained two items, and 1.9% three items.

Stomachs contained prey from 17 families of teleost fishes, three families of crustaceans and one of squid (Table 5.2). The most commonly occurring prey groups were the Leiognathidae (pony fish), Clupeidae (herrings) and Teraponidae (banded grunters), prawns of the family Penaeidae, and squid of the family Loliginidae. Although the Leiognathidae was the most commonly occurring group, the Clupeidae and Teraponidae were more important in terms of percentage weight. Penaeid prawns, although commonly occurring, were less important to the diet in terms of weight. The prey items with the highest R_W values (in decreasing order) were the Leiognathidae, Clupeidae, Teraponidae, Loliginidae, Penaeidae, Engraulidae, Hemiramphidae and Synodontidae (Table 5.2). 66.3% of the food items identified were considered demersal, and 33.7% pelagic (Table 5.2).

The cumulative diversity of the stomach contents was calculated for 79 specimens that had identifiable contents in their stomachs. Although the rate of increase of the cumulative diversity after 60 stomachs was low, there was no indication that the diversity had reached a maximum (Figure 5.1).

Table 5.2: Dietary information for 430 *Rhizoprionodon taylori* caught in Cleveland Bay. The prey were divided into demersal (D) and pelagic (P) forms (prey category) based on Stevens and Wiley (1986); N is the number of stomachs in which each item was found; O is the percentage occurrence; W is the percentage weight; and R_W is the weighted Resultant Index.

Prey group	Prey category	N	O	W	R_W
Empty		242			
Teleosts					
Apogonidae	D	1	0.61	0.28	0.38
Chirocentridae	P	1	0.61	5.38	0.70
Clupeidae	P	15	9.09	15.15	11.62
Cynoglossidae	D	1	0.61	0.57	0.77
Engraulidae	P	4	2.42	1.28	1.84
Haemulidae	D	1	0.61	1.41	0.72
Hemirhamphidae	P	3	1.82	1.14	1.57
Leiognathidae	D	21	12.73	9.24	13.63
Mullidae	D	2	1.21	0.20	0.26
Pempheridae	P	1	0.61	3.47	0.70
Plotosidae	D	1	0.61	2.28	0.70
Polynemidae	D	1	0.61	0.96	0.73
Sillaginidae	D	1	0.61	0.61	0.76
Syngnathidae	D	1	0.61	0.18	0.24
Synodontidae	D	2	1.21	2.10	1.55
Teraponidae	D	8	4.85	14.09	5.90
Triacanthidae	D	1	0.61	0.35	0.47
Unidentified		97	58.79	35.84	50.75
Crustaceans					
Penaeidae	D	14	8.48	2.27	2.98
Portunidae	D	1	0.61	-	-
Stomatopoda	D	1	0.61	-	-
Cephalopods					
Loliginidae	P	5	3.03	2.20	3.18
Total			112.17		100.00

The mean wet weight of items in the stomachs was 14.1g (sd= 17.95g), and they ranged in wet weight from 0.3g to 124.4g, for all size ranges of *R. taylori*. 74% of the items individually weighed less than 15g, and 95% weighed less than 50g (Figure 5.2a). The corrected wet weight of stomach contents (based on the SDI values) had a mean of 29.5g (sd= 35.07g), and ranged from 0.9g to 234g. 79% of corrected wet weights of individual items were less than 40g, and 90% were less than 65g (Figure 5.2b). The uncorrected wet weights represented between 0.03 and 8.34% of body weight of the specimens (mean=1.11%), and the corrected wet weights between 0.13 and 16.10% (mean=2.28%) (Figure 5.3).

The distribution of the SDI values 1 to 5 did not differ significantly from a 1:1:1:1:1 ratio (Figure 5.4; Chi square test, $\chi^2=0.7439$, d.f.=4, p=0.9458). This even distribution of SDI values suggests that feeding occurred throughout the day as crepuscular feeding, or feeding during other specific times of the day or night, would have resulted in distinct mode, or modes, in the distribution of SDI values as all specimens were collected at the same time of day (see Chapter 2). There were no significant differences in the distribution of SDI values between sexes, maturity states, years, sampling locations (excluding Middle Reef) and seasons (Table 5.3).

Table 5.3: Results of Chi^2 contingency tables testing proportions of SDI values between sex, maturity, year, sampling location and season for *Rhizoprionodon taylori* caught in Cleveland Bay.

Variation tested	Categories	Chi^2 value	d.f.	p
Sex	Male Female	1.530	4	0.8214
Maturity	Juvenile Adult	5.404	4	0.2483*
Year caught	1987 1988 1989	3.116	8	0.9269
Sampling location**	Strand Southern Bay	4.175	4	0.3829
Season	Dec-Feb Mar-May Jun-Aug Sep-Dec	15.26	12	0.2277*

* result tentative as more than 20% of expected values were less than 5.

**Middle Reef was excluded from the analysis due to the small sample size.

5.3.2 Variation in liver weight and body condition

Mean monthly HSI values for both adult males and adult females varied considerably throughout the year (Figure 5.5). Too few immature specimens were caught to enable analysis of juvenile data. There were significant differences in the mean monthly HSI values for both adult males and adult females (ANOVA; males, d.f.=10, F=5.73, $p<0.0001$; females, d.f.=9, F=38.87, $p<0.0001$). Mean monthly values of HSI for adult males were normally between 5 and 7, but were

lower in the early part of the year (Figure 5.5a) with the lowest mean monthly HSI value of 2.7 recorded in February. Mean values of HSI for adult females varied more than those for males, with a rapid decrease from July to November. There was a small increase in December followed by another decrease to the lowest values of approximately 2.4 which occurred in January and February. From March through to July female mean HSI values increased (Figure 5.5b).

Mean monthly values of body condition showed similar patterns of variation between the sexes in *R. taylori* (Figure 5.6). Highest monthly means occur in March and October and lowest values in February. There were significant differences in the mean monthly values of K for males (ANOVA, d.f. = 11, $F = 5.45$, $p < 0.0001$) and females (Kruskal-Wallis test, $p < 0.0001$).

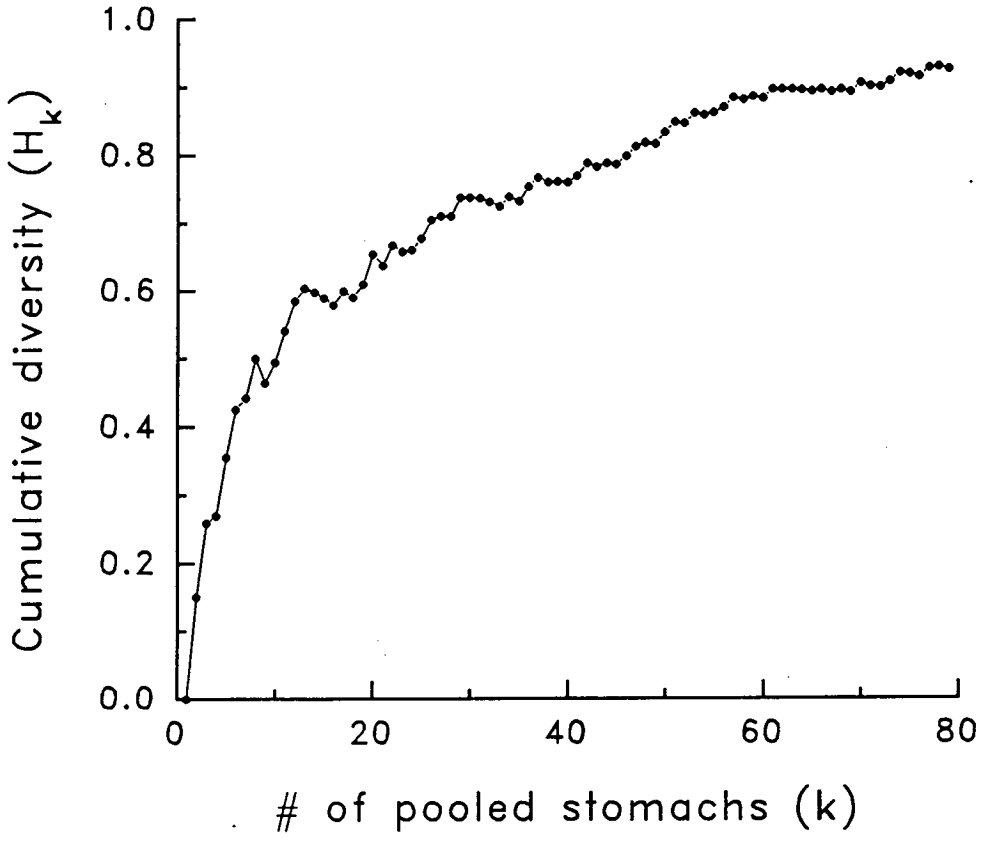


Figure 5.1: Cumulative diversity of stomach contents of *Rhizoprionodon taylori* from Cleveland Bay using the method of Hoffman (1979).

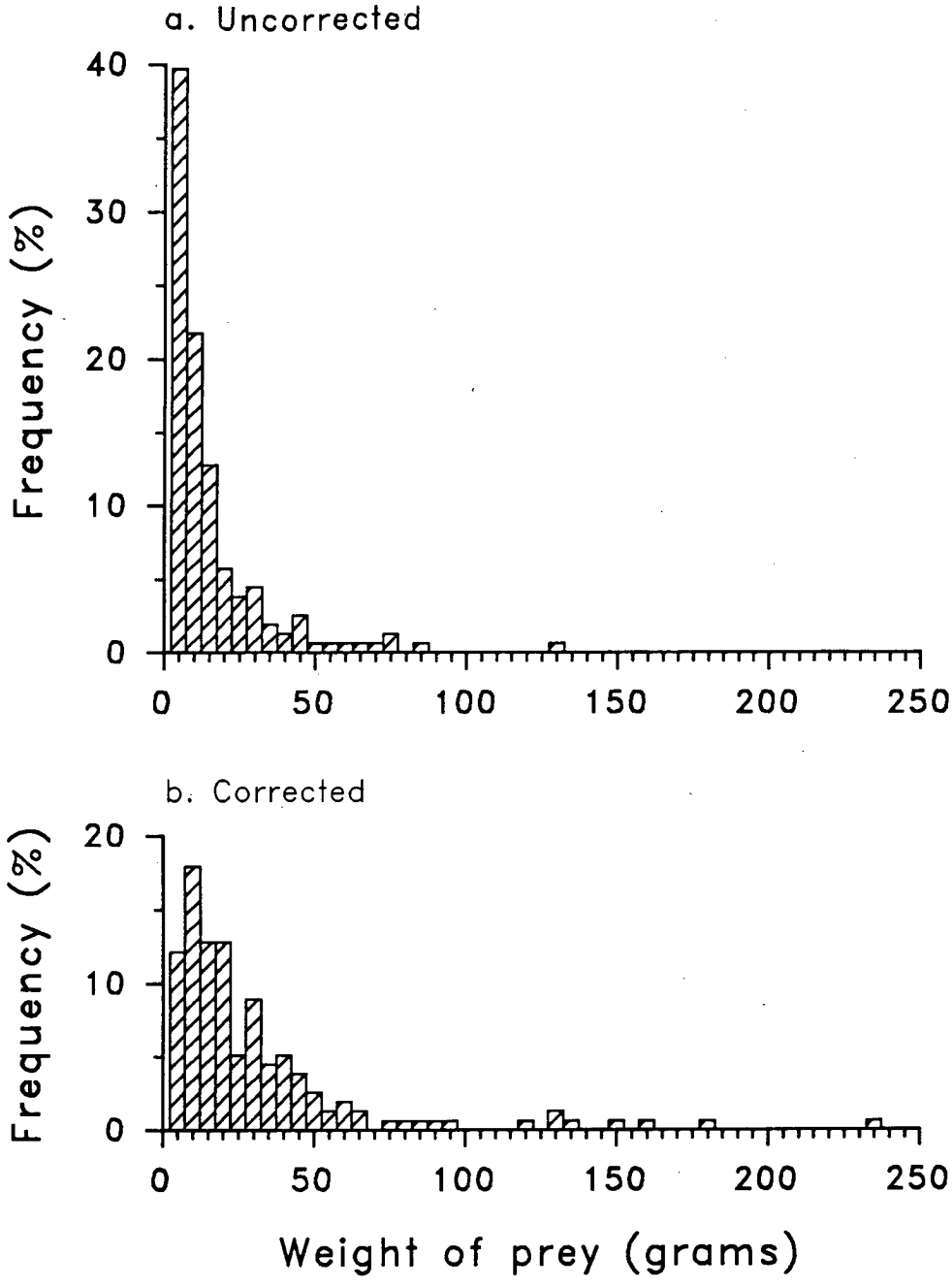


Figure 5.2: Wet weight of stomach contents from 149 *Rhizoprionodon taylori* specimens: (a) raw wet weight data; and (b) estimated original weight of prey based on correction factors for different digestive states (see text for description of method).

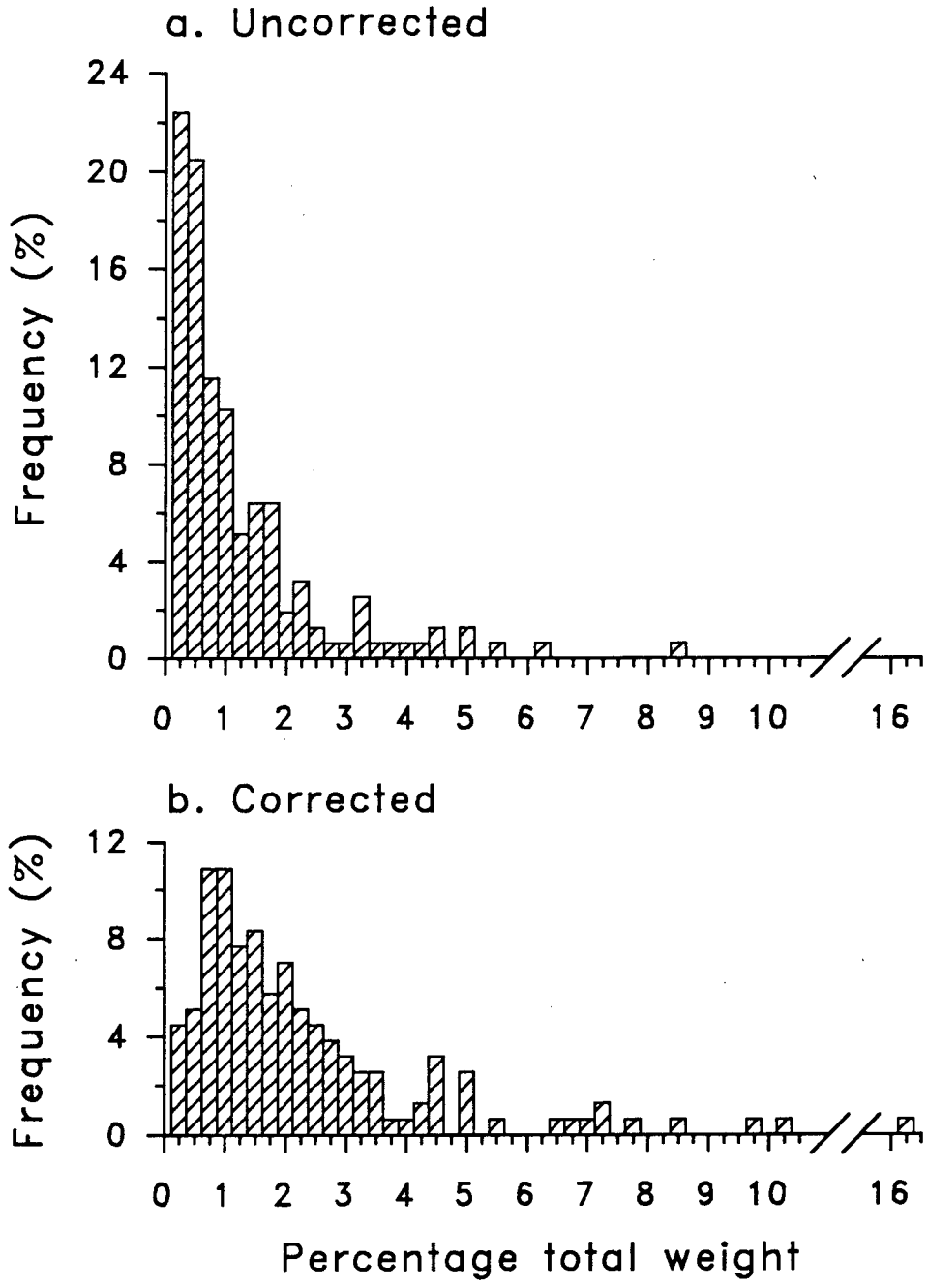


Figure 5.3: Wet weight of (a) stomach contents, and (b) estimated original prey weight, as a proportion of total body weight, of 149 *Rhizoprionodon taylori* specimens from Cleveland Bay. Weights of prey determined from digestive state (see text for description of method).

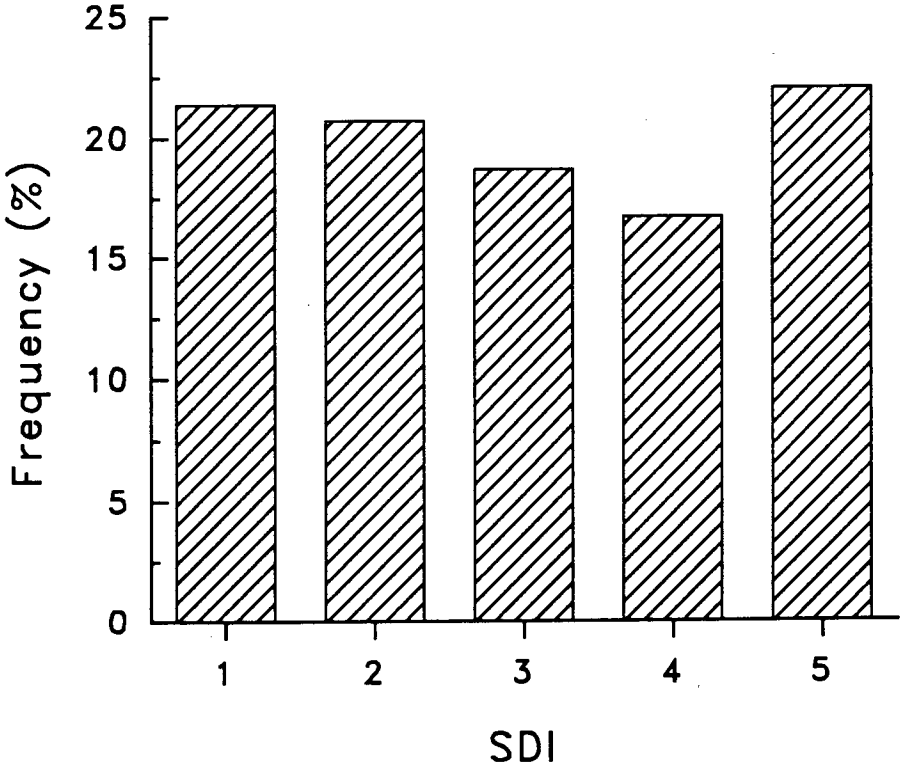


Figure 5.4: Frequency distribution of state of digestion index (SDI) for 149 *Rhizoprionodon taylori* from Cleveland Bay (see text for details of SDI values).

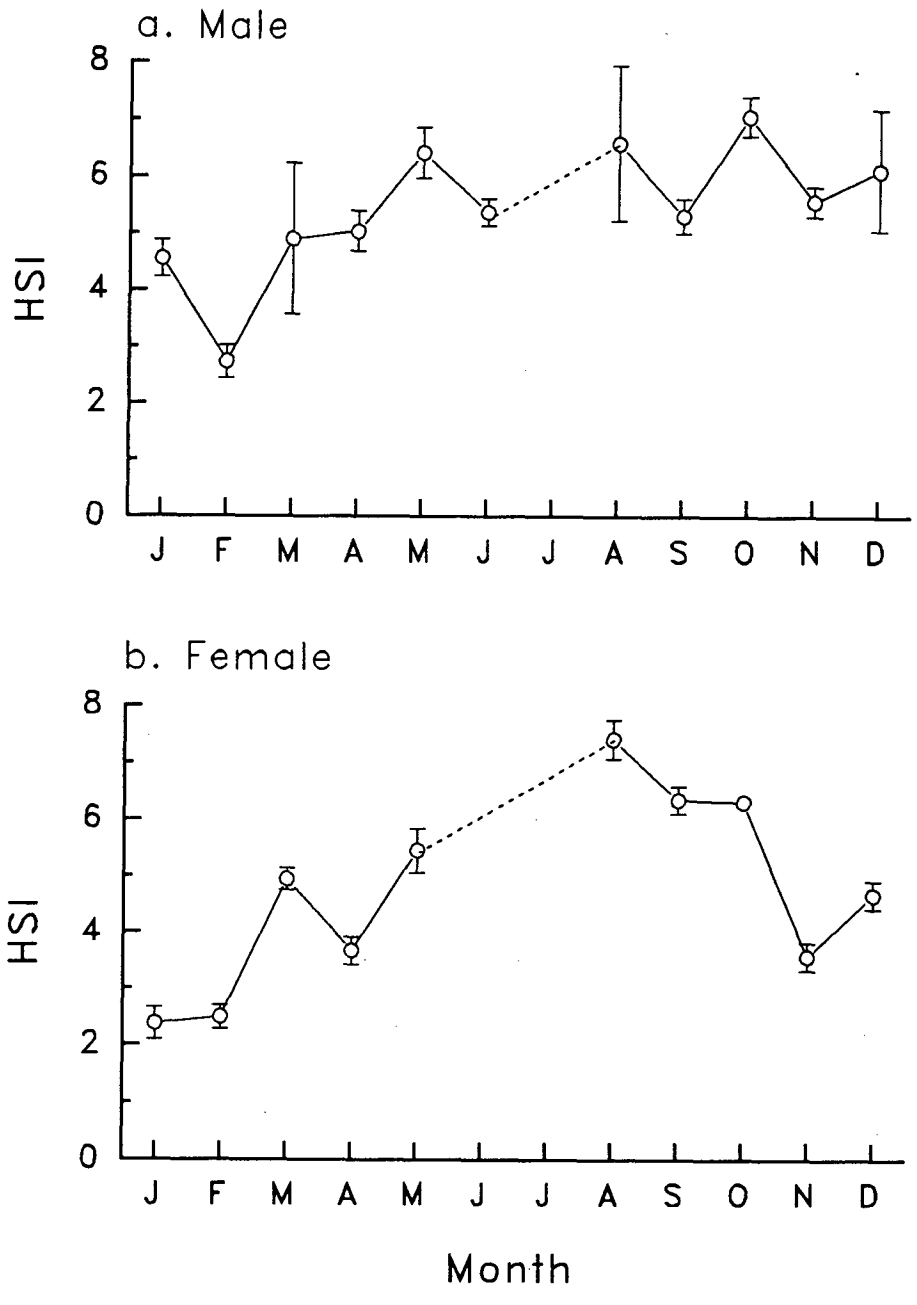


Figure 5.5: Monthly variation in the hepatosomatic index (HSI) of (a) male, and (b) female, *Rhizoprionodon taylori* specimens from Cleveland Bay. Circles represent monthly means, bars plus and minus one standard error.

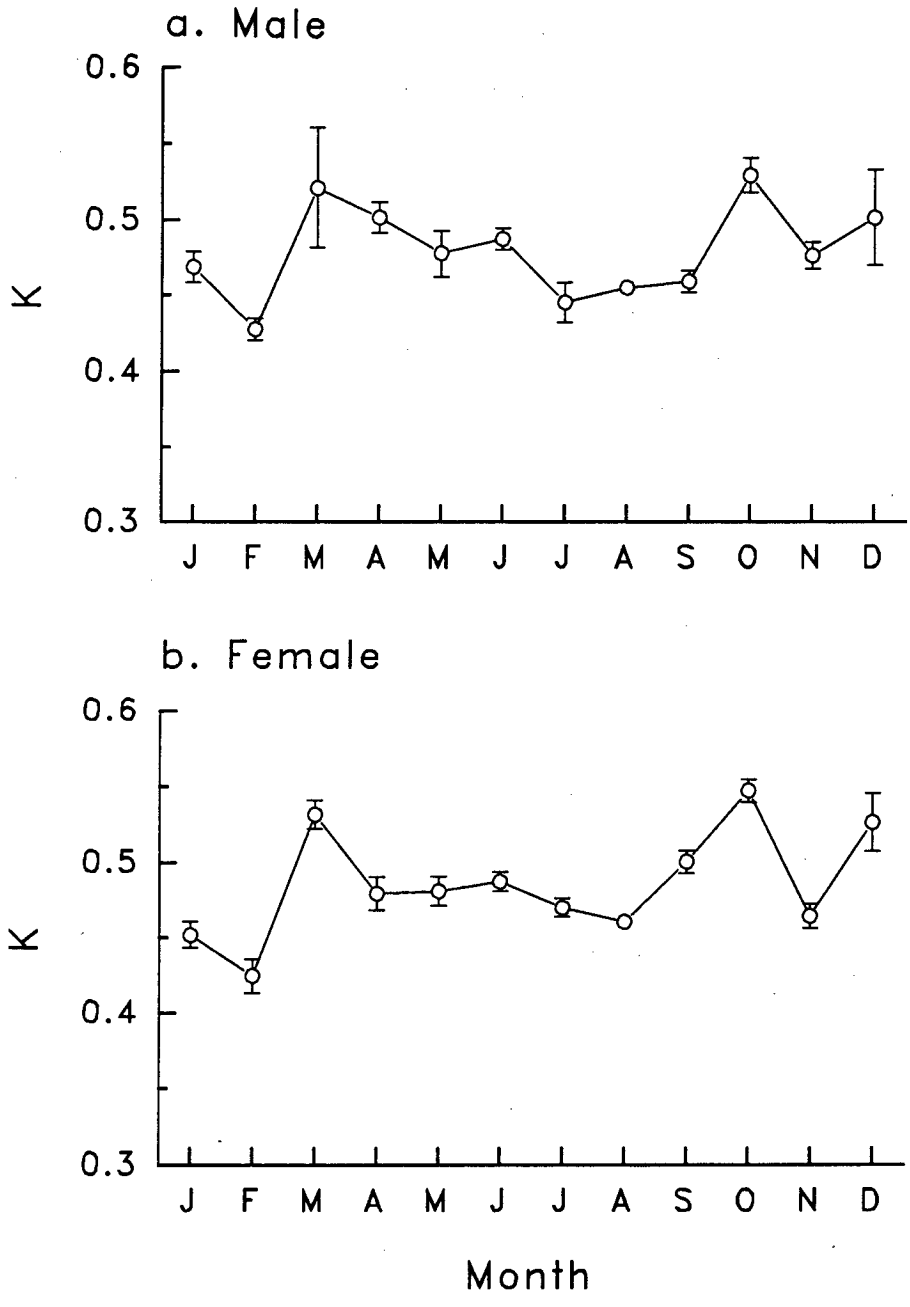


Figure 5.6: Monthly variation in the condition factor (K) of (a) male, and (b) female, *Rhizoprionodon taylori* specimens from Cleveland Bay. Circles represent monthly means, bars plus and minus one standard error.

5.4 Discussion

The diet of *R. taylori* from Cleveland Bay was dominated by small demersal teleosts and prawns. Although the Clupeidae were classified as pelagic by Stevens and Wiley (1986), Longhurst and Pauly (1987) suggested that in inshore tropical waters they are difficult to formally separate from demersal groups. From extensive trawl sampling in Cleveland Bay Milward (pers. comm.) noted that all of the prey items recorded from the sampled sharks may occur throughout the water column in the shallow nearshore waters but that, from echo sounder traces, the main aggregations of fish are close to the bottom. Thus, even though *R. taylori* occurs vertically throughout the water column (see Chapter 3) it is evident from its prey and their distribution that it feeds mainly near the bottom. This conforms with the suggestion of Lyle and Griffin (1987) that this species has a demersal habit.

Stevens and McLoughlin (1991) reported a diet for *R. taylori* from northern Australia that included clupeids, leiognathids, scombrids, stomatopods, prawns and crabs as the most common prey. Simpfendorfer (1986) reported a similar diet for specimens from Cleveland Bay, noting that juveniles consumed greater proportions of penaeid prawns than adults. These reports of diet are similar to that recorded in the present study. The small number of juveniles, however, meant that it was not possible to confirm Simpfendorfer's (1986) hypothesis regarding their preference for prawns.

The sample size of the present study was large enough to provide a good indication of the diet of *R. taylori*. More stomachs, however, would probably have provided additional data on rarer food items of *R. taylori* in Cleveland Bay because of the diversity of potential prey groups. The large number of empty stomachs, significant regurgitation rate and large numbers of unidentifiable prey greatly increased the

number of stomachs needed to be examined to provide a good estimation of the diet. The use of family level classification in the calculations also means that greater numbers of specimens would need to be examined if the diet of *R. taylori* was to be studied at the genus or species level.

The demersal teleost fauna of Cleveland Bay is very diverse (>200 spp.) and has been studied over a number of years (Milward pers. comm.). However, quantitative information on the abundance of teleost, prawn and squid species in Cleveland Bay is limited. Cabanban (1991) provided quantitative data on the teleost families caught in demersal fish trawls for a two day period in 1989. These data indicated that the family Leiognathidae made up nearly 50% of the catch (numerical abundance) and no other family made up more than 10%. The next 12 families in order of abundance were Pomadasyidae, Gerreidae, Triacanthidae, Bothidae, Synodontidae, Clupeidae, Teraponidae, Mullidae, Carangidae, Silliganidae, Nemipteridae and Tetraodontidae. Similar results were recorded from demersal trawls in the inshore waters of the Townsville region (JCUNQ 1975). The main food groups consumed by *R. taylori* thus include many of the most abundant groups of demersal teleosts. A number of families, however, were less common in stomachs of *R. taylori* than in the trawls (e.g. Bothidae, Carangidae, Gerreidae, Pomadasyidae and Triacanthidae). Several factors may account for these differences: some, or all, species within the families are of a size too large for *R. taylori* to consume; species within the families are able to avoid predation by being strong swimmers (e.g. Carangidae) or by possessing defensive structures (e.g. spines on fishes of the family Triacanthidae); and some groups may be digested more rapidly than others to the point where they are not identifiable (e.g. the Gerreidae have very soft flesh and

are rapidly digested). The apparent differences may also result from the limited nature of the teleost data. On the basis of the available information it is not possible to determine whether feeding of *R. taylori* was selective or non-selective.

There have been a variety of studies undertaken on components of the marine communities in Cleveland Bay (e.g. Beumer 1971; Lyle 1977; Gunn and Milward 1985; Lofthouse 1987; Robertson and Duke 1987, 1990), but there has never been a synthesis of information on the food web(s) present. The diversity of the fauna within the Bay means that trophic relationships are very complex, with seasonal and annual changes further complicating relationships. On the basis of the available information (much of which is unpublished) a simple food chain for the Bay is given in Figure 5.7. *R. taylori* fits into this food chain at the level of medium sized predator. This is the penultimate trophic position and is shared with species from groups such as the Chirocentridae, Lutjanidae, larger Polynemidae, Scombridae, Serranidae, and Synodontidae, amongst the teleost fishes, other small sharks, some seabirds and some dolphins.

The feeding habits of *R. taylori* appear to be similar to those reported for *Carcharhinus plumbeus* and *Negaprion brevirostris* (see 5.1.1). These species displayed no preference for feeding at a particular time of day, and when they did feed they normally consumed a single prey item. The average weight of a meal in *R. taylori* was larger than the daily rations estimated for juvenile *C. plumbeus* and *N. brevirostris*. Assuming that the daily ration of *R. taylori* is of a similar magnitude to these species (1-2% body weight per day) the average time between meals would be 1-2 days. This estimation of time between meals for *R. taylori*, however, cannot be considered as more than an approximation, which should be further tested in the future.

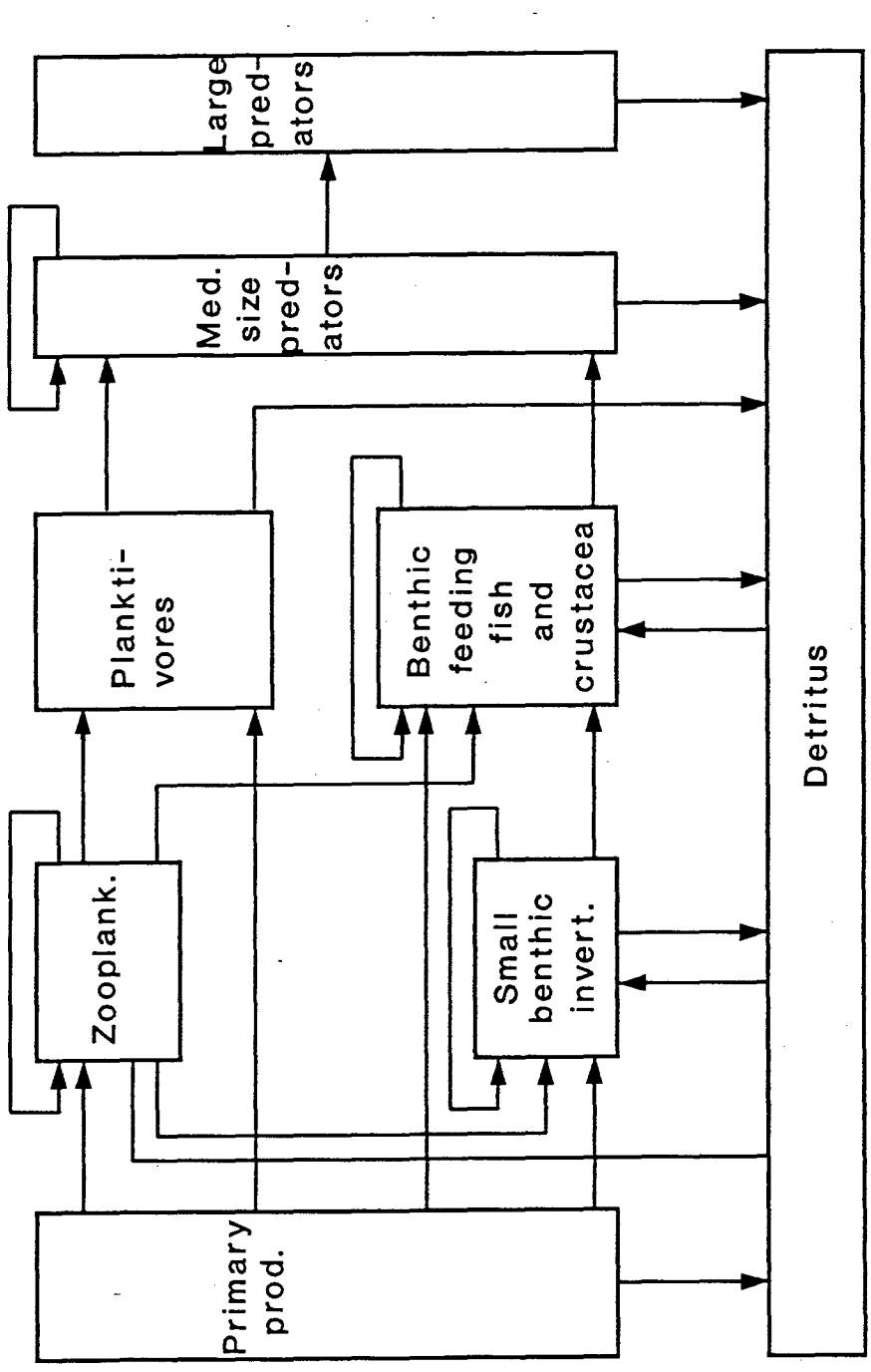


Figure 5.7: Simple food chain for Cleveland Bay.

Empty stomachs are common in *R. taylori*. Simpfendorfer (1986) reported that 51% of specimens caught in gillnets in Cleveland Bay had empty stomachs, and Stevens and McLoughlin (1991) found 44% of specimens caught in gillnets and on lines in northern Australia had empty stomachs. It was difficult to determine if the estimate of 56% empty stomach in the present study was accurate as regurgitation may have been under-estimated. If a specimen had regurgitated but the stomach had remained in the body cavity and was not extremely flaccid then it would have been recorded as empty, not regurgitated. It was also not possible, using the criteria from the present study, to identify specimens that had only partly regurgitated their stomach contents. Given these potential sources of error the number of specimens that had regurgitated may have been under-estimated. To what extent this error may have influenced the results cannot be determined without a thorough study of regurgitation in *R. taylori*. The likelihood of other methodological errors (e.g. time of sampling and sampling location) producing the large numbers of empty stomachs is low given the uniform SDI distributions recorded.

The difficulty of estimating the rate of regurgitation by individuals of *R. taylori* in Cleveland Bay prevented accurate information being obtained on the proportion of time spent without food in their stomachs. However, it is evident that a large proportion of the population had empty stomachs (other than through regurgitation). If an estimate of 50% for this is used then the sharks would have empty stomachs for approximately the same time that they spend digesting food (i.e 0.5-1 day).

The HSI and K values indicate that for some periods during the year both male and female *R. taylori* in Cleveland Bay did not meet their energy requirements by feeding. The lowest values of HSI and K in both males and females corresponded with the mating season (see Chapter 6) and may reflect increases in physical activity (e.g.

mate location, courtship and copulation) and a concomitant decrease in feeding to avoid consuming their own young (Springer 1967). The simultaneous decrease in both indices during this period strongly suggests that these reproductive activities place *R. taylori* under considerable physiological stress. The decrease in female HSI during the period from August to November most likely resulted from a combination of both providing nourishment to developing embryos within the uteri and vitellogenesis in the maturing eggs within the ovary (see Chapters 6 and 7). It is unlikely that during this period females are stressed since there was no decrease in mean K values. Variations in the mean HSI and K values of male and female *R. taylori* in the latter part of the year (particularly increases in HSI in December, and increases in K during October and December) are difficult to explain, but may reflect increased availability of food and the need to build up stored reserves in association with reproductive requirements.

Chapter 6.

Reproductive biology of *Rhizoprionodon taylori*

6.1 Introduction

Simpfendorfer (1986) presented a limited amount of data on the reproduction of *Rhizoprionodon taylori* from Cleveland Bay, suggesting that breeding was non-seasonal, litter sizes ranged from 2 to 8, and males matured at 560mm TL and females at 600mm TL. Stevens and McLoughlin (1991) have also provided some reproductive data for this species from northern Western Australia, the Northern Territory and far northern Queensland. In this study they reported a similar litter size (1-8) to that reported by Simpfendorfer (1986), but concluded that breeding was seasonal. They also noted that all adult females breed each year, that the sex ratio was even for embryos and post-partum individuals, and that males mature at 430mm TL and females at 450mm TL.

The purpose of this section of the work was to acquire further information on the reproductive biology of *R. taylori* from Cleveland Bay. This information provided data relevant to understanding the life history and population dynamics of *R. taylori*, and was also used to compare its reproductive strategy to that of other members of the family Carcharhinidae. With sampling carried out only in Cleveland Bay this study enabled the examination of reproductive traits removed from geographical variation. In studies conducted over a wide area the timing and magnitude of some aspects of reproduction can be obscured by geographical variations. Reproductive

traits examined in this study included: the sizes at birth and maturity, seasonality of breeding, timing of mating and parturition, gestation period, number of young produced, and sex ratio.

6.1.1 A review of reproduction in sharks from the family Carcharhinidae

All sharks have a similar reproductive style: fertilisation of the eggs is internal, facilitated by males possessing claspers, and the production of a small number of well developed offspring, with a high survival rate (Compagno 1990a). This reproductive style contrasts with many other types of marine organisms that fertilise eggs externally and produce large numbers of offspring that have a larval stage during which survival rates are low. The implication for shark populations of this type of reproduction is that recruitment is largely dependent upon the number of reproducing females. A knowledge of the reproductive biology of sharks is therefore important to enable the understanding of their population dynamics. This review examines reproductive traits of carcharhinid sharks. Physiological and behavioural aspects of reproduction were beyond the limits of the present study, and were not reviewed. Aspects of reproduction associated with embryonic development are reviewed in Chapter 7.

The general reproductive biology of carcharhinid species has been reported upon by numerous authors. In Australian waters Stevens (1984b) has provided data on *Prionace glauca*, *Galeocerdo cuvier* and *Carcharhinus brevipinna*; Stevens and Wiley (1986) examined *Carcharhinus sorrah* and *C. tilstoni*; and Lyle (1987a) studied *Carcharhinus cautus*, *C. fitzroyensis* and *C. melanopterus*. More recently Stevens and McLoughlin (1991) have given an overview of reproduction in most of the species present in the waters off northern Australia. Further afield Randall (1977) investigated *Triaenodon obesus* from islands in the Pacific Ocean, Tester

(1969) and Wass (1973) species from Hawaiian waters, and Cailliet and Bedford (1983) from the eastern Pacific Ocean off the west coast of the United States of America. Teshima and Mizue (1972) examined *Carcharhinus dussumieri* from Indonesian waters, Teshima *et al.* (1978) *Scoliodon laticaudus* from Malaysia, and Taniuchi (1971) *Carcharhinus plumbeus* from the East China Sea. Carcharhinid species from Indian waters were investigated by Sarangdhar (1943, 1949), Setna and Sarangdhar (1948, 1949a,b), Nair (1976), Devadoss (1979, 1988) and Krishnamoorthi and Jagadis (1986). Bass *et al.* (1973, 1975), Cliff *et al.* (1988), Cliff and Dudley (1991), and Walter and Ebert (1991) examined species from the waters off southern Africa. Carcharhinid species occurring at islands in the Indian Ocean were investigated by Fourmanoir (1961), Wheeler (1962) and Stevens (1984a). Studies on the species inhabiting the western Atlantic Ocean include those by Springer (1960), Clark and von Schmidt (1965), Pratt (1979), Branstetter (1981, 1987a), Parsons (1983a), Snelson *et al.* (1984), Ferreira (1988) and Lessa (1988). These studies have produced a relatively large body of data on reproductive traits, and these are summarised in Appendix C. Correlations between the values of reproductive parameters given in Appendix C were analysed to show trends within the family, particularly in relation to the size of a species.

Size at birth

The size of carcharhinid species at birth varies from 14cm TL in *Scoliodon laticaudus* (Teshima *et al.* 1978) to a maximum of 100cm TL in *Carcharhinus obscurus* (Clark and von Schmidt 1965; Bass *et al.* 1973), with the size at birth of most species being between 45 and 80cm TL. Larger species tend to produce larger young (Figure 6.1a; $r^2=0.569$, d.f.$=70$, $p<0.0001$). However, the young produced by smaller species tend to be larger, relative to the maximum size of the species (Figure 6.1b;

$r^2=0.586$, d.f.$=70$, $p<0.0001$). This inverse relationship suggests that smaller species invest a greater proportion of their energy in individual offspring than do larger species.

Size at maturity

Maturity in carcharhinid species occurs at a size between 40% and 90% of the maximum size for males and females, with most maturing between 60% and 90% (Figure 6.2). These values agree with estimates of 60% to 90% made by Holden (1974) for elasmobranchs in general. As would be expected the size at maturity is directly related to the maximum size, with larger species maturing at larger sizes (males, $r^2=0.928$, d.f.$=62$, $p<0.0001$; females, $r^2=0.946$, d.f.$=60$, $p<0.0001$). However, there is no significant relationship between the size at maturity, relative to maximum length, and the maximum length (males $r^2=0.0013$, d.f.$=62$, $p=0.7797$; females, $r^2=0.0036$, d.f.$=60$, $p=0.6454$).

Reproductive cycle

Once mature most carcharhinid species mate either annually or biennially. Small and medium sizes species tend to mate annually, while large species more often mate biennially (Clark and von Schmidt 1965; Bass *et al.* 1973; Branstetter 1981; Stevens and McLoughlin 1991). In most species mating occurs during a distinct period, however, populations of some species, especially those that live close to the equator, breed year-round. For example, *Carcharhinus dussumieri* in the waters off Borneo (Teshima and Mizue 1972) and *Scoliodon laticaudus* from Malaysia (Teshima *et al.* 1978) show no seasonality in their reproductive cycle. In seasonally reproducing species mating most often occurs in summer or spring (e.g. Clark and von Schmidt 1965; Taniuchi 1971; Bass *et al.* 1973,1975; Wass 1973; Pratt 1979;

Branstetter 1981; Parsons 1983a; Stevens and Wiley 1986; Lyle 1987a), with only a few mating during winter and autumn (e.g. Lyle 1987a; Stevens and Wiley 1986; Ferreira 1988).

