The reproductive biology and movement patterns of the draughtboard shark, *(Cephaloscyllium laticeps)*: implications for bycatch management

by

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Draughtboard shark, *Cephaloscyllium laticeps*
I hereby declare that this thesis is my own work except where due acknowledgement is given, and that the material presented here has not been submitted at another university for the award of any other degree diploma.

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Cynthia Andrea Awruch

January 2007
The draughtboard shark (*Cephaloscyllium laticeps*) is the most common shark on temperate reefs in southeastern Australia. In order to implement adequate management plans its reproductive biology and movement patterns were studied.

Females developed a single external-type ovary with a maximum follicle diameter of 35 mm. Vitellogenesis commenced at 10 mm follicle diameter. The male reproductive tract consisted of paired testis with spermatocysts undergoing diametric development.

The hormones testosterone, 17-β estradiol, progesterone and 11-ketotestosterone (males only) were examined to determine their role in reproduction. Testosterone and estradiol showed major changes during follicle development. Estradiol increased as the follicle developed before declining as the follicle reached maturity. Testosterone remained low during the first stages of follicular development and increased as the follicle reached maturity. Progesterone showed a peak just prior to ovulation. Testosterone was the only hormone that varied with maturity in males and no levels of 11-ketotestosterone were detected.

Females were able to store sperm for at least 15 months and eggs were laid in pairs at monthly intervals. Juveniles hatched after 12 months.

The size at maturity and seasonality of reproduction were estimated using reproductive parameters obtained from dissected animals and from steroid hormones. The sizes at onset of sexual maturity by both methods were similar. Females laid eggs throughout the year with a peak in deposition between January and June. Elevated values of testosterone and progesterone coincide with this period of egg deposition. Males showed no seasonal pattern in reproduction although both testosterone and the amount of sperm in the seminal vesicle were marginally higher in the first semester of the year.

Movement studies were undertaken using conventional and acoustic tagging. The area of study included a marine reserve and the adjacent bays of southeast Tasmania. Both
methods demonstrate that the majority of sharks remained in the same region in which they were tagged, although a few sharks moved large distances. Sharks were active throughout the day and night with peak activity during dawn and dusk. This species could remain stationary on the bottom for periods up to five days. No correlation was found between activity and lunar patterns and both sexes showed similar activity patterns.

This study has provided the first information on reproduction and movement of draughtboard shark and demonstrated the potential for hormones to provide reproductive information necessary for management without the need to sacrifice the shark.
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**GENERAL CONCLUSIONS**

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General introduction
The practice of harvesting sharks has a long history. Although sharks were considered edible prior the twentieth century, records associated with shark captures were more likely to be related with rituals rather than eating habits (Budker, 1971; Taylor et al., 2005). In the early part of the 1900s, sharks supported small regional artisanal fisheries that targeted individual species for specific products, while towards the 1920s the advance in fishing technology resulted in an increase in harvesting of chondrichthyan globally (Budker, 1971; Taylor et al., 2005). The commercial exploitation of sharks dates from the period between the two world wars (1930s to 1940s) when attention was drawn to the demand for shark liver oil stimulating a rapid growth in shark fisheries (Budker, 1971; Taylor et al., 2005). Since the mid 1980s the demand for shark products had greatly increased and by the late 1980s shark fisheries were widespread. A further escalation in exploitation of sharks occurred in the mid-1990s with the high price and increased demand for shark fins in Asian markets (Castro and Brudek., 1999).

Currently, chondrichthyan populations around the world are harvested by commercial, artisanal and recreational fisheries, and are mostly caught as bycatch (discarded after capture) in the world’s fisheries which target teleost species (Walker, 1998; Stevens et al., 2000). As such, understanding incidental catch and mortality of bycatch species is becoming an increasing requirement of future ecosystem management plans (Hall et al., 2000; Pope et al., 2000). Furthermore, because most sharks exist at or near the top of the food chain (Cortés, 1999; Heithaus, 2004), the removal of upper trophic level predators from their ecosystem can have food web consequences. In addition to their primary prey items, trophic cascades can have significant impacts on non-prey species (Pauly et al., 1998; Stevens et al., 2000; Schindler et al., 2002; Scheffer et al., 2005). With the fear of rapid depletion of world fish stocks because of over exploitation, together with the reduction in sharks as top predators, the effects on marine ecosystems of overfishing could result in an important loss of marine
biodiversity (Coleman and Williams, 2000; Mullon et al., 2005). Therefore, understanding the role of the top predator sharks in the ecosystem are primary requirements for management and conservation of the shark species but also to accurately address an ecosystem based fisheries management framework.