Gestation period

The gestation period of most carcharhinid species is 10-12 months. However, in some larger species (e.g. *Carcharhinus brevipinna*, *C. longimanus*, *C. plumbeus* and *Galeocerdo cuvier*) the gestation period may be longer than 12 months (e.g. Backus *et al.* 1956; Clark and von Schmidt 1965; Bass *et al.* 1973; Wass 1973). Only *Carcharhinus cautus* (7-9 months), *C. fitzroyensis* (7-9 months) and *Scoliodon laticaudus* (5-6 months) have been reported to have gestation periods less than 9 months (Lyle 1987a; Devadoss 1979). The normal length of the gestation period in carcharhinid species means that in annually reproducing species mating follows soon after parturition.

Litter size

Intra-specific variation in litter size has often been correlated with maternal length (e.g. *Carcharhinus cautus*, *C. fitzroyensis*, *C. limbatus*, *C. longimanus*, *C. plumbeus*, *C. tilstoni* and *Rhizoprionodon terraenovae*), with larger females producing larger numbers of offspring (Backus *et al.* 1956; Wheeler 1962; Bass *et al.* 1973; Wass 1973; Parsons 1983a; Stevens and Wiley 1986; Lyle 1987a; Cliff *et al.* 1988). A number of species (e.g. *Carcharhinus albimarginatus* and *Rhizoprionodon lalandei*) do not, however, show such a relationship (Wheeler 1962; Lessa 1988). There are conflicting reports for some species as to the existence of relationships between maternal length and litter size. For example, Krishnamoorthi

and Jagadis (1986) reported that *Rhizoprionodon acutus* lacked a significant relationship, whereas Stevens and McLoughlin (1991) found the relationship was significant.

Maximum litter sizes for species within the family vary from two, in many smaller species (e.g. *Carcharhinus dussumieri* - Teshima and Mizue 1972, Setna and Sarangdhar 1949; *C. macloti* - Setna and Sarangdhar 1949a, Stevens and McLoughlin 1991; *C. sealei* - Bass *et al.* 1973; *Loxodon macrorhinus* - Bass *et al.* 1975, Stevens and McLoughlin 1991), to a maximum of 135 in *Prionace glauca* (Gubanov and Grigor'yev 1975 in Pratt 1979). Most species have maximum litter sizes of less than 20. Compagno (1988) recognised that there is a significant relationship between the maximum litter size and maximum length for placentotrophic species of the families Carcharhinidae, Hemigaleidae and Sphyrnidae. Analysis of the literature values given in Appendix C indicates a strong correlation between maximum litter size and maximum length for the family Carcharhinidae (Figure 6.3a), especially when species with maximum litter sizes larger than 20 (e.g. *Galeocerdo cuvier* and *P. glauca*) are excluded ($r^2=0.622$, d.f.$=61$, $p<0.0001$). Similarly there is a significant correlation between mean litter size and maximum length (Figure 6.3b; $r^2=0.504$, d.f.$=33$, $p<0.0001$), however, fewer data were available. Both the mean and maximum litter size graphs indicate that it is both the largest and smallest species that deviate most from the normal pattern of litter sizes within the family.

Sex ratio

The sex ratio of embryos for most species of carcharhinid sharks is even (e.g. Stevens and McLoughlin 1991). Reports of post-partum sex ratios are more variable (e.g. Parsons 1983a; Lyle 1987a; Lessa 1988; Stevens and McLoughlin 1991). Interpretation of post-partum sex ratios in carcharhinid populations, however, is

difficult. Springer (1967) noted that in many populations males and females are segregated for most of the year. As a result obtaining accurate post-partum sex ratios is dependent upon samples being collected equally from areas inhabited by males and females. Further confounding the interpretation of post-partum sex ratios are seasonal changes in distributions (e.g. adult females migrate to nursery areas to give birth, while adult males do not normally occur in nursery areas (Springer (1967)) and differences in longevity between the sexes (females normally live longer than males). Studies that specifically address the problems facing the interpretation of post-partum sex ratios are not available. Literature values of sex ratio can therefore only be interpreted as values that are probably representative of the areas sampled, but not as being representative for a species as a whole.

Conclusion

The values of many of the reproductive traits of sharks from the family Carcharhinidae vary in a predictable way, especially being closely correlated with the maximum size of a species. Such relationships provide a rapid method of estimating the values of traits in species where only size data are available. However, it should be noted that some species deviate markedly from the normal pattern. As a result such relationships should be used cautiously, and the values confirmed by measurements of the traits where possible.

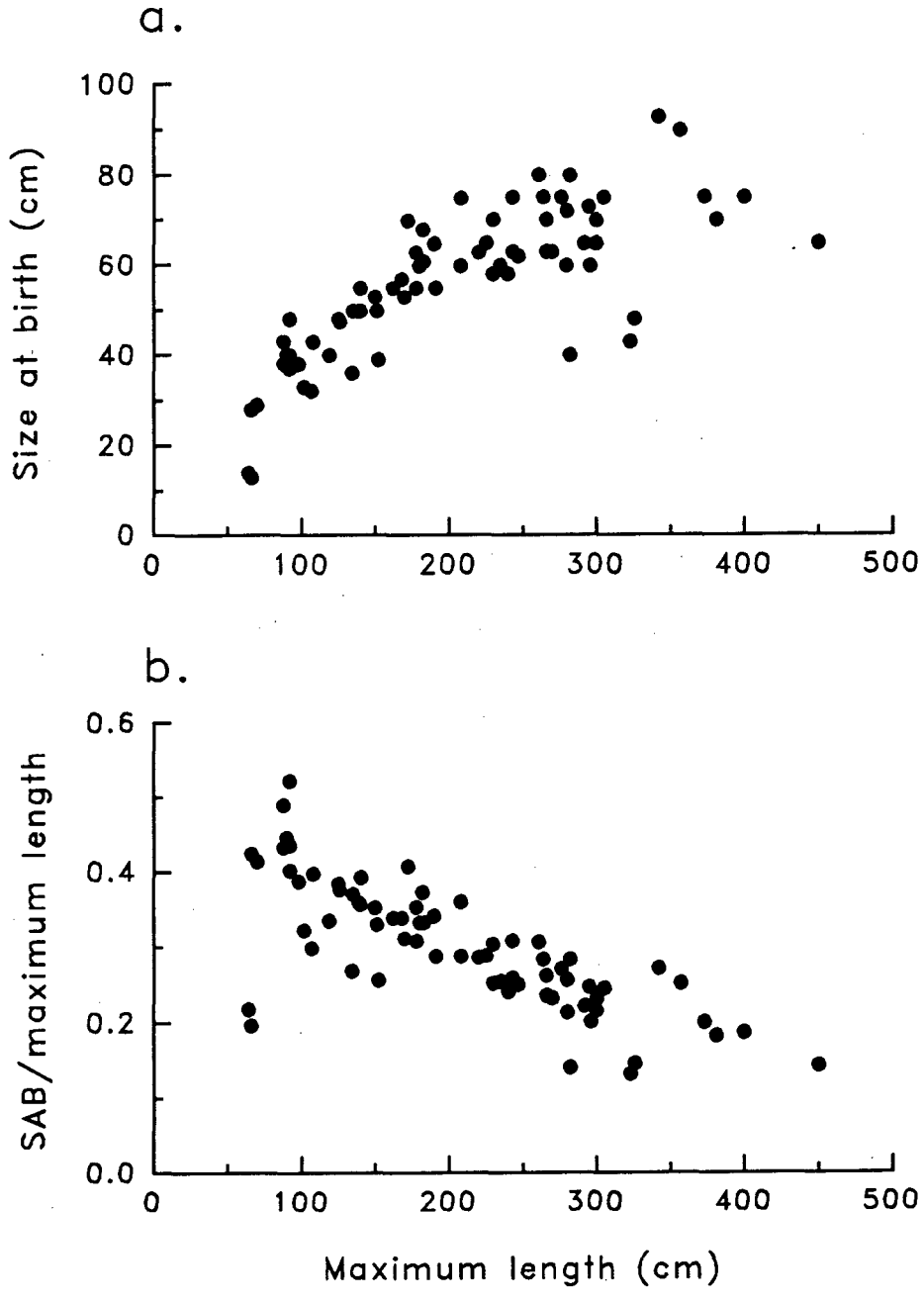


Figure 6.1: Relationships between, (a) the maximum length and the size at birth (SAB), and (b) the maximum length and the size at birth relative to maximum size, for species of the family Carcharhinidae. Where more than one report exists for a species all points are shown. Data from Appendix C.

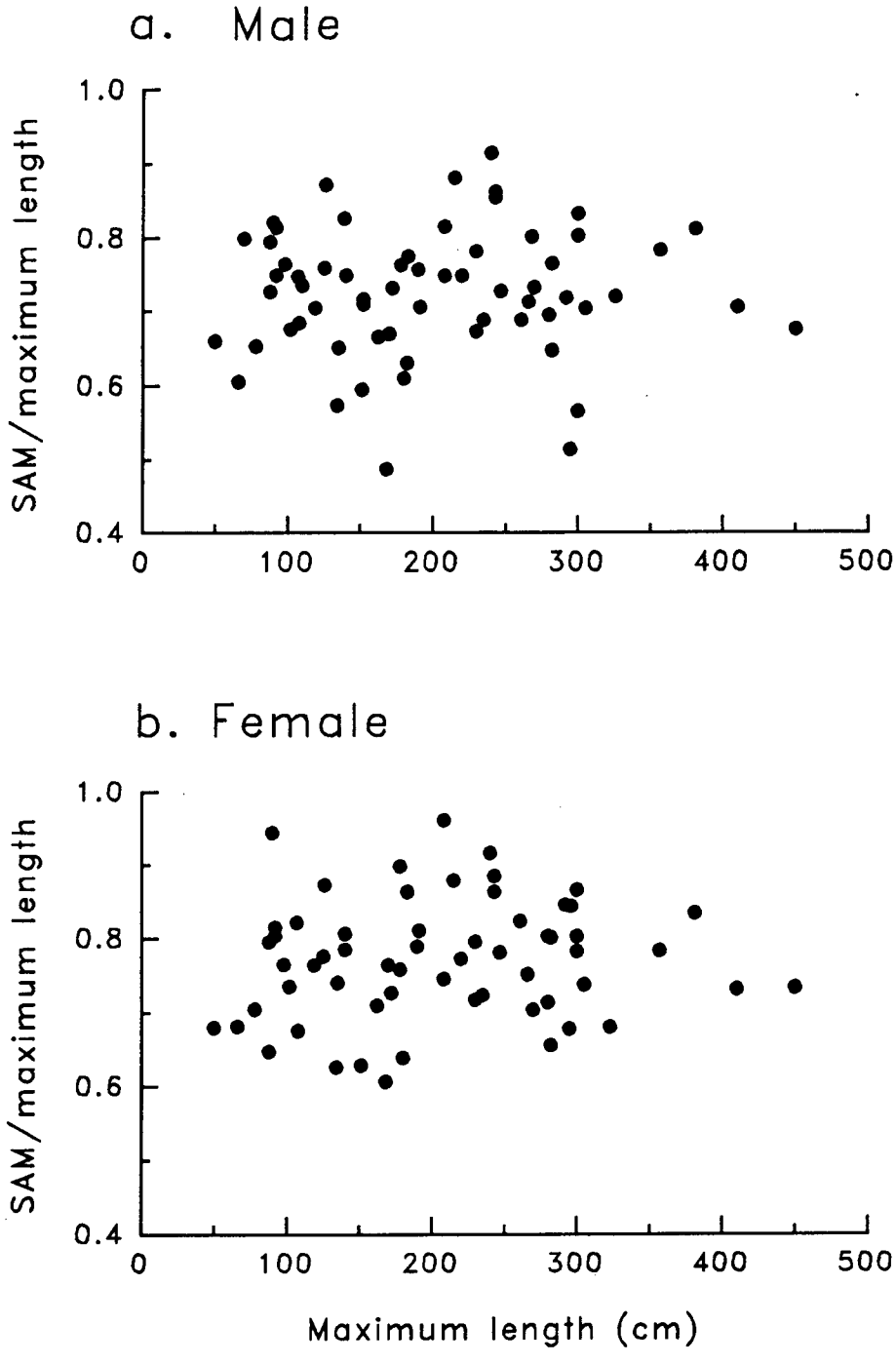


Figure 6.2: Size at maturity (SAM) as a proportion of maximum length in sharks of the family Carcharhinidae, (a) males, and (b) females. Where more than one report exists for a species all points are shown. Data from Appendix C.

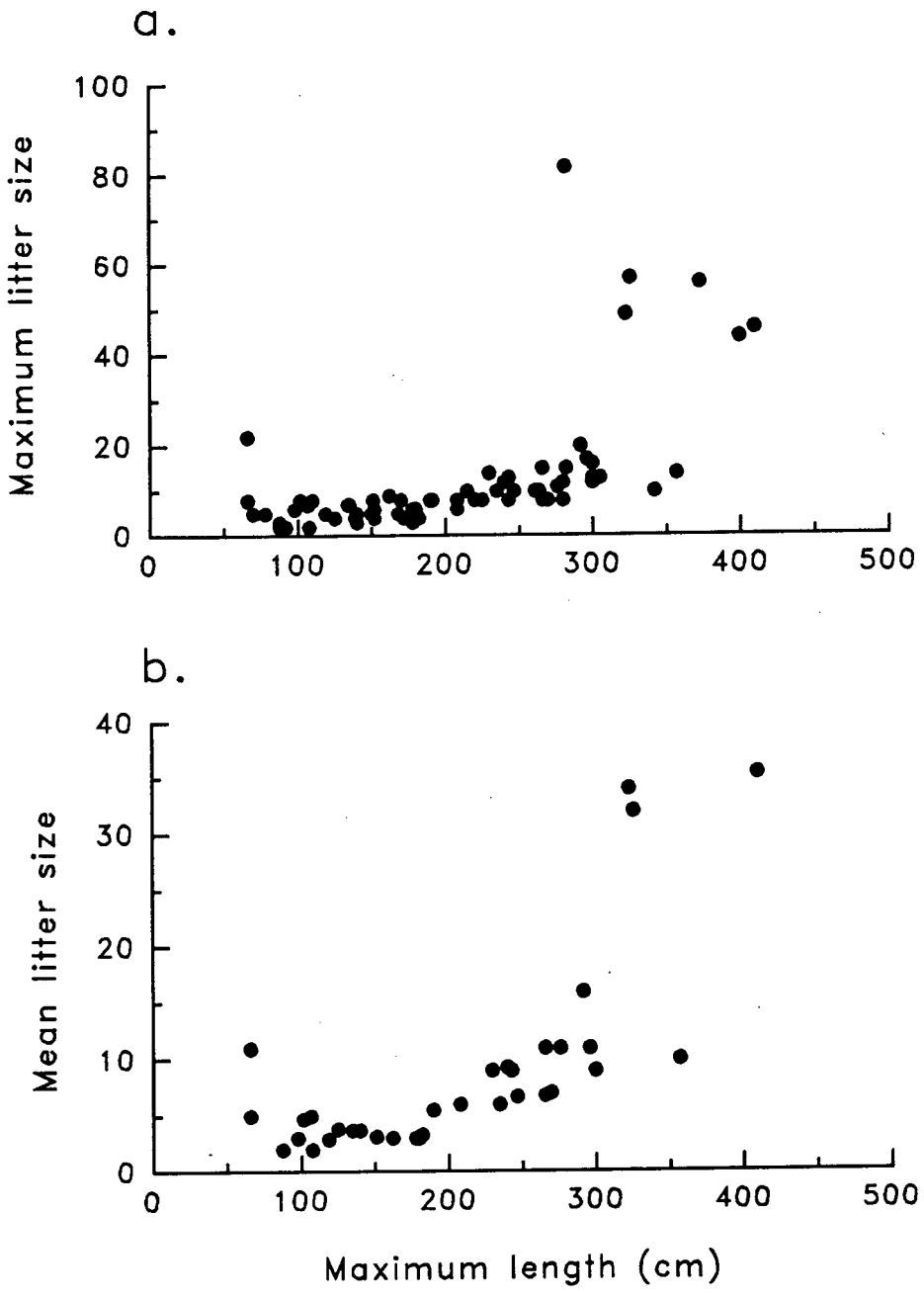


Figure 6.3: Relationships between, (a) the maximum litter size and maximum length, and (b) mean litter size and maximum length, for species of the family Carcharhinidae. Where more than one report exists for a species all points are shown. Data from Appendix C.

6.2 Materials and Methods

Collection of data

A total of 454 *Rhizoprionodon taylori* specimens were examined to provide data on reproductive biology. Information on the sampling techniques and sampling locations for this study are given in Chapter 2.

The sex of specimens collected was recorded along with their state of maturity. Males were adjudged mature if the claspers were elongate and calcified; otherwise, they were considered juveniles. The length of the claspers, from the tip to the junction with the pelvic fin, was measured to the nearest millimetre to assist in the determination of maturity of males. Mature females were distinguished by the presence of large yolky ova in the ovary, or by the presence of intra-uterine embryos. A sub-adult category was used to differentiate females in which the ova were undergoing vitellogenesis, but that had not mated as shown by the presence of a hymen. All other females were considered juveniles.

The gonads were dissected out of a sub-sample of specimens and weighed on an electronic balance to the nearest 0.1g. Measurements of maximum ova diameters (MOD) from the ovaries of a small sub-sample of mature females were made using callipers accurate to the nearest 0.1mm. The number of maturing ova in the ovary were counted, as were the number of intra-uterine embryos. Specimens that had recently been born were recognised by the presence of an open umbilical scar between the pectoral fins.

The timing of the mating period was determined from male specimens by examining for the presence of large quantities of spermatozoa in the ductus epididymidis. To

examine the contents of these ducts, a transverse cut was made across the kidney region on the dorsal wall of the body cavity. A large amount of milky white fluid running from the cut indicated that spermatozoa were present and was taken as an indicator that mating was soon to occur. Females that had recently mated were identified by the presence of mating scars on the dorsal surface of the body, mostly between the dorsal fins.

Analysis

Gonad weights were converted to gonadosomatic indices (*GSI*) using the formula:

$$GSI = \frac{gonad\ \ weight}{body\ \ weight} \cdot 100 \qquad 6.1$$

The method of collection of body weight data is provided in Chapter 2. Variations in the GSI values between months for males and females were tested using a single factor analysis of variance (ANOVA). Due to the irregularities of sampling GSI data were not collected for each month in each year of this study. Samples were pooled for all years to provide sufficient data for analysis, but as a result precluded analysis of inter-annual variations.

The proportions of individuals of different sexes and maturity states were tested between sampling sites using a Chi square contingency table. Yates' correction was applied to tests with a single degree of freedom. If more than 20% of the expected values of these tables were less than five then the results were regarded as tentative (Zar 1984).

6.3 Results

6.3.1 Maturation

The claspers of male *R. taylori* became elongate at approximately 510mm TL, and most were fully calcified at 560mm TL (Figure 6.4). All males over 570mm TL had fully calcified claspers. Elongate claspers were between 7 and 9% of body length. Males with claspers less than 7% body length were only recorded between January and September.

All female *R. taylori* under 510mm TL were juveniles, while those between 520 and 540mm TL were all sub-adults (Figure 6.5). Females normally matured at sizes between 540mm TL and 630mm TL, with 50% of females mature at around 575mm TL (Figure 6.5). Sub-adult females as large as 665mm TL were caught.

The proportions of juvenile, sub-adult female and adult *R. taylori* differed significantly between the southern Bay and Strand sites (Chi square contingency table, $\chi^2=33.98$, d.f.$=2$, $p<0.001$), with non-adults (juveniles and sub-adult females) more commonly caught at the southern Bay site (Figure 6.6). The sample size at the Middle Reef site was too small to statistically test the proportions relative to the other sites.

6.3.2 Reproductive cycle

There were significant differences in mean values of male and female GSI between months (males, ANOVA, d.f.$=10$, $F=24.61$, $p=0.0001$; females, ANOVA, d.f.$=11$, $F=11.54$, $p=0.0001$). Monthly mean values of male GSI were highest in December and January, while in females they were highest in February (Figure 6.7). Mean monthly GSI values fell sharply in males and females following these

peaks and remained low for most of the year. Increases in the male GSI values were noted in November, while in females GSI values showed a significant rise only in January (Figure 6.7). Concomitant with the increase in the female GSI there was an increase in mean monthly MOD starting in December and reaching a maximum of 10.4mm in February (Figure 6.8).

Observations of the ovary throughout the year corroborate the results of the GSI and MOD data. During early February, when female GSI and MOD were high, large yolky ova occurred in the ovary (Plate 6.1), however, by late in the month no large ova were present, and eggs were present in the uterus (Plate 6.2). In all post-ovulatory ovaries there was a single corpora atretica (Plate 6.2) that was present until August or September. At about the same time as the corpora atretica disappeared a group of ova started to accumulate yolk indicating that vitellogenesis had commenced. The number of yolky ova produced in the ovary ranged from 6 to 14, with a mean of 9.1. There was a significant relationship ($r^2=0.3395$, d.f.$=14$, $p=0.0179$) between the maternal size and the ovarian fecundity, with large females producing greater numbers of yolky ova.

A more accurate estimate of the timing of the mating period in *R. taylori* was obtained from examination of specimens from all years of this study. During the period from mid January to early February, large amounts of spermatozoa were present in the ductus epididymus of male specimens. During the same period female specimens frequently had light scarring on the dorsal surface of the body consistent with the mating scars reported for other species of carcharhinid shark (Springer 1967; Pratt 1979).

Embryos were observed in the uteri of all mature females from February (after ovulation) until mid-January of the next year. Adult females caught in January that

did not contain intra-uterine embryos had enlarged flaccid uteri, indicating that parturition had recently occurred. Data from January 1989 show that no females had pupped prior to the 11th, but by the 17th, 70% of females had given birth, and that by the 1st of February no gravid females were observed. The period during which parturition occurred in Cleveland Bay was thus no more than three weeks, and probably lasted for only two weeks, in mid to late January.

Using the date when approximately 50% of females had mated (February 5th) and the date when approximately 50% of females had pupped (January 15th) the gestation period for *R. taylori* in Cleveland Bay was calculated to be approximately 345 days (11.5 months).

6.3.3 Litter size

The litter sizes of *R. taylori* from Cleveland Bay ranged from 1 to 10, with a mean value of 4.5. There was a significant relationship between the litter size and body size of individual females (Figure 6.9; $r^2=0.3062$, d.f.$=218$; $p<0.001$), with the largest females producing the largest litters. The mean litter size was significantly lower than the mean number of yolky ova in the ovary prior to ovulation (t-test, $t=10.67$, d.f.$=271$, $p<0.001$).

6.3.4 Size at birth

Full-term embryos from females caught during mid-January ranged in size from 220 to 261mm TL. The smallest post-partum specimens examined were three juveniles ranging in size from 232 to 249mm TL, and bearing open umbilical scars, caught on the 24th of January 1989 in demersal otter trawls on the north side of

Magnetic Island (Dr. G. Jackson, Department of Marine Biology, James Cook University). These data indicate that the size at birth of *R. taylori* in Cleveland Bay spans at least 220-260mm TL.

6.3.5 Sex ratios

The sex ratio of *R. taylori* embryos and juveniles (including sub-adult females) did not differ significantly from 1:1 (Chi square tests: Embryos, $\chi^2=0.42$, d.f.$=1$, $p=0.517$; juveniles, $\chi^2=0.72$, d.f.$=1$, $p=0.395$). Adult females out-numbered adult males at a ratio of 1.86:1, which was significantly different from a 1:1 ratio (Chi squared test, $\chi^2=4.01$, d.f.$=1$, $p=0.045$). The monthly sex ratio (for all years pooled) of adults favoured females in all months except October and November (Figure 6.10).

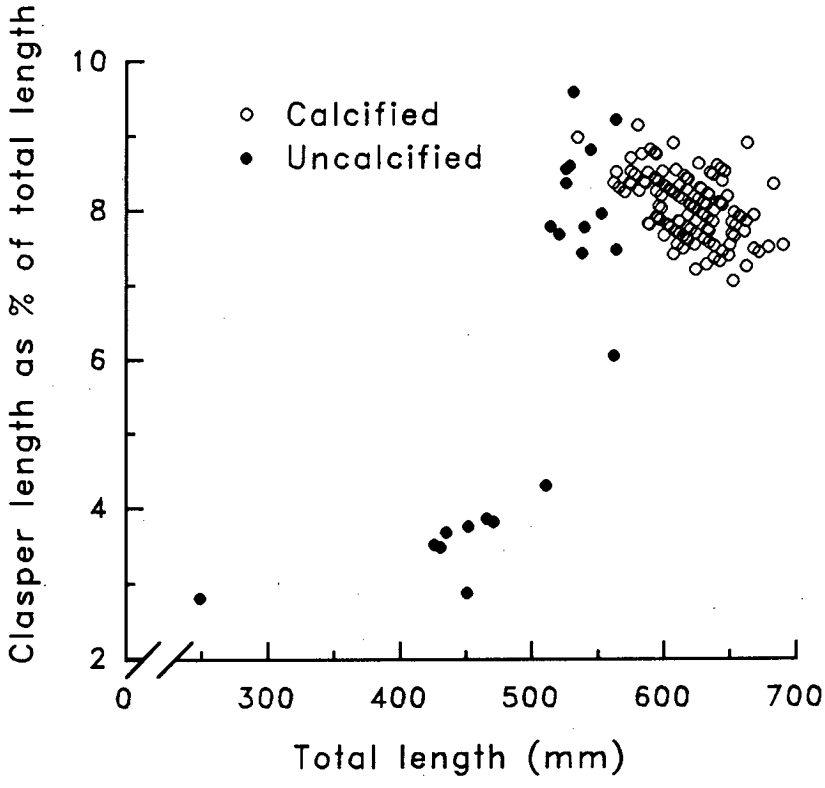


Figure 6.4: Relationship of clasper length (as a percentage of total length) to total length in 165 male specimens of *Rhizoprionodon taylori* from Cleveland Bay.

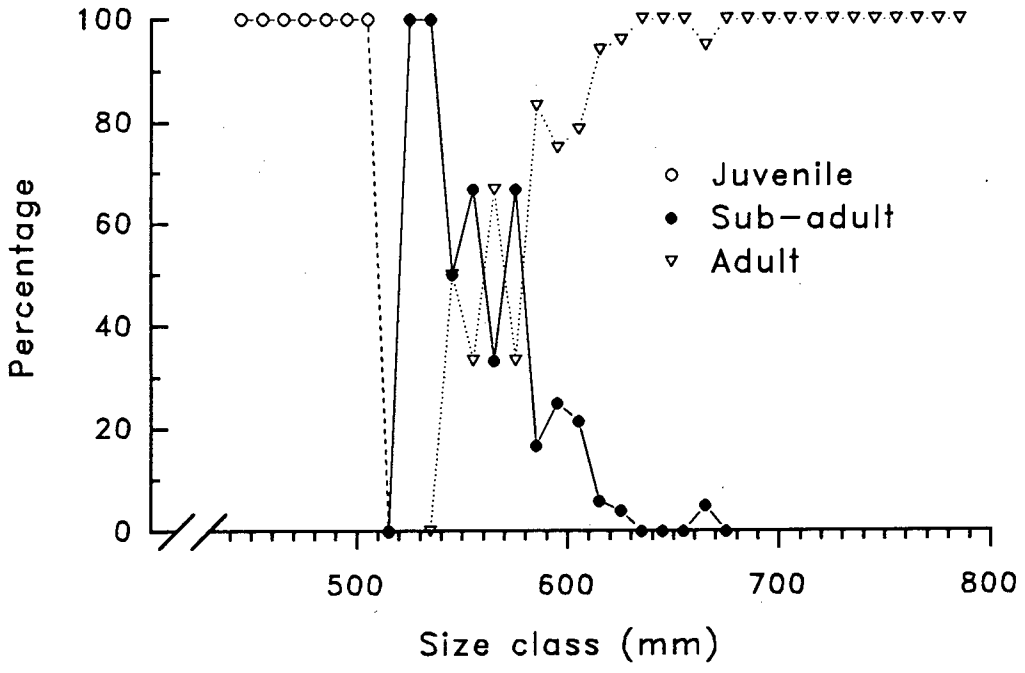


Figure 6.5: Percentage occurrence of maturity groups (juvenile, sub-adult and adult) in 10mm size classes of the female *Rhizoprionodon taylori* population from Cleveland Bay. See text for description of maturity groups.

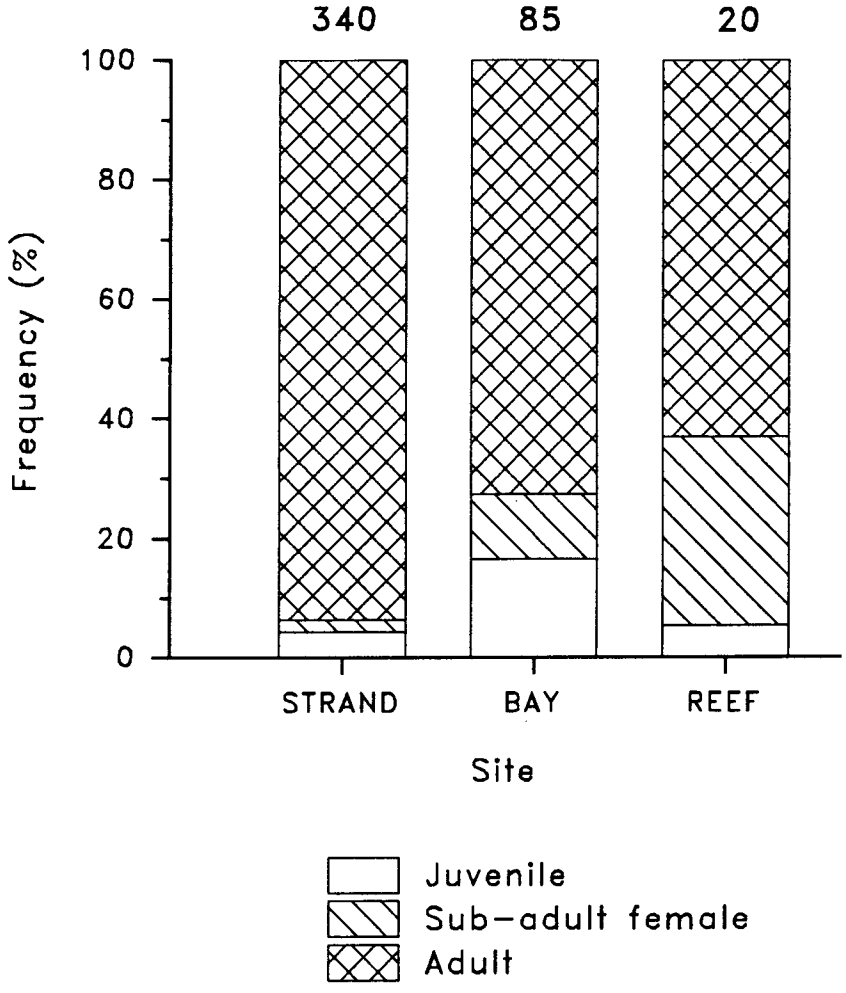


Figure 6.6: Percentage occurrence of maturity groups (juvenile, sub-adult female and adult) of *Rhizoprionodon taylori* at the three principal sampling sites in Cleveland Bay. Numbers above bars are sample sizes.

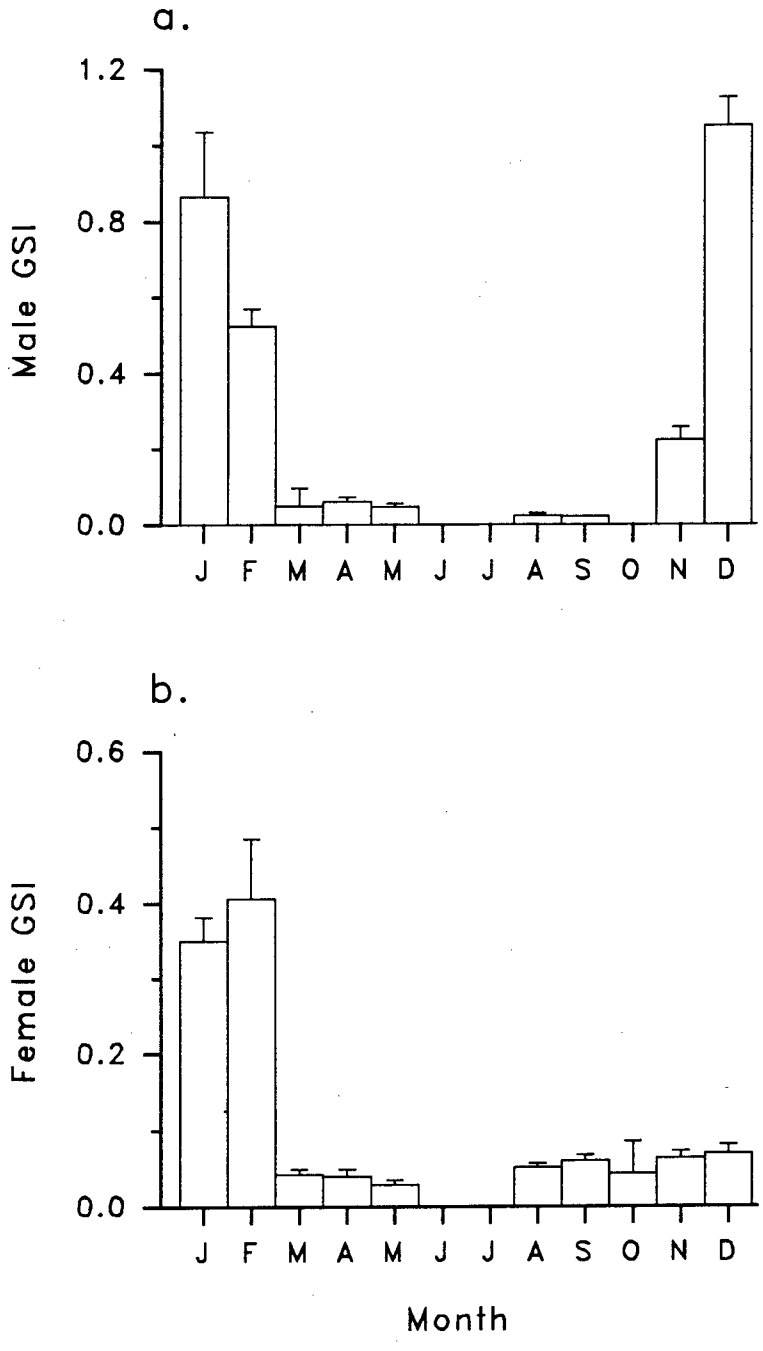


Figure 6.7: Mean monthly values (all years pooled) of gonadosomatic index (GSI) for, (a) male, and (b) female, *Rhizoprionodon taylori* from Cleveland Bay. Error bars, plus one standard error.

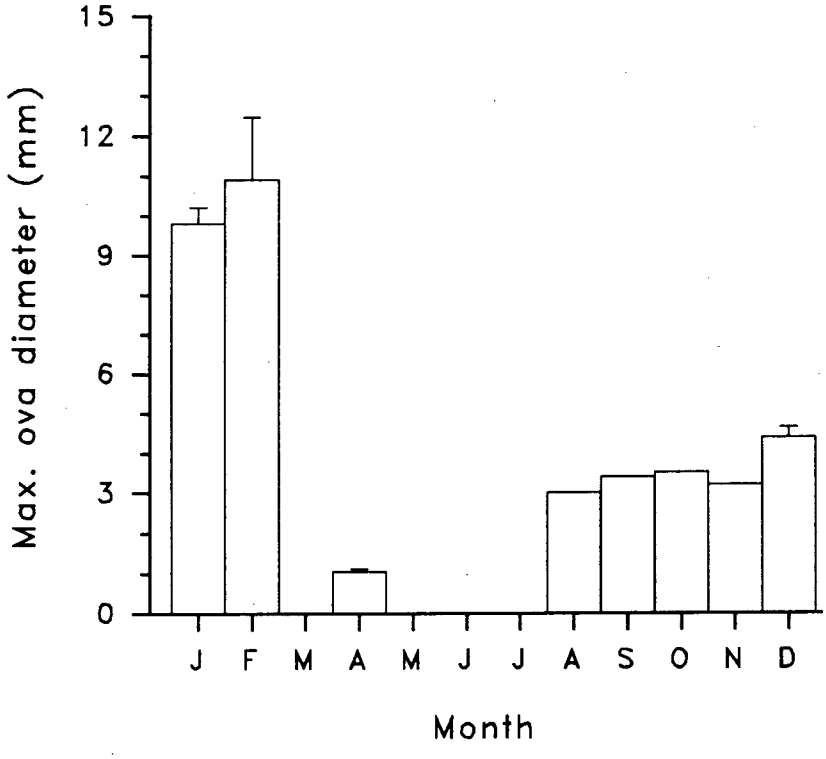


Figure 6.8: Mean monthly values of maximum ova diameter (MOD) for 29 adult female *Rhizoprionodon taylori* from Cleveland Bay. Error bars, plus one standard error.

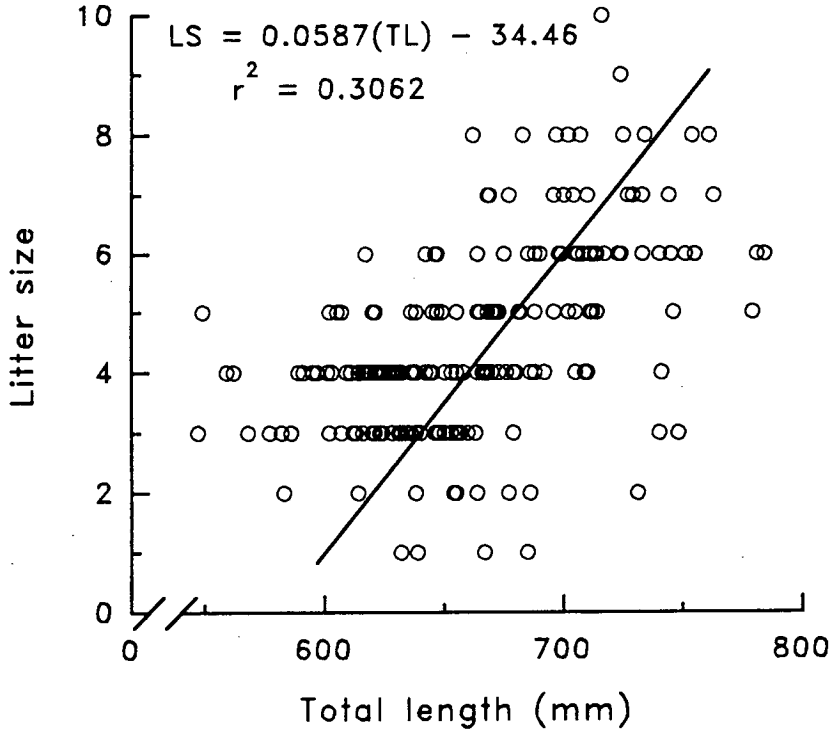


Figure 6.9: Relationship between maternal length and litter size in 221 female *Rhizoprionodon taylori* from Cleveland Bay.

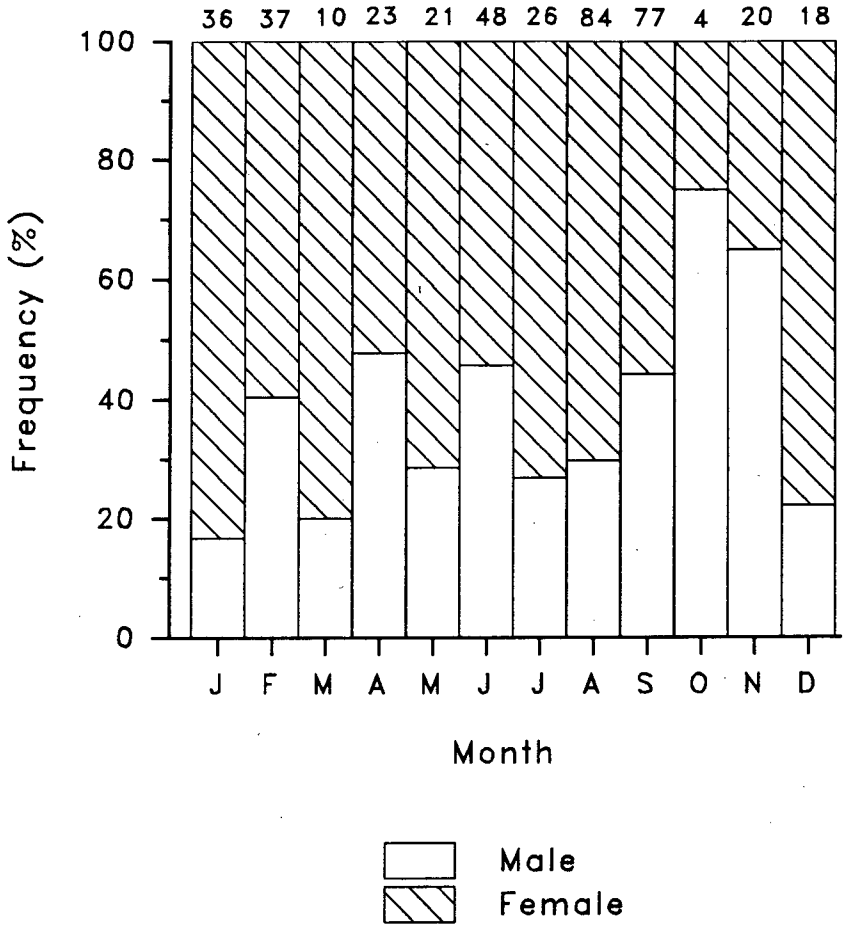
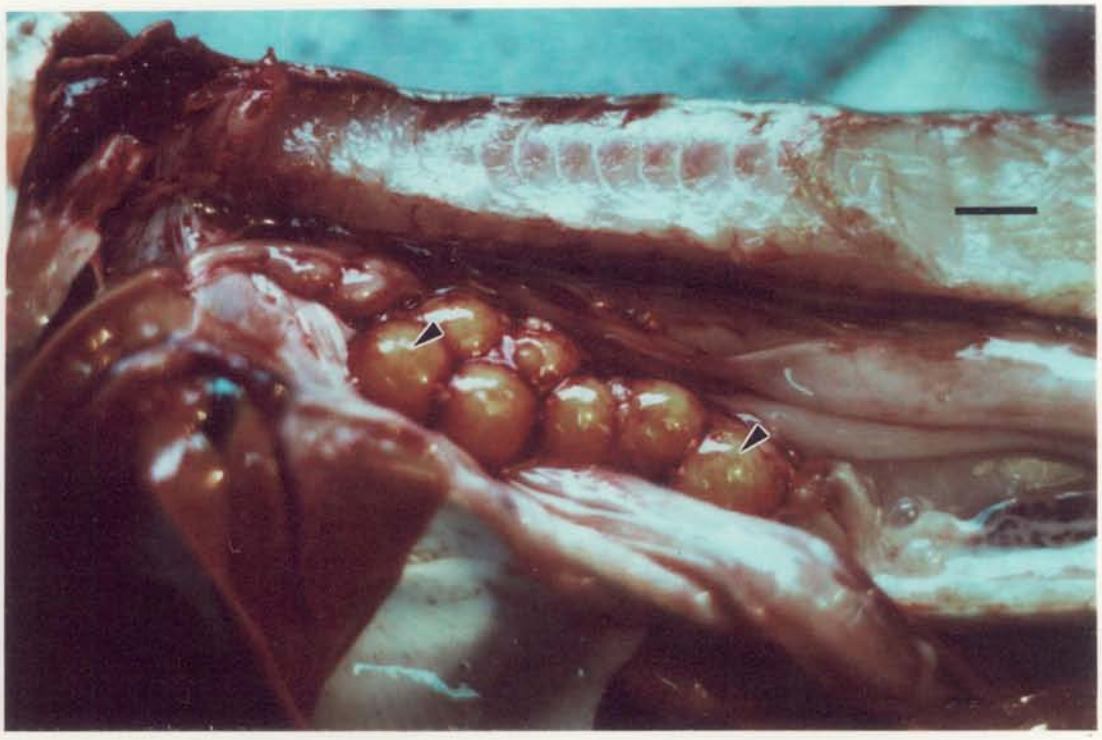
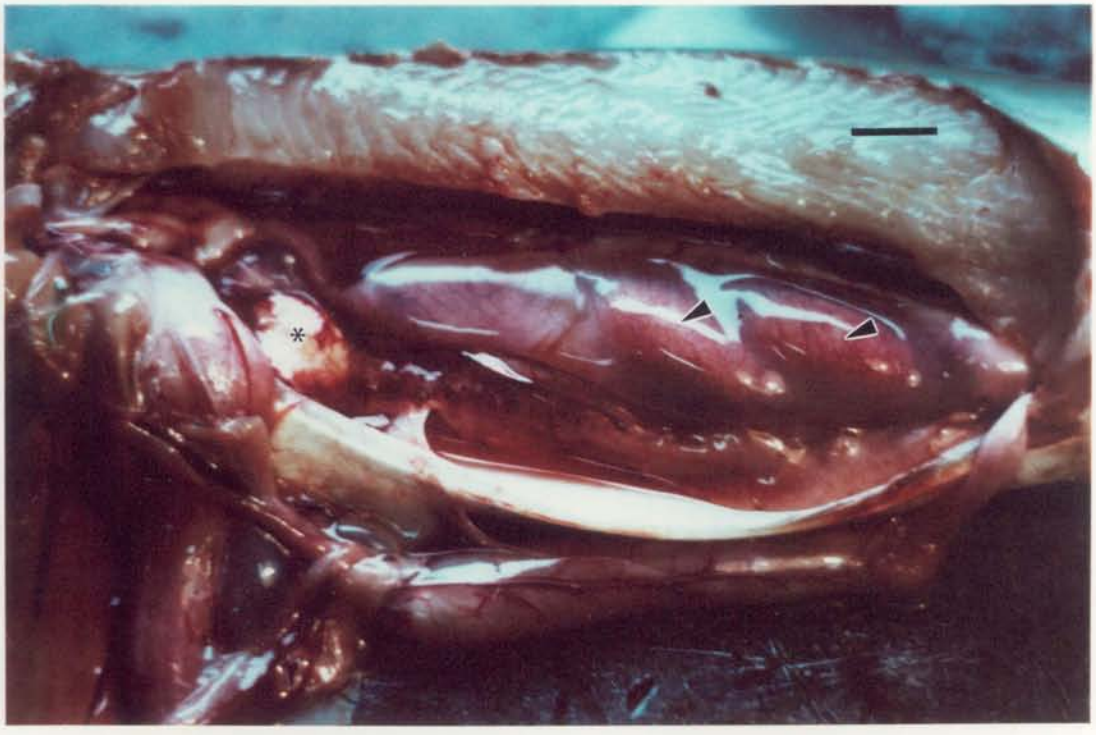


Figure 6.10: Proportions of mature male and female *Rhizoprionodon taylori* caught each month in Cleveland Bay during this study. Numbers above bars are sample sizes for each month (all years pooled).

Plate 6.1: Dissected adult female *Rhizoprionodon taylori* caught in early February showing the presence of large yolky ova (arrow heads) in the ovary prior to ovulation. Scale bar, 10mm.

Plate 6.2: Dissected adult female *Rhizoprionodon taylori* caught in late February. The yolky ova have been ovulated and eggs are present in the uteri (arrow heads). A large corpora atretica is present in the ovary (asterisk). Scale bar, 10mm.





6.4 Discussion

On the basis of the reproductive data obtained over the study period, maturity in *R. taylori* from Cleveland Bay occurs at approximately 560mm TL in males and 575mm TL in females. These sizes agree closely with the estimate of 550mm TL, for both males and females, based on growth data presented in Chapter 4. Using similar reproductive criteria for determining maturity, Stevens and McLoughlin (1991) reported that specimens of *R. taylori* from northern Western Australia, the Northern Territory and far northern Queensland mature at 430 and 450mm TL, for males and females respectively. These sizes are significantly smaller than those observed in Cleveland Bay. The maximum sizes of *R. taylori* observed in Cleveland Bay (males 691mm TL, females 784mm TL) were also larger than that reported by Stevens and McLoughlin (1991) (males 550mm TL, females 660mm TL). The sizes at maturity, relative to the maximum sizes, in each area, however, are similar (82% versus 78% for males, 73% versus 68% for females). Growth differences between these two areas therefore probably account for the observed differences in sizes at maturity.

Male *R. taylori* from Cleveland Bay started to mature in September, when the claspers grew rapidly from less than 4% TL to over 7% TL. Calcification of the claspers did not appear to be complete until after February despite the presence of large amounts of spermatozoa in the ductus epididymidis of maturing specimens. Thus either the claspers were not fully calcified at first mating in *R. taylori*, or the determination of the calcification state could have been improved. It is not known if a lack of full calcification of the claspers would effect mating success.

The GSI, MOD, and observations of the ovaries, indicate that vitellogenesis began in female *R. taylori* from Cleveland Bay during September of each year and lasted

until February. Vitellogenesis thus occurred over a period of approximately five months. Stevens and Wiley (1986) and Stevens and McLoughlin (1991) have reported similar vitellogenic periods for a number of carcharhinid species from northern Australia based on GSI and MOD data. Juvenile female *R. taylori* from Cleveland Bay must therefore have become sub-adults during September so that they were ready to mate during the next breeding season. This agrees with observations in Chapter 4 that maturation in males and females begins in September. The capture of sub-adult females up to 665mm TL suggests that a small proportion of females did not successfully mate in their first mating season, or that there were large differences in growth between individuals.

The reproductive cycle of *R. taylori* from Cleveland Bay appears to be very similar to that of other small and medium sized carcharhinid species (e.g. Clark and von Schmidt 1965; Branstetter 1981; Stevens and Wiley 1986; Stevens and McLoughlin 1991). The results of GSI and MOD analysis, and the observations of the reproductive tracts, indicate that this species has an annual reproductive cycle with mating occurring each year in late January and early February. All adult females ovulated in February, soon after mating had occurred. The young were carried for approximately 11.5 months and were born in mid-January. There appeared to be a high degree of synchrony in the timing of this cycle in Cleveland Bay, both within and between years. There is little information on the reproductive cycle of this species over the rest of its range. Stevens and McLoughlin (1991) concluded that reproduction was probably seasonal, based mostly on embryological data, with female GSI and MOD values possibly highest in February, suggesting they mate in summer. Previous work in Cleveland Bay suggested that *R. taylori* was a

non-seasonal breeder (Simpfendorfer 1986). However, this conclusion was based on embryological data that was misleading because of a period of embryonic diapause (see Chapter 7).

The fact that the mean number of maturing ova in the ovary prior to ovulation was higher than the mean number of intra-uterine embryos indicates that not all ova are ovulated. It is presumed that they remain in the ovary after ovulation forming the corpora atretica. Since all mature female specimens caught between February (post-ovulation) and August possessed a corpora atretica it is possible that excess ova were produced purposefully. The fact that the corpora atretica remained until just prior to the commencement of vitellogenesis suggests a possible function in the control of the reproductive cycle. However, further investigation of the properties of the corpora atretica will be required to properly resolve its function.

The only previous estimate of the size at birth for *R. taylori* was made by Stevens and McLoughlin (1991) who estimated it to be 250-300mm TL. This estimate, however, was only inferred from embryological data and the specimens examined by Springer (1964). The size at birth of 220-260mm TL observed in Cleveland Bay is lower than that suggested by Stevens and McLoughlin (1991), but probably represents a more accurate estimate since it is based on both embryos and young with umbilical scars. This size at birth is one of the smallest recorded for species of the family Carcharhinidae. However, it represents approximately 33% of the maximum length of *R. taylori* from Cleveland Bay which is within the normal range for the family (see Figure 6.1).

Compagno (1984) suggested that the litter size of *R. taylori* was normally two, presumably based on the observation of Whitley (1939) of a specimen with two intra-uterine embryos. The specimen examined by Whitley (1939), however, was

identified by Springer (1964) as *Rhizoprionodon acutus*. A litter size of two was also predicted by the equation given by Compagno (1988) for the relationship between maximum litter size and maximum length. However, the observations of Simpfendorfer (1986), Stevens and McLoughlin (1991), and the present study, all indicate that maximum litter size is larger than two. The high value of maximum litter size in *R. taylori* indicates that it does not conform to the normal pattern of reproduction in the family Carcharhinidae. In addition three other species do not conform to the normal range of litter sizes: *Galeocerdo cuvier*, *Prionace glauca* and *Scoliodon laticaudus*. In all four species, litter sizes are larger than would have been expected.

Both of the small species that deviate from the carcharhinid maximum size-litter size relationship have small sizes at birth: 140mm for *Scoliodon laticaudus* (Teshima *et al.* 1978; Devadoss 1979) and 220-260mm for *R. taylori*. These are the smallest sizes at birth recorded for the family. Given that development in carcharhinid sharks is internal, and thus that space within the mother is limited, there is a direct trade-off between litter size and the size of the young. Most smaller species of the family Carcharhinidae have resolved this trade-off in favour of a few large young. *R. taylori* and *S. laticaudus* have apparently evolved a strategy that produces a larger number of smaller young.

The relationship between litter size and maternal length in *R. taylori* is typical of many species in the family Carcharhinidae in which larger females produce more young than smaller ones. *R. taylori* from northern Western Australia, the Northern Territory, and far northern Queensland, show a similar relationship between these two factors (Stevens and McLoughlin 1991). Maternal length, however, only accounts for approximately 30% of the variation observed in litter size in *R. taylori*

from Cleveland Bay. Other influences on the size of litters were not identified.

Stevens and McLoughlin (1991) reported an even sex ratio in post-partum individuals, but did not distinguish between juveniles and adults. The even sex ratio observed in juvenile *R. taylori* from Cleveland Bay, and an even sex ratio in embryos, suggest that equal numbers of males and females are produced, and that survival of juveniles may not be different between the sexes. Since female *R. taylori* live longer than males it would be expected that there would be more females in the population. Although this was observed in Cleveland Bay it is not known how accurate the determined sex ratio was given the difficulties in interpretation (see 6.1.1).

The variation in the proportions of juveniles, sub-adults and adults between areas in Cleveland Bay indicates that these components of the population may utilise the Bay in different ways. Adults were probably distributed throughout the Bay, but non-adults occurred more frequently at the southern Bay site. The occurrence of non-adults in this section of the Bay may be due to the greater availability of preferred food. Simpfendorfer (1986) reported that the diet of juvenile *R. taylori* contains a significant proportion of prawns, which Robertson and Duke (1987) noted were abundant in the seagrass and mangrove habitats in the southern and eastern sections of Cleveland Bay.

The results of this Chapter indicate that some aspects of reproduction in *R. taylori* differ from those expected, based on the correlations given in the Introduction for a species of the family Carcharhinidae. In particular the litter size is much higher than predicted by the species' maximum size. The effect of the reproductive traits on the life history of *R. taylori*, and how this compares to other species of the family, are discussed in more detail in Chapter 9.

Chapter 7.

Embryonic development of *Rhizoprionodon taylori*

7.1 Introduction

Aspects of the development of *Rhizoprionodon taylori* have been briefly mentioned by Simpfendorfer (1986), Hamlett (1987), and Stevens and McLoughlin (1991). In reporting the reproductive biology of *R. taylori* Simpfendorfer (1986) observed, "Fertilised eggs were present in the uteri of mature females from all samples [May-August], however no distinct embryos were observed" (p.55). This brief note was the first indication that this species has an unusual developmental pattern, and was subsequently corroborated by Stevens and McLoughlin (1991).

Little has been recorded of the embryonic nutrition of *R. taylori*. For other species of *Rhizoprionodon* authors have recorded the presence of the entire type of placenta (Mahadevan 1940; Setna and Sarangdhar 1949a; Compagno 1988). Hamlett (1987) reported that embryos of *R. taylori* possess appendiculae on the umbilical cord, an indication that they utilise uterine secretions as a source of nutrition.

In this chapter the embryonic development of *R. taylori* is described. Here the term "embryonic development" encompasses not only the changes in the morphology of the embryo as it develops, but also those processes associated with the development of the embryos. These processes include the role that the oviducal gland plays in egg formation, the accommodation of the embryos within the female, and how the embryos obtain their nourishment.

The unusual development pattern reported by Simpfendorfer (1986) has been further investigated, and its possible occurrence in other species of elasmobranchs is examined. Structure and function of the oviducal gland in relation to embryonic development is reported. Morphological development of the embryos and their accommodation within the uterus are described, together with the structure of the tissues associated with possible sources of embryonic nutrition. On the basis of the structure of these tissues, and histochemical tests, the involvement of these structures in embryonic nutrition of *R. taylori* is hypothesized. It was not, however, an aim to demonstrate the function of these tissues, such an aim was beyond the resources of this project.

7.1.1 A review of the embryonic development of carcharhinid sharks

7.1.1.1 Introduction

This review primarily examines the literature pertaining to the embryonic development of the family Carcharhinidae. However there is a paucity of information on some aspects of development in this family, particularly in relation to early stages. Accordingly the taxonomic scope of the review has in places been broadened. With the present study directed at investigating the lack of embryonic development in uterine eggs in *R. taylori* this review concentrates on the early stages of embryonic development. Development of embryos from gastrulation onwards is not covered in detail, and information on these stages can be found in Nelsen (1953) and Pasteels (1958).

A diverse range of reproductive modes is recognised within the elasmobranchs, determined largely by the type of embryonic nutrition (Wourms 1981; Otake 1990; Compagno 1990a). These modes have been divided into oviparous (those that lay

eggs) and viviparous (those that retain eggs). The term ovoviviparous has at times been used in the literature to refer to those modes where the eggs are retained by the mother, but that do not receive any nutrients beyond the yolk in the egg (e.g. Amorosa 1960; Breder and Rosen 1966; Budker 1971). The use of this term, however, has been argued against because of the lack of information about the metabolic relationships between mother and embryo (e.g. Hogarth 1976; Wake 1992), and the presence of a continuum between mothers supplying no nourishment and complete nourishment (e.g. Breder and Rosen 1966; Wourms *et al.* 1988). Since the term ovoviviparous is not normally used Wourms (1981, p.480) defined viviparity as, "a process in which eggs are fertilised internally and are retained within the maternal reproductive system for a significant period of time, during which they develop to an advanced state and then are released." Similar definitions have been applied to groups other than elasmobranchs (e.g. Hogarth 1976). Such definitions include any mode that involves egg retention as viviparity. For consistency I follow the definition of viviparity that includes "ovoviviparous" forms that has become widely used in the literature (Balon 1981).

Wourms *et al.* (1988) divided viviparous elasmobranchs into 5 types (Table 7.1). Compagno (1990a) also described a reproductive mode as "retained oviparous" in which eggs are retained in the body. However, by the definition given above this mode is infact viviparous since the eggs are retained for a significant amount of time. Compagno (1990a) suggested that lecithotrophy represents the earliest stage in the phylogenetic progression through viviparous forms, possibly giving rise to adelphophagy/oophagy, trophodermy and placentotrophy as independent lineages, sometimes more than once. Within the family Carcharhinidae all but one species is placentotrophic (Compagno 1988), the exception being *Galeocerdo cuvier* which is lecithotrophic (Sarangdhar 1949).

Table 7.1: Trophic patterns of viviparous elasmobranchs listed by Wourms *et al.* (1988). Orders of elasmobranchs possessing these trophic patterns from Compagno (1990a).

Trophic pattern	Source of nutrients	Order of sharks
Lecithotrophy	Yolk stored in yolk sac	Hexanchiformes Squaliformes Pristiophoriformes Squantiniformes Orectolobiformes (some) Carcharhiniformes (some) Rhinobatiformes Pristiformes Torpediniformes
Adelphophagy	Ingestion of other embryos in the uterus	Lamniformes
Oophagy	Ingestion of yolky "eggs" ovulated by mother	Lamniformes
Trophodermy	Absorption of maternal secretions	Myliobatiformes
Placentotrpohy	Yolk sac placenta	Carcharhiniformes (some)

Most authors have based their descriptions of reproductive modes on a single form of embryonic nutrition. This, however, ignores the fact that depending on the stage of development at which a species is examined different methods of nutrition may be utilised. For example, placentotrophic species will normally rely on the yolk for their nutrition (i.e. lecithotrophy) before the placenta develops, and may also use uterine secretions during some stages of development (i.e. trophodermy) (Hamlett 1986). Thus the names of the reproductive modes can often belie the complexities of the arrangement of embryonic nutrition on which they are based.

7.1.1.2 Function of the oviducal gland in relation to embryonic development

The oviducal gland of elasmobranchs plays an important role in the packaging of the ova after ovulation. The only detailed study of the structure of the oviducal glands of carcharhinid sharks is that of Prasad (1944), although their general form has been described by a number of authors (e.g. Pratt 1979; Parsons 1983a). A number of detailed structural and functional investigations of the glands have been undertaken on oviparous species (e.g. Threadgold 1957; Rasaouen 1976; Rasaouen *et al.* 1976; Rasaouen-Innocent 1990). These studies have shown that the glands are made up of numerous tubules, which can be classified on the basis of their structure and secretion. Three possible functions have been suggested for different tubule types found in the oviducal gland: (1) egg case production, (2) albumen production, and (3) seminal receptacle. It has been postulated that the majority of tubules are concerned with the production of the egg case (Prasad 1944; Rasaouen-Innocent 1990). In oviparous species the egg case is thick, to protect the eggs in the external environment, and is composed of collagen with phenolic cross linkages produced by a quinone tanning process (Rasaouen 1976; Wourms 1977). Viviparous species on the other hand have a thin, membranous egg case (Sarangdhar 1949; TeWinkel 1950; Springer 1960; Gilbert and Schlernitzauer 1966; Teshima and Mizue 1972; Hamlett *et al.* 1985a). The exact nature of the egg case in carcharhinids has not been reported (Hamlett *et al.* 1985a). In all of the carcharhinids, the egg occupies approximately 10% of the egg case, the remaining space being produced to accommodate the growth of the embryo (TeWinkel 1950; Teshima and Mizue 1972; Baranes and Wendling 1981; Hamlett 1986).

It has also been hypothesized that the gland produces albumen, which is incorporated around the ova within the egg case (Prasad 1944). However, Rasaouen (1976)

suggested that the areas identified by early workers (e.g. Threadgold 1957) as albumen secreting do not produce protein (the major component of albumen) and may instead be responsible for sealing the egg case. Where albumen is produced is therefore unknown.

Finally the oviducal gland may serve as a seminal receptacle (Metten 1939; Prasad 1944; Wourms 1977), a function particularly important in oviparous forms that produce eggs for long periods after copulation (Metten 1939), and also in the carcharhinid species that delay fertilisation (Prasad 1944; Pratt 1979). Metten (1939) reported that sperm were stored in the shell secreting tubules of *Scyliorhinus canicula*. Prasad (1944) observed that sperm were only stored in viviparous species around the time of fertilisation, but did not identify the type of tubule involved in the storage.

7.1.1.3 Early embryonic development

The eggs of elasmobranchs are telolecithal. The largest of the eggs are found in the forms that rely totally on the yolk to provide energy for development (i.e. oviparous and lecithotrophic viviparous species). Other viviparous species, where the mother supplies energy to the young during pregnancy, have smaller eggs at ovulation. The smallest elasmobranch eggs occur in the carcharhinid species *Scoliodon laticaudus* which have eggs with little yolk as a placenta develops at a very early stage of pregnancy and so dependence on yolk is minimal (Setna and Sarangdhar 1948).

Most published accounts of elasmobranch embryology appeared in the late nineteenth and early twentieth centuries (e.g. Balfour 1878; Zielger 1902 in Nelson 1953; Vandebroek 1936; Gudger 1940; Smith 1942; Nelson 1953), and some more recently

(e.g. Mellinger *et al.* 1986). All of these accounts are based on oviparous species as eggs are easily obtained and it is possible to keep the eggs viable for long periods of time in the laboratory. On the other hand viviparous species are more difficult to study as the eggs must be obtained from a dissected female, and their maintenance in the laboratory for any useful period of time has to date proved extremely difficult, if not impossible (TeWinkel 1950). For the purposes of this review it is assumed that the early embryonic development of viviparous species is the same, or similar, to that described for oviparous species.

Protoplasm in an ovulated ovum is concentrated at the animal pole (TeWinkel 1950; Wourms 1977) in a small disc referred to as the germinal disc or blastodisc (Balfour 1878; Nelsen 1953). The germinal disc is normally recognised by having a distinctive red or orange appearance (Balfour 1878; Smith 1942). The ovum is fertilised in the cranial section of the oviduct or as it passes through the oviducal gland (Wourms 1977). The fertilisation of elasmobranch eggs involves polyspermy (Nelsen 1953), as is the case in other species with telolecithal eggs due to the presence of large amounts of yolk. However, although many spermatozoa penetrate the germinal disc it is the one closest to the female pronucleus that fuses with the male pronucleus to form the zygote (Wourms 1977).

Cleavage of the eggs of elasmobranchs is meroblastic. In the early stages two distinct groups of cells are formed, one being the blastoderm and the other the syncitial periblast or trophoblast (Nelsen 1953). The periblast is located around the periphery of the blastoderm and has a nutritive function (Nelsen 1953). As the embryo develops the periblast tissue lines the internal wall of the yolk sac and is termed the yolk syncitium (Hamlett and Wourms 1984). It has been suggested that the non-fertilising spermatozoa that penetrate the egg may contribute part, or all,

of the nuclei in the periblast tissue (Nelson 1953) but this has not been proven. During the stages of early cleavage of the eggs of *Mustelus canis* TeWinkel (1950) observed that the blastoderm moved from a posterior position to a location on the equator of the ellipsoid shaped eggs.

The developing blastoderm forms two layers comprising surface blastomeres and deep blastomeres. The surface blastomeres enclose the segmentation cavity (which forms only in the caudal section of the blastoderm), whereas the deep blastomeres are located within the segmentation cavity (Wourms 1977). Vandebroek (1936) produced a detailed account of the origin of the early tissues, but Wourms (1977) questioned the validity of this work based on the techniques used.

As the developing embryo grows it differentiates from the blastoderm, eventually only being attached by a thin yolk stalk (Wourms 1977). The remaining blastoderm tissue grows around the yolk, forming the yolk sac.

7.1.1.4 Gestation periods and embryonic growth rates of carcharhinid sharks

Gestation periods of viviparous elasmobranchs are typically long, varying from six months (e.g. *Hemigaleus microstoma* - Stevens and McLoughlin 1991; *Scoliodon laticaudus* - Devadoss 1979; *Sphyrna tiburo* - Parsons 1987) to two years (e.g. *Squalus acanthias* - Ford 1921). In the carcharhinid sharks they normally vary from 5 to 12 months (Table 7.2), with large species (e.g. *Galeocerdo cuvier*) possibly having slightly longer periods of gestation (Compagno 1984).

The birth size of carcharhinid species is typically 40-70cm (Table 7.2), but can be as small as 14cm in *Scoliodon laticaudus* (Setna and Sarangdhar 1948; Devadoss 1979). The average length increase varies from approximately 2cm $month^{-1}$ to

7cm month^{-1} (Table 7.2). The lowest monthly length increases recorded occur in small species such as *Rhizoprionodon* spp. *Prionace glauca*, a relatively large species, also has a low growth rate which may be the result of the exceptionally high litter sizes that occur in this species (Pratt 1979). Interestingly the non-placental *Galeocerdo cuvier* has a comparable growth rate to the placental species. There is a considerable degree of intraspecific variation in the average monthly length increase of embryos between studies in different areas (Table 7.2). In *Carcharhinus plumbeus*, for example, the average length increase ranged from 4.5cm month^{-1} to 6.8cm month^{-1}.

Table 7.2: Growth rates of carcharhinid sharks reported in the literature. G, gestation period in months; L, total length at birth in centimetres; and L/G, average monthly length increase.

Species	G (mon)	L (cm)	L/G (cm mon^{-1})	Source
Carcharhinus amblyrhynchoides	10	55	5.50	Stevens & McLoughlin 1991
C. amblyrhynchos	9	63	7.00	Stevens & McLoughlin 1991
C. amboinensis	9	63	7.00	Stevens & McLoughlin 1991
C. brevipinna	12	65	5.42	Branstetter 1981
	12	75	6.25	Stevens & McLoughlin 1991
C. cautus	8	40	5.00	Lyle 1987
C. fitzroyensis	8	50	6.25	Lyle 1987
C. limbatus	12	55	4.58	Branstetter 1981
C. longimanus	12	70	5.83	Backus *et al.* 1956
C. macloti	12	43	3.58	Stevens & McLoughlin 1991
C. melanopterus	11	50	4.54	Stevens 1984
	9	48	5.33	Lyle 1987
C. plumbeus	9	61	6.80	Springer 1960
	12	64	5.33	Wass 1973
	12	54	4.50	Cliff *et al.* 1988
	12	60	5.00	Stevens & McLoughlin 1991
C. sorrah	10	50	5.00	Stevens & Wiley 1986
C. tilstoni	10	60	6.00	Stevens & Wiley 1986
Galeocerdo cuvier	12	65	5.42	Stevens & McLoughlin 1991
Prionace glauca	12	40	3.33	Pratt 1979
Rhizoprionodon taylori	11	28	2.54	Stevens & McLoughlin 1991
R. terraenovae	11	35	3.18	Branstetter 1981
	11	32	2.91	Parsons 1983
Scoliodon laticaudus	6	14	2.33	Devadoss 1979

7.1.1.5 Delays during the early stages of embryonic development

Observations of Simpfendorfer (1986) and Stevens and McLoughlin (1991) regarding the lack of visible embryos in uterine eggs of *R. taylori* from northern Australia suggest that development after ovulation may be delayed. In the

elasmobranch literature there are several other reports of species in which there was no visible development of embryos for some time following ovulation. Lessa *et al.* (1986) observed that in the shovel-nose ray, *Rhinobatus horkelli*, ovulation (and presumably fertilisation since the egg case is present) occurs in April but no development was visible until December. A similar phenomenon was reported by Snelson *et al.* (1989) for the stingray *Dasyatis sayi*. In *D. sayi* ovulation occurred in June and development was visible only from April of the next year. Gilmore *et al.* (1983) suggested that *Carcharias taurus* may also have a period of delayed development, but thought that it was more likely that fertilisation occurred over an extended period of time.

Snelson *et al.* (1989) suggested two hypotheses for the lack of development after ovulation in *D. sayi*, either that spermatozoa may be stored in the female and fertilisation delayed or, alternatively, that zygote development is arrested. Snelson *et al.* (1989) pointed out that the presence of the egg case suggests that the second of these is more likely. However, long term storage of spermatozoa occurs in some oviparous species of elasmobranchs (e.g. *Scyliorhinus retifer* - Castro *et al.* 1988), and also in some viviparous species (e.g. *Prionace glauca* - Pratt 1979). In the latter cases spermatozoa are stored in the oviducal glands and fertilisation of the eggs is thought to occur as the ova pass through the gland. It would thus be expected that any eggs that have passed through the oviducal gland would be fertilised, as is the case for *D. sayi* and *R. horkelli*.

None of the foregoing authors provided information about the embryo, if there was one, during the period when development was not observed after ovulation. If, in fact, the eggs are fertilised, then the inability to observe embryos suggests that development is stopped, or greatly slowed, at an early stage.

7.1.1.6 The accommodation of pregnancy

Young of all species of viviparous elasmobranchs develop in the paired uteri (Wourms 1977). Several authors have differentiated two types of uterus in viviparous species: those that form individual compartments for each of the young, and those that do not (e.g. Teshima and Mizue 1972; Otake 1990). Otake (1990) reported that all placental species, along with some of the lecithotrophic viviparous species, have uterine compartments. All of the family Carcharhinidae have uterine compartments, including *Galeocerdo cuvier* which is yolk sac viviparous (Sarangdhar 1949). The normal orientation of the embryos within the compartments is to face anteriorly (Baranes and Wendling 1981).

Uterine compartments are formed by the growth of dorsal and ventral septa from the uterine mucosa (Mahadevan 1940; Baranes and Wendling 1981). Initially the septa run transversely, but as the embryos grow they become longitudinal (Mahadevan 1940; Baranes and Wendling 1981). A number of authors have reported that extending off each of these compartments is a storage chamber in which the excess egg case is housed (e.g. Setna and Sarangdhar 1949b; Teshima and Mizue 1972; Baranes and Wendling 1981; Hamlett 1987) (Figure 7.1). Teshima and Mizue's drawings showed that in *Carcharhinus dussumieri* the storage chamber has only one section. On the other hand Baranes and Wendling (1981) indicate that in *Carcharhinus plumbeus* it is composed of two sub-chambers. The wide spread occurrence of the storage chamber lead Baranes and Wendling (1981) to conclude that they probably occur in at least all species of *Carcharhinus*, and Hamlett (1987) suggested that they were a feature common to all carcharhinid species.

Hamlett (1987) considered that the uterine compartments served at least two purposes. He proposed that: (1) septa separating compartments increase the surface

area for gas exchange and secretion; and (2) compartments prevent tangling and knotting of egg cases, yolk stalks, and umbilical cords of different embryos. The storage chambers may serve a similar function, but stop tangling and knotting between the egg case and umbilical cord of a single embryo.

Structure and function of the uterus

There have been a number of studies on the structure and function of the uterus of elasmobranchs, including several on carcharhinid species. These studies on carcharhinid sharks have shown that the uterus is comprised of four layers: mucosa, submucosa, muscularis and a serosa (e.g. Mahadevan 1940; Baranes and Wendling 1981) . This organisation appears to be similar in viviparous species from other families (e.g. Jollie and Jollie 1967).

The uterine mucosal cells of some species of carcharhinid sharks (e.g. *Scoliodon laticaudus* and *Rhizoprionodon acutus*) have been reported to produce secretions that are utilised as a source of nutrients by the developing embryos (Southwell and Prashad 1919; Mahadevan 1940; Wourms 1977; Otake 1990). These secretions, however, do not occur in all species of the family Carcharhinidae. For example, Baranes and Wendling (1981) did not report any secretion in *Carcharhinus plumbeus*, nor did Otake and Mizue (1986) for *Prionace glauca.*

Also producing uterine secretions in carcharhinid species of shark are glands with a tubular appearance (Baranes and Wendling 1981). Glands in the submucosa of *Rhizoprionodon acutus*, *R. oligolinx* and *Scoliodon laticaudus*, were described by Mahadevan (1940, p.11) as "a sheet like structure made up of glandular cells". A similar type of gland was described by Ranzi (1934) in association with the placenta of *Mustelus laevis*. It has been suggested that the secretion of the sheet or tubular

glands is also utilised as a source of nutrients by embryos (Mahadevan 1940). However, their presence in species which are not thought to utilise uterine secretions as a source of nutrients (e.g. *Carcharhinus plumbeus* - Baranes and Wendling 1981) suggests that they may have some other function.

Ranzi (1934) has provided the most complete analysis of the composition of the uterine secretions of elasmobranchs. He found that *Mustelus laevis*, a placental species, produces a secretion which has an organic content of 9.1%, most of which was mucus. In the myliobatoids (sting rays), which rely heavily on uterine secretions as a source of nutrients for the young, Ranzi found the secretion to be mostly lipid with an organic content of up to 13.3%. Although Ranzi did not report findings from any species of carcharhinid sharks it is likely that their uterine secretions would be most similar to those of *Mustelus laevis*.

7.1.1.7 Embryonic nutrition

Carcharhinid sharks are considered placentally viviparous, with the exception of *Galeocerdo cuvier*. Only in *Scoliodon laticaudus*, however, does the placenta provide nourishment for the embryo throughout its development (Setna and Sarangdhar 1948). In all of the other placental carcharhinid species the direct connection between mother and foetus only occurs after a number of months (Mahadevan 1940; Teshima and Mizue 1972; Baranes and Wendling 1981). Prior to the development of the placenta, embryos of these species rely upon other sources of nutrients. Mechanisms of embryonic nutrition have received increasing attention in recent years (e.g. Jollie and Jollie 1967; Hamlett and Wourms 1984; Hamlett *et al.* 1985a,b,c,d; Otake and Mizue 1986) and have been reviewed by Hamlett (1986,

1987, 1989) and Wourms *et al.* (1988). These studies have revealed that in the carcharhinids three possible sources of nutrients for embryos: yolk, uterine secretions and placentae.

Yolk

The eggs of all species of carcharhinid shark contain at least some yolk which the embryo uses as an energy source. The amount of yolk in the egg varies. In *Scoliodon laticaudus* the eggs are only a few millimetres in diameter and contain little yolk (Mahadevan 1940; Setna and Sarangdhar 1948; Devadoss 1979). The eggs of all other species are large (up to 30mm diameter) and contain large amounts of yolk (Mahadevan 1940; Hamlett 1989). The yolk is stored in the external yolk sac that connects to the embryo via the yolk stalk that contains three channels: vitelline artery, vitelline vein, and ductus vitello-intestinalis (Gilbert and Schlernitzauer 1966; Hamlett 1989). Hamlett (1989) reported that the ductus of *Rhizoprionodon terraenovae* is lined with three types of cells. Ciliated cells that may move yolk platelets to the embryo; cells with microvilli that possibly absorb yolk metabolites; and "Enteroendocrine cells that ... may exert a paracrine regulator effect on other cells in the ductus" (Hamlett 1989, p.39). It has been reported that in *Scoliodon laticaudus*, where there is little yolk, the ductus is not formed (Setna and Sarangdhar 1948).

In the earliest stages of development the yolk is probably absorbed by phagocytosis by the periblast tissue (Baranes and Wendling 1981). As the development continues it is absorbed by the embryonic gut after transport from the yolk sac via the ductus vitello-intestinalis, and through the yolk sac into the vitelline blood vessels (TeWinkel 1943). Hamlett (1989) also suggested the possibility of yolk being

absorbed by epithelial cells in the ductus. Heming and Buddington (1988) suggested that most of the digestion of the yolk occurs in the embryonic gut after passing up the ductus.

Uterine secretions

Another source of nutrients utilised by embryos of carcharhinid species is thought to be uterine secretions. Not all species of carcharhinid sharks produce uterine secretions that may be utilised as a source of nutrients by the embryos. In species that produce uterine secretions their production begins early in development (Setna and Sarangdhar 1948). The production and nature of the uterine secretions has already been discussed in the section on the accommodation of pregnancy (7.1.1.6). In this section the possible mechanisms employed by embryos to procure nutrients from these secretions in *R. taylori* are considered.

Two mechanisms of absorption have been identified in carcharhinid species. The first of these is via numerous appendiculae that cover the yolk stalk/umbilical cord (Southwell and Prashad 1919; Mahadevan 1940; Hamlett 1986). The form of appendiculae varies from short and club-shaped, to elongate, flattened, and branching structures (Hamlett 1986). Southwell and Prashad (1919) described four types of appendiculae from the carcharhinid genera *Rhizoprionodon* and *Scoliodon*. These varied from simple folding of the epithelium of the umbilical cord, to elongate thread-like structures.

All appendiculae have the same general structure, being composed of an outer epithelium underlaid by an inner core of connective tissue through which a blood capillary may run. Hamlett (1989) identified two types of cells in the epithelium

of the appendiculae of *R. terraenovae*: absorptive cells characterised by the presence of microvilli, and secretory cells. The type or function of the secretion from the appendiculae has yet to be determined (Hamlett 1989).

Appendiculae have been reported from only four genera in the family Carcharhinidae: *Carcharhinus*, *Loxodon*, *Rhizoprionodon* and *Scoliodon* (Mahadevan 1940; Setna and Sarangdhar 1948, 1949a,b; Compagno 1988). The appendiculae form on the yolk stalk early in development and normally persist until parturition. Only one species of *Carcharhinus* (*C. acronotus*) has been recorded as having appendiculae (Hamlett 1986). However, in this species they are transient, occurring only during early development (Hamlett 1986).

The second mechanism suggested for the absorption of uterine secretions in carcharhinid sharks is via the external branchial filaments or external gills (Hamlett *et al.* 1985d). External branchial filaments are found only in the early stages of development prior to the formation of the placenta in all carcharhinid species except *Scoliodon laticaudus*, in which the early development of the placenta results in the external branchial filaments forming after implantation. As in the appendiculae, the external branchial filaments are composed of a connective tissue core with an overlaying epithelium (Kryvi 1976; Hamlett 1986). Hamlett *et al.* (1985d) have shown that proteinaceous macromolecules can be transported by the epithelium of the filaments.

Ingestion through the mouth is another mechanism that has been postulated for the utilisation of uterine secretions (Graham 1967). Although the mouth is open during much of development it has not been demonstrated that this is an avenue of absorption.

Placentae

Formation of the placenta in carcharhinid species normally occurs at about the mid-point of development when a section of the embryonic yolk sac becomes intimately associated with part of the maternal uterine mucosa (Teshima and Mizue 1972; Wourms 1977; Hamlett 1986, 1989). This form of placenta is known as a yolk sac placenta (Springer 1960; Mossman 1987; Wourms *et al.* 1988). During the implantation stage both the yolk sac and the uterine mucosa undergo cytological and functional changes (Hamlett 1986). An exception to this general form is found in *Scoliodon laticaudus* in which the placenta forms at a very early stage of development (Mahadevan 1940; Setna and Sarangdhar 1948; Teshima *et al.* 1978).

The placental structure within the carcharhinids is variable, with three forms being recognised: entire, discoid and columnar (Compagno 1988). The structures of these three types of placenta are shown in Figure 7.2. The columnar type is considered to be a highly specialised form found only in *Scoliodon laticaudus* (Setna and Sarangdhar 1948). This highly specialised type of placenta is not considered further in this review. Wourms *et al.* (1988) further divided the discoidal type, separating the placenta of *Prionace glauca* from the others on the basis of the maternal-embryonic interface. The discoid placenta is found in some members of the genus *Carcharhinus*, including *C. falciformis* (Gilbert and Schlernitzauer 1966), *C. longimanus* and *C. melanopterus*, as well as *Lamiopsis temmincki*, *Prionace glauca* and *Triaenodon obesus* (Compagno 1988). The remaining genera, and some species of *Carcharhinus* have entire placentae.

The discoid and entire types of placenta both have two components:

a. A smooth proximal portion that contains few blood vessels (Hamlett 1986). Hamlett (1989) reported finding measurable levels of steroid hormones in this portion of the placenta of *Rhizoprionodon terraenovae*, suggesting that it may be a site of steroid synthesis. Further investigation, however, will be required to confirm this hypothesis.

b. A rugose distal portion that is adjacent to the maternal uterine tissue. This portion contains large numbers of blood vessels (Mahadevan 1940; Hamlett *et al.* 1985a) and tracer studies using horse radish peroxidase have shown that it is this portion that is responsible for nutrient transfer (Hamlett *et al.* 1985a; Hamlett 1989).

The entire type of placenta is characterised by having the distal portion enclosed within a cup of maternal tissue (Mahadevan 1940; Compagno 1988), while in the discoid form the distal portion is disc shaped and lies opposed to the maternal tissue but is not enclosed in it (Gilbert and Schlernitzauer 1966). In the entire type of placenta there is complex interdigitation between the maternal tissue and the distal portion of embryonic tissue (Mahadevan 1940; Setna and Sarangdhar 1949b). In the discoidal placenta the amount of interdigitation appears variable. Gilbert and Schlernitzauer (1966) reported that in *Carcharhinus falciformis* there was no interdigitation, while Hamlett *et al.* (1985a) observed it in *Carcharhinus plumbeus*.

It appears that within the carcharhinids not only the forms, but also the nutrient transport mechanisms, of the placenta are different. Hamlett *et al.* (1985c) reported the placenta of *Carcharhinus plumbeus* was haemotrophic and there was no evidence for the secretion of macromolecular products. On the other hand Otake (1990) observed evidence for the secretion of macromolecular substances from the maternal portion of the placenta of *Prionace glauca* indicating that it may have an histotrophic

function. Setna and Sarangdhar (1948) suggested from their observations of the placenta of *Scoliodon laticaudus* that it was initially histotrophic, but became haemotrophic.

A number of authors have also noted that in many species within the family Carcharhinidae (e.g. *Carcharhinus dussumieri* and *Rhizoprionodon acutus*) the thin egg case persists between the maternal and foetal tissues in the placenta (Setna and Sarangdhar 1949b; Teshima and Mizue 1972; Mossman 1987; Otake 1990). Retention of the egg case presumably acts as a restriction to the passage of some products, especially larger molecules (Wourms *et al.* 1988). However, tracer studies using horse radish peroxidase and radioisotopes have shown that the egg case is permeable to at least some organic molecules (e.g. Graham 1967; Hamlett *et al.* 1985a). It has been reported that in some species the egg case is broken down (e.g. Setna and Sarangdhar 1948; Gilbert and Schlernitzauer 1966; Otake 1990) which may allow more efficient exchange.

7.1.1.8 Conclusions

It is clear from this review that we possess only a rudimentary knowledge of the embryonic development of sharks in the family Carcharhinidae. There have been no studies that have examined in detail the morphological development of the embryos, especially at the early stages. Our best knowledge is of the pathways of embryonic nutrition, but even this is relatively basic work and only relates to a very limited range of species. In future detailed studies covering a broad range of species are required to provide a thorough understanding of embryonic development in the family Carcharhinidae.

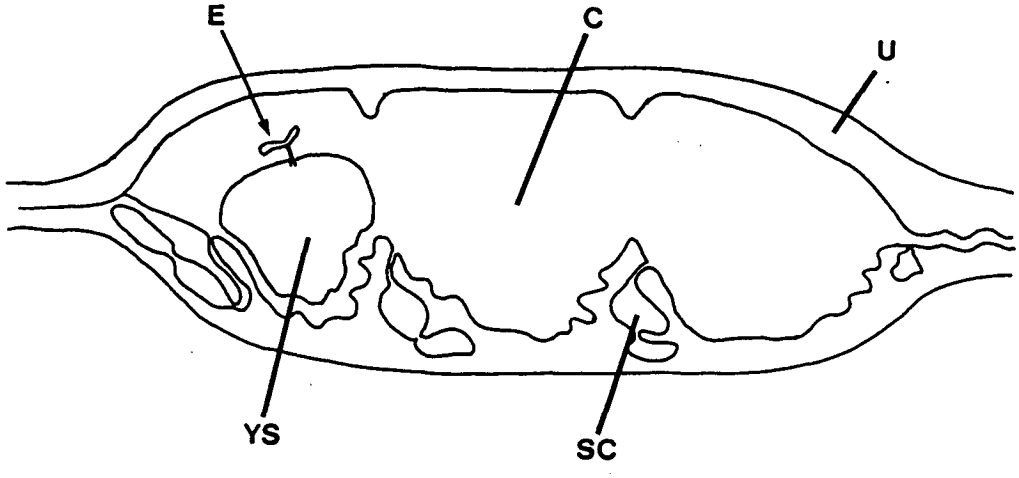


Figure 7.1: Longitudinal section of the uterus of *Carcharhinus plumbeus* containing three embryos (only the one in the left compartment is shown) showing the location of a compartment (C), embryo (E), storage chamber (SC), uterine wall (U), and yolk sac (YS). Redrawn from Figure 12b in Baranes and Wendling (1981).