It is increasingly being recognized that the life history characteristics of chondrichthyans (long lived, slow growth and producing few offspring) make this group a fragile marine resource that is vulnerable to exploitation (Walker, 1998; Dulvy et al., 2003). Several species of sharks, rays and skates either targeted or caught as bycatch or byproduct (retained after capture) have demonstrated substantial population declines over the last 20 years (Pauly et al., 1998; Stevens et al., 2000; Graham et al., 2001; Baum et al., 2003). By 2006, of the 547 chondrichthyan species listed in the International Union of the Conservation of Nature’s (IUCN) Red List, 20% are threatened with extinction; confirming that this taxonomic group is extremely vulnerable to overfishing and is disappearing at an unprecedented rate across the world (IUCN, 2006). In recognition of the expanding global catch of chondrichthyans and the potential negative impacts on chondrichthyan populations, an International Plan of Action for the Conservation and Management of Sharks (IPOA-Sharks) was adopted by the 23rd session of the United Nations Food and Agriculture Organisation’s (UN FAO) Committee on Fisheries in 1999 (FAO, 1999; FAO, 2005).

Australia has an extremely rich chondrichthyan fauna with the most recent taxonomic review estimating that of the 1025 species of chondrichthyans worldwide, at least 297 species inhabit Australian waters. Of these species more than half (48% of sharks, 73% of rays) are endemic to Australia (Last and Stevens 1994). In 2001 a Shark Assessment Report, commissioned by the Department of Agriculture, Forestry and Fisheries (DAFF), listed 5 species as protected, 6 species as endangered, 6 species as vulnerable, 21 species as near threatened and 3 species as conservation dependent
As a member of the UN FAO, Australia committed to producing its own National Plan of Action for the Management and Conservation of Sharks (referred to as Shark-plan). The Shark-plan was endorsed by the Natural Resource Management Ministerial Council on 16 April 2004 (DAFF, 2004). The Shark-plan recognises that while Australia is not a major shark fishing nation, it is acknowledged that sharks are an important part of the total quantity of Australia’s wild fish production and that Australian vessels regularly take sharks as target and non-target catch (DAFF, 2004).

This thesis has focused on an endemic Australian shark species, the draughtboard shark *Cephaloscyllium laticeps* (Duméril, 1853); that within the order Carcharhiniformes, belongs to the Scyliorhinidae family. This family, the largest of the shark families, includes up to 17 genera and about 100 species. Particularly, 8 genera and 32 species are found in Australia (Springer, 1979; Last and Stevens, 1994). The entire family lives in marine habitats, feeding mainly on small fish and invertebrates. Most of scyliorhinids are near-bottom dwellers in shallow waters, although a few genera include species that occur along the continental slopes to depths exceeding 2000 m (Springer, 1979; Compagno, 1984; Last and Stevens, 1994).

The draughtboard shark is the most common catshark in the coastal areas of southern Australia, where it is a higher trophic level predator of temperate reefs. It is particularly found inshore on the continental shelf of southern Australia from the Recherche Archipelago (Western Australia) to Jervis Bay (New South Wales) down to at least 60m (Last and Stevens, 1994). Common names for this species are: Australian swell shark, sleepy joe, and nutcracker shark, and local synonymies are: *Cephaloscyllium isabella laticeps* and *Cephaloscyllium isabella nascione* (Last and Stevens, 1994).

Draughtboard sharks form a significant component of the southeastern Australian shark fishery where they are caught as accidental bycatch from rock lobster traps, demersal trawls, long-lines and gillnets (Frusher and Gibson, 1999; Walker *et al.*, 2005).
This species is usually returned to the water and fishing mortality is low due to its resilience (Brickhill, 2001). In Bass Strait (southern Australia), there was a reduction of approximately 54% in *C. laticeps* between 1973-1976 to 1998-2001 period. However, this reduction has been attributed to commercial fishers avoiding fishing grounds where these animals are abundant (Walker et al., 2005). There is currently no targeted commercial fishery for draughtboard shark, although it has recently been marketed in some areas of Tasmania, where there has been a trend for draughtboard sharks that have been caught in commercial gillnets to be retained for local consumption as “flake” (J. Lyle, TAFI Marine Research Laboratories, Hobart. pers. comm). Although caught as a bycatch, draughtboard sharks are potentially vulnerable to population reduction through fishing due to their high catchability in either pots or gillnets. Despite being a common bycatch species, the lack of commercial value has resulted in this species not being the subject of scientific study. Furthermore, assessing the potential impact of fishing mortality of this top-level predator in a temperate reef ecosystem is currently hindered by the poor knowledge of its biology.