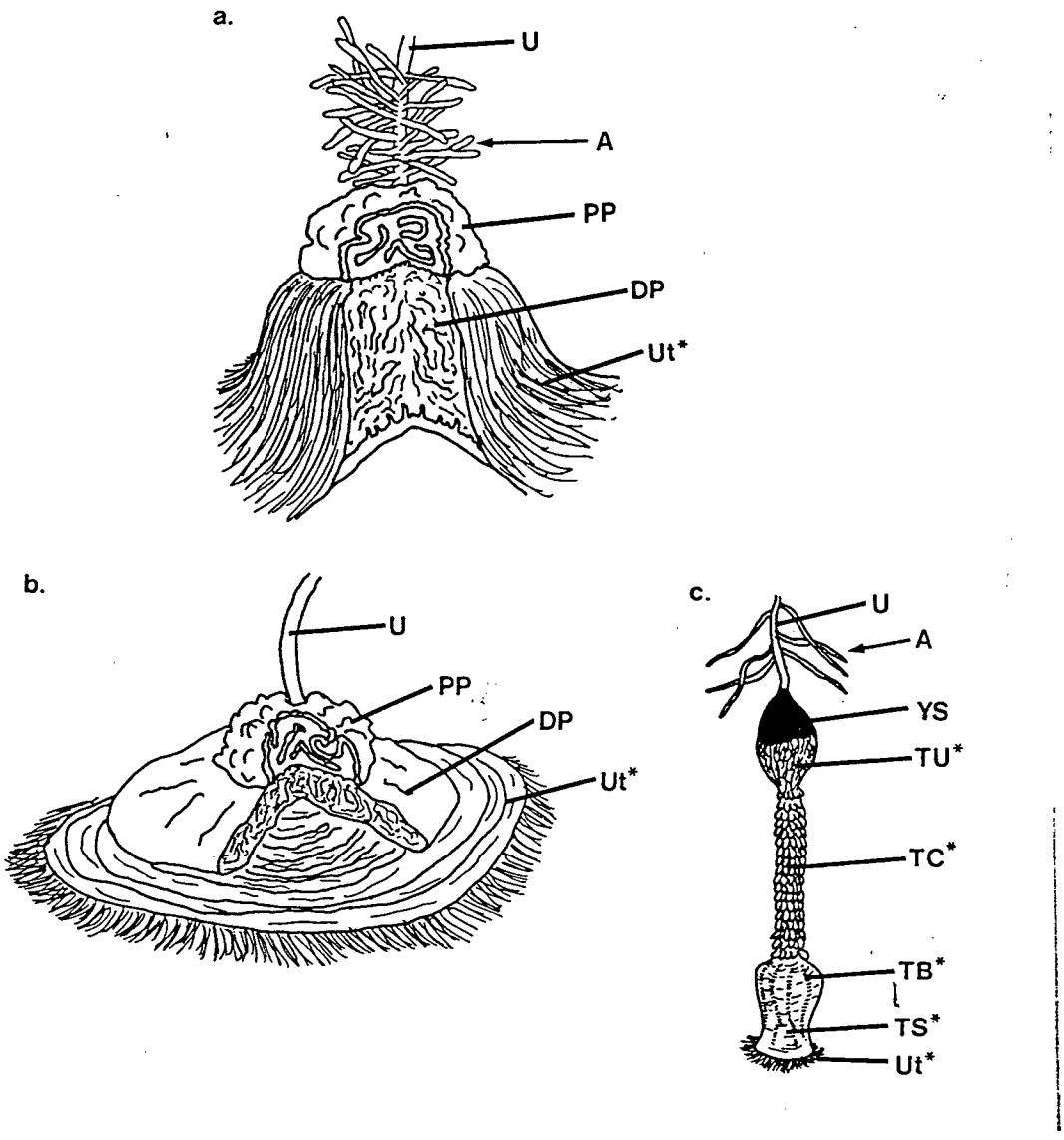


Figure 7.2: The three types of placenta that occur in the family Carcharhinidae: (a) entire (mature), (b) discoidal (mature) and (c) stalked (early, redrawn from Setna and Sarangdhar 1948). A, Appendiculae; DP, distal portion of placenta; PP, proximal portion of placenta; TB, trophonematous bulb; TC, trophonematous cord; TS, trophonematous stalk; TU, trophonematous cup; U, umbilical cord; Ut, uterus; YS, yolk sac. Asterisk indicates tissues of maternal origin.

7.2 Materials and Methods

Material for the examination of embryonic development in *Rhizoprionodon taylori* was obtained from specimens caught in the field. The collection of these specimens has been described in Chapter 2. The number of eggs or embryos in the uteri of all mature females were counted, and a representative portion were excised for further examination. Two forms of uterine eggs were identified: Stage I eggs which were those that displayed no macroscopic evidence of development, and Stage II eggs which were those where there was macroscopic evidence of development of the embryo. Once the embryo was attached to the yolk sac by only a yolk stalk or umbilical cord it was considered a free embryo (post Stage II). Stage I and II eggs were weighed with the yolk intact and preserved in formaldehyde-calcium acetate (4% formaldehyde, 2% calcium acetate) as it provides rapid fixation of the lipid rich eggs. Free embryos were weighed (without the yolk-sac and yolk stalk, or placenta and umbilical cord, as it was not possible to divide maternal and embryonic tissue in all specimens), measured to the nearest millimetre and sexed.

The development of Stage I eggs was examined by histological staining of serial sections. The eggs were processed to paraffin wax, serially sectioned at 8-10 micrometres, mounted on slides and stained. A number of stains were used including Mayer's Haematoxylin and Eosin, Mallory-Heidenhain Trichrome, Martius Scarlet Blue (MSB) with a haematoxylin nuclear stain, and Iron Haematoxylin. References for the histological procedures and techniques used are provided in Appendix D.1. The best results were obtained using the MSB staining method.

Oviducal gland, uterine, yolk stalk, umbilical cord and placental tissues were collected from a number of specimens for histological examination. After fixation in 4% formaldehyde or formaldehyde-calcium acetate tissues were embedded in

wax, sectioned at 6-7 micrometres and stained. For general structural examination tissues were stained with Mallory-Heidenhain Trichrome, Mayer's Haematoxylin and Eosin, or 0.1% Toluidine Blue. Information on the secretions from the oviducal glands and uteri were obtained using a number of histochemical procedures. These included Alcian Blue-Periodic Acid Schiff method (AB-PAS) for detection of acidic and neutral mucopolysaccharides (mucins); Catechol, Diazo, Masson-Fontana and Methanamine Silver techniques for detection of compounds associated with quinone tanning; Van Gieson's stain for the presence of collagen; and dinitrofluorobenzene method for general protein. References for these histological procedures can be found in Appendix D.2. The size of tissues, or components of tissues, in histological sections was measured using an optical micrometer on a high power microscope.

Whole uteri from a small sample of pregnant females were fixed in 4% formaldehyde to allow examination of the organisation of the embryos within the uteri. Following dehydration via an ascending alcohol series whole uteri were rendered transparent using methyl salicylate. Drawings were then made of the uteri and their contents.

The surface structure of the appendiculae of the yolk stalk or umbilical cord was observed using scanning electron microscopy (SEM). Specimens for SEM were selected from tissues fixed in either 4% formaldehyde or formaldehyde-calcium acetate. Specimens were dehydrated in an ascending ethanol series, then in a critical point dryer, mounted on aluminium stubs, sputter coated with gold, and viewed using a Philips XL20 scanning electron microscope at 15kV.

Umbilical tissue from a single specimen caught in October were fixed in 2% gluteraldehyde for transmission electron microscope (TEM) observations. After two hours of fixation tissues were washed in cacodylate buffer and post-fixed in 1% osmium tetroxide for one hour. Specimens were then dehydrated in an ascending

ethanol series, infiltrated and embedded in Spurr's epoxy resin. Ultra-thin sections were cut using a LBK-Nova Ultratome. Sections were stained with uranyl acetate and lead citrate and viewed using a JEOL 2000 transmission electron microscope at 80kV.

7.3 Results

7.3.1 Ovulation and the functions of the oviducal gland

In Chapter 6 it was shown that ovulation occurs in February, soon after mating. At this time the ova are released from the ovary and move down the cranial section of the oviduct and into the oviducal gland. After passing through the oviducal gland eggs in the caudal section of the oviduct and uterus had an ellipsoid shape, measuring approximately 23mm long and 9mm in diameter. Mean total weight of eggs was 1.64g. Encapsulating each egg was a thin membranous egg case. An egg only occupied a small portion of the egg case (Plate 7.1a) and the excess egg case was tightly coiled at the anterior of the egg (Plate 7.1b).

The oviducal gland of *R. taylori* is illustrated in Figure 7.3. The size of the gland varies throughout the year, with the largest sizes attained in February around the time of ovulation (Figure 7.4). The gland has two parts: the basal region, dorso-ventrally flattened, laterally bulbous and continuous with the oviduct; and two lateral horns which spiral anteriorly from the basal section on either side of the descending cranial oviduct.

Histological examination of the tubules that form the gland indicate the presence of three types of tubules here designated A, B and C. All three types of tubule are present in the basal section and the lateral horns (Figure 7.3). The diameters of each type of tubule are different - type A tubules being the smallest and type C the largest (Table 7.3). During the reproductive season (January-February) all types of tubule are larger than in the non-reproductive season (Table 7.3). The results of the histological and histochemical staining techniques for the three tubule types are given in Table 7.4. Histological results are presented only for oviducal glands

taken from ovulating females, observations of glands from other females produced inconsistent results. This suggests that oviducal glands are only fully functional at the time of ovulation. Descriptions of the structure and function of each of the tubule types follow, and are summarised in Table 7.4.

Table 7.3: Comparison of tubule sizes in the oviducal glands of four individual *Rhizoprionodon taylori* caught at different time of the year. Tubule width is given as range and mean (in parentheses).

			Width of tubule (micrometres)		
Specimen	Month caught	Position	A	B	C
RT89-005	January (pre-ovulatory)	Horn	NA	29-42(35.5)	39-63(51.9)
		Basal	18-32(25.0)	NA	47-53(50.0)
RT90-015	February (ovulating)	Horn	37-63(50.6)	79-99(87.3)	87-116(105.9)
		Basal	47-60(51.9)	52-107(88.1)	84-124(105.7)
RT89-044	February (post-ovulatory)	Horn	NA	39-53(46.0)	63-66(65.1)
		Basal	42-53(48.0)	47-73(58.5)	68-74(71.0)
RT89-049	April	Horn	26-42(34.8)	29-34(30.9)	34-50(41.4)
		Basal	NA	32-47(39.4)	34-39(36.2)

Tubule type A

Type A tubules are the least common in the oviducal glands of *Rhizoprionodon taylori*. They are located on the periphery of both the basal portion and lateral horns (Figure 7.3), but open mostly into the lumen in the caudal section of the basal portion. During the period between mating and ovulation spermatozoa were observed to be present in these tubules (Plate 7.2). The cells stain positive for acid mucins with AB-PAS staining (Table 7.4). The cells also have a metachromatic reaction with toluidine blue, indicating that the acid mucin is possibly a sulphated mucopolysaccharidae.

Table 7.4: Results of histological and histochemical techniques for oviducal glands from ovulating *Rhizoprionodon taylori*. -, negative; + positive.

		Tubule type		
Technique	Substance	A	B	C
Mallory-Heidenhain trichrome	collagen	-	-	secretion + (blue)
Van Geison's	collagen	-	secretion + granules +	secretion + granules +
AB-PAS	acid mucins	+	-	-
	neutral mucins and a range of other substances	-	granules +	secretion + granules +
0.1% toluidine blue	sulphated mucins?	metachromatic	-	-
Catechol	polyphenol oxidase	-	-	-
Diazo	polyphenols	-	-	-
Methanamine silver	polyphenols	+ (colour?)	granules + (colour?)	granules +, immature secretion + (colour??)
Masson-Fontana	diphenols	-	-	-

Tubule type B

The locations of type B tubules in the oviducal glands are shown in Figure 7.3. Although they occur in both parts of the gland it is only in the lateral horns that the tubules open into the lumen. The tubules enter the lumen at the base of lamellal folds (Plate 7.3) located at the medial end of the transverse region of the lumen of the lateral horn. In ovulating specimens granules were present in the cytoplasm of some tubule epithelium. These granules stained positively for neutral mucins (AB-PAS), positive for collagen (Van Geison's), Methanamine silver was also positive, but there were negative reactions for di- and polyphenols, and polyphenol oxidase (Masson-Fontana, Diazo, and Catechol techniques).

Tubule type C

Type C tubules are the most common in the oviducal glands of *R. taylori* (Figure 7.3). They open into the lumen in both the lateral horns and basal portion at the base of tufted lamellae (Plate 7.4). Type C tubules from ovulating specimens contain large amounts of secretion that form a sheet in the lumen of the gland (Plate 7.4). This secretion stained blue with Mallory-Heidenhain trichrome, and bright red with Van Geison's stain, indicating the probable presence of collagen (Table 7.4). The positive reaction for collagen, and the sheet like nature of the secretion, suggest that type C tubules are responsible for the production of the egg case. Masson-Fontana, Diazo and Catechol techniques, for the tubules and secretion, were all negative suggesting that there is little evidence for quinone tanning of the egg case. There was a positive reaction for the Methanamine silver technique (Table 7.4) but this stain is not specific enough to provide firm evidence of quinone tanning on its own. AB-PAS and Methanamine silver staining of type C tubules was variable in ovulating specimens, with some areas staining more than others. This may indicate that these tubules can be subdivided into different regions. Insufficient data were available from the present study to determine the significance of this variation.

7.3.2 Development of embryos

Stage I eggs (those without macroscopic signs of development) were present in the uteri of mature females from just after ovulation through until September (Figure 7.5). Stage II eggs were observed only in September, after which time only free embryos were present until parturition in January. The presence of stage I eggs for a seven month period suggests that the eggs are not fertilised or their development is in some way arrested or slowed. The same pattern of development occurred in

each year of this study and always with a high degree of synchrony within the population. The commencement of normal development in September coincides with increasing sea temperature, and parturition occurs at the time of maximum sea temperature (Figure 7.5).

7.3.2.1 Description of embryos

Diapausing blastoderm

Embryos were only located in Stage I eggs after extensive observations and sectioning. Sectioning of the eggs was hindered by their delicate nature and large amounts of yolk. Eventually serial sections of two stage I eggs from two different females, one caught in May and the other in August, revealed that embryos had developed to a blastoderm stage approximately 1.9mm in length (Plate 7.5). Both blastoderms had a similar size suggesting that development of embryos is delayed at this stage. Such a delay in development is normally referred to as a period of embryonic diapause (Renfree 1978; Renfree and Calaby 1981). The diapausing blastoderms were located near the equator of the ellipsoid shaped egg between the yolk and an outer non-cellular layer (Plate 7.5). This outer layer stains positively for general protein using the dinitrofluorobenzene method.

The structure of the diapausing embryo from the female caught in May was reconstructed from serial sections. A diagrammatic representation of this reconstruction is shown in Figure 7.6. Histologically two distinct regions were identified, one anterior and the other posterior. The anterior region is composed of a solid mass of cells that appears to lie at the junction of the yolk and protein layers of the egg (Plate 7.5a). The posterior region is composed of a single layer

of cells surrounding the segmentation cavity (Plate 7.5b). The segmentation cavity is filled mostly with acellular material with a granular appearence that stains the same as the acellular protein layer.

6mm embryo

The embryo is laterally compressed and lies flat against the yolk sac (Plate 7.6a). The gill slits and spiracle are not visible, nor are the fin buds or mouth. There is no pigmentation.

16mm embryo

The gill slits, spiracle and fin buds have started to develop, but the mouth is not visible (Plate 7.6b). The forming eyes are visible anteriorly as bulbous protrusions from the head. There is no pigmentation. The embryo is attached to the yolk sac by a short (8mm) yolk stalk. The yolk stalk is devoid of appendiculae, but the vitelline artery, vitelline vein and ductus vitello-intestinalis are all present (Plate 7.7a).

38mm embryo

Embryos of this size are characterised by the presence of long external branchial filaments which extend out of the gill slits and spiracles (Plate 7.6c). Paired and median fin buds are present, and individuals can be sexed as there is evidence of clasper formation in males. There is no pigmentation. The yolk stalk is approximately 24mm in length and is covered with densely packed, short (approximately 0.5mm), branching, knob-like appendiculae (Plate 7.7b).

68mm embryo

At this stage the embryos begin to look more like post-partum specimens. The rostrum is short, but the head is not reflexed as in early forms (Plate 7.6d). External branchial filaments still extend out of the gill slits and spiracles, but are relatively smaller than in the 38mm embryo. There is no pigmentation on the fins or body. The yolk stalk is approximately 49mm in length and the appendiculae have become longer (approximately 3mm) and flattened. The yolk sac is loosely associated with the uterine mucosa, but still contains a significant amount of yolk.

133mm embryo

The body form is similar to post-partum specimens (Plate 7.6e). The fins are close to fully formed and the spiracle has been lost. Pigmentation is visible on the tips of the fins, but is not present on the body. The external branchial filaments have been lost. The yolk sac is closely associated with the uterine mucosa and little yolk remains, suggesting that implantation is nearly complete and the placenta is an important source of nutrients for the embryo. With the implantation of the placenta nearly complete the yolk stalk functions as an umbilical cord. The blood vessels that served as the vitelline artery and vein, are now functional as the umbilical artery and vein. The ductus vitello-intestinalis is still present (Plate 7.7c), but probably has only a minor function as no yolk is present in the yolk sac. The appendiculae have increased in size to 7-8mm in length and remain flattened and branching. The umbilical cord is approximately 96mm long.

235mm embryo

Embryos of this size were obtained from females caught in mid-January, indicating that they were full-term. They had the appearance of the adult and the body is fully

pigmented (Plate 7.6f). The umbilical cord is approximately 116mm in length and the appendiculae are similar in size to those of the 133mm embryo (Plate 7.7d). However, the appendiculae appear more scarse especially near the embryo where they have probably been removed by rubbing against its body.

The mean wet weight of full-term embryos (without the umbilical cord and placenta) was 48.8g. This represents a 2980% increase in the wet weight over the ovulated eggs. (This would have been higher if the placenta and uterus could have been separated in all specimens to allow accurate weighing). There was a significant relationship between the mean weight of pups in a litter and the number of pups in the litter ($r^2=0.526$, d.f.$=14$, $p=0.0009$), with pups from small litters being heavier than those from large litters (Figure 7.7).

7.3.3 Accommodation of pregnancy

R. taylori embryos remain in the uteri throughout the gestation period. Each is enclosed within a separate compartment in the uterus. These compartments are present during both the diapause and developmental periods. The septa forming the compartments are composed of mucosa and submucosa (Plate 7.8). Within each compartment there is a small store chamber where some of the excess egg case is stored, however, most of the egg case remains outside the store chamber tightly coiled on the egg (see Plate 7.1).

Within the compartments the embryos are normally orientated longitudinally and face anteriorly. The accommodation of embryos within the uteri of females with diapausing, mid-term and full-term embryos, is illustrated in Figure 7.8. The septa forming the compartments are transverse in the uteri that contain the diapausing

embryos (Figure 7.8a), while in the later forms they are longitudinal. In the half-term female one embryo is orientated opposite to normal, facing posteriorly. The placentae are located at the caudal end of each of the compartments (Figure 7.8b,c).

The uterus of *Rhizoprionodon taylori* is composed of 4 layers: the mucosa, submucosa, muscularis and serosa (Plate 7.9). The form of these layers varies depending on the stage of development, as well as the location in the uterus.

7.3.3.1 Mucosa

The mucosa of the uterus is composed of a juxtaluminal layer of columnar epithelium (Plate 7.10). During the diapause period the cells appear inactive and have a high nuclear to cytoplasmic ratio (Plate 7.10a). During the developmental period the cells elongate and beads of secretion appear at their apicies (Plate 7.10b). The beaded form of the secretion suggests that it is produced in an apocrine manner. This secretion stains positive for acid mucins with AB-PAS staining. All mucosal cells produce this secretion prior to the implantation of the placenta. The implantation of the placenta results in cytological changes to the mucosal cells at the point of implantation (described in 7.3.4.4). Mucosal cells not incorporated into the placenta continue their pre-implantation secretions until parturition.

7.3.3.2 Submucosa

The submucosa is composed mostly of loose connective tissue in which blood vessels, glands and smooth muscle occur (Plate 7.9). During the developmental period an almost continual layer of capillaries forms a network subjacent to the mucosa (Plate 7.10b). During the diapause period there are fewer capillaries subjacent to the mucosa (Plate 7.10a).

The glands present in the submucosa of *R. taylori*, although having a tubular appearance, were continuous throughout the uterus and intrauterine septa, indicating a sheet-like structure composed of many tubules (Plates 7.7 and 7.11). The glands' secretions stain positively for both acid and neutral mucins with AB-PAS staining. Cells secreting neutral mucins were more common than those secreting acid mucins. The latter of these cells were metachromic when stained with toluidine blue, indicating the presence of sulphated mucins. The sheet-like glands were inactive during the diapause period, but became active at approximately the same time as recommencement of embryo development.

7.3.3.3 Muscularis and serosa

The muscularis is composed of inner circular and the outer longitudinal layers of smooth muscle (Plate 7.9). The mean thickness of the muscularis in females with diapausing embryos of 79.2 micrometres was not significantly different to the 86.1 micrometres in females with developing embryos (t-test, $p=0.6385$). There was no observable change in the form or size of the serosa between the diapause and developmental periods.

7.3.4 Structure of tissues possibly associated with embryonic nutrition

7.3.4.1 Yolk sac and stalk

The yolk sac of *R. taylori* is thin walled and initially contains all of the yolk reserves. The yolk sac is connected to the embryo via the yolk stalk. Later in development, when the placenta forms, the yolk stalk functions as an umbilical cord. The yolk stalk of *R. taylori* contains three channels: vitelline artery, vitelline vein, and ductus vitello-intestinalis (see Plate 7.7). The two blood vessels carry embryonic blood to and from the blood vessels in the yolk sac. The ductus vitello-intestinalis connects

the yolk sac to the embryonic gut. Cells lining the ductus vitello-intestinalis have large numbers of microvilli (Plate 7.12); are often, but not always, ciliated (Plate 7.12b); and often contain vesicles the contents of which stain darkly, and appear to have a crystalline structure, in TEM sections (Plate 7.12a,b).

7.3.4.2 Appendiculae

The general morphology of the appendiculae during development of the embryos has been described in section 7.3.2.1, and illustrated in Plate 7.7. Histological sections of appendiculae show that they are composed of an epithelium overlaying an inner core of connective tissue through which a capillary loop often runs (Plate 7.13). During early- and mid-gestation the epithelial cells are columnar (Plate 7.13a,b), while in late gestation they become more cuboidal (Plate 7.13c). The epithelial cells in early gestation appear inactive, have a high nuclear to cytoplasmic ratio, and are undifferentiated (Plate 7.13a). However, in mid-gestation there is evidence of differentiation. Some of the cells appear to form apocrine secretions (Plate 7.13b). Others appear to have apical tufts of microvilli (Plate 7.13b) and are considered to be absorptive cells. The apocrine secretions stain red with Mallory-Heidenhain Trichrome indicating an acidophilic substance. In late-gestation secretory cells are rare, with most epithelial cells being absorptive (Plate 7.13c).

The SEM observations of the surface of the appendiculae support the histological data. There was no evidence of absorptive cells with apical microvilli on the appendiculae of a 38mm embryo (Plate 7.14a). Absorptive cells were present on the appendiculae from a 90mm embryo, but were less common than secretory cells (Plate 7.14b). The appendiculae from a full-term embryos contained more absorptive cells than secretory cells (Plate 7.14c).

7.3.4.3 External branchial filaments

External branchial filaments are transitory structures occurring only when the embryos are between approximately 20mm and 75mm (see 7.3.2.1). Structurally they are similar to appendiculae, being composed of a layer of epithelium cells over a capillary loop (Plate 7.15a). This structure gives the external branchial filaments a characteristic "figure of eight" shape when viewed using SEM (Plate 7.15a,b). The mean diameter of histologically sectioned filaments was 79.8 micrometres (SE=3.4 micrometres). The epithelial cells of the filaments are cuboidal (Plate 7.15a), but do not possess structures, such as microvilli, that would indicate a major absorptive function. The thin epithelium of the filaments should enable efficient gas exchange.

7.3.4.4 Placentae

The full term placenta of *R. taylori* is composed of two parts: the rugose distal portion and smooth proximal portion (Plate 7.16). The distal portion is enveloped in a cup of uterine tissue, above which is the proximal portion surrounding the umbilical cord. The form of each of the portions is described below.

Proximal portion

This portion is composed of two layers: an inner layer of connective tissue which is continuous with part of the umbilical cord, and an outer layer of columnar epithelium (Plate 7.17). The connective tissue layer contains many small capillaries and eventually joins the distal portion of the placenta. The columnar epithelium produces an apocrine secretion (Plate 7.17).

Distal portion

Development of the distal portion of the placenta commences when the embryos are 50-60mm in length. At this stage the yolk sac becomes closely associated with the uterus and the epithelial cells of the uterine mucosa change from columnar (see Plate 7.10), to squamous (Plate 7.18). The capillary network that underlaid the maternal epithelium prior to contact with the yolk sac remains and provides the maternal blood supply to the placenta. The yolk sac at this stage, and throughout the development of the placenta, is composed of a layer of squamous epithelium, a thin connective tissue layer in which blood capillaries are located, and a layer of endoderm (Plates 7.18b and 7.19b). The distal portion of the placenta develops as the maternal and embryonic tissues form folds that increase in size interdigitating the maternal and embryonic tissue. Areas in which yolk platelets remain are not occupied by folded tissue (Plate 7.18a). Maternal tissue forming the folds of the immature placenta contain a layer of connective tissue, which is the remains of the submucosa of the uterus. This layer of connective tissue is absent in the mature placenta (Plate 7.19). In the placenta of full-term embryos the interdigitations are extremely complex and the surface area of exchange between mother and embryo is enormous (Plate 7.19a). At all times during development the egg case remains intact, separating the maternal and embryonic tissues in the distal portion of the placenta (Plates 7.18b and 7.19b).

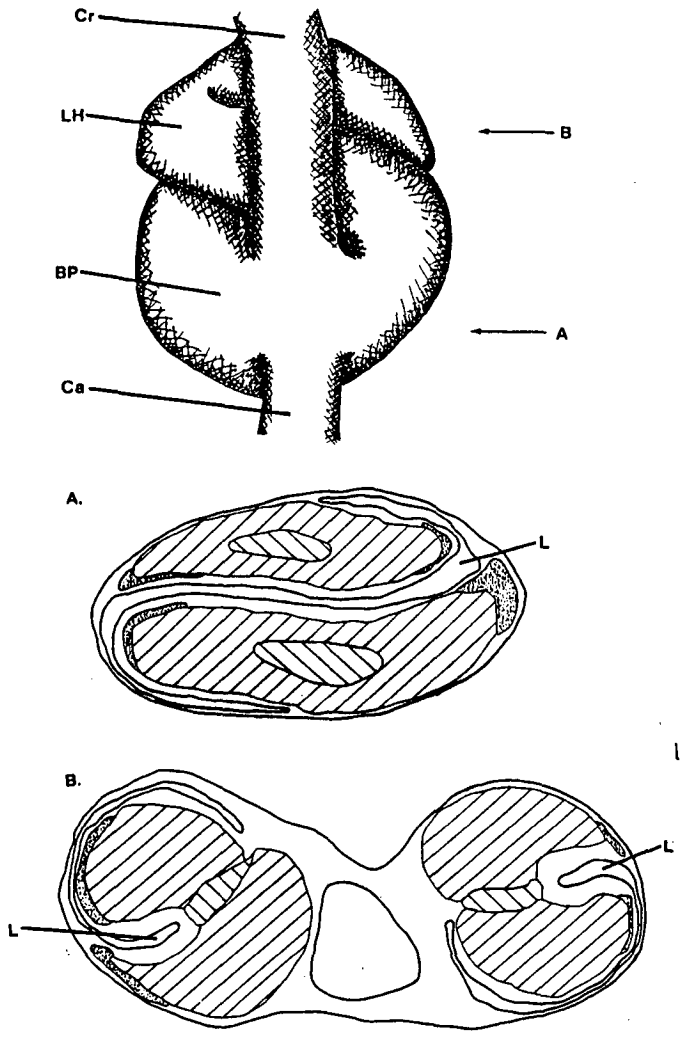


Figure 7.3: General morphology of the oviducal gland of *Rhizoprionodon taylori*, and distribution of tubule types A (stippled), B (hatch rising right) and C (hatch rising left) in the basal portion (section A) and the lateral horns (section B). BP, basal portion; Ca, caudal oviduct; Cr, cranial oviduct; L, lumen; LH, lateral horn.

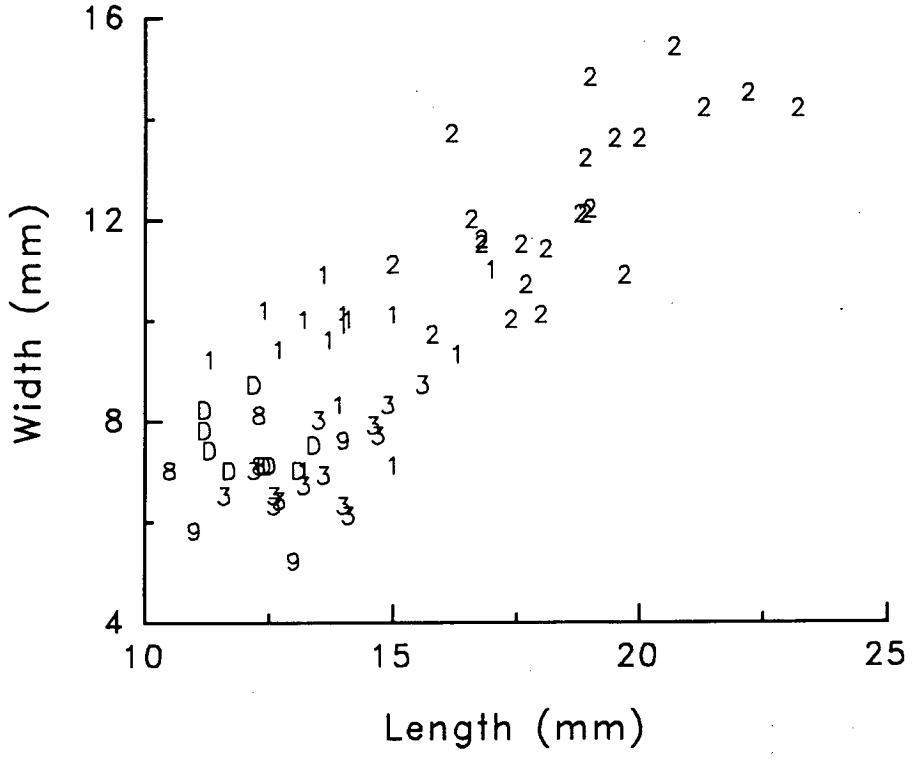


Figure 7.4: Variation in the size (length and width) of the oviducal glands of *Rhizoprionodon taylori* by month. 1, January; 2, February; 3, March; 8, August; 9, September; D, other.

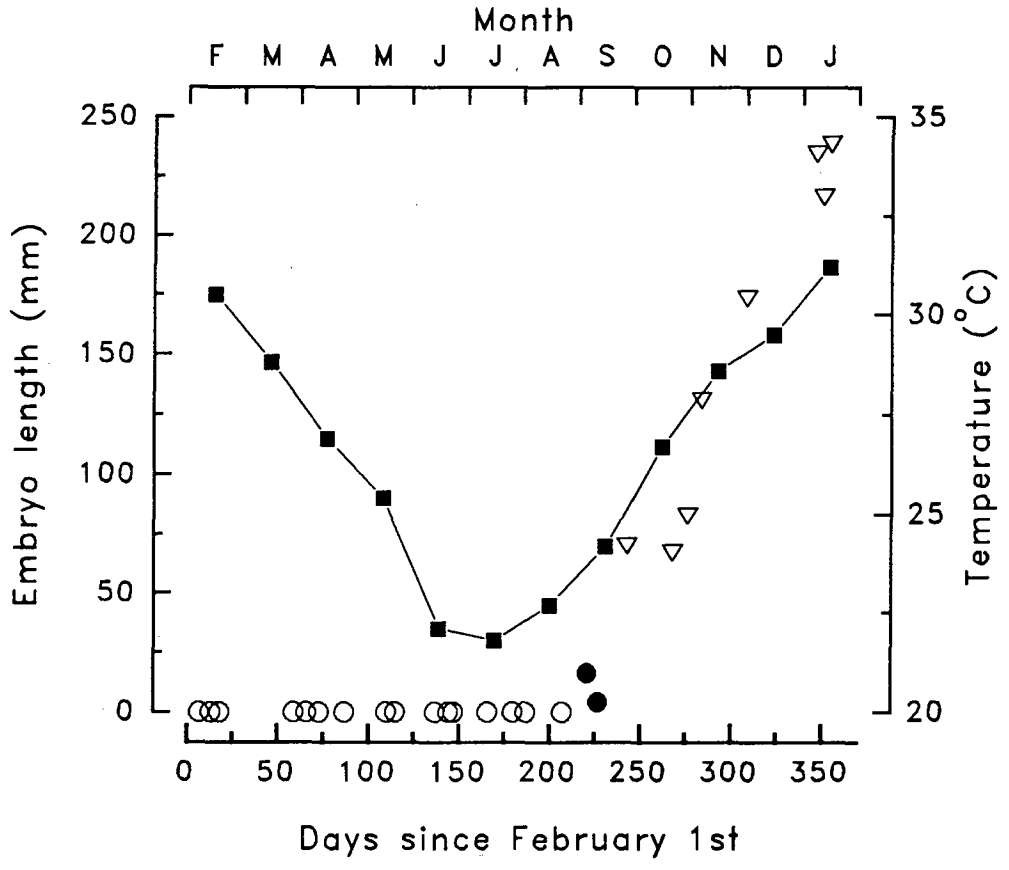


Figure 7.5: Growth of *Rhizoprionodon taylori* embryos. Open circles, stage I embryos; closed circles, stage II embryos; open triangles, free embryos; solid squares, mean monthly sea surface temperature for Townsville (from Kenny 1974).

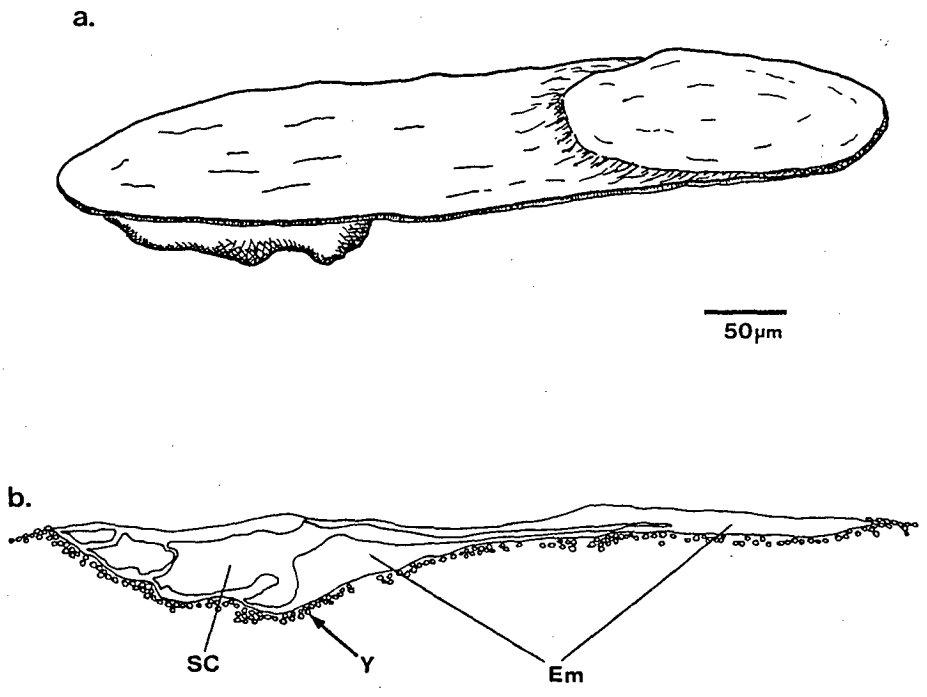


Figure 7.6: Oblique view (a), and longitudinal section (b), of a diapausing blastoderm of *Rhizoprionodon taylori* based on a reconstruction from serial sections of an embryo from a female caught in May 1988. E, embryo; SC, segmentation cavity; Y, yolk. Anterior of embryos is to the right.

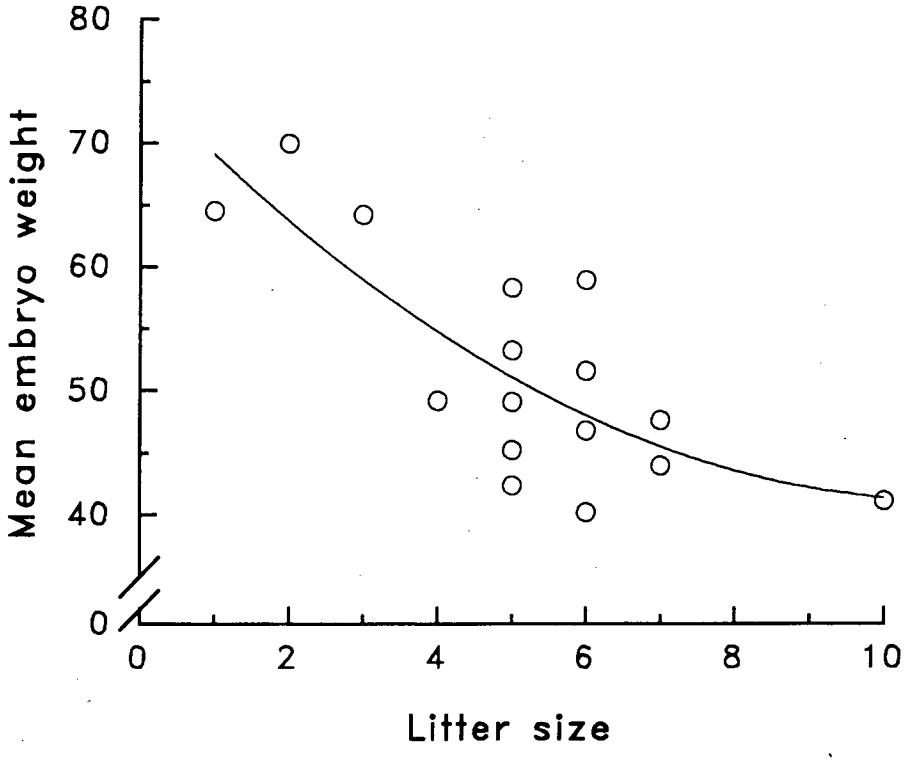


Figure 7.7: Relationship between the mean weight of individuals in litters and the litter size, indicating that females that have smaller litters produce larger young.

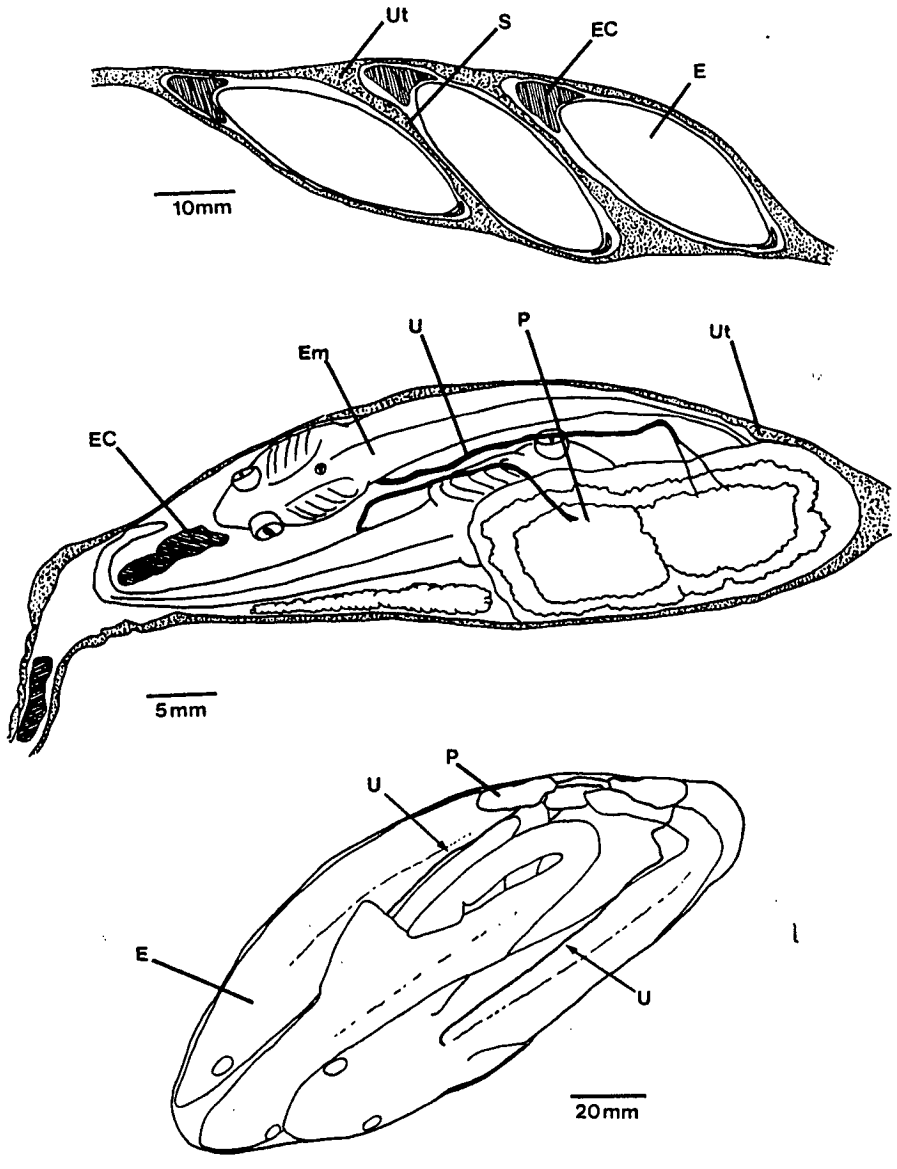
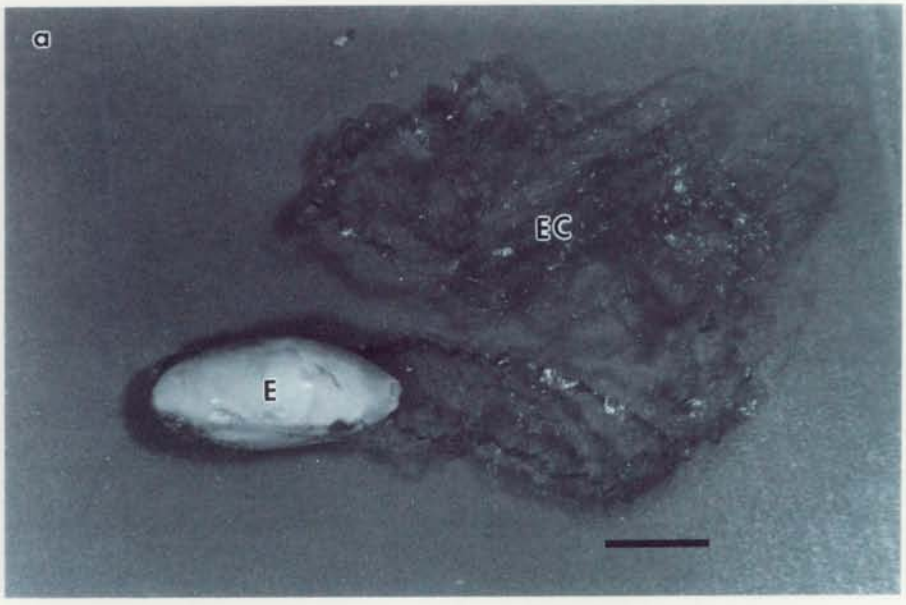


Figure 7.8: Accommodation of (a) diapausing, (b) mid-term, and (c) full-term, embryos within the uteri of *Rhizoprionodon taylori*. E, egg; EC, egg case; Em, embryo; P, placenta; S, septa; U, umbilical cord; Ut, uterus.

Plate 7.1: Eggs from the uterus of a female *Rhizoprionodon taylori* showing the egg case (a) uncoiled, and (b) in natural position. E, egg; EC, egg case. Scale bars, 10mm.


a
EC
E

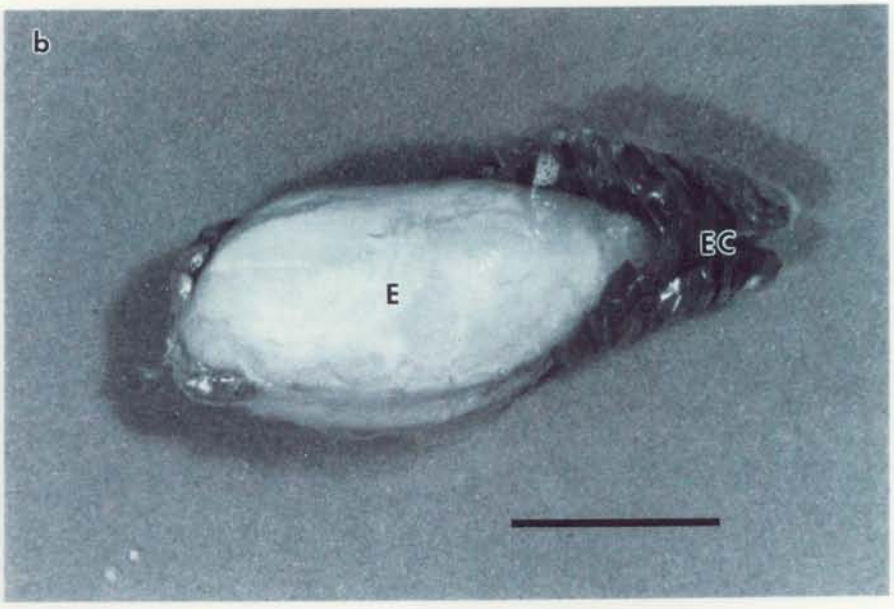

b
EC
E

Plate 7.2: Histological section through type A tubules of an adult female *Rhizoprionodon taylori* caught in early February, showing the presence of spermatozoa (arrow heads) in the lumen. Scale bar, 0.02mm.

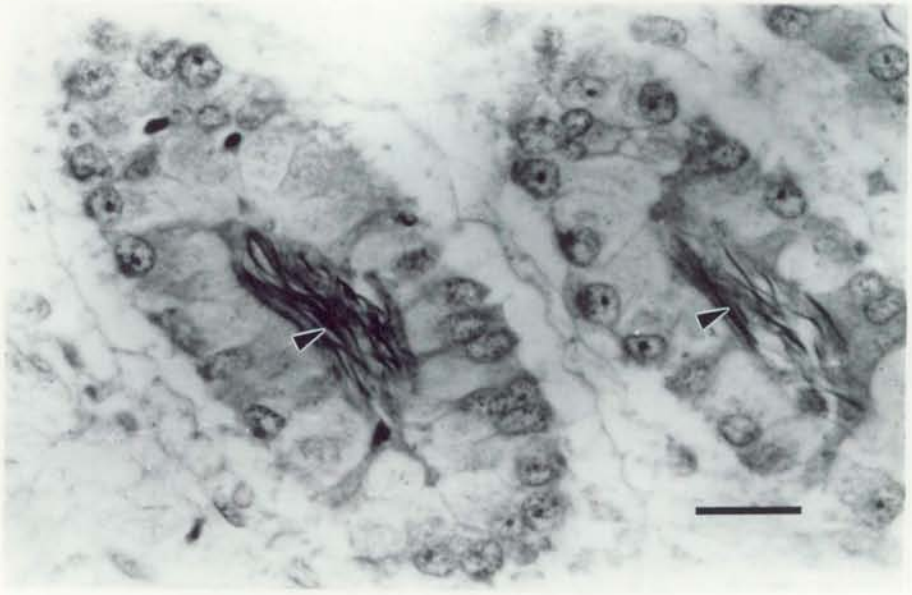
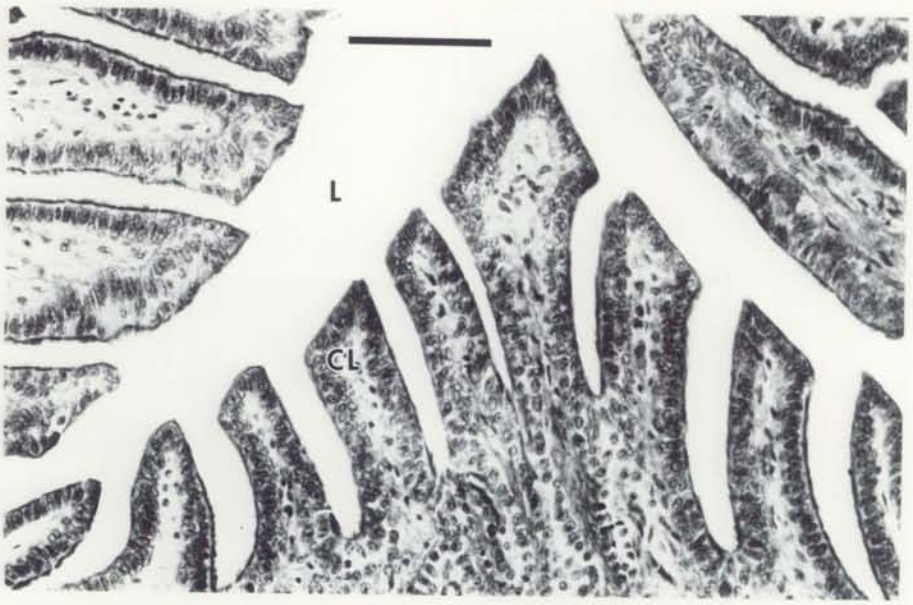


Plate 7.3: Histological section of the lumen of the lateral horn from the oviducal gland of *Rhizoprionodon taylori*, showing the column shaped lamellae at the base of which type B tubules open. CL, columnar lamellae; L, lumen of gland. Scale bar, 0.1mm.

Plate 7.4: Histological section of part of the lateral horn of *Rhizoprionodon taylori* during ovulation showing the tufted lamellae and production of the sheet-like secretion (arrow heads) in type C tubules. L, lumen of gland; TL, tufted lamellae. Scale bar, 0.2mm.


L
CL


L
TiL

Plate 7.5: Sagittal histological sections through a diapausing blastoderm of *Rhizoprionodon taylori* from a female caught in April 1988. Section (a) shows the anterior portion of the embryo, and (b) the posterior section. Em, embryo; SC, segmentation cavity; y, yolk. Scale bars, 0.1mm (a) and 0.25mm (b)

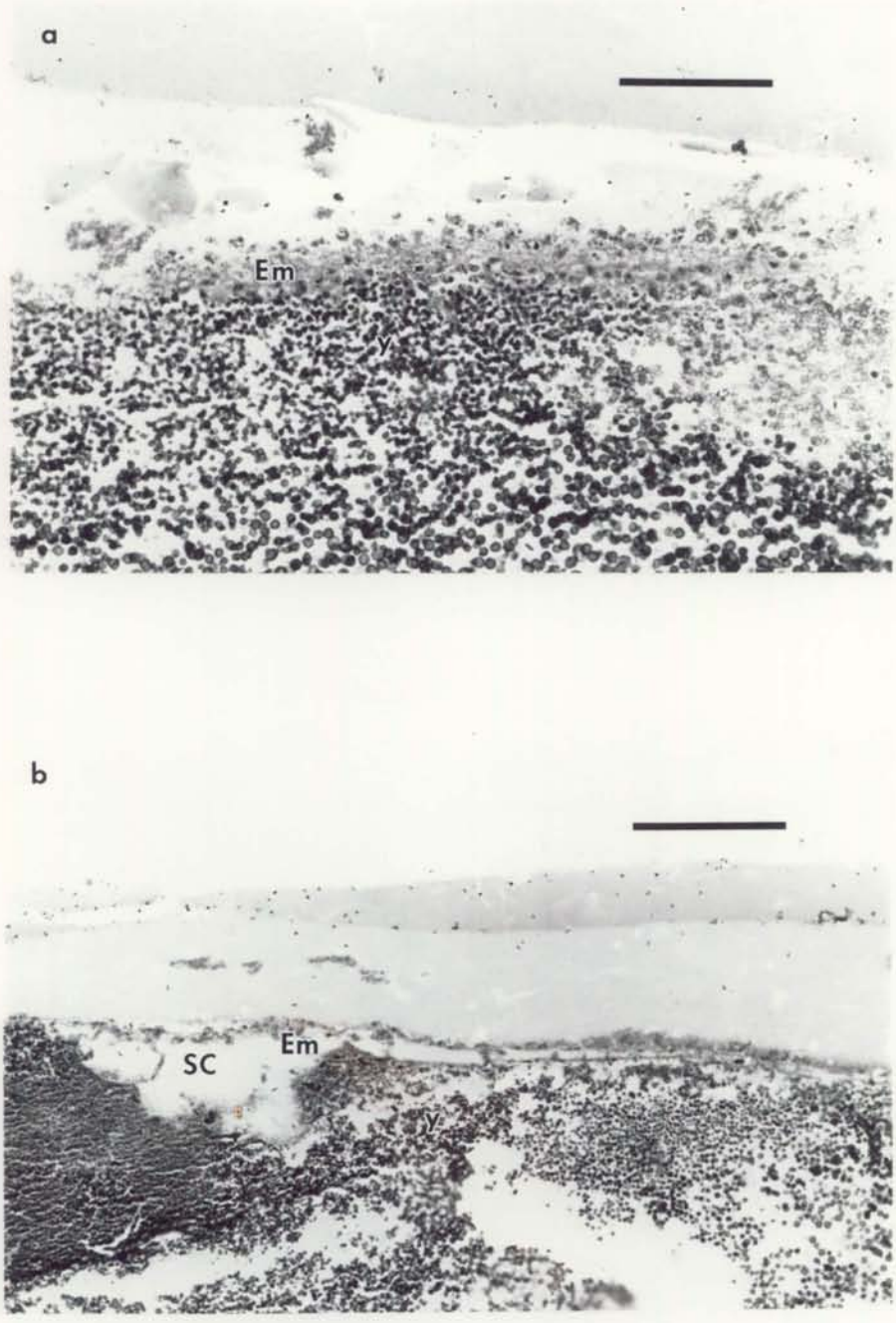
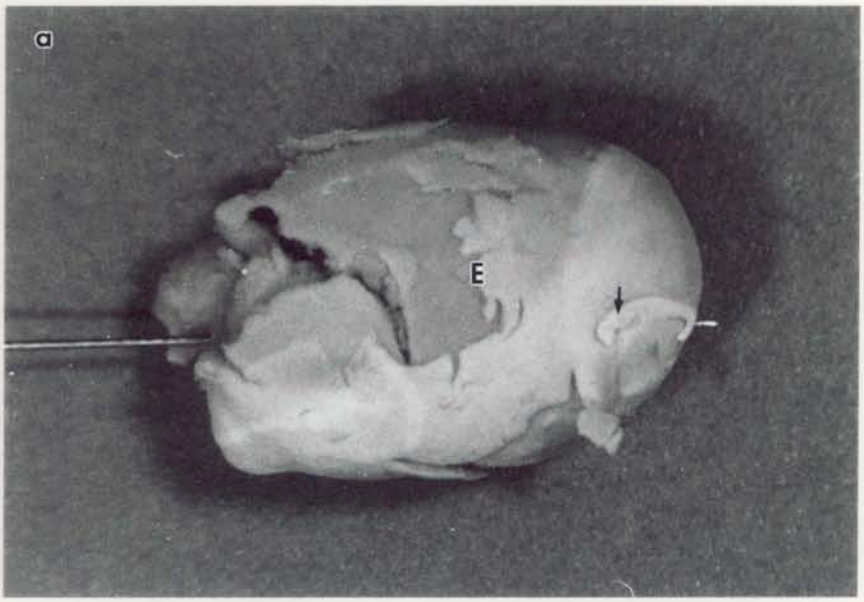
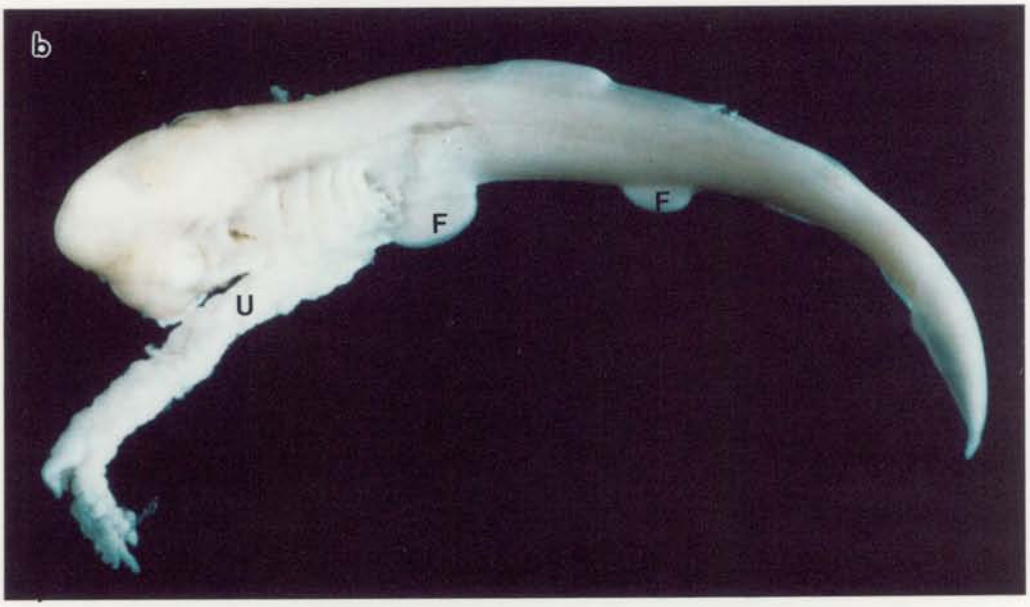
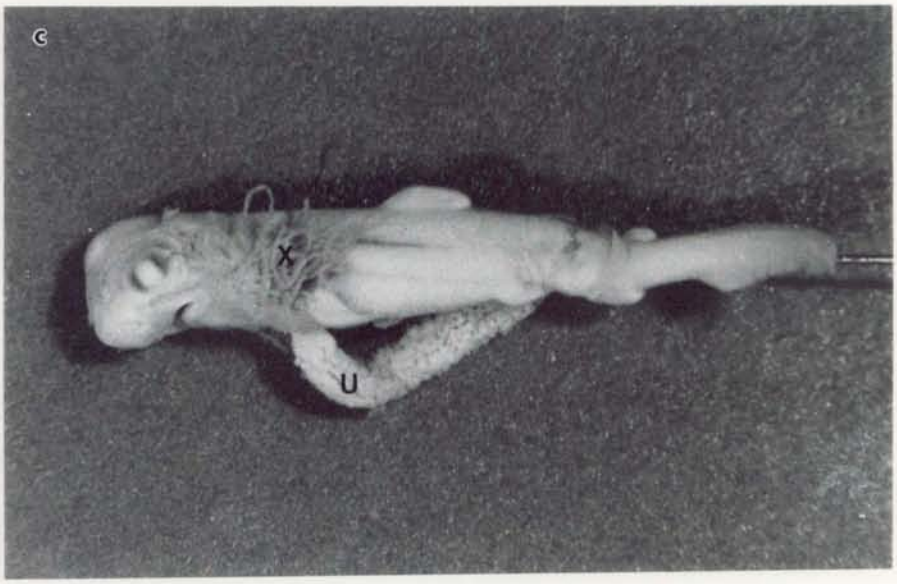
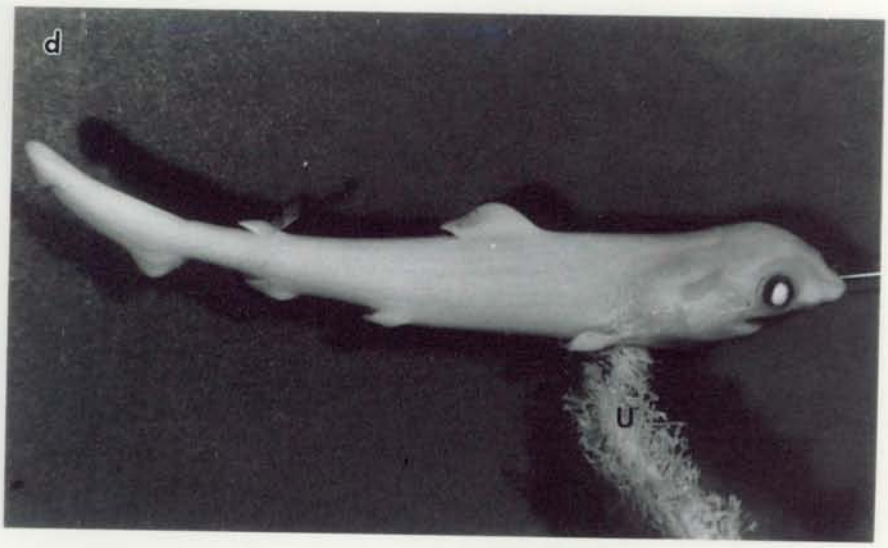

a
Em
y
b
SC
Em
y

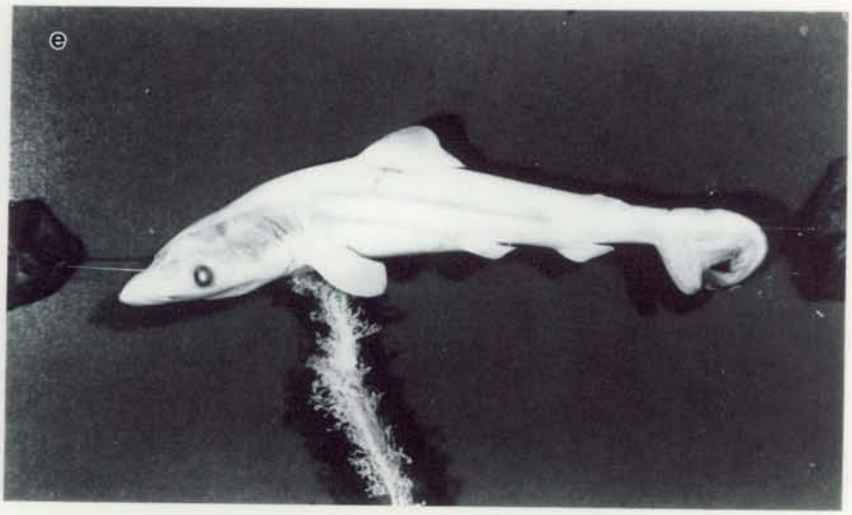
Plate 7.6: Development of *Rhizoprionodon taylori* embryos. (a) 6mm embryo (arrow shows location of embryo), (b) 16mm embryo, (c) 38mm embryo, (d) 68mm embryo, (e) 133mm embryo, and (f) 235mm embryo. E, egg; F, fin buds; U, umbilical cord (or yolk stalk); X, external branchial filaments.


a
E


b
F
F
U


c
X
U


d
U


e

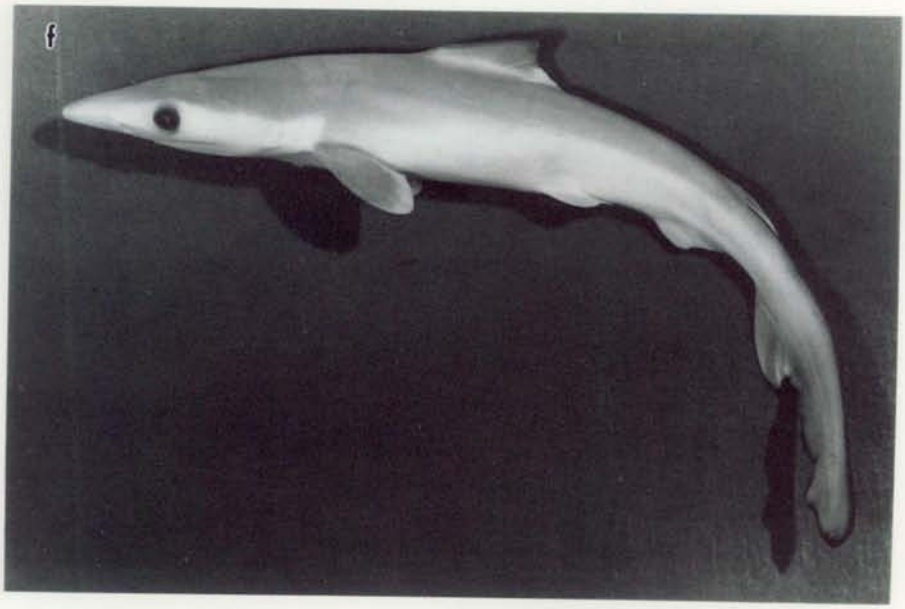

f

Plate 7.7: Histological section showing the development of the yolk stalk and umbilical cord in *Rhizoprionodon taylori* embryos. (a) 16mm embryo, (b) 38mm embryo, (c) 133mm embryo, and (d) 235mm embryo. A, appendiculae; D, ductus vitello-intestinalis; VA, vitelline or umbilical artery; VV, vitelline or umbilical vein. Scale bars, 0.2mm (a), 0.5mm (b), 0.3mm (c) and 0.4mm (d).

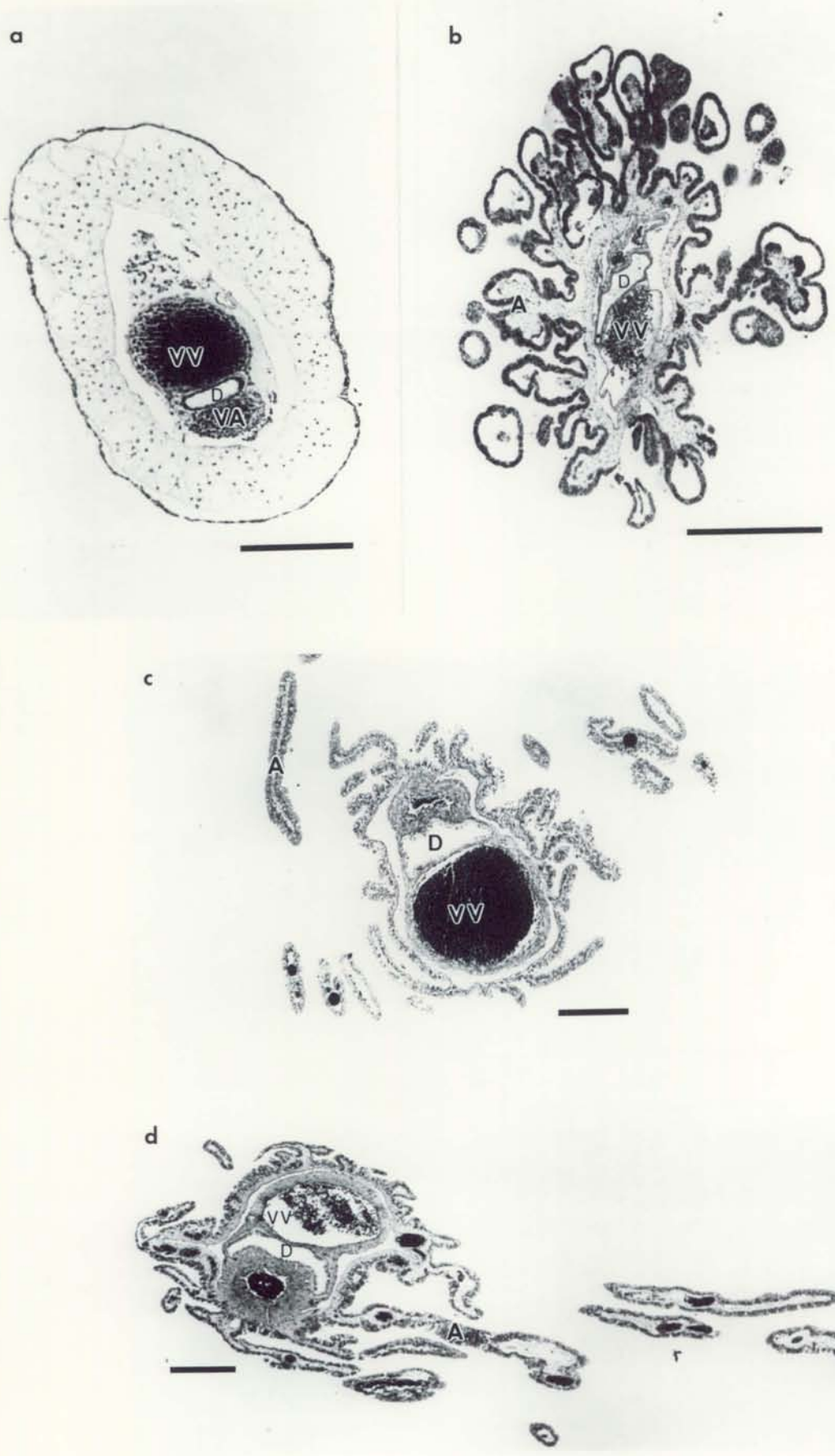
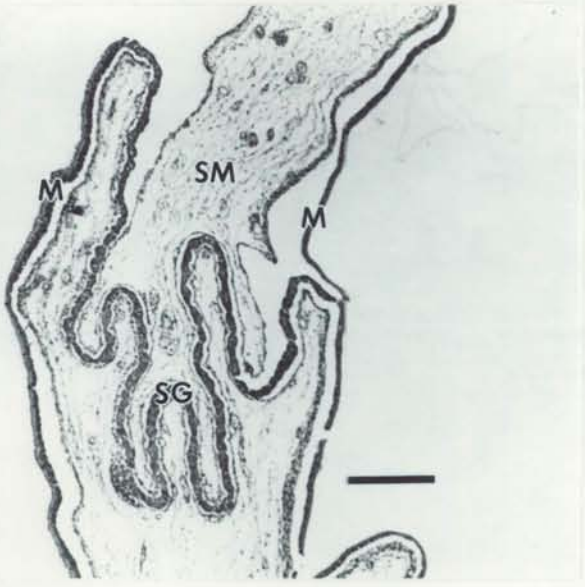

a
VV
D
VA
b
A
D
VV
c
A
D
VV
d
VV
D
A
r

Plate 7.8: Histological section through a uterine septum of *Rhizoprionodon taylori*. M, mucosa; SG, sheet-like gland; SM, submucosa. Scale bar, 0.25mm.

Plate 7.9: Histological section through the uterus of a mid-term *Rhizoprionodon taylori* illustrating the four tissue layers. EC, egg case; M, mucosa; Mu, muscularis; Se, serosa; and SM, submucosa. Scale bar, 0.08mm.


M
SM
M
SG

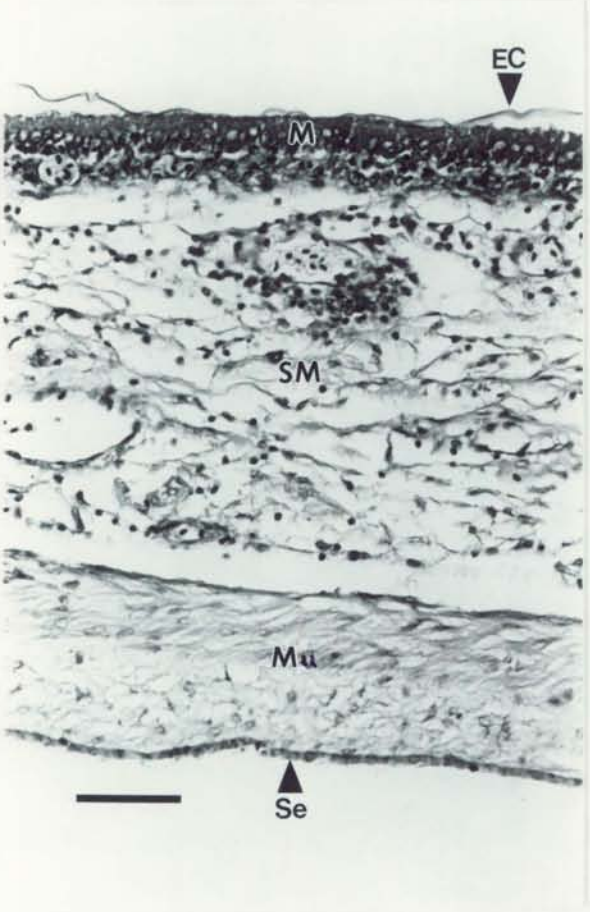
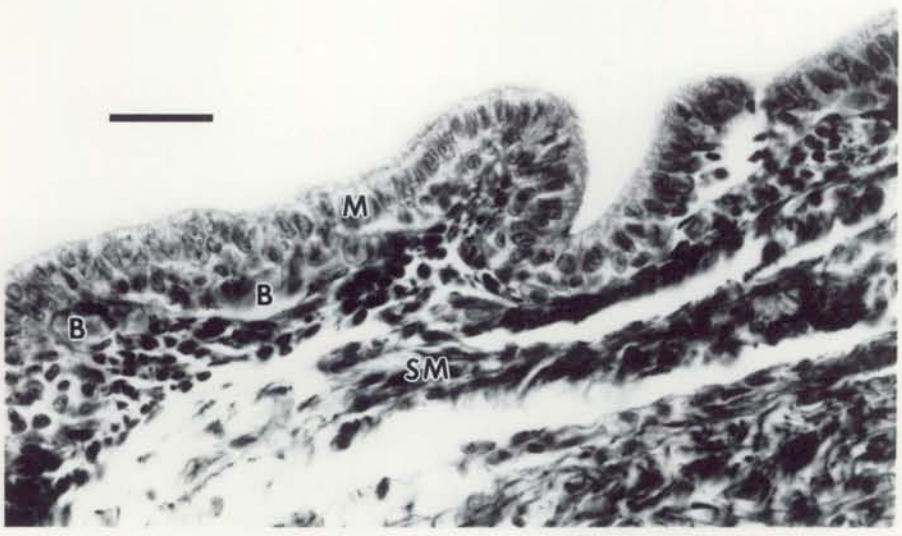

EC
M
SM
Mu
Se

Plate 7.10: Histological sections of the uterine mucosa of *Rhizoprionodon taylori* from (a) the diapause, and (b) the developmental, periods. The epithelial cells of the mucosa produce an apocrine secretion into the lumen of the uterus. B or arrows, blood vessels; EC, egg case; M, mucosa (epithelium); SM, submucosa. Scale bars, 0.05mm.

a



b

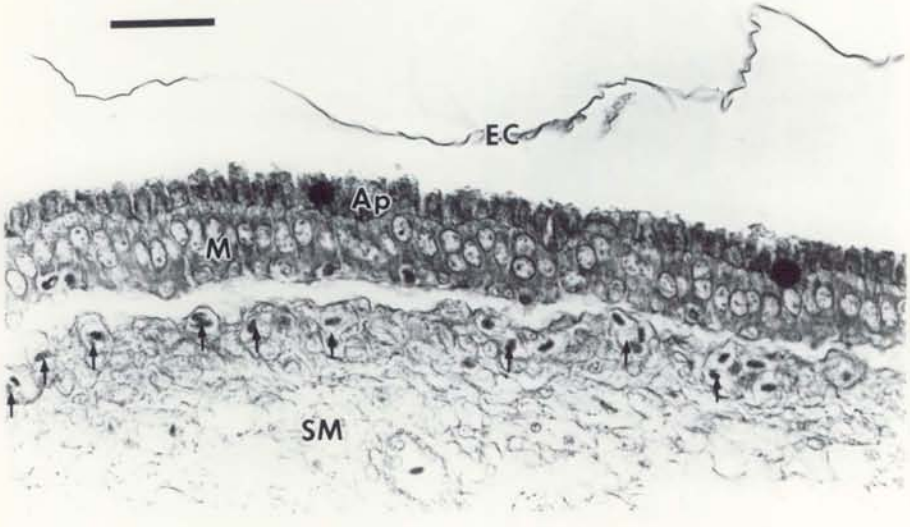


Plate 7.11: Histological section of the sheet-like gland (see text for description) present in the submucosa of the uterus of *Rhizoprionodon taylori*. Arrows indicate tubules that make up the gland. Scale bar, 0.04mm.

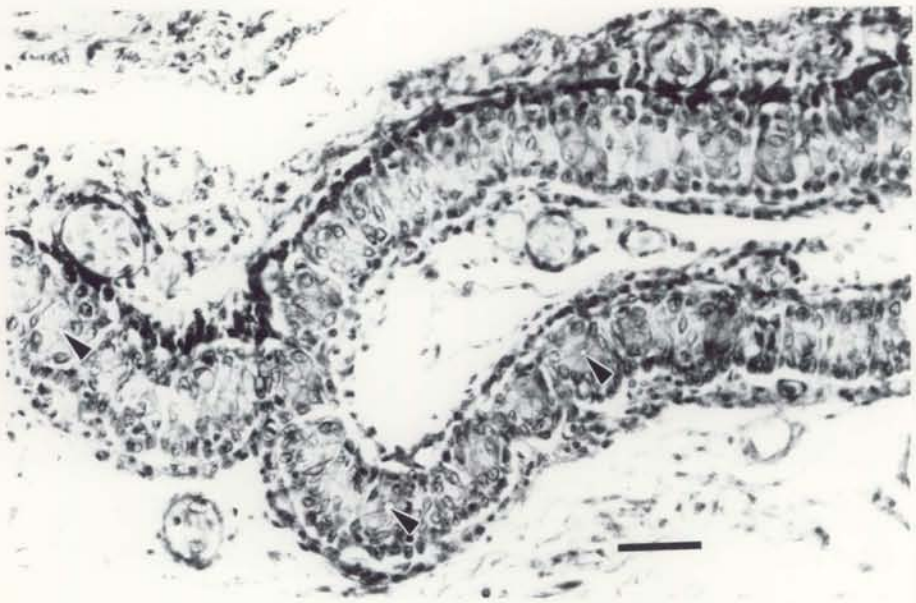


Plate 7.12: Transmission electron micrographs of the epithelial cells of the ductus vitello-intestinalis of *Rhizoprionodon taylori* showing vesicles (asterisk), microvilli (arrows) and cilia (arrow heads). (a) 10000 times, and (b) 5000 times, magnification.


a

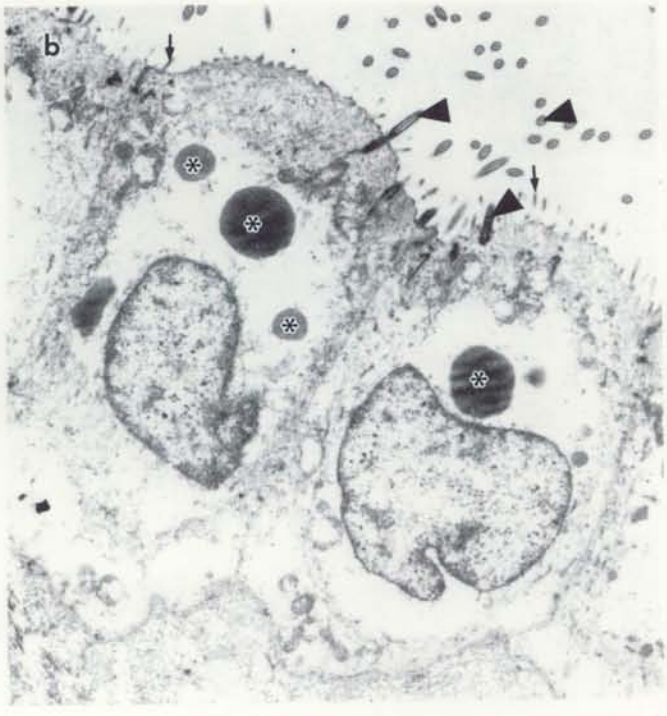

b

Plate 7.13: Histological sections of appendiculae of *Rhizoprionodon taylori*, showing the form of the epithelial cells. (a) 36mm embryo, (b) 68mm embryo, and (c) 235mm embryo. Arrows, apocrine secretion; Arrow heads, microvilli; B, blood vessel; CC, connective tissue; Ep, epithelium. Scale bars, 0.02mm.

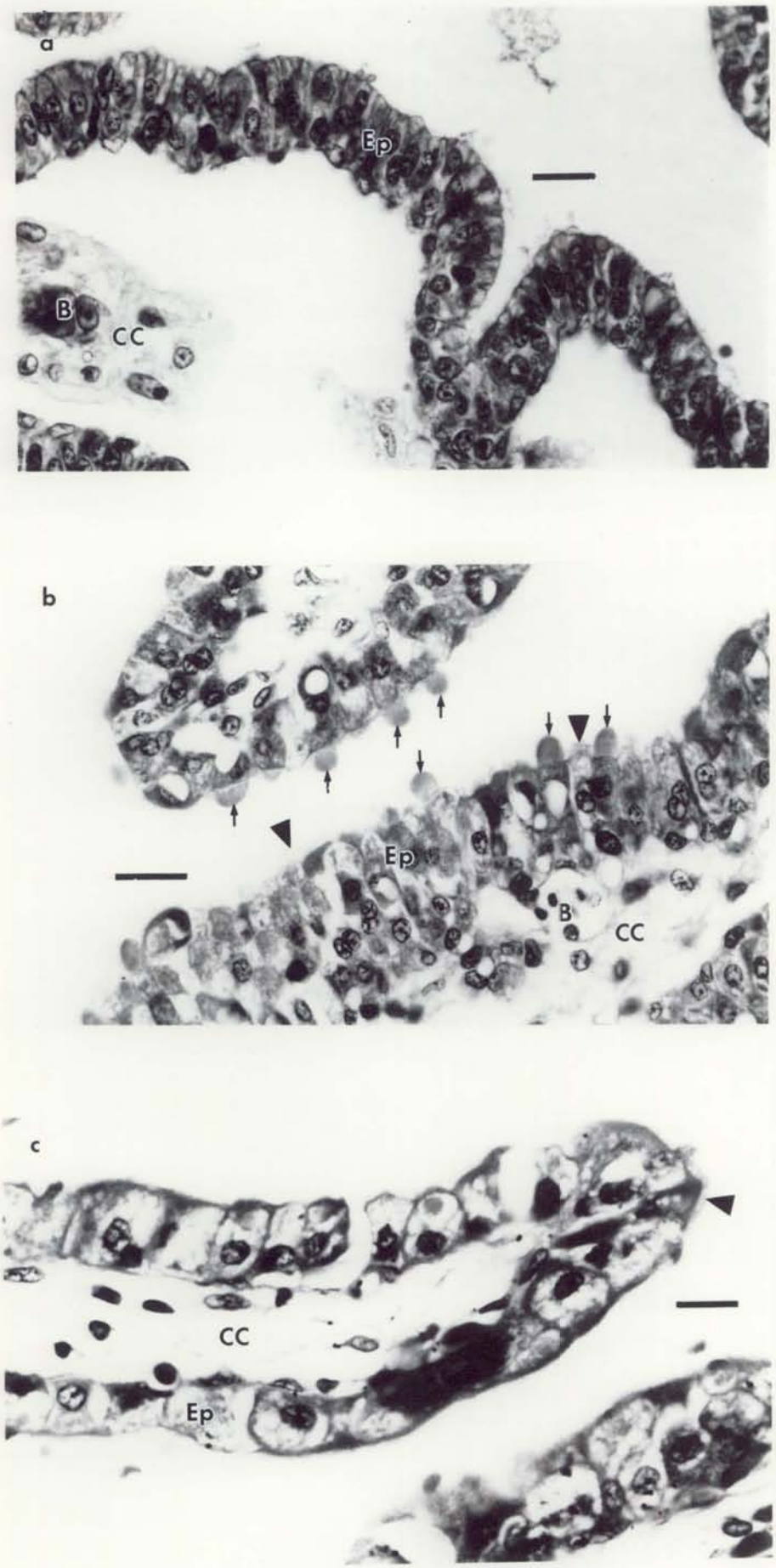
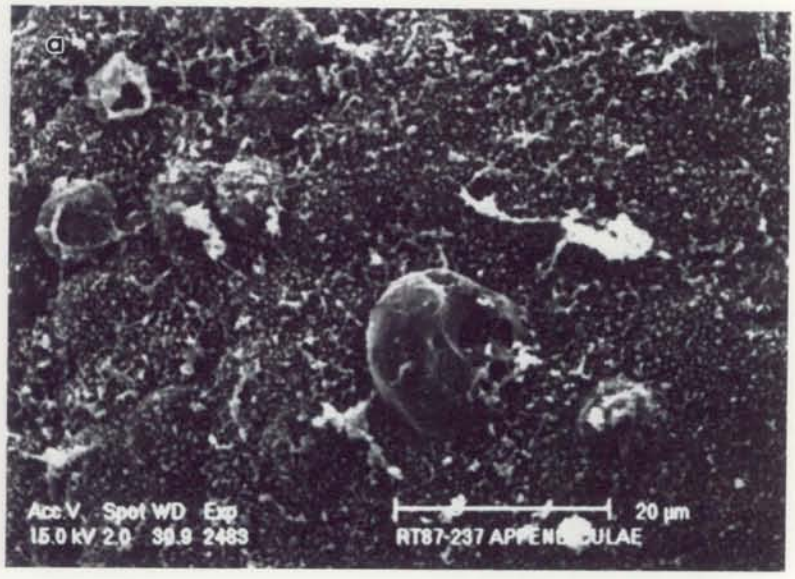
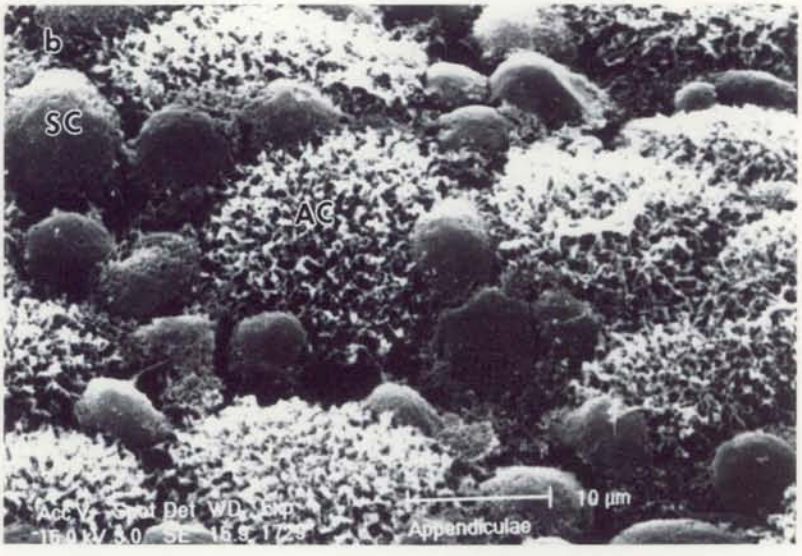

a
Ep
B
CC
b
Ep
B
CC
c
CC
Ep

Plate 7.14: Surface view of the epithelial cells of appendiculae of *Rhizoprionodon taylori* showing absorptive cells (AC) with apical microvilli and secretory cells (SC) . (a) 36mm embryo, (b) 90mm embryo, and (c) 235mm embryo. Scales indicated on photographs.


a
Acc.V Spot WD Exp
15.0 kV 2.0 30.9 2483
20 µm
RT87-237 APPENDICULAE


b
SC
AC
10 µm
Appendiculae

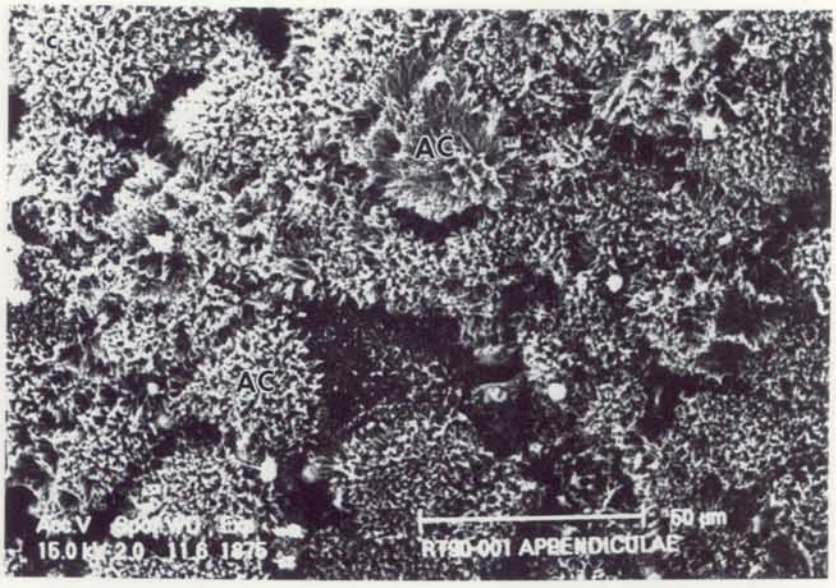
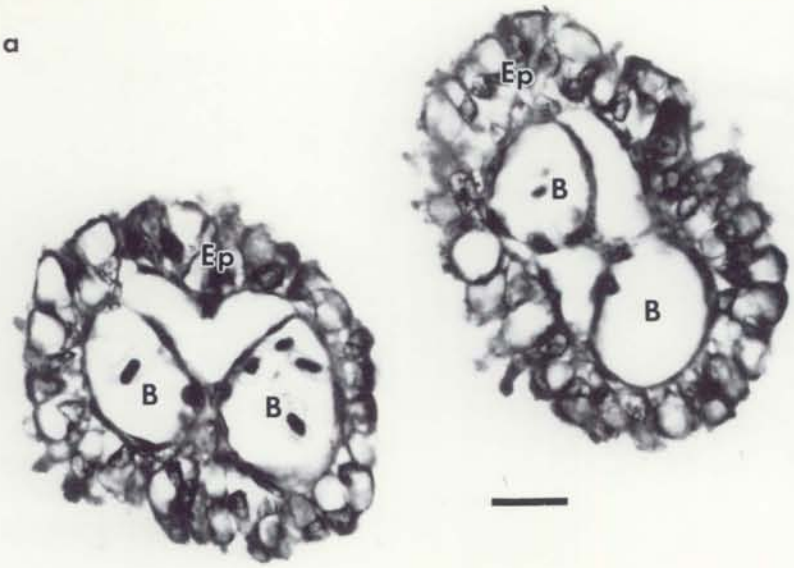

AC
AC
50 µm
RT90-001 APPENDICULAE

Plate 7.15: External branchial filaments of *Rhizoprionodon taylori*. (a) transverse histological sections, and (b) scanning electron micrograph. B, blood vessel; Ep, epithelial cells. Scale bar for (a) 0.03mm, scale for (b) on photograph.

a


Ep
B
B
Ep
B
B

b

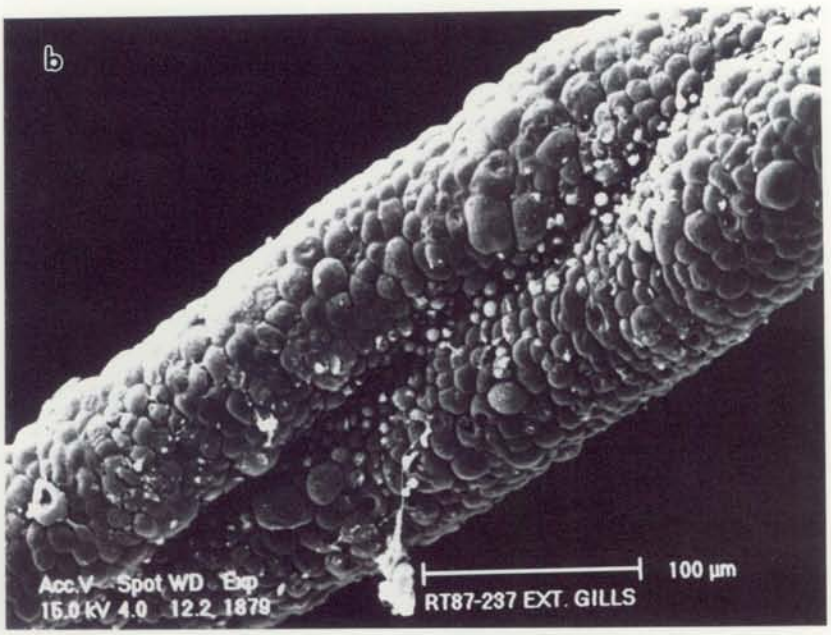
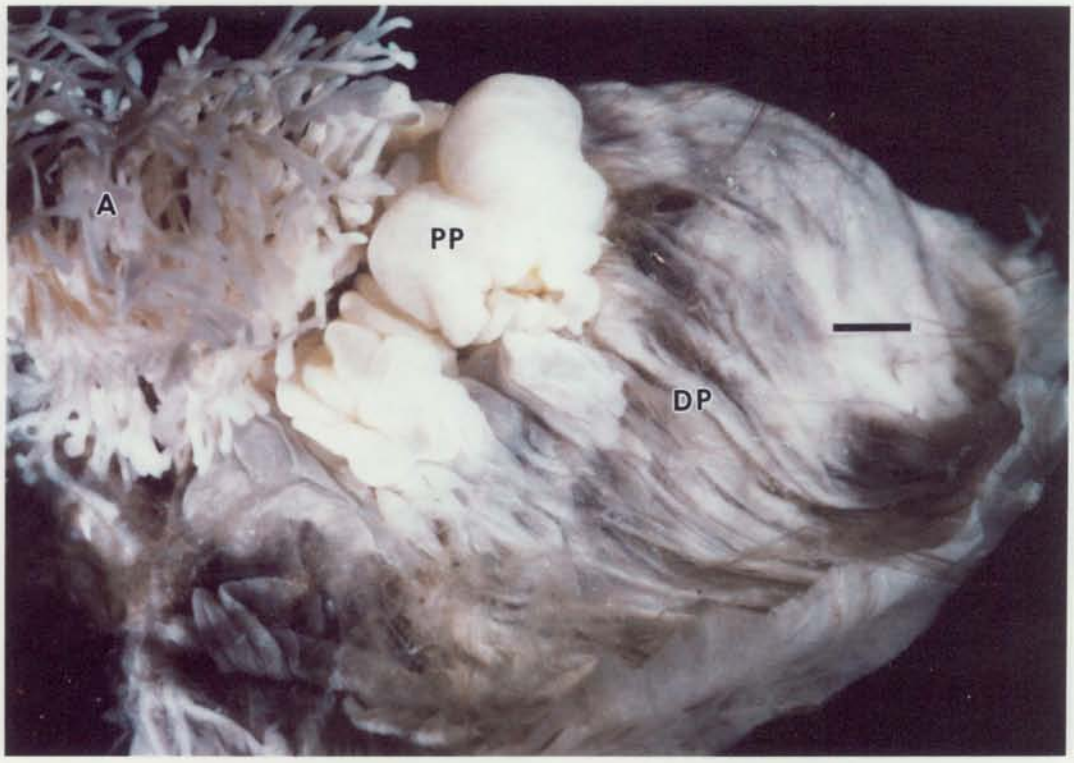

Acc.V Spot WD Exp
15.0 kV 4.0 12.2 1879
100 µm
RT87-237 EXT. GILLS

Plate 7.16: General morphology of a mature placenta of *Rhizoprionodon taylori*. A, appendiculae; DP, distal portion of placenta (enveloped in uterine tissue); PP, proximal portion of placenta. Scale bar, 2mm.