The aims of this thesis were to address the biology, ecology and ecosystem role of draughtboard sharks by studying their reproductive biology, movement patterns and habitat utilisation. The results of this thesis will both, increasing the knowledge of draughtboard sharks, but will also be essential to accurately addressing ecosystem based fisheries management programs in Australian temperate reefs, where draughtboard sharks share the habitat with other marine species of very high commercial value, such as the rock lobster *Jasus edwardsii* (S. Frusher, TAFI Marine Research Laboratories, Hobart. pers. comm). In addition, as this shark is, in general, not retained as a byproduct there is a need for a non-destructive sampling methodology to study reproduction on this species. In consequence, steroid hormones were used as a tool to assess draughtboard sharks reproductive biology. These results will not only help to
understand reproduction of this species, but will also increase the knowledge of the reproductive endocrinology of chondrichthyan species; an area within this marine group that still remains highly unknown. Furthermore, because assessing and protecting threatened species or species living in marine protected areas has become an important part of global conservation activities (Powles et al., 2000; Blyth-Skyrme et al., 2006), there is a need to find methodology of addressing the reproductive stage of chondrichthyans species without killing the animals.

The thesis consists of the general introduction, four descriptive chapters and the general conclusions. The next chapter, chapter one, describes the area of study. Chapter two initially describes the reproductive characteristics and development of C. laticeps obtained thorough anatomical and histological examination of the reproductive organs. The second part of chapter two correlates reproductive hormones with basic reproductive parameters to explore the role of steroid hormones on oviparous sharks. Finally, in the last part of chapter two the seasonal reproductive cycle and embryo development are described. Chapter three evaluates the use of reproductive hormones as a non-destructive method to describe reproduction in sharks, and the hormones are explored in the context of providing information required for fisheries management and conservation. Chapter four explores the use of acoustic tagging techniques to assess habitat utilisation and movement patterns and to compare this data with a conventional tagging project that was also undertaken at the same time. Finally, in the general conclusions, the information from the previous chapters was used to understand the linkages between reproduction, movements and habitat selection, essential for understanding the role of draughtboard sharks in the reef habitat and therefore address future ecosystem management programs.
CHAPTER ONE:

Area of study
Tasmania is an island located at 39-44°S and 144-149°E (Fig. 1.1 and 1.2a). Three main bodies of water influence the island (Fig. 1.2b). The East Australian Current (EAC) flows down the eastern seaboard to the southern tip of Tasmania where it converges with colder subantarctic waters in a subtropical convergence zone (STC). The Zeehan Current is an extension of the Leeuwin and flows down Tasmania’s west coast and around southern Tasmania (Cresswell, 2000). Both the EAC and Zeehan Current are nutrient poor water originating in sub-tropical regions. In contrast, the sub-antarctic water mass is nutrient rich (Harris et al., 1987). Water temperatures in Tasmania range from 10.7 to 18.6 (°C) between summers to winters (Cresswell, 2000).

The main marine habitat surrounding Tasmania is rocky reef formed of sandstone and granite. While the reef supports a diverse and abundant fauna, seaweed and seagrass is the predominant living flora (Edgar, 2001).

Fig. 1.1: Map of Australia. Tasmania is located in the south east of Australia.
Fig. 1.2: Map of Tasmania showing (a) the location of the main study site and (b) a satellite image demonstrating the main currents influencing Tasmania during autumn. The EAC and ZC extend further south during summer and retreat further north during winter. **EAC**: East Australian Current, **ZC**: Zeehan Current, **STC**: Subtropical Convergence, **SAW**: Subantarctic Water. NOAA 14 NLSSTC MOSAIC 11 MAR 1998 05 11Z-0653Z COPYRIGHT 1998 CSIRO.
CHAPTER TWO:

Reproduction
2.1 Introduction

Reproduction involves one of the most important events in the life of any living organism. The primary requirement for successful propagation of any species and their individuals is the availability to reproduce. Understanding of the overall process of reproduction requires knowledge of the morphology and physiology of the reproductive tract, and reproductive strategies and cycles.