Plate 7.17: Histological section of a proximal portion of the mature placenta of *Rhizoprionodon taylori*. Arrows, apocrine secretion; CC, connective tissue; Ep, epithelium. Scale bar, 0.06mm.


A
PP
DP

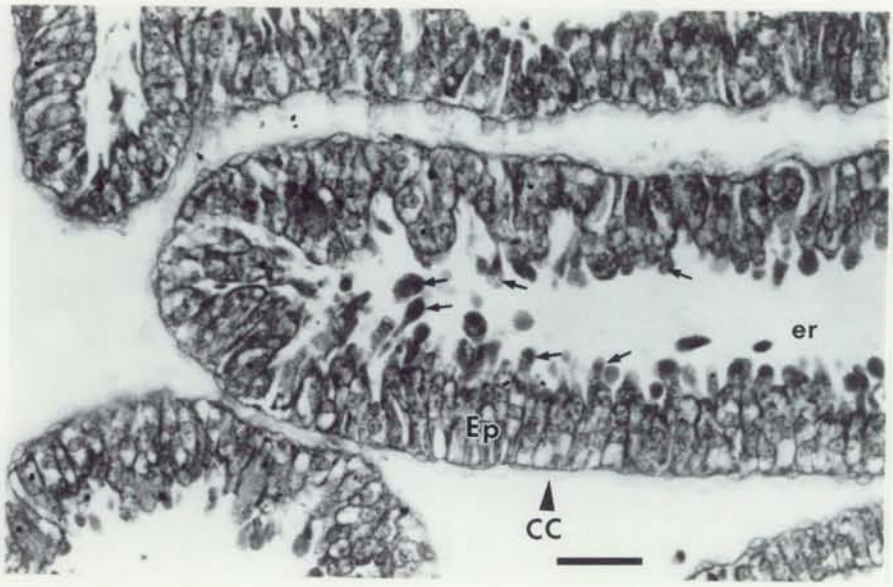

er
Ep
CC

Plate 7.18: Histological sections of the early placenta of *Rhizoprionodon taylori* showing the developing interdigitation (a) and the tissue layers present at the maternal and embryonic interface (b). B, blood vessels; EC, egg case; EE, embryonic endoderm; ES, embryonic squamous epithelium; MS, maternal squamous epithelium; Y, Yolk. Scale bars, 0.3mm (a) and 0.04mm (b).


a
y
y
RS

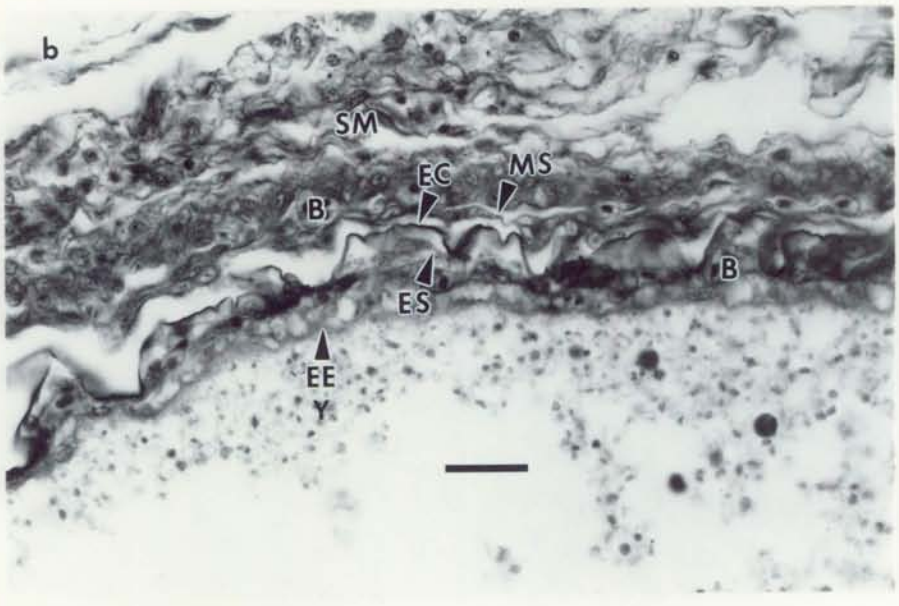
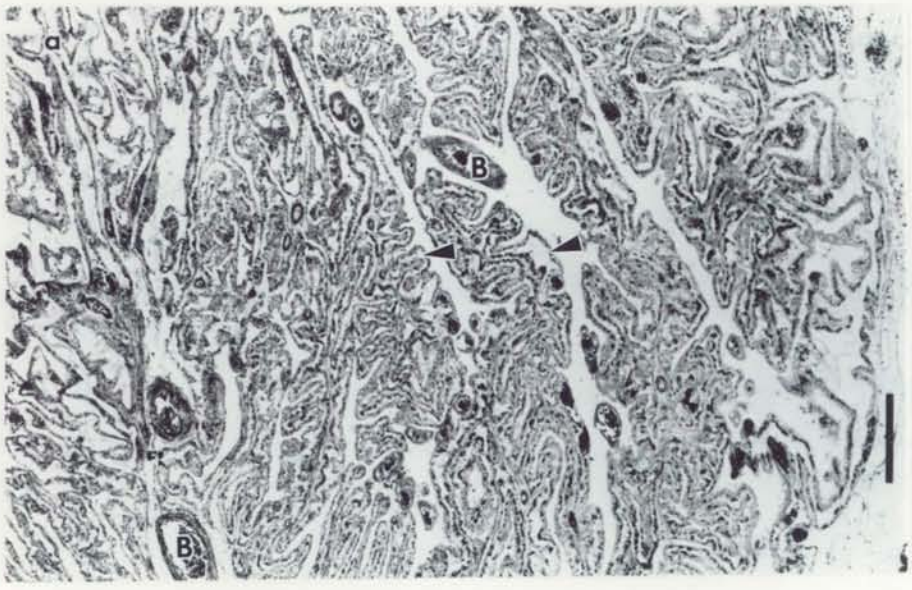
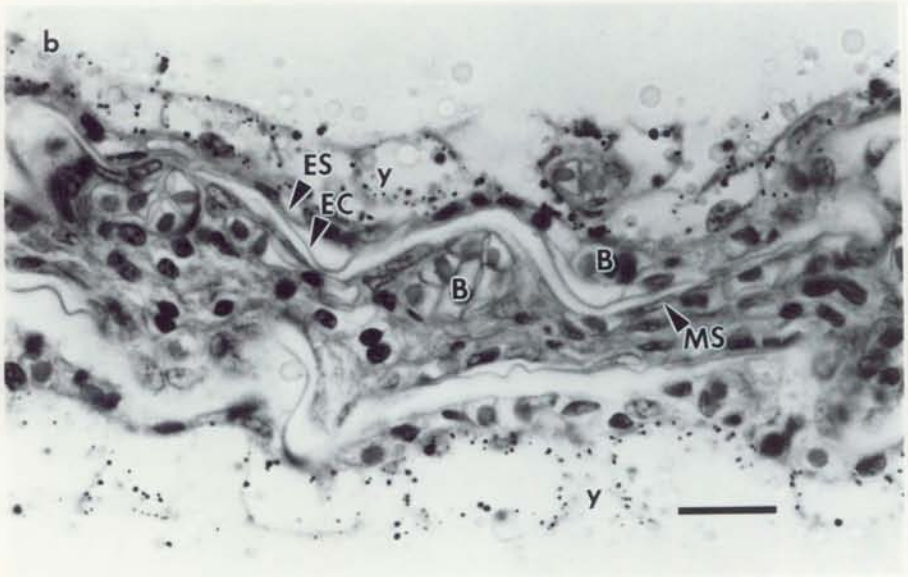

b
SM
EC
MS
B
B
ES
EE
Y

Plate 7.19: Histological sections of the mature placenta of *Rhizoprionodon taylori* showing the complex interdigitation of the uterus and yolk sac (a) and the cellular detail at the maternal-embryonic interface (b). Arrow heads, maternal-embryo interface; B, blood vessel; EC, egg case; RS, remnant submucosa; Y, yolk. Scale bars, 0.3mm (a) and 0.015mm (b).


a
B
B


b
ES
EC
y
B
B
MS
y

7.4 Discussion

7.4.1 The oviducal gland and its functions

From size and histological data, it is evident that the oviducal glands of *R. taylori* are functional only for a short period each year around the time of mating and ovulation. When the gland becomes functional the tubules increase in size as they become active and as a result the whole gland increases in size. The size of the oviducal gland can therefore be used as an indicator of the time of ovulation.

The general form of the oviducal gland of *R. taylori* is similar to that described for other species of *Rhizoprionodon* by Prasad (1944). On the basis of their location tubule types B and C described in the present study correspond to the albumen and shell secreting tubules of Prasad (1944), respectively. Prasad, however, did not describe tubules equivalent to type A, although he did note the presence of spermatozoa in the glands of some specimens.

The primary functions of the oviducal gland in *R. taylori* are the production of the egg case and the storage of spermatozoa. Type B tubules were described by Prasad (1944) as being albumen secreting, but he did not provide any evidence for this. In *R. taylori* type B tubules produced similar histochemical results to those of type C tubules, although they had a smaller diameter. The positive staining for collagen like compounds suggests a function in production of the egg case for type B tubules. Rasaouen (1976) has suggested that similar tubules in *Scyliorhinus canicula* may be involved in sealing the edges of the egg case. Such a function in *R. taylori* is possible but needs to be investigated. The results of staining diapausing eggs indicate the presence of a proteinaceous layer external to the yolk. This may be a layer of albumen, but this needs to be confirmed by further study. There was no evidence

from the tests conducted for the production of this acellular layer in the oviducal gland of *R. taylori* suggesting that it is either produced elsewhere (possibly the oviduct) or the techniques used were not effective in identifying albumen secretions.

The egg case of *R. taylori* is composed of collagen. However, unlike oviparous species (e.g. Rasaouen 1976) there was little evidence that the egg case underwent quinone tanning in the oviducal gland. However, the negative reaction to histochemical tests for products involved in quinone tanning may also have been the result of using fixatives that included acetic acid. Johri and Symth (1956) suggested that such fixatives reduce the chances of obtaining positive reactions from the Catechol method. Further study using fixative not containing acetic acid will be required to determine if the egg case of *R. taylori* undergos quinone tanning. The fact that the eggs are retained in the body, and so protected by the mother, may explain the lack of quinone tanning if it does not occur.

The storage of spermatozoa within specialised tubules of the oviducal gland of carcharhinid sharks has not previously been reported. Prasad (1944) noted the presence of spermatozoa in the oviducal gland of several carcharhinid species, including *Rhizoprionodon oligolinx* a sibling species of *R. taylori* (Compagno 1988), but did not indicate that the tubules in which they occurred were specifically for this purpose. Pratt (1979) recorded the storage of spermatozoa in *Prionace glauca*, but again did not describe the tubules in which they were stored. The acid mucin produced by these tubules in *R. taylori* is presumed to be a nutritive secretion for the spermatozoa, but this requires confirmation. Spermatozoa are stored only for a few weeks or days in the oviducal glands of *R. taylori* since none were observed in the oviducal glands after February.

7.4.2 Embryonic diapause

In Chapter 6 it was demonstrated that the gestation period of *R. taylori* was approximately 11.5 months. However, the existence of a seven month period of embryonic diapause reduces the time for development of the embryos to four and a half months. During the diapause period the embryos remain at the blastoderm stage. Diapause at the blastoderm stage, or its equivalent, has also been reported in mammals (Renfree 1978; Renfree and Calaby 1981).

A similar period after ovulation during which embryonic development does not occur, was reported by Stevens and McLoughlin (1991) for *R. taylori* in northern Western Australia, the Northern Territory and far northern Queensland. Although Stevens and McLoughlin (1991) were unable to demonstrate that diapausing embryos were present it is assumed that the lack of development they observed was attributable to embryonic diapause. The occurrence of embryonic diapause in *R. taylori* over the whole of its range (northern Australia) indicates that it is a characteristic of this species. The phenomenon is synchronous across northern Australia, and between different years in the Townsville region. This indicates that embryonic diapause in *R. taylori* is obligatory (i.e. always occurs and for the same period) rather than facultative (i.e. variable in duration in response to maternal or environmental factors). There is no evidence that *R. oligolinx* (the suggested sibling species of *R. taylori*) has a period of embryonic diapause based on the descriptions of reproduction in this species by Mahadevan (1940) and Setna and Sarangdhar (1949a). Further information on the embryogenesis of *R. oligolinx* would be extremely valuable as an aid for elucidating the true relationship between these two taxa.

R. taylori is the only species of elasmobranch in which embryonic diapause has been confirmed. However, the absence of embryonic development following

ovulation in *Rhinobatus horkelli* (Lessa *et al.* 1986) and *Dasyatis sayi* (Snelson *et al.* 1989) appears similar to that observed in *R. taylori*, and thus may also be attributable to embryonic diapause. This can only be confirmed by further research. The probable occurrence of embryonic diapause in three divergent groups of elasmobranchs (Rhinobatidae, Dasyatidae and Carcharhinidae) indicates that it has evolved separately on each occasion in both placentotrophic (*R. taylori*) and lecithotrophic (*R. horkelli* and *D. sayi*) species.

Embryonic diapause in *R. taylori* possibly evolved in conjunction with the small size at birth and large litter size (see Chapter 6). The small size at birth would permit decreased development time and so open up the possibility for a period of diapause. The reason for the evolution of diapause as opposed to functionally similar mechanisms observed in other species of viviparous sharks - reduced gestation period (e.g. *Sphyrna tiburo* - Parsons 1987) or delayed fertilisation (e.g. *Prionace glauca* - Pratt 1979) - is unknown.

The timing of the diapause period may have evolved to enable *R. taylori* to produce its young when environmental conditions are most favourable. Such a mechanism has been reported in most other groups in which embryonic diapause has been identified (e.g. mammals - Renfree 1978; teleosts - Kroll 1984; insects - Braune 1973; and copepods - Ianora and Santella 1991). Young of *R. taylori* are born when sea temperatures are highest, and thus when growth rates would be maximal. This is particularly important in species having young with a small size at birth since rapid growth after birth reduces the risk of predation (Branstetter 1990). In Chapter 4 it was shown that *R. taylori* in Cleveland Bay achieve very high growth rates after birth, doubling in size in a few months.

On the basis of the present study it is not apparent by what means embryonic diapause is controlled. Two different hypotheses can be postulated. Firstly, diapause may be controlled by a factor (presumably chemical) that inhibits development of the embryo beyond the blastoderm stage. Diapause would thus be terminated when this factor was removed. The existence of the corpora atretica in the ovary throughout the diapause period (see Chapter 6) may play some role in the production of this inhibiting factor. The production of hormonal substances (e.g. progesterone) by follicles such as this is well known in a variety of vertebrate groups, including the elasmobranchs (Browning 1973; Callard *et al.* 1992). A second possible mechanism of control could be by some critical factor being absent. In this mechanism diapause would be terminated by the production of the critical factor. The most obvious source for such a factor would be the uterus.

Given the highly synchronous timing of the diapause period in *R. taylori* it is likely that the stimulus for the termination of diapause is external, probably associated with environmental cues such as sea temperature, moon phase, photoperiod, etc. Further research on the control of diapause in *R. taylori* is required to test these hypotheses and to determine the mechanism by which it is controlled.

7.4.3 Developing embryos

Morphological development of *R. taylori* embryos is apparently similar to that in other species of elasmobranchs, except that they have a period of diapause. The diapause period results in a significant reduction in the time available for embryos to develop. In Table 7.2 it was estimated that *R. taylori* had the lowest growth rate of any carcharhinid species, growing 2.91cm month^{-1}. Calculation of the embryonic growth rate using the actual development period (not the gestation period) and the mean size at birth for individuals from Cleveland Bay gives a value of 6.00cm

month^{-1}. This value is comparable with that of larger species of carcharhinids, and is higher than for other species of small carcharhinids (e.g. *Rhizoprionodon terraenovae*).

Life history theory predicts that litter sizes will be such that future reproduction will be maximised (Stearns 1992). That is if producing a large litter reduces the chances of a female surviving to the next year to produce another litter, then through evolution younger females would produce smaller litters. Similarly, older females would produce larger litters as the greater investment would be less likely to influence their survival since they are close to maximum age. An evolutionary process such as this has possibly occurred in *R. taylori* and may account for the difference in size at birth between mothers of different sizes. Such an evolutionary strategy may also account for the differences in the size at birth between small and large mothers. To produce large litter sizes older mothers may have to trade off the size of young against the number produced due to the limited space in the uteri. It is not known whether the smaller size at birth of the young from older mothers affects survival.

The accommodation of the developing embryos within the uterus of *R. taylori* is similar to that reported in other carcharhinid species (e.g. Teshima and Mizue 1972; Baranes and Wendling 1981). However, unlike all other species examined to date all of the excess egg case is not sequestered in a stored chamber. A store chamber does exist in *R. taylori*, but only a small proportion of the egg case is stored in it. The reason for this difference is unknown.

7.4.4 Embryonic nutrition

The results of the structural observations of maternal and embryonic tissues in *R. taylori* provide valuable information on the possible pathways by which the embryos

obtain nutrients from the mother. However, it is not possible to prove the functioning of these pathways solely on structural grounds. The results of this section of work therefore provide testable hypotheses with regard to embryonic nutrition in *R. taylori*.

On the basis of the structural observations, both histological and ultrastructural, there appears to be three sources of nutrients in *R. taylori*. These are yolk (lecithotrophy), uterine secretions (trophodermy) and a yolk sac placenta (placentotrophy). A similar series of nutritional sources have been reported for *R. terraenovae* by Hamlett (1989).

The first nutritional source utilised by *R. taylori* embryos is the yolk stored in the egg. This is the only source of nutrients available to the embryos during the diapause period and early development prior to the differentiation of structures for the absorption of other types of nutrients. The reliance of embryos on lecithotrophy continues until all of the yolk has been absorbed, which occurs when they reach approximately 60-70mm in length, probably sometime during November.

Hamlett (1989) illustrated two forms of pre-implantation yolk sac, but did not distinguish between these two types. The yolk sac of *R. taylori* appears similar to the second of these, a three layered structure composed of squamous epithelium, connective tissue, and endothelium. Since Hamlett *et al.* (1987) have demonstrated, in *R. terraenovae*, that there is a yolk syncitial layer inside the endothelium through which some yolk absorption takes place, a similar mechanism possibly exists in *R. taylori*. The existence of such a mechanism in *R. taylori*, however, requires confirmation.

Yolk is carried to the embryonic gut of *R. taylori* for digestion via the ductus vitello-intestinalis. The ciliated cells in the ductus facilitate the movement of the yolk. Hamlett (1989) has suggested that yolk may also be absorbed by epithelial cells in the ductus of *Rhizoprionodon terraenovae*. The TEM observations of the ductus vitello-intestinalis epithelium in *R. taylori* suggest that it also absorbs yolk. In particular the presence of microvilli on the epithelium is characteristic of absorptive cells, and the dark staining vesicles in the cells are probably droplets of yolk (which are high in lipid content and so stain darkly with osmium tetroxide in TEM preparations). The crystalline structure of these vesicles also suggests that they are yolk, or yolk derivative, since yolk-like material is known to have a para-crystalline structure (Hamlett 1989). They could, however, be steroids which have a similar structure.

The second source of nutrients for embryos to be utilised by *R. taylori* is probably secretions from the uterus. The production of large amounts of organic secretion in the uterus *R. taylori* begins at about the same time as diapause is terminated, and is achieved by both the apocrine production of acid mucins by mucosal cells, and secretion of acid and neutral mucins by the sheet-like glands. The exact functions of these secretions is unknown. Since apocrine secretion by the mucosal cells has only been observed in species that have appendiculae on the yolk stalk/umbilical cord, it suggests that it is these secretions that are utilised as the main source of histotrophic nutrition (see 7.1.1.6).

The appendiculae present on the yolk stalk and umbilical cord of *R. taylori* correspond to the fourth type described by Southwell and Prashad (1919) since they contain blood vessels. The two types of cells present in the epithelium appear to correspond to those illustrated by Hamlett (1989) from *R. terraenovae*. The

increasing proportion of absorptive cells during gestation suggests that the amount of absorption increases as embryos grow. The function of the secretion formed during the middle of the development period by the secretory cells, which Hamlett (1989) referred to as granulated cells, is unknown. However, given that it is formed at approximately the same time as the yolk sac is forming the placenta suggests it may fulfil some role in this process. Alternatively it may be a mechanism for waste elimination, or some other as yet undetermined function.

On the basis of histological and SEM observations in the present study it was not possible to determine if the external branchial filaments of *R. taylori* play a role in embryonic nutrition. The structure of the filaments appears similar to those in *R. terraenovae* in which Hamlett *et al.* (1985d) demonstrated the ability to absorb macromolecules. Although this provides evidence that embryos of *R. taylori* may absorb uterine secretions with their external branchial filaments, such a hypothesis needs to be tested.

The final source of nutrients for *R. taylori* embryos is the placenta, which functions during the second half of the development period. The cellular structure of the placenta of *R. taylori* is the same as that reported by Mahadevan (1940) for *Rhizoprionodon oligolinx*, and by Schlernitzauer and Gilbert (1966) for the sphyrnid shark *Sphyrna tiburo*. All three species, *R. oligolinx*, *R. taylori* and *S. tiburo*, have entire types of placentae. All reports of the cellular structure of the discoid type of placenta (the other commonly occurring type in the family Carcharhinidae) reveal a columnar maternal epithelium, a reduced embryonic epithelium in the distal portion, and cuboidal epithelium in the proximal portion (e.g. Gilbert and Schlernitzauer 1966; Hamlett *et al.* 1985a,b,c). Consistent differences therefore occur in the cytology of the placental types found in the family Carcharhinidae.

Further research on the placentae throughout the order Carcharhiniformes is required to elucidate the structural relationships between the types of placentae that occur within this group.

The structural similarity of the entire type of placenta between families (Carcharhinidae and Sphyrnidae) within the Carcharhiniformes reflects the phylogeny of this group suggested by Compagno (1988) using cladistic analysis. The entire type of placenta is found in the species from the less apomorphic groups (e.g. *Hemigaleus*, *Sphyrna*, *Loxodon* and *Rhizoprionodon*), while the discoid form occurs in the more derived groups. Within the most derived groups, the tribe Carcharhinini, only one of the least derived species groups, Compagno's *porosus* group (including *Carcharhinus porosus*, *C. borneensis*, *C. dussumieri*, *C. sealei*, *C. macloti*, *C. sorrah* and *C. hemidon*), possess entire placentae. Only one species within this group, *C. sorrah*, has a discoid placenta (Compagno 1988). All other groups within the tribe Carcharhinini probably have discoid placentae (Compagno 1988). The occurrence of the entire placenta in the least derived of the groups of the Carcharhinidae suggests that it is probably the pleisomorphic type, with the discoid form being apomorphic. On the basis of this phylogenetic evidence the discoid placenta is presumably more efficient, a view stated by Mahadevan (1940) since species with entire placentae also rely on uterine secretions for embryonic nutrition. Confirmation of this hypothesis will require a comparative study of the efficiency of entire and discoid placentae within the family Carcharhinidae.

On the basis of structural information the pattern of embryonic nutrition in *R. taylori* and *R. terraenovae* appears very similar. However, in *R. taylori* the rate of embryonic growth is much higher resulting from the occurrence of the diapause period. This suggests that *R. taylori* is able to move nutrients to the embryos more rapidly and efficiently than is the case in *R. terraenovae*.

Both Graham (1967) and Hamlett *et al.* (1985a) have demonstrated that placental sharks are capable of exchanging organic molecules across the maternal-embryonic interface despite the presence of the egg case in many species. What, if any, limitations the retention of the egg case has on the movements of nutrients across the interface in *R. taylori* is unknown, but warrants further study.

Further work needs to be undertaken to enable a better understanding of the pathways involved in embryonic nutrition of *R. taylori* and other sharks. In particular, studies which explicitly demonstrate the functioning and efficiency of the nutritional mechanisms would seem the most useful. Such work would allow for a comparison of the nutritional mechanisms and would provide information on the proportion of nutrition that each mechanism contributes both within and between different species.

Section III.

Other species of the family Carcharhinidae

Chapter 8.

Biology of other carcharhinid species from Cleveland Bay

8.1 Scope and aims

The primary objective of the research reported upon in this thesis was to examine the biology of *Rhizoprionodon taylori* from Cleveland Bay. However, during the collection of specimens for this project a number of other species of shark from the family Carcharhinidae were commonly collected. Data on the abundance, distribution and biology of these species were recorded and the results are presented in this Chapter.

In a preliminary study of the sharks that occur in Cleveland Bay Simpfendorfer (1986) reported briefly on the biology of 14 species, including *R. taylori*. In particular he noted that several of the species apparently utilise Cleveland Bay as a nursery area. The primary goal of the collection of data on other species of carcharhinid sharks in the present study was the further investigation of this phenomenon. This included examination not only of distribution and abundance data, but also reproductive and feeding information. The specific aims of this Chapter were: 1) to determine the species of sharks from the family Carcharhinidae that occur in Cleveland Bay; 2) to investigate the spatial and temporal distribution of carcharhinid species in Cleveland Bay, especially in relation to the utilisation of

the Bay as a nursery area by populations of these sharks; and 3) briefly examine the biology of carcharhinid species, in addition to *R. taylori*, that occur in Cleveland Bay.

8.1.1 A review of the literature on the occurrence and utilisation of nursery areas in the family Carcharhinidae

Information on the structure of carcharhinid populations is scarce. The most comprehensive coverage of the subject was given by Springer (1967) in a paper entitled "Social organisation of shark populations". Springer based this paper on a long period of study of shark populations along the eastern seaboard of the USA (e.g. Springer 1940, 1948, 1950a,b, 1960,1963; Baughman and Springer 1950). In this paper he presented many ideas on the structure of shark populations, but provided little supporting data. Despite this, Springer's work has provided a theoretical framework which subsequent studies have been able to test. One of the key ideas put forward by Springer was that populations of sharks commonly segregate by size, sex and maturity. This review deals with the last of these, the segregation by maturity.

Segregation of populations by maturity implies the separation of juveniles and adults, as well as segregation by size. As with other marine organisms the areas which juvenile sharks inhabit are referred to as nursery areas. Nursery areas have been reported for at least 16 species of shark from the family Carcharhinidae (Table 8.1). In all of the neritic species (i.e. inhabiting the continental shelves) the nurseries occur in areas close to shore, especially bays, estuaries, river mouths, mangrove channels and coastal lagoons.

Table 8.1: Reports of nursery areas in populations of sharks from the family Carcharhinidae.

Species	Location	Habitat	Source
Carcharhinus amblyrhynchos	Aldabra Atoll	reef lagoon	Stevens 1984a
C. ambionensis	South Africa	inshore	Bass 1978
	South Africa	inshore	van der Elst 1979
C. brevipinna	South Africa	inshore	Bass 1978
	South Africa	inshore	van der Elst 1979
C. cautus	N.W. Australia	mangroves	Blaber *et al.* 1985
C. falciformis	N.W. Atlantic	outer shelf & oceanic banks	Springer 1967
C. galapagensis	Clipperton Island	around island	Limbaugh 1963
	St. Paul's Rock	oceanic rock	Edwards & Lubbock 1982
C. leucas	Texas	nearshore	Baughman & Springer 1950
	Gulf of Mexico	river mouth	Springer 1963
	Louisiana	bay	Caillouet *et al.* 1969
	South Africa	rivers	Bass *et al.* 1973
	South Africa	estuary	Wallace & van der Elst 1975
	South Africa	inshore	van der Elst 1979
	Florida	coastal lagoon	Snelson & Williams 1981
	Florida	coastal lagoon	Snelson *et al.* 1984
C. limbatus	Texas	nearshore	Branstetter 1981
	South Africa	inshore	Bass 1978
	South Africa	inshore	van der Elst 1979
	N.W. Australia	mangroves	Blaber 1986
	N.W. Australia	inshore	Blaber *et al.* 1985
C. obscurus	South Africa	inshore	Bass *et al.* 1973
	South Africa	surf zone	van der Elst 1979
C. plumbeus	Eastern USA	inshore	Springer 1960
	Virginia	bay	Hoese 1962
	South Africa	inshore	Bass *et al.* 1973
	Virginia	coastal lagoon	Medved & Marshall 1981
	Florida	coastal lagoon	Snelson & Williams 1981
	South Africa	inshore	Cliff *et al.* 1988
Galeocerdo cuvier	Florida	inshore	Springer 1940
Negaprion acutidens	N.W. Australia	mangroves	Blaber *et al.* 1985
N. brevirostris	Florida	inshore	Springer 1940
	Eastern USA	inshore	Springer 1963
	Florida	bay	Gruber 1982
	Florida	shallow seagrass	Schmidt 1986
	Bahamas	reef lagoon	Gruber *et al.* 1988
Rhizoprionodon lalandei	Brazil	inshore	Ferreira 1988
R. porosus	Brazil	inshore	Ferreira 1988
R. terraenovae	Gulf of Mexico	littoral zone	Parsons 1983a

Springer (1967) was the first to discuss the occurrence of nursery areas in populations of carcharhinid sharks. He suggested that the young of carcharhinid sharks are born in specific nursery areas that are in shallower water than normally inhabited by the adults, and that a number of behavioural mechanisms ensure that the adults and juveniles remain separated. These behavioural mechanisms are:

a. adult females only enter the nursery areas when gravid and ready to pup,

b. adult females do not feed while they are in nursery areas,

c. adult males do not normally occur in nursery areas,

d. the young remain in the nursery areas until they are near sexual maturity, and

e. the juveniles may migrate from one area to another if forced by adverse environmental conditions, but these migrations are normally shorter than those undertaken by the adults.

Springer (1967) formulated these mechanisms in terms of a theoretical population, and stated, "the degree to which ... larger carcharhinids conform to the hypothetical one [model] is not uniform" (p.154).

On the basis of a large amount of work in South Africa Bass (1978) supported Springer's theoretical model and added to it by dividing the nursery areas into two types: primary nurseries where the young are born and live for a short time after birth, and secondary nurseries where the juveniles spend the rest of the time until near maturity. In some species the primary and secondary nursery areas occur in

the same place (e.g. summer nurseries of *Carcharhinus leucas* - Snelson *et al.* 1984), while in others they are separate (e.g. *Negaprion brevirostris* - Gruber *et al.* 1988).

Two functions have been suggested in the literature for the utilisation of nursery areas by shark populations. The first is that nursery areas reduce predation on the juveniles. The behavioural mechanisms suggested by Springer (1967, see above) would be responsible for a reduction in predation by conspecifics. However, as Springer also noted nursery areas would provide a greater reduction in predation if they were free of large sharks of all species. Van der Elst (1979) demonstrated that for the nursery area of *Carcharhinus obscurus* off the Natal coast of South Africa large sharks of other species do frequently occur in some nurseries and result in significant levels of predation. Alternatively, in species such as *Carcharhinus leucas* the nursery areas are relatively free from adults of other species (Springer 1967). To distinguish between these two situations Branstetter (1990) identified protected and unprotected nursery areas. He observed that protected nursery areas (e.g. estuaries and bays), where predation of juveniles is normally low, are utilised by species that have slow growing young as predation would have a marked effect on the population size. Alternatively, species that produce large or rapidly growing young may use unprotected nursery areas (e.g. open coastal areas and surf zones) as predation on their offspring is low. On the basis of van der Elst's and Branstetter's work it would appear that the predation reduction hypothesis regarding nursery areas is true mostly for those species that have protected nursery areas.

The second suggested function of nursery areas is that they provide an abundance of food on which the juveniles feed (Branstetter 1990). This factor may account

for the occurrence of nursery areas in coastal waters, especially highly productive areas such as seagrass beds and mangroves (see Table 8.1) where food resources are normally abundant.

Study on the nursery areas of other marine organisms has also identified another possible benefit of nursery areas - physiological advantage. Such a mechanism may allow individuals that use nursery areas during the Spring and Summer to grow more rapidly than if they did not because water temperatures in shallow coastal areas increase more rapidly than deeper areas (Miller *et al.* 1985). Whether such a mechanism applies to some sharks species is not known, but warrants further study.

Given the advantages of nursery areas to shark populations evidence in the literature suggests that their evolution may have been closely associated with the evolution of reproductive style. All sharks share a common reproductive style: internal fertilisation and a large investment in a small number of well developed offspring. This reproductive style is believed to have evolved in chondrichthyans over 350 million years ago (Young 1981; Compagno 1990a), and Lund (1990) has reported nursery areas in chondrichthyan populations from fossilised marine communities up to 320 million years old. A consequence of their reproductive style is that recruitment can be directly related to the number of reproducing females. Thus, the success of a population with this reproductive style is dependent upon high juvenile survival to maintain the size of the reproducing portion of the population. Nursery areas are therefore an advantage as they reduce predation and/or increase the availability of food.

Conclusions

Evidence from the literature indicates that nursery areas are an important part of the life history strategy of many species of carcharhinid sharks. Despite this, investigations into the occurrence and functioning of nursery areas remain scarce, and those studies that have been undertaken have generally examined them at the species level. However, evidence suggests that in some areas several species use the same nursery (e.g. Sadowsky 1965, 1967; Simpfendorfer 1986). As a result research into nursery areas may need to be focussed more on the community level rather than the species level. Such studies would enable a greater understanding of factors such as the advantages of sharing nursery areas, and competition within, and for, nursery areas.

8.2 Materials and methods

Materials and methods used for this chapter have previously been described in other chapters and these should be consulted for information. Chapter 2 describes the study area and sampling methods; Chapter 3 the analysis of catch data; Chapter 5 examines the feeding habits; and Chapter 6 the reproductive biology.

Specimens, additional to those caught in gillnets and demersal otter trawls, were obtained from other sources (e.g. Reef Wonderland Aquarium, Townsville; and James Cook University Teaching Collection material) to supplement the range of species examined during this study. Little information was available on the capture of these specimens.

8.3 Results

8.3.1 Abundance and distribution

A total of 12 species from the family Carcharhinidae (excluding *Rhizoprionodon taylori*) were obtained from Cleveland Bay (Table 8.2). Four of these species (*Carcharhinus cautus*, *C. leucas*, *C. melanopterus* and *Galeocerdo cuvier*) were obtained from other sources, and their status in the nearshore waters of the Bay is considered uncertain. Only two species, excluding *R. taylori*, were commonly caught in the 10cm gillnets: *C. tilstoni* and *R. acutus*.

Table 8.2: Numbers of sharks of the family Carcharhinidae examined from Cleveland Bay (excluding *Rhizoprionodon taylori*). * designates species obtained from sources other than sampling trips.

Species	Number caught
Carcharhinus brevipinna	1
C. cautus	1*
C. dussumieri	7
C. fitzroyensis	8
C. leucas	1*
C. limbatus	15
C. macloti	2
C. melanopterus	1*
C. sorrah	16
C. tilstoni	134
Galeocerdo cuvier	1*
Rhizoprionodon acutus	128

The catch rate data for *C. tilstoni* and *R. acutus* from night set 10cm gillnets were examined to investigate patterns of distribution using analysis of variance, in the same manner to the data from *R. taylori* (see Chapter 3). Catch rate data used in

analyses were transformed using a logarithmic function as it provided an improvement in the Shapiro-Francia statistic (*C. tilstoni*, untransformed 0.2442, transformed 0.3593; *R. acutus*, untransformed 0.5293, transformed 0.6065). Netting effort at each of the sites in Cleveland Bay is shown in Figure 2.2.

Carcharhinus tilstoni

Net type was excluded from the final ANOVA model comparing the catch rates between sites as it was not significant at any level (net type, d.f.=1, F=0.05, p=0.3925; net type*site, d.f.=2, F=1.12, p=0.3305). There was a significant difference in the mean catch rate of *C. tilstoni* between sites (ANOVA, d.f.=2, F=7.33, p=0.0009). The highest mean catch rate of *C. tilstoni* was 1.1 sharks $net^{-1}hr^{-1}$ at Middle Reef, while at the Strand and southern Bay sites it was less than 0.2 sharks $net^{-1}hr^{-1}$ (Figure 8.1).

Net type was excluded from the ANOVA model comparing catch rates at the Strand site between years and seasons as it was not significant at any level (net type, d.f.=1, F=0.02, p=0.8962; net type*year, d.f.=2, F=0.52, p=0.5987; net type*season, d.f.=3, F=0.03, p=0.9942; net type*year*season, d.f.=5, F=0.41, p=0.8376). Year was excluded from the resulting model, as it was not a significant factor (year, d.f.=2, F=2.54, p=0.0842; year*season, d.f.=5, F=1.85, p=0.1104), although the mean catch rate in 1988/89 was higher than in 1987/88 and 1989/90 (Figure 8.2a). The final model included only season. The mean catch rate was significantly different between seasons at the Strand site (d.f.=3, F=11.10, p>0.0001). No *C. tilstoni* were caught during Winter or Spring, while the catch rate was highest in Summer (Figure 8.2b). *C. tilstoni* was caught in Summer of each year of this study, with the highest catch rates in Summer 1988/89 (Figure 8.3).

The variance to mean ratio (VMR) of *C. tilstoni* catch rates for night set 10cm gillnets was 5.35.

Rhizoprionodon acutus

Mean catch rates of *R. acutus* in night set 10cm gillnets were highest at the southern Bay site (Figure 8.4). Net type was removed from the model as it was not significant at either level (net type, d.f.=1, F=0.70, p=0.4046; net type*site, d.f.=2, F=0.55, p=0.5803). When net type was removed the analysis indicated that there was no significant difference in the catch rates between the sites (ANOVA, d.f.=2, F=3.04, p=0.0511).

The mean catch rate of *R. acutus* in night set 10cm gillnets increased during each year of this study (Figure 8.5a), with highest mean seasonal catch rates occurring in Autumn and Winter (Figure 8.5b). *R. acutus* was caught in all seasons of this study except Spring and Summer 1987/88 (Figure 8.6), with the highest mean seasonal catch rates occurring between Summer 1988/89 and Spring 1989/90 (Figure 8.6). However, none of the factors were statistically significant in the ANOVA model comparing the catch rate for net type, year, and season at the Strand site (Table 8.3).

The VMR of the catch rates of *R. acutus* in night set 10cm gillnets was 0.81.

Other species

The temporal abundance of the less commonly occurring carcharhinid species is shown in Table 8.4. *C. fitzroyensis* and *C. limbatus* were caught during late spring and summer; *C. dussumieri* during autumn and winter; and *C. sorrah* throughout

Table 8.3: Results of three factor ANOVA for catch rate data of *Rhizoprionodon acutus* caught in night set 10cm gillnets in Cleveland Bay.

Factor(s)	d.f.	F	p
season	3	0.64	0.5920
year	2	0.80	0.4543
net type	1	2.17	0.1443
season*net type	3	0.43	0.7292
season*year	5	0.73	0.6030
year*net type	2	0.50	0.6082
year*season*net type	5	0.08	0.9954

the year. Too few *C. brevipinna* and *C. macloti* were caught to comment on temporal abundance in Cleveland Bay. *C. limbatus* were caught at all three locations; *C. dussumieri*, *C. fitzroyensis* and *C. sorrah* at the Strand and southern Bay.

Table 8.4: Monthly catches of uncommonly occurring species of carcharhinid sharks in Cleveland Bay.

	Month											
Species	J	F	M	A	M	J	J	A	S	O	N	D
Carcharhinus brevipinna	-	-	-	1	-	-	-	-	-	-	-	-
C. dussumieri	-	-	-	-	1	1	3	-	1	-	-	-
C. fitzroyensis	-	2	-	-	-	-	-	-	-	-	3	2
C. limbatus	2	2	-	-	-	-	-	-	-	-	7	2
C. macloti	-	1	-	-	-	-	-	-	1	-	-	-
C. sorrah	7	-	-	-	-	4	1	-	3	-	4	-

8.3.2 Size distributions

The majority of *C. tilstoni* specimens were between 580mm TL and 680mm TL, close to the size at birth suggested by Stevens and Wiley (1986), while very few larger specimens were caught (Figure 8.7a). Most *R. acutus* specimens measured between 400mm TL and 650mm TL (Figure 8.7b), which covered most of the size range of juveniles as reported by Stevens and McLoughlin (1991). Size frequency distributions were not produced for other species because of insufficient data.

8.3.3 Reproduction

Varying amounts of reproductive data were gathered from six species of carcharhinid shark depending upon the sex and life cycle stage of the specimens. The results are presented below for each species.

Carcharhinus dussumieri

Six specimens were examined, five juveniles and an adult female. The adult female, caught in September, contained uterine eggs without visible development of embryos, an indication normally that mating had recently occurred. The juveniles were caught between May and August, and ranged in size from 502mm TL to 629mm TL. None of the juveniles had distinct umbilical scars.

Carcharhinus fitzroyensis

Four specimens examined were adults - three male and one female. The mature males were caught in December and February (2). Spermatozoa were present in the ductus epididymus of the specimen caught in December, and one specimen caught in February appeared to have recently mated. The mature female was caught in December and had enlarged uteri indicating she had recently pupped. This

specimen also contained large (25mm diameter) yolky ovarian follicles suggesting she was close to mating. These data from adult specimens suggest a summer breeding season and annual reproductive cycle.

Three juveniles caught during November and December all had distinct umbilical scars. This indicates an early summer pupping season. Specimens with umbilical scars ranged in size from 477mm TL to 528mm TL.

Carcharhinus limbatus

Thirteen specimens were examined, of which only one was mature. The single adult was a male caught in January with enlarged testes and spermatozoa in the ductus epididymus. These characters suggest that mating was imminent. Eight juvenile specimens ranging in size from 683mm TL to 776mm TL had umbilical scars and all were caught between December and February. These data suggest a summer pupping season, possibly followed by mating.

Carcharhinus sorrah

Nineteen specimens, all juveniles, were caught throughout the year. Four juveniles with umbilical scars were caught in January and ranged in size from 524mm TL to 579mm TL.

Carcharhinus tilstoni

136 specimens were caught, of which 97% were juveniles. Two mature females carrying litters of four and five near-term embryos were caught in early November. The near-term embryos measured between 539mm TL and 593mm TL. The ovaries

of the mature females contained large yolky follicles, suggesting preparation for mating after parturition, probably during summer. The adult males caught provided no useful reproductive data.

Juveniles with distinct umbilical scars were caught between November and February, but mostly in December and January. This suggests a late spring and summer pupping season. The size of juveniles with umbilical scars ranged from 556mm TL to 692mm TL (mean = 617mm TL).

Rhizoprionodon acutus

Juveniles also dominated the catch of 128 *R. acutus*, making up 97% of the specimens. Juveniles ranged in size from 354mm TL to 684mm TL. Those with umbilical scars ranged from 354mm TL to 504mm TL, and were caught in September, October, January and February. This suggests either an extended pupping season over most of Spring and Summer, or year round parturition.

Four adults were caught, all of them males. Two were caught in August, and two in November. Spermatozoa were present in the ductus epididymus of all mature specimens, suggesting that mating may occur at least during the second half of the year. On the basis of the state of the claspers the size at maturity in males is estimated to be between 650mm TL and 720mm TL (Figure 8.8).

8.3.4 Feeding

Diets of six species of carcharhinid sharks from Cleveland Bay are described briefly in Table 8.5. More than half of the specimens of all species examined, except *C. dussumieri*, had empty stomachs. Stomachs from four species contained only teleost remains, while two contained teleosts as well as crustaceans and cephalopods. The

most commonly observed teleost families observed in stomachs from *C. tilstoni* and *R. acutus* were the Clupeidae and Leiognathidae. Information on details of the diet in other species were not recorded.

Table 8.5: Dietary information for six species of carcharhinid sharks caught in the Townsville region. N represents the number of each species examined; R is the number that had regurgitated; E is the percentage that were empty; T, Cr and Ce are the percentages of non-empty stomachs that contained teleosts, crustaceans and cephalopods respectively.

Species	N	R	E	T	Cr	Ce
C. dussumieri	7	2	40	100	0	0
C. fitzroyensis	6	0	66.7	100	0	0
C. limbatus	14	0	50	100	0	0
C. sorrah	16	0	75	100	0	25
C. tilstoni	118	5	73.4	93.3	10	3.3
R. acutus	123	2	71.9	100	8.8	2.9

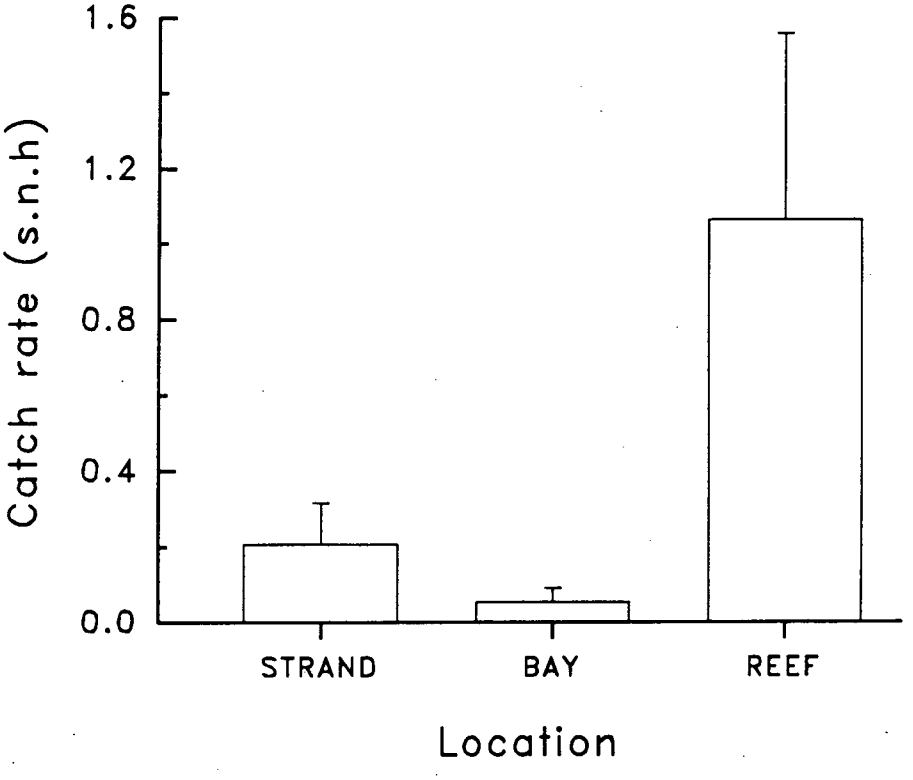


Figure 8.1: Mean catch rates of *Carcharhinus tilstoni* at three sites in Cleveland Bay. Error bars, plus one standard error; s.n.h., sharks $net^{-1}hr^{-1}$.

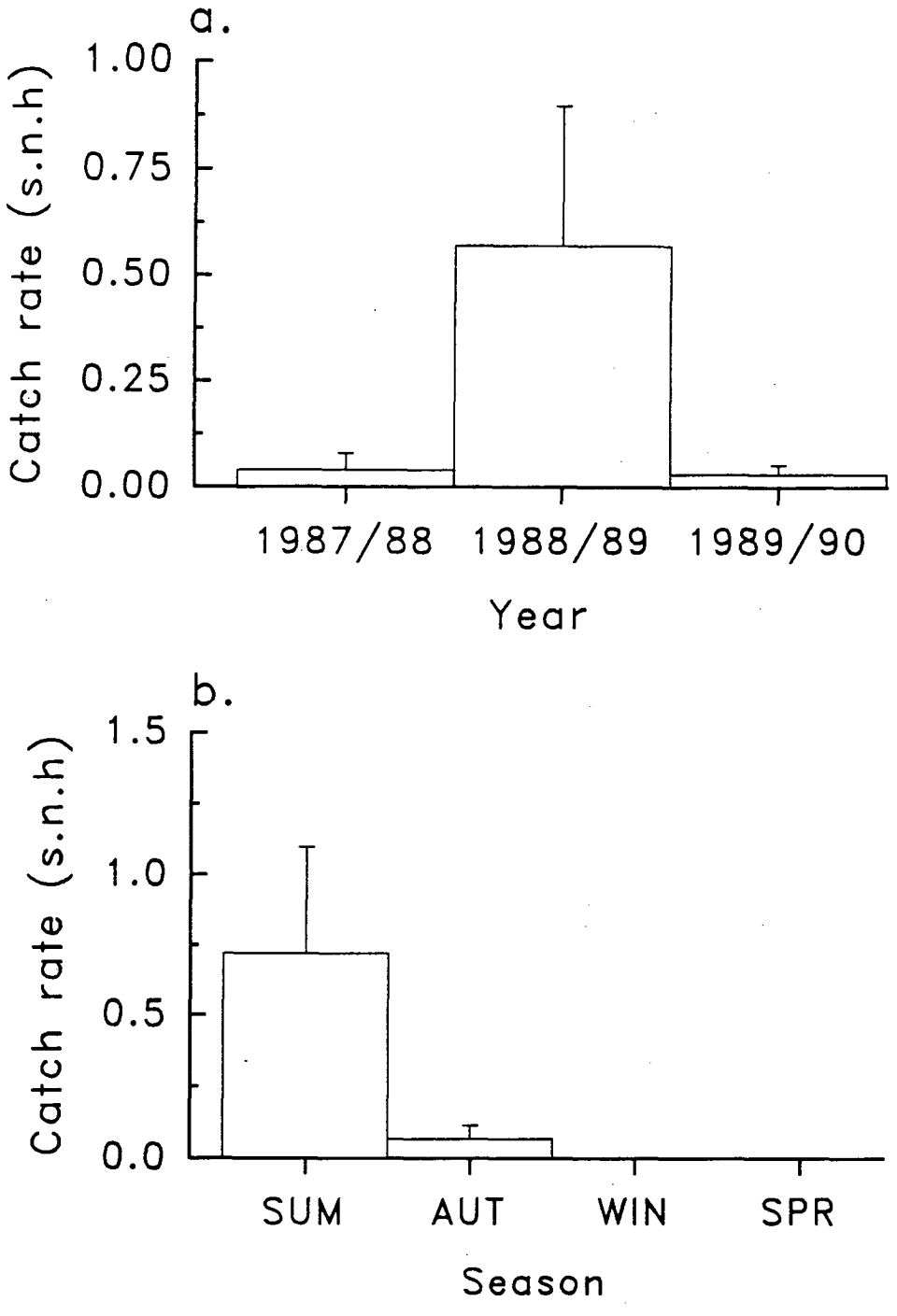


Figure 8.2: Mean (a) annual, and (b) seasonal, catch rates of *Carcharhinus tilstoni*, in night set 10cm gillnets at the Strand site. Error bars, plus one standard error; s.n.h., sharks $net^{-1}hr^{-1}$.

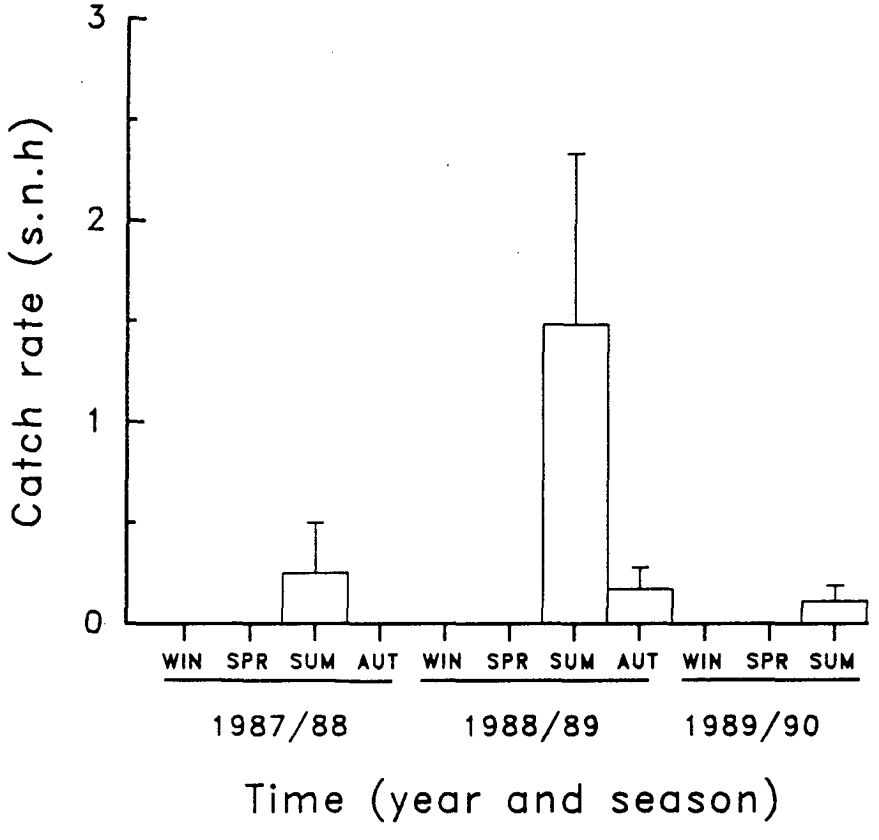


Figure 8.3: Mean seasonal catch rates of *Carcharhinus tilstoni*, caught in night set 10cm gillnets at the Strand site, for each year of the study between winter 1987 and summer 1990. Error bars, plus one standard error; s.n.h., sharks $net^{-1}hr^{-1}$.

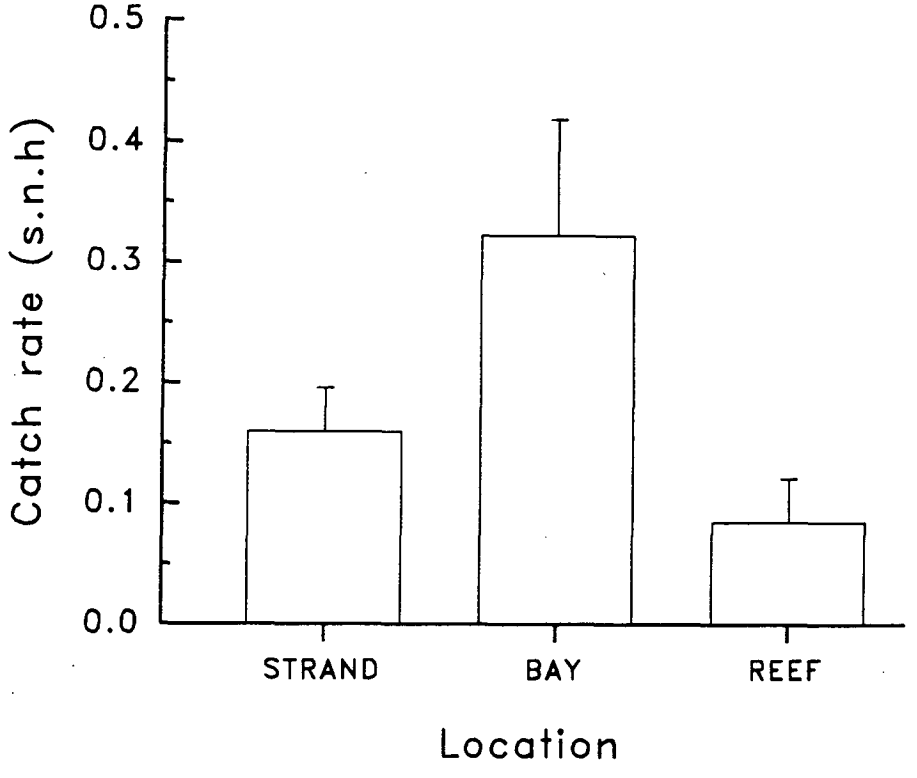


Figure 8.4: Mean catch rates of *Rhizoprionodon acutus* at three sites in Cleveland Bay. Error bars, plus one standard error; s.n.h., sharks $net^{-1}hr^{-1}$.

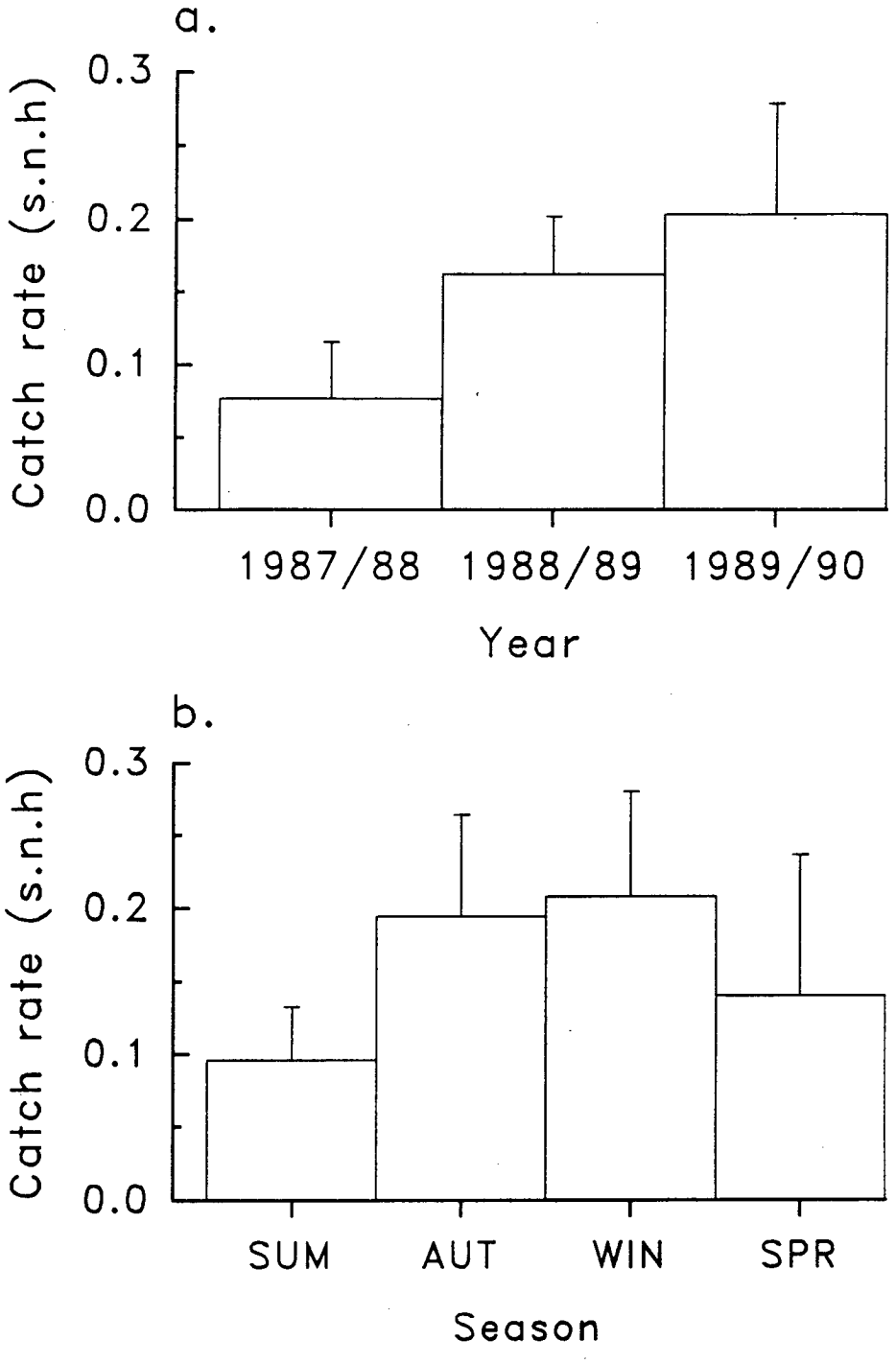


Figure 8.5: Mean (a) annual, and (b) seasonal, catch rates of *Rhizoprionodon acutus*, in night set 10cm gillnets at the Strand site. Error bars, plus one standard error; s.n.h., sharks $net^{-1}hr^{-1}$.

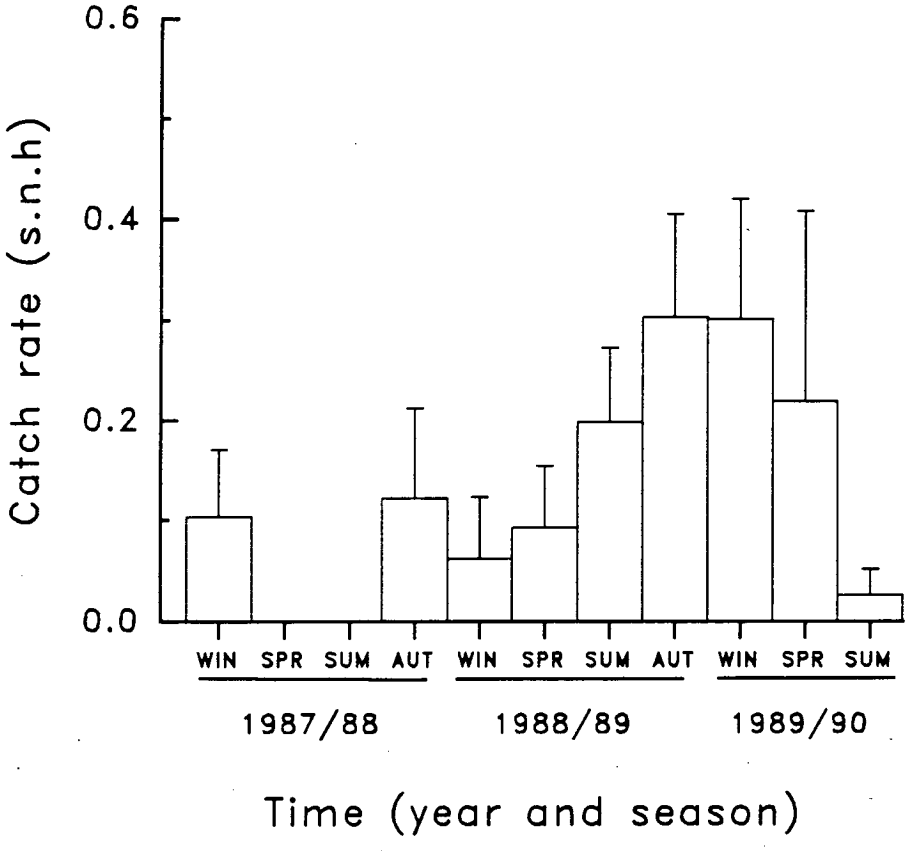


Figure 8.6: Mean seasonal catch rates of *Rhizoprionodon acutus*, caught in night set 10cm gillnets at the Strand site, for each year of the study between winter 1987 and summer 1990. Error bars, plus one standard error; s.n.h., sharks $net^{-1}hr^{-1}$.

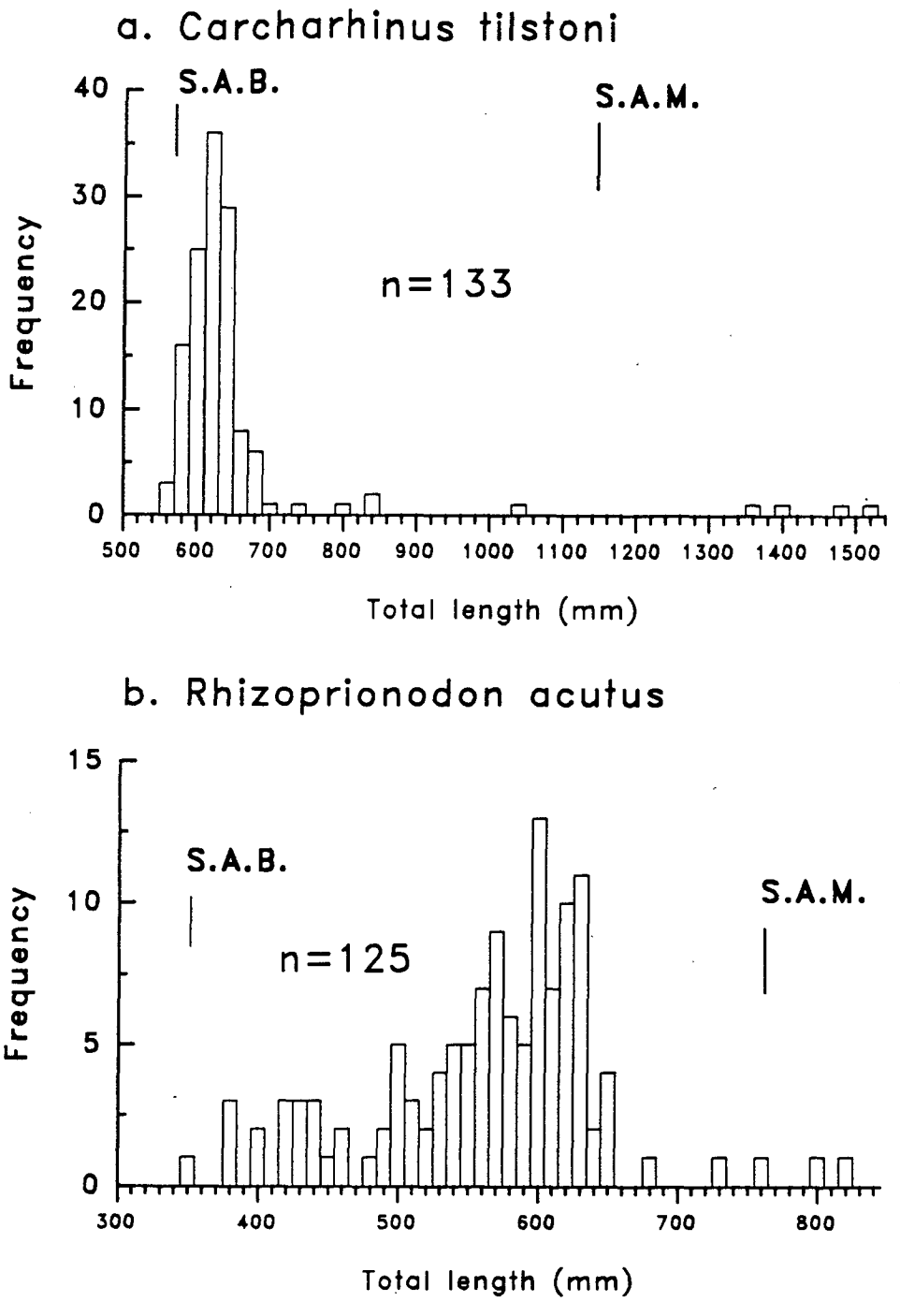


Figure 8.7: Length frequency distributions of (a) *Carcharhinus tilstoni* and (b) *Rhizoprionodon acutus* caught in Cleveland Bay using 5 and 10cm gillnets. S.A.B., size at birth; S.A.M., size at maturity. Estimates of the sizes at birth and maturity based on data from Stevens and Wiley (1986), Stevens and McLoughlin (1991), and the present study.

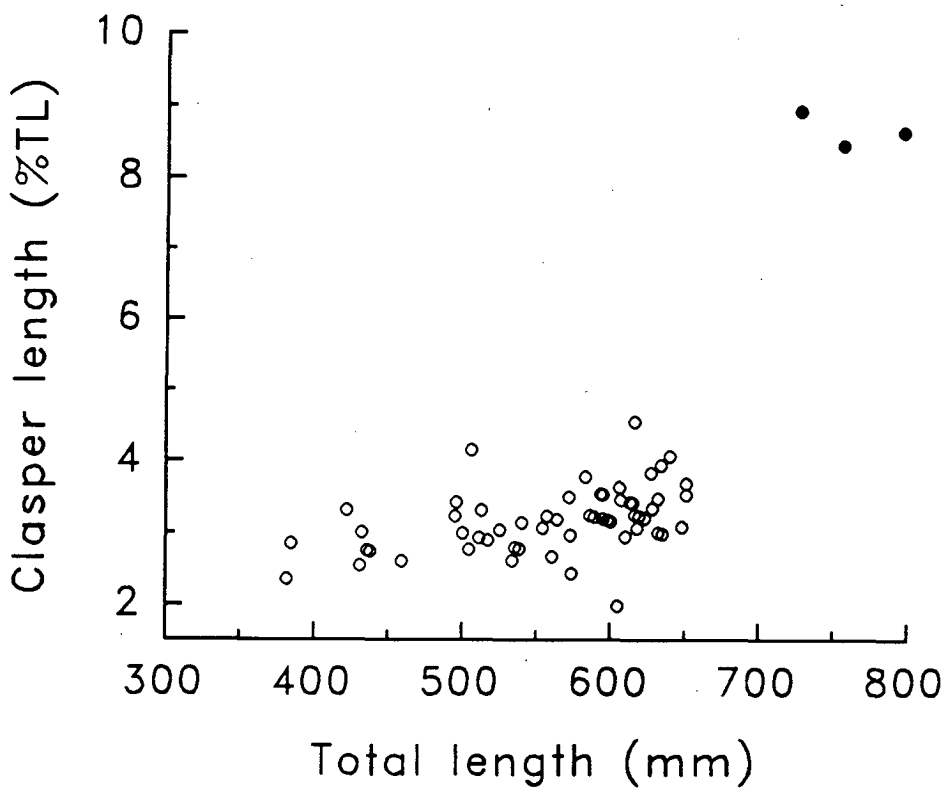


Figure 8.8: Clasper length as a proportion of total length to demonstrate the size at maturity of male *Rhizoprionodon acutus* from Cleveland Bay. Open circles, uncalcified claspers; solid circles, calcified claspers.

8.4 Discussion

Investigations in northern Australia have identified the presence of at least 29 species of shark from the family Carcharhinidae (see Table 1.1). The present study identified 13 species (including *R. taylori*) from Cleveland Bay. Three other species probably also occur in the Bay - *Carcharhinus amblyrhynchoides*, *Loxodon macrorhinus* and *Negaprion acutidens* - from reports by Simpfendorfer (1986) based on records from the Queensland Museum. *N. acutidens* has also been recorded in small numbers from Cleveland Bay by the Queensland Shark Meshing Program (QSMP), a scheme that uses large mesh gillnets and set lines to catch sharks as a protective measure (Simpfendorfer 1992; QSMP logbook data). Data from the QSMP also indicate that *Galeocerdo cuvier* occurs regularly in Cleveland Bay (Simpfendorfer 1992). The size selectivity of the gillnets used in the present study, however, excluded the capture of *G. cuvier* because of its large size. Examination of two of the three *L. macrorhinus* specimens recorded from the Cleveland Bay area and held by the Queensland Museum revealed that one was *R. taylori* and the other *R. acutus*. The third specimen could not be located in the collection and so its status is unknown. No direct evidence could therefore be found to confirm the occurrence of *L. macrorhinus* in Cleveland Bay. However, the Queensland Museum does hold specimens of *L. macrorhinus* from other areas of the Queensland coast and so its occurrence in Cleveland Bay is possible, but is considered at best rare.

The sixteen species of carcharhinid sharks that have been reported from Cleveland Bay are all known to occur in northern Australia and probably represent a nearshore subset of the shark fauna. During a study in thewaters of northern Australia Church (1981) noted that there were differences in the species between nearshore and offshore areas. Most of the species known from northern Australia, that have not

been recorded from Cleveland Bay, occur in habitats that are not present in the Bay. In particular open water species (e.g. *Carcharhinus falciformis*, *C. obscurus*, *C. plumbeus*, and *Prionace glauca*), deeper water species (e.g. *C. altimus*), and species normally associated with coral reefs (e.g. *C. albimarginatus*, *C. amblyrhynchos* and *Triaenodon obesus*) were not present (see Table 1.1). The lack of reef associated species, despite sampling adjacent to a coral reef (Middle Reef) was probably because of the nearshore location and small size of the reef. Species that occurred in Cleveland Bay only at large sizes would also have been under-represented in samples because of the size selectivity of the gillnets used.

8.4.1 Utilisation of Cleveland Bay as a nursery area

Simpfendorfer (1986) identified four species from the family Carcharhinidae that utilise Cleveland Bay as a nursery area (*C. dussumieri*, *C. fitzroyensis*, *C. sorrah* and *Rhizoprionodon acutus*). Data from the present study support these findings and suggest that another two species (*C. limbatus* and *C. tilstoni*) also utilise the Bay as a nursery area.

The utilisation of a single area as a nursery by several species of carcharhinid sharks may be advantageous by reducing predation on juveniles. As Springer (1967) noted, adults tend to remain separate from their own juveniles. However, van der Elst (1979) demonstrated that this does not stop other species of sharks from causing significant amounts of predation in some nursery areas. Thus if several species of sharks use the same area as a nursery then adults of all of those species will not normally occur in it and the potential level of inter-specific, as well as intra-specific, predation will be reduced. Communal nursery areas should therefore provide a low predation environment for juvenile sharks.

The size selectivity of the sampling techniques used in the present study made it impossible to accurately determine the extent to which larger adult sharks enter Cleveland Bay, and so subject the juveniles to predation. Data from the QSMP logbooks, however, does provide some information. Adults of several species do enter the nearshore areas of the Bay, most are pregnant females, and many are carrying full-term embryos. The occurrence of these adults probably does not represent a major threat to juveniles since it is thought that adult females rarely feed around the time of parturition (Springer 1967). Other large sharks do occur in the Bay, but as the numbers caught are low and sharks occur in less than 1% of the stomachs of them (Simpfendorfer 1992; QSMP logbook data), they may not represent a major predatory threat. On the basis of this information the communal nursery area in Cleveland Bay can probably be considered a protected area as described by Branstetter (1990), since there is no evidence to suggest high levels of predation on juvenile sharks.

A possible disadvantage of several species utilising the same area as a nursery is that there may be a greater chance of competition for food resources compared to areas utilised by only one species. However, this does not appear to be the case in Cleveland Bay. Thus, although little dietary information was gathered the stomach contents of the shark species co-occurring in Cleveland Bay do suggest consumption of mainly small to medium sized demersal teleosts, which are very abundant and are unlikely to be resource limiting. This may be attributed to the highly productive mangrove and seagrass habitats in the Bay that support large populations of teleosts and prawns (Robertson and Duke 1987, 1990; Milward personal data). Also, over the period when juvenile sharks are most common in the Bay (i.e. summer) the abundance of their main foods are also at their highest (Robertson and Duke 1987; Staples 1979). This is the case for all seasonally occurring species except *C.*

dussumieri that use Cleveland Bay as a nursery during autumn and winter. This is at a time when food resources are least abundant, but owing to the temporal separation from most other juvenile sharks this may not be critical. A more detailed study of feeding habits of sharks and teleosts would be required to determine if food resources are partitioned by species within Cleveland Bay.

Two patterns of nursery area utilisation were observed in the carcharhinid species that occur in Cleveland Bay. The first pattern of utilisation is as a seasonal primary nursery, where juveniles are present for only a few months each year at a size close to that at which they are born. The second pattern of utilisation is as a year-round combined primary and secondary nursery, where juveniles of a species occur throughout the year at sizes from birth to close to maturity.

Four species displayed the seasonal primary nursery pattern: *C. dussumieri*, *C. fitzroyensis*, *C. limbatus* and *C. tilstoni*. One of these species (*C. dussumieri*) occurred during autumn and winter, while the other species occurred during late spring and summer. Analysis of catch rate data for *C. tilstoni* indicates that at least at the Strand site this pattern of utilisation did not differ between years. There were insufficient data to determine if there was any inter-annual variation in this pattern for the other three species. To be considered a true primary nursery as defined by Bass (1978) adult females of these species should migrate into Cleveland Bay to give birth at a time when the nursery is in use. The capture of two adult female *C. tilstoni* carrying full-term embryos in Cleveland Bay during November indicates that such immigrations do take place. However, the size selectivity of the gear used in the present study meant that because of their large size few adult sharks were caught. Further evidence of these migrations is provided by the data from the

that indicate blacktip sharks (mostly *C. limbatus* and *C. tilstoni*) are common only during spring and summer, and that most of these sharks are adult females carrying full-term embryos (QSMP logbook data).

The year-round combined primary and secondary nursery pattern was displayed by at least *R. acutus*. Juvenile *R. acutus* were present throughout the study period and ranged in size from birth to maturity. This suggests that juveniles emigrated from Cleveland Bay as they approach maturity. The year round occurrence of juveniles was most likely the result of an extended, or year round, reproductive season (see section 8.3.3). This pattern of nursery area utilisation may also have occurred in *C. sorrah*, but insufficient data were available to confirm this. Species that have combined primary and secondary nursery areas are those in which the larger juveniles do not represent a predatory threat to the smaller ones. As a result combined primary and secondary areas probably only occur in smaller sharks, such as *Rhizoprionodon* spp.

It was not possible to determine the normal distribution of the adult components of the populations for the species that utilised Cleveland Bay as a nursery area based on information gathered in the present study. Previous workers have suggested that the adults normally occur further offshore in deeper water (e.g. Springer 1967; Bass 1978; van der Elst 1979; Gruber *et al.* 1988). Data presented by Simpfendorfer (1986) suggest that this is probably the case for *C. dussumieri*, *C. fitzroyensis*, *C. sorrah* and *C. tilstoni* from Cleveland Bay. The location of the secondary nursery areas for species that only utilise Cleveland Bay as a primary nursery are also unknown, but again are most likely to be further offshore. More extensive sampling will be required to determine the distribution of all sections of the populations that utilise Cleveland Bay as a nursery.

The results presented confirm the observations of other workers that many species of sharks utilise inshore habitats as nursery areas. The data also suggest that in these nursery areas at least one (*C. tilstoni*), but not all, of the species studied form aggregations. Holland *et al.* (1992) have reported aggregating behaviour in the sphyrnid species *Sphyrna lewini* in a nursery area off Hawaii. Little else, however, is known of the spatial distribution of juvenile carcharhinid sharks in nursery areas, and further research is required in this field.

8.4.2 Other aspects of carcharhinid biology

The present study was able to provide only limited information on the reproductive biology of carcharhinid species other than *R. taylori* due to the small sample sizes, and restricted temporal distributions within Cleveland Bay of adults. Interpretations drawn from the data should therefore be regarded with caution, but do provide testable hypotheses for future study.

The majority of species examined (*C. fitzroyensis*, *C. limbatus*, *C. sorrah* and *C. tilstoni*) probably have seasonal reproductive patterns in the Townsville region. Such patterns concur with the information available for these species from other areas in northern Australia (Stevens and Wiley 1986; Lyle 1987; Stevens and McLoughlin 1991). The exact timing of parturition and mating could not be determined for any of the species. However, the data suggest that in most species parturition occurs between November and January, and mating between December and February. The exception to this was *C. dussumieri* which probably gives birth in autumn and mates in winter or spring. Only one species, *R. acutus*, has either an extended reproductive season, or reproduces aseasonally.

The summer mating season of *C. fitzroyensis* in Cleveland Bay is contrary to that reported by Lyle (1987a) from the Northern Territory. Lyle found that this species mates from May to July, approximately six months from the time of mating by the species in Cleveland Bay. The occurrence of a late Spring or Summer pupping season in Cleveland Bay was also suggested by Simpfendorfer (1986) on the basis of the occurrence of juveniles close to the size at birth during February. Why such a large difference in mating season occurs between areas in this species is unclear, but may represent latitudinal variation in habits perhaps related to climatic or other environmental factors. Confirmation of the summer breeding season in Cleveland Bay is required especially given the lack of specimens from March to October in the present study.

In northern Australia *R. acutus* reproduces aseasonally (Stevens and McLoughlin 1991), but in other parts of its distribution it has been recorded as a seasonal breeder (Bass *et al.* 1975; Cadenat and Blache 1981; Compagno 1984). The occurrence of adult males in mating condition in Cleveland Bay over an extended period suggests that in this area it conforms to the pattern observed in northern Australia. The size at maturity of male *R. acutus* estimated for specimens from Cleveland Bay agreed closely with that for specimens from northern Australia (Stevens and McLoughlin 1991). Further study will be required to determine if *R. acutus* reproduces seasonally or aseasonally in this region of the Queensland coast.

The estimates of the size at birth of the five carcharhinid species from this study are similar to those previously reported by other workers (Table 8.6). The one exception to this is *C. limbatus* which was born in Cleveland Bay at sizes larger than previously reported. It should be noted that the umbilical scar persists for a

number of weeks after birth which means that the use of such specimens may produce over-estimates of sizes at birth. The magnitude of this error will depend upon the growth rate of the juveniles and the length of time that the scar persists.

Table 8.6: Estimates of the size at birth for five species of sharks from the family Carcharhinidae caught in Cleveland Bay. Estimates are based on the size of individuals with umbilical scars, and full-term embryos.

	Size at birth (mm)		
Species	Cleveland Bay	Previous estimates	Sources of previous estimates
C. fitzroyensis	477-528	510-520	Simpfendorfer 1986
C. limbatus	683-776	620	Bass *et al.* 1973
		500-600	Branstetter 1981
C. sorrah	524-579	500-600	Bass *et al.* 1973
		500	Stevens and Wiley 1986
C. tilstoni	556-692	600	Stevens and Wiley 1986
R. acutus	354-504	300-350	Bass *et al.* 1975
		250-390	Randall 1986
		380	Stevens and McLoughlin 1991

The diets reported for several of the species caught in this study differ substantially from those recorded by Simpfendorfer (1986) from Cleveland Bay. For example, Simpfendorfer (1986) reported that the stomach contents of *C. dussumieri* and *C. fitzroyensis* both contained substantial amounts of crustaceans (especially prawns). In reporting the diet of *C. dussumieri* from northern Australia Stevens and McLoughlin (1991) observed that 25% of the diet was crustaceans and 20% cephalopods, and Lyle (1987) reported that nearly 20% of the diet of *C. fitzroyensis* was crustaceans. The small sample sizes of these species from Cleveland Bay in the present study may account for at least some of the variation recorded in the

diets. The high proportions of teleosts in the diets of *C. tilstoni* and *R. acutus* in Cleveland Bay are similar to those given by Stevens and Wiley (1986) and Stevens and McLoughlin (1991) for these species, respectively.

Section IV.

General Discussion

Chapter 9.

General Discussion

The aim of this study, identified in the General Introduction, was to gather biological information that would increase the understanding of populations of sharks from the family Carcharhinidae in nearshore tropical waters. This was achieved by investigating the life history of the most common species in Cleveland Bay - *R. taylori* - and by examining the utilisation of nearshore waters by all carcharhinid species caught in the Bay during the study. These two aspects are discussed below, drawing together the information presented throughout this thesis. The applicability of the main findings of this study to management of shark stocks and nearshore waters are then discussed.

9.1 Life history of *Rhizoprionodon taylori*

The relative abundance of *Rhizoprionodon taylori* in Cleveland Bay waters enabled a detailed study of the biology of this species, particularly in relation to its reproduction, age and growth. The findings from the study enable a full description of the life history of *R. taylori*, a comparison of this with those of other members of the Carcharhinidae, and a consideration of the factors that may influence the life history patterns of species within this family of sharks.