In life-history theory, reproductive strategy is defined as a complex mixture of adapted characteristics designed by natural selection to solve ecological problems (Stearns, 1976). A series of reproductive strategies has been developed by chondrichthyan during their long evolutionary history. The general trend in chondrichthyans reproductive evolution is a progression from oviparity to viviparity, but within this there is still a great diversity of morphological and physiological adaptations (Wourms, 1977; Carrier et al., 2004). These reproductive strategies are expressed through reproductive cycles, which are regulated by a combination of physical and biological variables to ensure that young fish are produced in the best environment for their survival (Bromage et al., 2001; Pankhurst and Porter, 2003). Four categories of reproductive cycles can be defined for chondrichthyan females: 1) species that are reproductively active throughout the year, 2) species that are reproductively active throughout the year, but exhibit seasonal periods where a greater proportion of reproductive activity occurs, 3) species with a well defined seasonal cycle, where animals are reproductively active for only a portion of the annual cycle, and 4) species that are pregnant for approximately a full year, after which they spend a year or two non-pregnant (Wourms, 1977; Hamlett and Koob, 1999; Koob and Callard, 1999). In chondrichthyan males, sperm production can be either seasonal or occur throughout the year and can be coupled or not with the mating period (Parsons and Grier, 1992).
Among oviparous elasmobranchs, both seasonal and non-seasonal reproductive activity has been observed. For example, while a clear seasonal reproductive period for both females and males has been reported in *Hemyscyllium ocellatum* (Heupel et al., 1999), in *Amblyraja radiata* both sexes are reproductively active all year round (Sulikowski et al., 2005a). Furthermore, male and females of the same species may differ fundamentally in their reproductive tactics. In species such as *Leucoraja ocellata*, females are capable of continuously laying eggs but show a seasonal peak in activity, while males are able to continuously reproduce throughout the year (Sulikowski et al., 2004).

In the family Scyliorhinidae, species show single or multiple oviparity, as well as aplacental yolk sac viviparity. Single oviparity, where only one egg case develops in each uterus, has been recorded in the most primitive genera: *Cephaloscyllium*, *Apristurus*, *Scyliorhinus* and some species of *Galeus*. Multiple oviparity, where several egg cases develop in the uterus and the egg capsule is laid when the embryo reaches a certain length, has been found in the genus *Halaelurus* and some species of *Galeus* (Nakaya, 1975; Springer, 1979; Compagno, 1984). Aplacental yolk sac viviparity, where embryos are retained in the uterus during the entire period of development and depend solely on yolk sac reserves, has been reported in some species of *Galeus* and in *Cephalurus cephalus* (Nakaya, 1975; Springer, 1979).

Studies on reproduction of the scyliorhinids are limited to only a few species. In the oviparous scyliorhinids, both sexes of *Apristurus brunneus* and *Parmaturus xaniurus* are reproductively active throughout the year (Cross, 1988) whereas females of *S. canicula* (Craik, 1978; Sumpter and Dodd, 1979) and *Galeus melastomus* (Costa et al., 2005) although capable of producing eggs throughout the year, demonstrate seasonal periods of reproductive activity. Embryo development in the scyliorhinid shark, *Scyliorhinus retifer* (Castro et al., 1988) showed an incubation time of 256 days in captivity, while embryo development in *S. canicula* (Capapé, 1977; Mellinger et al., 1986; Lechenault et al., 1993)
varied (in captivity) from 185 days in warm temperatures to 285 days in colder temperatures.

Histological examinations of chondrichthyans show that follicular organization in females is very similar to that of other vertebrate species (Fasano et al., 1989). However, in chondrichthyan males, the testicular organization has been classified into three different categories (radial, diametric and compound) (Pratt, 1988) depending on the origin and propagation of the spermatocysts (the unit of structure and function of the testis (Callard, 1991b)). The lamnid-alopiid testis type is radial and the testis is comprised of lobes. The germinal zone is localized in the centre of each lobe and development of spermatocysts proceeds radially from the germinal zone towards the end of the lobes. The carcharhinid-sphyrnid testis is diametric; the development of the spermatocysts proceeds from the germinal zone across the diameter of the testis. The rajid testis is compound, combining both the radial and the diametric organization of the testis (Pratt, 1988; Girard et al., 2000).