The unique feature of the life history of *R. taylori* is the occurrence of an obligatory period of embryonic diapause. This involves the development of embryos being arrested, or greatly slowed, at the blastoderm stage. The embryos remain in this

state for approximately seven months after which development recommences and continues until birth after a further four and a half months. This is the only confirmed report of embryonic diapause in the Elasmobranchii, although it possibly occurs in two species of the Batoidea (Lessa *et al.* 1986; Snelson *et al.* 1989). The reasons for the evolution of embryonic diapause in *R. taylori* are unclear, although it may be hypothesized that it does provide several advantages. Thus, as the diapause period extends over the autumn and winter months the embryos resume development when temperatures are rising. This permits more rapid growth of the embryos and, in turn, effectively a shorter time for the mothers to carry the growing young. It also synchronises the maximal growth of the embryos with the time of increasing food availability for the mother, thus optimising the support she can afford them. Similarly, the sequence of development of the young ensures that they are born when their food sources are most abundant and water temperatures highest, so that post-natal growth may also be maximised. Collectively these factors can be expected to lead to increased numbers of viable offspring and elevated survival following birth.

The other reproductive life history traits of *R. taylori* in the study region are as follows. The reproductive cycle is annual. Mating occurs in late January and early February, and parturition occurs in mid-January of the following year. The results indicate that all mature females mate each year. The gestation period is 11.5 months, being divisible into the 7 month diapause period and the 4.5 month development period. The young are born at 220 to 260mm TL and mature when they are 550 to 570mm TL. Litter sizes vary from 2 to 10, with larger females producing larger litters. During development embryos probably derive their nutrition from three sources: yolk, uterine secretions, and placentae. The sex ratios of embryos and juveniles are even, but in adults favoured females.

Rhizoprionodon taylori is characterised by rapid growth and a short life-span. The oldest male examined was approximately six years old, and the oldest female seven years. Both males and females mature at the end of their first year. The von Bertalanffy growth parameters determined for males were: t_0=0.41yr, K=1.337, L_∞= 652mm TL, and for females: t_0=0.46yr, K=1.013, L_∞= 733mm TL. Growth is initially very rapid, but slows after maturity. Little growth is observed after three or four years of age.

The findings of this study have indicated how many of the individual life history traits of *R. taylori* differ from those normally observed within the family Carcharhinidae. When the life history strategy of this species is considered as a whole it is evident that it diverges significantly from that of most carcharhinid species. In particular the following features were considered atypical for a carcharhinid species: size at birth (small), age at maturity (low), litter size (large), embryo development time (short), von Bertalanffy's K (high), initial growth rate (high), and embryonic diapause (unique).

The divergence of *R. taylori*'s life history strategy from that typical of carcharhinid species can probably be attributed to several factors. These include the species' size and the occurrence of the diapause period, together with consideration of geographic and environmental factors.

Correlations between a species' maximum size and the values of a number of reproductive traits discussed in Chapter 6 illustrate size effects on components of life history strategies. Pratt and Casey (1990) have also demonstrated a size effect for values of von Bertalanffy's K. Size effects occur because of physical constraints (e.g. limited space for embryos), physiological constraints (e.g. changes in surface area to volume ratio, size related metabolic factors), and ecological constraints (e.g.

small species subject to higher predation than large species). A consequence of size effects is that large species typically have different life histories compared with small species. Large carcharhinid species (e.g *Carcharhinus falciformis*, *C. leucas*, *C. obscurus*, *C. plumbeus* and *Negaprion brevirostris*) have life histories that are characterised by a long life span, slow growth (small von Bertalanffy K values), maturity after many years, and production of large offspring (Springer 1960; Casey *et al.* 1985; Branstetter 1987a; Branstetter and Stiles 1987; Brown and Gruber 1988; Hoenig and Gruber 1990; Pratt and Casey 1990). Small to medium sized species (e.g *Carcharhinus sorrah*, *C. tilstoni*, *Rhizoprionodon taylori* and *R. terraenovae*) on the other hand have shorter life spans, grow more rapidly (higher von Bertalanffy K values), mature after a couple of years, and produce smaller offspring (Parsons 1985; Branstetter 1987b; Davenport and Stevens 1988).

While it is not possible to causally relate the period of embryonic diapause to other life history traits in *R. taylori*, it is valuable to draw comparisons with the species *R. oligolinx* which is very closely related (Springer 1964; Compagno 1988), grows to a similar size, and occurs in similar habitats (Compagno 1984). Table 9.1 compares the life history traits of these two species based on the data presently available. Two data sets are presented for *R. taylori* - one from the present study and another from Stevens and McLoughlin (1991) - because of growth differences identified in Chapters 4 and 6. The size at birth of *R. taylori* reported by Stevens and McLoughlin (1991) is probably an over-estimate (see Chapter 6). The main differences in life history traits appear to be the litter size and, possibly, the size at birth. This suggests that the diapause period especially affects the trade-off between the litter size and offspring size. The data for *R. oligolinx* are limited and further studies, particularly on age and growth, would be valuable. It also needs to be confirmed that these two species are closely related, which is probably best achieved

using molecular techniques. However, from a consideration of the differences in life histories, Compagno's (1988) suggestion that *R. oligolinx* may be a subspecies of *R. taylori* is unlikely to be valid.

Table 9.1: Comparison of life history data for *Rhizoprionodon oligolinx* (Setna and Sarangdhar 1949a) and *Rhizoprionodon taylori* (from this study and Stevens and McLoughlin 1991).

Life history trait	*R. oligolinx*	*R. taylori*	
		This study	Stevens & McLoughlin 1991
maximum size (Lmax, cm)	70	80	66
size at birth (SAB, cm)	27-30	22-26	25-30
SAB/Lmax	0.39	0.28	0.38
size at maturity (SAM, cm)	56	55	40
SAM/Lmax	0.80	0.69	0.61
litter size	3-5	2-10	1-8

Geographic location probably also affect life histories in the Carcharhinidae. Comparisons within the family indicate that species which occur commonly in temperate and sub-tropical regions (e.g. *Carcharhinus brachyurus*, *C. obscurus* and *C. plumbeus*) are mostly large, slow growing, and long lived (e.g. Casey *et al.* 1985; Hoenig and Gruber 1990; Walter and Ebert 1991). Some large carcharhinid species are common in tropical waters, but these typically grow more rapidly than those more common at higher latitudes. For example, Branstetter *et al.* (1987) reported that *Galeocerdo cuvier*, a common species in tropical waters which grows to at least 5.5m, had a von Bertalanffy K value above 0.1, while Walter and Ebert (1991) found that *C. brachyurus* had a K value of 0.04 and Casey *et al.* (1985)

reported that *C. plumbeus* had a value of 0.04-0.05. Although large species may occur in tropical waters, it is the small and medium sized carcharhinids that grow more rapidly, and have shorter life spans, which dominate. The size differences observed between *R. taylori* from northern Australia and Cleveland Bay indicate that similar effects may also occur between populations of the same species in different geographic regions. These effects (both intra- and inter-specific) in being primarily latitudinal are most probably caused by differences in temperature influencing life history features, such as growth rate and longevity.

The environment in which a species lives has obvious effects on life history. Factors such as predation, food availability, and competition, can provide selective pressures that influence the evolution of life history (Stearns 1992). The abundance of *R. taylori* in the nearshore waters of Cleveland Bay indicates that its life history is suited to such an environment. Its small size at birth, and low maximum size, are particularly suitable in places such as nursery areas, where predation is normally low. In areas where predation is greater these characteristics would possibly result in levels of mortality that are too high to sustain a population. A consequence of the smaller size at birth is that more young can be produced, and the development time can be reduced. *R. taylori* is not the only small (< 1m) inshore species that has evolved these types of characteristics. *Scoliodon laticaudus*, a common carcharhinid species in South East Asia, and *Hemigaleus microstoma*, a hemigaleid species with an Indo-West Pacific distribution, both have many similar life history traits. However, not all small inshore species share these features. At least five carcharhinid species - *Carcharhinus dussumieri*, *C. macloti*, *C. sealei*, *Loxodon macrorhinus* and *Rhizoprionodon lalandei* - are small, but have larger sizes at birth and longer development periods. Why this dichotomy in life histories of small

inshore sharks exists is not clear, but may be the result of different predation rates on the young. However, both represent successful strategies for life in inshore waters.

9.2 Nearshore waters and sharks of the family Carcharhinidae

The results of this study indicate that the nearshore waters of Cleveland Bay are of considerable importance to several species of sharks from the family Carcharhinidae. The thirteen species studied utilise the waters of the Bay in a number of different ways, but especially as nursery areas. For some species the nearshore waters are occupied only briefly, the young using them as a primary nursery area for only a few months each year; juveniles of other species remain in them for longer periods, often until near maturity, before emigrating to other, probably deeper, waters; and in a single species the young persist in them until they mature, reproduce, and eventually die.

The findings indicate that juvenile sharks use Cleveland Bay as a nursery area because it offers a refuge from predation by larger sharks, while also providing abundant food in the form of demersal teleosts and crustaceans. To what extent other parts of the coast of northern Australia, similar to Cleveland Bay, act as nursery areas is not well known. However, it is fair to suggest that they may also act as nurseries. Given the development of commercial shark fisheries in northern Australia, and the susceptibility of nearshore areas to heavy fishing, more research is required on the occurrence of nursery areas and their importance to shark populations.

Not all of the carcharhinid species caught in Cleveland Bay use it only as a nursery area. In fact, the most abundant species (*R. taylori*) was caught mostly as adults,

and may occur throughout its life in nearshore waters. (Stevens and McLoughlin (1991) have reported that it also occurs in other inshore waters up to 111m deep). Cleveland Bay probably provides an ideal habitat for *R. taylori* because of the small size of this species, which allows it to obtain the same benefits as the juveniles of other species (reduced predation and abundant food) without itself being a predatory threat to its own, or other, species.

The high abundance of small sharks in Cleveland Bay makes them an integral part of the communities within the Bay. Predation by the small carcharhinid sharks may play an important role in the dynamics of the populations of demersal teleosts (especially leiognathids and clupeids) and crustaceans (mainly penaeid prawns), and in turn, in maintaining the community structure in Cleveland Bay. Further quantitative work on the feeding habits and densities of the species (both predator and prey) that occur within the Bay is warranted, in order to determine to what extent predation by small sharks is important.

The above paragraphs raise two separate, but inter-related, conservation issues in relation to the nearshore waters and carcharhinid sharks. The first concerns the maintenance of shark nurseries in nearshore waters, which requires: (a) the conservation of the habitats, and (b) the conservation of the prey communities that provide food for the juvenile sharks. Without appropriate habitat and prey communities the advantages of nursery areas would be diminished and the survival of populations reduced. The second conservation issue is the requirement for effective management of shark stocks so as to maintain the community structure within the nearshore waters. The failure to manage shark populations so that they decline to a point where the community structure of nearshore waters changed significantly could exacerbate the situation, as well as changes to stocks of other

important species (Parsons 1992).

Much has been written about the need for the protection of habitats utilised as nurseries by teleosts and crustaceans (e.g. Newel and Barber 1975; Young 1978; Pollard 1981; Boesch and Turner 1984; Coles and Lee Long 1985; Staples *et al.* 1985). Such habitats are vital to the continued survival of species, and for the maintenance of catches from commercially exploited stocks. Nursery areas for non-commercial species also need to be maintained, as these species are usually integral parts of the food chain for commercial species (Robertson and Duke 1987). The same arguments can be applied to the nursery areas of sharks. As it happens many of the habitats identified as important to shark nurseries (e.g. mangroves and seagrasses) are also important as nurseries for teleosts and crustaceans. The large size and mobility of sharks means that they are more loosely associated with these areas than are juvenile fish and crustaceans. However, since sharks are higher up the trophic pyramid than juvenile fish and crustaceans they are probably more sensitive to changes in the community structure. The conservation of both the habitats and the communities associated with them will therefore be critical for the optimum management of shark nursery areas.

9.3. Application of the results to the management of shark stocks

Over-fishing (and its consequences) is a problem symptomatic of the commercial exploitation of shark populations (e.g. Anderson 1990; Compagno 1990b; Pratt and Casey 1990; Taniuchi 1990; Simpfendorfer 1991). Much of the susceptibility to over-fishing has been attributed to the reproductive mode of sharks (see Chapter 5). However, the lack of adequate management and the application of control practices more appropriate to teleost fishes have also contributed to declines in

stocks (Hoenig and Gruber 1990; Anderson 1990). It is now recognised that the successful long-term exploitation of shark stocks requires the early implementation of management strategies based on sound biological information (Anderson 1990).

The present study contributes some of the first information applicable to the development of management strategies for the shark fishery off the east coast of Queensland. Information on the life history of *R. taylori* provides a good understanding of the population dynamics of this species. Rapid growth, early maturity, and large litter sizes, may make *R. taylori* better suited to withstand fishing pressure than other species of the family Carcharhinidae. Despite this recruitment remains proportional to stock size, so strict management will still be required to produce long-term sustainable yields.

Identification of nursery areas for populations of commercially exploited carcharhinid species is also important for management of shark stocks. The necessity for the preservation of habitats used as nurseries has been discussed above (9.2). The importance of low levels of juvenile mortality also requires that fishing for sharks in nursery areas needs to be controlled. It is a widespread practice in fisheries management that individuals should be allowed to reproduce before being subject to capture in the fishery (Royce 1972), particularly if recruitment and stock size are closely related. One factor that makes such a management practice difficult to implement in a shark fishery is that many species of carcharhinid sharks are born at a size suitable for capture and processing while they remain several years from maturity.

The scope for further research on Queensland's sharks is enormous when it is considered that the species of main focus of this project - *R. taylori* - is only one of sixteen species of the family Carcharhinidae that occur in Cleveland Bay, and

that there are other species of this and other families which occur in other parts of the State. The utilisation of habitats other than those in the nearshore waters also needs to be examined to understand the life cycle of many species. This study has produced information relevant to the management of shark stocks off the east coast of Queensland, which, it is hoped, will provide a firm foundation for this further research.

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Appendix A.1: Details of individual gillnet sets.

Date	Loc[1]	Net[2] type	Start time	Set time (hours)	By-catch	Species[3] Cb	Cd	Cf	Cl	Cm	Cs	Ct	Ra	Rt
1/5/87	SB	5	0930	3.00	2								4	
		5	0935	3.00	2								1	
19/5/87	ST	5	1810	7.00	84									7
		5	1805	7.50	46									
		10M	1800	7.30	1									2
15/6/87	ST	10B	1530	7.00	2									
		5	1535	7.00	248									1
		10T	1540	7.00	0									6
		5	1545	7.50	96						1			3
22/6/87	SB	10B	1415	6.00	6						1		1	4
		5	1420	5.66	9									1
		10T	1425	5.30	8								1	2
		5	1430	5.00	20									3
25/6/87	SB	10B	1535	5.40	0								3	7
		5	1540	5.30	12									2
		10T	1545	4.75	3		1						4	16
		5	1550	4.15	20								2	3
2/7/87	ST	10B	1630	2.85	1								1	
		5	1635	2.67	1									
		10T	1640	2.50	0									
14/7/87	ST	10B	1545	5.33	10									12
		10T	1540	5.00	2									6
28/7/87	SB	10B	1515	5.00	4						1		8	3
		5	1520	5.40	33								2	4
		10T	1525	5.75	3								11	1
4/8/87	ST	10B	1715	4.25	0								2	49
		5	1720	3.66	6								1	7
		10T	1725	3.00	1									28
7/9/87	ST	10B	1550	4.66	4									36
		10T	1555	3.66	0					1				31
29/9/87	ST	10B	1615	1.25	0									
		10T	1810	1.33	1									3
		10B	1800	1.75	2									6
13/10/87	RR	10T	1645	2.00	0									
5/11/87	ST	10B	1105	2.40	1									
		10B	1110	2.50	0									
19/11/87	ST	10B	1635	2.00	0									
		10T	1640	1.66	0						1			
26/1/88	MR	10T	1800	2.66	0							7		
		10B	1805	3.00	0							29		1
		10B	1810	3.25	0							20		1
		5	1815	3.00	0							2	1	
2/2/88	ST	10T	1845	2.25	0									1
		10B	1850	2.00	2							2		4
16/2/88	ST	10B	1835	0.75	1									
		10B	1840	0.80	0									3
21/2/88	MR	10T	1840	2.00	1							1		
		10B	1845	2.08	0							5		
		10B	1850	2.15	1							6		

Date	Loc[1]	Net[2] type	Start time	Set time (hours)	By-catch	Species[3] Cb	Cd	Cf	Cl	Cm	Cs	Ct	Ra	Rt
29/3/88	ST	10T	1850	2.00	2									5
		10B	1840	2.17	1									1
		10B	1835	2.50	2								2	4
5/4/88	ST	10T	1840	1.58	1									5
		10B	1835	1.85	7									3
		10B	1830	2.17	7									1
12/4/88	SB	10T	1550	3.92	8								1	4
		10B	1545	3.75	12								2	2
		10B	1540	4.08	9								4	3
3/5/88	BB	15	1910	3.67	0									
		10B	1925	3.50	0									
		10B	1930	3.25	1									
		10T	1935	3.00	0									
10/5/88	MR	15	1835	3.50										
		10B	1845	3.25	1									
		10B	1850	3.00	1								1	1
		10T	1855	2.83										
17/5/88	SB	10B	1730	4.50	11		1						1	
		10B	1735	4.08	13								2	
		10T	1740	4.66	2									
2/5/88	ST	10B	1755	3.40	0									3
		10B	1800	3.10	4								1	2
		10T	1805	2.90	1								1	9
19/7/88	ST	10B	1815	2.75	3									
		10B	1820	3.00	0		3							
26/7/88	ST	10T	1645	4.25	0									
		10B	1650	4.25	0									
		10B	1655	4.25	0									
24/8/88	ST	10B	2215	2.50	5									2
		10B	2225	2.33	1								1	3
13/9/88	ST	10T	1955	3.00	1		1							1
		10B	2000	3.42	1								1	11
		10B	2005	3.75	3									3
18/10/88	MR	10B	2200	4.17	1									
		10B	2205	4.00	0									
		10T	2215	3.59	1									
24/10/88	ST	10T	2040	2.50	0									
		10B	2035	2.75	1									3
		10B	2030	3.17	1									1
9/11/88	SB	15	1945	3.75	0				3		1			
		10B	1950	3.18	1			2						7
		10B	1955	3.39	1			1						9
		10T	2000	2.75	3						2			6
16/11/88	MR	15	2120	3.83	1									
		10B	2125	3.66	1									1
		10B	2130	3.50	1								1	2
		10T	2135	3.17	1								1	5
28/11/88	ST	10B	1945	2.25	1								1	
		10B	1950	2.00	0									

Date	Loc[1]	Net[2] type	Start time	Set time (hours)	By-catch	Species[3] Cb	Cd	Cf	Cl	Cm	Cs	Ct	Ra	Rt
7/12/88	SB	10T	2000	2.00	0									
		10B	1955	2.33	3									1
		10B	1950	2.50	0			1	1					
		15	1940	2.66	0			1						
12/12/88	ST	10T	1950	2.66	3							27		11
		10B	1945	3.08	0							7		
		10B	1940	3.33	1							9		
11/1/89	ST	10B	1940	2.83	2						1		1	4
		10B	1935	3.16	0				1		1	2		4
		10T	1930	3.50	2				1			3		9
19/1/89	ST	15	1940	1.80	1									
		10B	1935	2.16	2							1	1	13
		10B	1930	2.50	2							3	1	
		10T	1925	2.80	3						2		2	1
1/2/89	MR	10T	2015	2.16	1				1	1				1
		10B	2010	2.33	2									4
		10B	2005	2.57	1									1
		15	2000	2.84					1					
8/2/89	ST	10T	1905	2.25	1								1	1
		10B	1900	2.58	3									2
		10B	1855	2.83	2									1
		15	1850	3.00	1									
12/4/89	ST	10T	1930	1.83	2									1
		10B	1925	2.08	1								1	
		10B	1920	2.33	6							1	1	1
26/4/89	MR	10T	1945	3.33	0									
		10B	1940	3.42	2								2	2
		10B	1935	3.50	4	1						2	1	1
31/5/89	ST	10B	1900	3.08	5								1	
		10B	1855	3.33	6									
		10T	1850	3.41	3							2	2	
7/6/89	SB	5	1930	1.25	15									4
		10T	1905	2.58	3									1
		10B	1900	2.83	0									
		10B	1855	3.00	5						2			2
13/6/89	ST	10B	1905	2.00	0								4	
		10B	1900	2.25	0								1	
		10T	1855	2.42	0									
3/7/89	ST	10T	1915	2.86	0									
		10B	1910	3.00	0									
		10B	1905	3.14	1									
5/7/89	ST	10T	1845	2.14	6									
		10B	1840	2.42	2									
		10B	1835	2.66	1								1	
25/7/89	ST	10T	1910	3.00	1									
		10B	1905	3.25	1									
		10B	1900	3.46	6									
2/8/89	ST	10B	1910	1.25	1									
		10B	1905	1.54	1									
9/8/89	CB	10B	1845	3.07	2									
		10B	1842	3.33	2									
		10T	1840	3.46	1									

Date	Loc[1]	Net[2] type	Start time	Set time (hours)	By-catch	Species[3] Cb	Cd	Cf	Cl	Cm	Cs	Ct	Ra	Rt
23/8/89	ST	10T	1930	2.58	3									
		10B	1925	2.83	9								1	
		10B	1920	3.08	4									
28/8/89	ST	10T	1910	2.58	4								1	
		10B	1905	2.75	7								3	
		10B	1900	3.00	9								3	
13/9/89	ST	10T	1935	1.25	1									
		10B	1930	1.50	2									
		10B	1925	1.75	6						1		3	
18/9/89	MB	10T	0745	1.58	0									
		15	0740	1.84	0									
		10B	0735	2.08	1									
		10B	0730	2.33	0									
18/9/89	MR	10B	1040	2.75	0									
		10B	1045	3.00	0									
20/9/89	SB	10B	1915	2.75	1							2		
		10B	1910	2.93	5									
		10T	1905	3.08	5						2			
25/9/89	ST	10B	1910	1.50	2									
		10B	1905	1.75	5									
		10T	1900	2.00	0									
5/10/89	ST	10B	1935	2.92	2								1	
		10B	1930	3.16	1								1	1
		10T	1925	3.42	1								1	
1/11/89	ST	10T	2000	2.66	0									
		10B	1955	2.75	0				2		2			1
		10B	1950	2.86	1				2				1	5
4/12/89	ST	10T	2000	2.00	5							1	1	
		10B	1955	2.25	6							1		2
		10B	1950	2.50	0				1					
15/1/90	ST	5	2005	1.75	4									
		10T	2000	1.84	1						1			
		10B	1955	2.08	2							1		2
		10B	1950	2.33	1							2		3
5/2/90	ST	5	2030	2.58	4									1
		10T	2025	2.83	0									5
		10B	2020	3.08	2									2
		10B	2015	3.25	4			2					1	6
16/2/90	ST	10T	0045	3.00	0									1
		10B	0040	3.13	3									1
		10B	0035	3.42	3									

1. Locations: BB, Bolger Bay, Magnetic Island; MB, mid Cleveland Bay; MR, Middle Reef; SB, southern Cleveland Bay; ST, The Strand, Townsville.
2. Net type: 5, 5cm monofilament gillnet; 10B, 10cm monofilament gillnet set on bottom; 10M, 10cm multifilament gillnet; 10T, 10cm monofilament gillnet set on surface; 15, 15cm monofilament gillnet.
3. Species: Cb, *Carcharhinus brevipinna*; Cd, *C. dussumieri*; Cf, *C. fitzroyensis*; Cl, *C. limbatus*; Cm, *C. macloti*; Cs, *C. sorrah*; Ct, *C. tilstoni*; Ra, *Rhizoprionodon acutus*; Rt, *R. taylori*.

Appendix A.2: details of demersal otter trawls that yielded specimens.

Date	Location	Species	Number
9/2/87	Near mouth of Ross River	*Rhizoprionodon acutus*	1
19/9/87	Near mouth of Ross River	*Rhizoprionodon acutus*	13
24/9/87	Near mouth of Ross River	*Rhizoprionodon acutus*	1
24/9/87	Seagrass beds near Cape Cleveland	*Rhizoprionodon acutus*	1
21/2/88	Near mouth of Ross River	*Rhizoprionodon acutus*	1
21/2/88	Near mouth of Ross River	*Rhizoprionodon acutus*	2
		Rhizoprionodon taylori	1
21/2/88	Near mouth of Ross River	*Rhizoprionodon acutus*	4
24/1/89	North of Horseshoe Bay, Magnetic Island	*Rhizoprionodon taylori*	3

Appendix A.3: Environmental parameters for sampling trips.

Date	Location	Depth (m)	Tide (m)	Sea temp. (°C)	Salinity (ppt)	Wind strenght (knots)	Sea state
1/5/87	SB	3	-	-	-	5-15	slight
19/5/87	ST	4	-	-	-	10-15	mod.
15/6/87	ST	4	-	-	-	-	-
22/6/87	SB	1.5	+2.4	26.5	-	5	slight
25/6/87	SB	1.0	+2.1	23.6	37.5	5	slight
2/7/87	ST	4	+0.3	21.0	37.0	5-10	slt-mod
14/7/87	ST	2.5	+1.2	21.0	37.0	5-10	slt-mod
28/7/87	SB	0.8	+1.5	21.3	37.3	5	slight
4/8/87	ST	5	-1.5	23.7	36.0	5-10	slt-mod
7/9/87	ST	3	+3.0	25.3	36.6	5-10	slt-mod
29/9/87	ST	3	-0.5	24.8	36.1	10	slt-mod
13/10/87	RR	2.5	-1.0	28.2	-	5-10	slt-mod
5/11/87	ST	5	-1.3	28.0	-	5	slight
19/11/87	ST	4	+1.0	27.8	36.1	10	slt-mod
26/1/88	MR	5	-	30.5	35.6	15	mod
2/2/88	ST	4	-	31.3	36.6	15	mod
16/2/88	ST	-	-	29.3	35.7	15	mod
21/2/88	MR	5	-	29.0	-	10	slt-mod
29/3/88	ST	-	-	26.0	34.8	10	mod
5/4/88	ST	3	+1.0	-	35.2	5	slight
12/4/88	SB	2	+2.3	29.0	36.1	5	slight
3/5/88	BB	1.5	+2.1	26.3	34.6	<5	calm
10/5/88	MR	5.5	-1.5	27.2	34.8	5	slight
17/5/88	SB	1.2	+2.3	27.0	35.9	5-10	slt-mod
2/5/88	ST	3	-0.4	25.8	35.1	5	slight
19/7/88	ST	2.2	+0.5	21.7	35.7	10-15	mod
26/7/88	ST	3.5	-1.4	22.0	35.6	10	mod
24/8/88	ST	5	-1.0	23.6	35.7	10	mod
13/9/88	ST	3.5	-0.5	25.3	35.9	10	slight
18/10/88	MR	4.5	+0.0	28.3	36.2	5	slight
24/10/88	ST	4.6	-1.0	28.6	36.2	10	slt-mod
9/11/88	SB	5.0	+1.2	27.2	35.6	5	slight
16/11/88	MR	4.8	+0.7	27.8	35.6	<5	calm
28/11/88	ST	2.2	-0.2	30.7	35.6	10-15	mod
7/12/88	SB	5	-0.6	29.0	35.5	5	slight
12/12/88	ST	3.2	+0.8	30.0	35.5	5	slight
11/1/89	ST	3.0	+1.0	30.5	33.6	5	slight
19/1/89	ST	4.6	-0.3	29.5	33.6	5-10	slt-mod
1/2/89	MR	5.1	-0.3	28.5	34.1	5-10	slt-mod
8/2/89	ST	3.2	+1.1	30.0	32.2	5-10	slt-mod
12/4/89	ST	3.3	-0.3	27.6	30.0	10-15	mod
26/4/89	MR	4.8	+0.8	27.8	30.9	<5	calm
31/5/89	ST	5.0	-1.0	24.4	31.4	5-10	slt-mod
7/6/89	SB	3.3	+1.0	21.8	32.1	<5	calm
13/6/89	ST	4.1	-0.3	20.6	32.5	5-10	slt-mod
3/7/89	ST	4.0	+1.4	21.4	33.2	5	slight
5/7/89	ST	3.2	+1.1	22.0	34.0	10-15	mod
25/7/89	ST	4.0	-0.7	19.8	34.0	5-10	slt-mod
2/8/89	ST	3.3	+1.1	21.8	34.0	10-15	mod
9/8/89	SB	5.0	-0.4	20.4	34.4	10	slt-mod
23/8/89	ST	4.2	-1.0	22.0	34.5	<5	calm
28/8/89	ST	5.3	-0.6	23.6	34.9	5-10	slt-mod
13/9/89	ST	5.6	-0.3	23.5	34.8	10	mod
18/9/89	MB	7.7	+1.6	24.0	34.9	5-10	slt-mod
18/9/89	MR	7.0	-0.5	24.0	34.9	5	slight
20/9/89	SB	4.4	-0.3	26.0	35.1	5	slight
25/9/89	ST	5.3	-0.6	27.5	35.8	5-10	slt-mod
5/10/89	ST	4.0	-0.3	26.6	35.4	5	slight
1/11/89	ST	3.6	+0.5	27.8	35.7	5	slt-mod
4/12/89	ST	3.5	-0.3	29.0	35.1	10	slt-mod
15/1/90	ST	3.0	+0.3	30.5	35.8	5	slight
5/2/90	ST	4.0	-1.2	30.5	36.0	<5	calm
16/2/90	ST	3.1	+0.2	31.0	36.8	5-10	slt-mod

Appendix B: Method for calculation of Weighted Resultant Index. Modified from Mohan and Sankaran (1988).

The occurrence index (o_i), and the weight index (w_i), can be calculated using the formaulae:

$$o_i = \frac{n_i}{\sum n_i} \times 100$$

$$w_i = \frac{m_i}{\sum m_i} \times 100$$

where n_i is the number of stomachs in which the *ith* food item occurs and m_i is the mass of the *ith* food item in all stomachs examined. The method relies on the values of o_i and w_i being the co-ordinates of a point on a plane. The distance from the origin to this point is:

$$\sqrt{(w_i^2 + o_i^2)}$$

And the direction from the origin to the point (θ_i) is:

$$\theta_i = \tan^{-1}\left(\frac{o_i}{w_i}\right)$$

When the values of o_i and w_i are similar θ_i will be close to 45°. The deviation of from 45° (Q_i) can be calculated using the formula:

$$Q_i = \frac{45 - |\theta_i - 45|}{45}$$

The same form of index as for occurrence or weight can then be calculated using the distance from the origin to the *ith* point. Correcting the distance values with the value Q_i enables points with a similar distance from the origin, but with different angles, to be distinguished, and gives the Weighted Resultant Index:

$$R_w = \frac{Q_i(w_i^2 + o_i^2)^{\frac{1}{2}}}{\sum Q_i(w_i^2 + o_i^2)^{\frac{1}{2}}} \times 100$$

Appendix C: Summary of the reproductive parameters of sharks from the family Carcharhinidae. Information taken from Compagno (1984) when no other data avaliable or accessible. L_{max}, maximum length; SAB, size at birth; SAM, size at maturity.

Species	L_{max} (cm)	SAB (cm)	Male SAM (cm)	Female SAM (cm)	Mating season	Pupping season	Gestation period (months)	Litter size	Periodicity of cycle	Location	Source
Carcharhinus acronotus	126	45-50	110	110	?Jun-Jul	Jun	-	-	annual	G. of Mexico	Branstetter 1981
	-	45	103	103	Jun	-	-	3-6	-	Florida	Clark & von Schmidt 1965
C. albimarginatus	280?	-	170-180	?200	-	-	-	-	-	Aldabra Atoll	Stevens 1984a
	208	70-80	160-180	200	-	-	-	6	-	South Africa	Bass et al. 1973
	261	80	180	215	Jan-Feb	Jan-Feb	12	1-10	-	Mauritius-Seychelles	Wheeler 1962
C. altimus	282	70-90	216	226	-	Sep-Oct	-	3-15	-	Madagascar	Fourmanoir 1961
	280	60	-	225	-	-	-	6-8	-	N. Australia	Stevens & McLoughlin 1991
C.amblyrhynchoides	162	55	108	115	Feb-Mar	Jan-Feb	9-10	1-9(3)	annual	N. Australia	Stevens & McLoughlin 1991
C. amblyrhynchos	172	70	112-140	125	Jun-Jan	-	12-13	1-4	-	Mauritius-Seychelles	Wheeler 1962
	178	63	-	135	-	Aug	9	2-3(3)	-	N. Australia	Stevens & McLoughlin 1991
C. amboinensis	280	71-72	195	200	-	-	-	-	-	South Africa	Bass et al. 1973
	243	63	208	215	-	Nov-Dec	9	6-13(9)	-	N. Australia	Stevens & McLoughlin 1991
C. brachyurus	292	60-70	200-220	247	-	-	-	13-20(16)	-	South Africa	Bass et al. 1973
C. brevipinna	-	60-80	215	?210	-	Mar-Apr	-	8-13	-	N.S.W.	Stevens 1984b
	225	60-70	-	-	Jun-Jul	May-Jun	11-12	6-8	biennial	G. of Mexico	Branstetter 1981
	266	65-75	180-200	200	-	Apr-May	12-15	6-15(11)	biennial	South Africa	Bass et al. 1973
	276	75	-	-	-	Mar-Apr	12	11	-	N. Australia	Stevens & McLoughlin 1991
C. cautus	119	40	84	91	Jan-Mar	Oct-Nov	7-9	1-5(2.9)	annual	N. Australia	Lyle 1987a
C. dussumieri	-	37-38	-	-	unseas.	unseas.	-	2	unseas.	Borneo	Teshima & Mizue 1972
	92	35-40	69	74	-	Nov-Apr	-	1-2	-	India	Setna & Sarangdhar 1949b
	88	38	70	70	unseas.	unseas.	-	1-3(2)	annual	N. Australia	Stevens & McLoughlin 1991
C. falciformis	-	75-85	214	?200	unseas.	unseas.	-	5-8(6.5)	unseas.	N.S.W.	Stevens 1984b
	305	75	210-220	>225	May-Jun	May-Jun	12	4-13	biennial	G. of Mexico	Branstetter 1987
	243	75(?)	210	210	unseas.	unseas.	-	8	annual	N. Australia	Stevens & McLoughlin 1991

C. fitz-royensis	135	50	88	100	May-Jul	Feb-Apr	7-9	1-7(3.7)	annual	N. Australia	Lyle 1987a
C. galapa-gensis	300+	57-80	170	235	-	-	-	6-16	-	various	Compagno 1984
C. isodon	139	49-51	115	140	-	-	-	4	-	G. of Mexico	Brastetter 1981
C. leucas	-	60-80	225	233	Jun-Jul	Jun-Jul	10-12	-	?biennial	Florida	Snelson et al. 1984
	266	-	217	228	-	-	-	5-10	-	G. of Mexico	Branstetter 1981
	300	60-70	250	260	-	Nov-Dec	-	10-13	-	South Africa	Bass et al. 1973
	264	74-75	-	-	Jun-Jul	Apr-Jun	11-12	5-10	-	Florida	Clark & von Schmidt 1965
	300	-	241	241	-	-	-	6-12(8.7)	-	South Africa	Cliff & Dudley 1991
	268	-	215	>225	-	-	-	-	-	G. of Mexico	Branstetter & Stiles 1987
	235	50-70	162	170	unseas.	unseas.	-	1-10(5.5)	-	Lake Nicaragua	Jensen 1976
C. limbatus	178	50-60	136	160	Jun	Jun	11-12	2-6	biennial	G. of Mexico	Branstetter 1981
	247	62	180	193	summer	Nov-Mar	12	1-10(6.7)	biennial	South Africa	Bass et al. 1973
	191	50-60	135	155	Jun-Jul	Apr-May	10-11	3-8	-	Florida	Clark & von Schmidt 1965
	183	61	142	158	-	Jan-Mar	-	-	-	India	Setna & Sarangdhar 1949b
	230	55-60	155	165	-	Apr-May	-	-	-	India	Devadoss 1988
C. longimanus	257	-	-	-	May-Jun	May-Jun	12	2-9(6)	biennial	G. of Mexico	Backus et al. 1956
	266	60-65	-	200	-	Jan-Mar	-	4-8(6.8)	-	N.S.W.	Stevens 1984b
	270	60-65	198	190	-	summer	-	6-8(7)	-	South Africa	Bass et al. 1973
C. macloti	92	45-50	-	-	-	-	-	1-2	-	India	Setna & Sarangdhar 1949b
	108	43	74	73	-	Jul	12	1-2(3)	biennial	N. Australia	Stevens & McLoughlin 1991
C. melano-pterus	140	50	105	110	Nov	Oct	10-11	2-5(3.7)	biennial	Aldabra Atoll	Stevens 1984a
	125	48	95	97	Jan-Mar	Oct-Nov	7-9	3-4(3.8)	annual	N. Australia	Lyle 1987a
C. obscurus	357	69-100	280	260-300	-	-	-	6-14(10)	-	South Africa	Bass et al. 1973
	342	85-100	-	-	Apr	-	16	6-10	biennial	Florida	Clark & von Schmidt 1965
C. perezi	295	<73	152	200	-	-	-	-	-	W. Atlantic	Compagno 1984
C. plumbeus	230	65-75	180	183	Mar-Aug	Jun	8-12	1-14(9)	?biennial	eastern USA	Springer 1960
	-	-	-	-	-	Jun-Jul	10-12	2-10	-	E. China Sea	Taniuchi 1971
	190	61-69	144	150	Jun-Aug	Jul-Sep	12	1-8(5.5)	annual	Hawaii	Wass 1973
	215	-	190	189	Jul	-	-	6-10	biennial	G. of Mexico	Branstetter 1981
	202	-	168	163	Oct-Jan	-	12	4-10	biennial	South Africa	Cliff et al. 1988
	220	60-65	160-170	170	-	-	-	8	-	South Africa	Bass et al. 1973
	230	-	-	185	-	?Mar	-	4-11	-	Florida	Clark & von Schmidt 1965
	208	60	156	155	Nov-Apr	Feb-Mar	12	3-8(6)	biennial	N. Australia	Stevens & McLoughlin 1991
C. porosus	134	31-40	75-78	84	-	-	10+	2-7	-	S. America	Compagno 1984
C. sealei	92	35-45	70-80	75	-	summer	9	1-2	-	South Africa	Bass et al. 1973

C. signatus	280	60	-	-	-	-	-	4-12	-	Atlantic	Compagno 1984
C. sorrah	140	50-60	-	110-116	-	summer	-	3	-	South Africa	Bass et al. 1973
	151	50	90	95	Feb-Mar	Jan	10	1-8(3.1)	annual	N. Australia	Stevens & Wiley 1986
	152	-	109	-	-	Mar-May	-	3-6	-	India	Setna & Sarangdhar 1949b
	-	-	115	120	-	Apr-May	-	2-6(4)	biennial	India	Devadoss 1988
C. tilstoni	180	60	110	115	Feb-Mar	Jan	10	1-6(3)	annual	N. Australia	Stevens & Wiley 1986
C. wheeleri	182	65-70	110-120	-	-	Jul-Feb	-	2-4(3.3)	biennial	Aldabra Atoll	Stevens 1984a
Galeocerdo cuvier	376	-	305	330	-	-	-	-	-	N.S.W.	Stevens 1984b
	400	75	-	-	-	-	-	27-44	-	India	Setna & Sarangdhar 1949b
	381	>70	310	315-320	-	-	-	-	biennial?	eastern USA	Branstetter et al. 1987
	373	70-80	-	-	May	Jun	12+	37-56	biennial	Florida	Clark & von Schmidt 1965
	410	-	290	300	-	-	-	23-46 (35.4)	-	South Africa	Bass et al. 1975
	450(?)	65(?)	305	330	-	Jan-Feb	12	-	-	N. Australia	Stevens & McLoughlin 1991
Isogomphodon oxyrhynchus	152	38-41	108	-	-	-	-	4	-	S.W. Atlantic	Compagno 1984
Lamiopsis temmincki	170	45-60	114	130	-	Apr-May	-	4-8	-	India	Setna & Sarangdhar 1949b
Loxodon macrorhinus	90	40	73-75	85	-	-	-	2	-	South Africa	Bass et al. 1975
	88	43	64	57	unseas.	unseas.	-	1-2(2)	annual	N. Australia	Stevens & McLoughlin 1991
Nasolamia velox	150	53	-	-	-	-	-	5	-	E. Pacific	Compagno 1984
Negaprion acutidens	240	55-60	220	220	Oct-Nov	Oct	10-11	6-12(9.3)	biennial	Aldabra Atoll	Stevens 1984a
N. brevirostris	296	60	-	250	May-Jun	Apr-Jun	12	5-17(11)	-	Florida	Clark & von Schmidt 1965
Prionace glauca	282	35-44	183	?185	May-Nov	Apr-Jul	9-12	?-82	annual	eastern USA	Pratt 1979
	326	46-51	220-250	?>220	Oct	Oct-Nov	-	4-57(32)	-	N.S.W.	Stevens 1984b
	323(?)	43	-	220	-	Dec-Mar	9-12	11-49(34)	-	N. Australia	Stevens & McLoughlin 1991
Rhizoprionodon acutus	102	30-35	68-70	70-80	Nov-Dec	Oct-Nov	11-12	2-8(4.7)	-	South Africa	Bass et al. 1975
	94	-	-	74	-	Nov-Dec	-	2	-	India	Setna & Sarangdhar 1949b
	83	-	-	65	-	-	-	1-6	-	India	Krishnamoorthi & Jagadis 1986
	98	38	75	75	unseas.	unseas.	-	1-6(3)	annual	N. Australia	Stevens & McLoughlin 1991
R. lalandei	-	-	50-60	50-60	-	-	11	3-5	annual	Brasil	Ferreira 1988
	78	-	51	53-56	-	-	-	2-5	-	Brasil	Lessa 1988
R. oligolinx	70	27-30	56	-	-	Jan-Feb	-	3-5	annual?	India	Setna & Sarangdhar 1949b

R. porosus	-	-	70-80	70-80	-	-	11	3-5	annual	Brasil	Ferreira 1988
R. taylori	66	25-30	40	45	-	Dec-Jan		1-8(5)	annual	N. Australia	Stevens & McLoughlin 1991
R. terraenovae	107	32	80	85-90	May-Jul	Jun	10-11	1-7(5)	annual	G. of Mexico	Parsons 1983a
	110	-	81	-	Jun-Jul	May	11	2-8	annual	G. of Mexico	Branstetter 1981
Scoliodon laticaudus	64	14	-	-	-	-	-	-	-	India	Nair 1976
	50	-	33	32-35	unseas.	unseas.	-	-	unseas.	Malaysia	Teshima et al. 1978
	66	13	-	-	unseas.	unseas.	-	2-22(11)	-	Bombay, India	Setna & Sarangdhar 1948
	-	14	33	38	-	Nov-Dec & Feb-Mar	5-6	6-18(13)	-	India	Devadoss 1979
Triaenodon obesus	168	52-60	82	102	-	-	-	1-5	-	Pacific Islands	Randall 1977

Appendix D.1: Histological techniques used for the examination of embryos.

Method	Reference
Mayer's haematoxylin and eosin	Winsor 1984
Mallory-Heidenhain trichrome	Winsor 1984
Martius Scarlet Blue (with haematoxylin nuclear stain)	Drury and Wallington 1980
Iron haematoxylin	Winsor 1987

Appendix D.2: Histological and histochemical techniques used to examine the structure and function of tisues.

Substance tested for	Method	Reference
general protein	dinitrofluorobenzene	Bancroft 1975
collagen	Mallory-Heidenhain trichrome Van Gieson's stain	Winsor 1984 Winsor 1984
acid and neutral mucins	alcian blue - periodic acid Schiff technique	Winsor 1984
sulphated mucins	0.1% toluidine blue	Bancroft 1975
polyphenol oxidase	catechol	Smyth 1954
polyphenols	diazo methanamine silver	Cook 1974 Cook 1974
diphenols	Masson-Fontana technique	Cook 1974