All scyliorhinid sharks share an external type of ovary (follicles are ovulated into the body cavity where they reach the ostium (Pratt, 1988)). Ovarian follicles are embedded under a single layer of generative tissue, and follicles are ovulated into the body cavity (Pratt, 1988). Some species contain only one functional ovary, while in others both ovaries are developed (Springer, 1979; Compagno, 1984; Cross, 1988). While the anatomy of the scyliorhinid males have been less studied than that of females, it is known that the entire family shares a pair of testes and accessory ducts, including epididymis, efferent and deferent ducts and seminal vesicles (Springer, 1979; Dodd, 1983; Compagno, 1984). In the only two histological studies of the gonads of scyliorhinids, Scyliorhinus retifer and S. canicula females had follicular organization similar to other vertebrates, and males had a diametric type of testis (Dodd, 1983; Pratt, 1988).

Knowledge of vertebrate endocrinology is an essential component of understanding reproductive processes, as reproductive hormones are involved as either triggers or
regulators of all aspects of reproduction. The brain-pituitary-gonadal axis is a cascade system that regulates the entire reproductive process, promoting gametogenesis and subsequent gamete maturation (Sherwood and Lovejoy, 1993; Gelsleichter, 2004; Pankhurst, 2006). The release of gonadotropin releasing hormone (GnRH) by the brain stimulates the production of the gonadotropins (GTH) from the pituitary gland. These gonadotropins are released into the circulatory system, reaching the target cell where they bind with membrane-bound receptors. This gonadotropin-receptor complex triggers adenyl cyclase, to form cAMP (cyclic adenosine monophosphate), which in turn activates protein kinases A, leading to the activation or de novo synthesis of steroid synthesizing enzymes resulting in production of steroid hormones (Eckert, 1988). In females, the ovary is the primary producer of steroid hormones, producing three main gonadal steroids: testosterone (T), 17β-estradiol (E₂) and progesterone (P₄) (Koob and Callard, 1991; Gelsleichter, 2004). Follicular estrogens are necessary for both hepatic vitellogenin synthesis and reproductive tract development. Progesterone has antagonistic actions with estrogens and is considered necessary for ovulation, the continued maintenance of pregnancy, egg retention, and the simultaneous inhibition of vitellogenin synthesis (Callard et al., 1991; Tricas et al., 2000; Gelsleichter, 2004). The role that androgens play in female elasmobranchs is less clear. As in teleost fishes, T is the precursor for biosynthesis of E₂ (Tsang and Callard, 1982; Selcer and Leavitt, 1991; Pankhurst et al., 1999; Tricas et al., 2000). While some authors have associated T only with the follicular cycle of oviparous elasmobranchs (Sumpter and Dodd, 1979; Koob et al., 1986), others have found high T concentrations during the egg retention and egg laying process (Rasmussen et al., 1999; Sulikowski et al., 2004).

In vertebrate males, the testis is the principal source of reproductive hormones (Fasano et al., 1989; Pankhurst, 2006) and although the presence of several gonadal steroids has been reported in male elasmobranchs (Simpson et al., 1964; Callard, 1991a; Manire et al., 1999), the function of most of these hormones remain uncertain.
Testosterone seems to be the primary androgen in elasmobranch males, and may play a role in development and maturation of spermatocysts, and stimulation of the development of secondary sex characteristics (Callard et al., 1985; Cuevas and Callard, 1992; Sourdaine and Garnier, 1993; Tricas et al., 2000; Gelsleichter, 2004). However, other androgens such as dihydroxytestosterone (DHT), 11-ketotestosterone (11-KT), and 11-ketoandrostenedione (11-KA) have also been reported to play a role in spermatogenesis (Callard et al., 1989; Garnier et al., 1999; Manire et al., 1999). Although male elasmobranchs do produce estrogens and P₄ (Cuevas and Callard, 1992; Manire and Rasmussen, 1997; Tricas et al., 2000) and both E₂ and P₄ receptors have been identified in elasmobranch testes (Callard et al., 1985; Cuevas and Callard, 1992), their roles in male reproduction remain unclear. Several authors (Callard, 1991a; Tricas et al., 2000; Sulikowski et al., 2004) have reported elevated E₂ levels with the middle stages of spermatogenesis, while others (Manire and Rasmussen, 1997; Garnier et al., 1999) have found no clear variation in E₂ concentrations within the male reproductive cycle.

Male elasmobranch production of P₄ has been associated with spermiogenesis and spermiation (Gelsleichter, 2004), which suggests that P₄ could act as a precursor for androgen synthesis (Manire and Rasmussen, 1997; Gelsleichter, 2004). In contrast, P₄ was found to peak independently of T in other shark species (Snelson et al., 1997; Garnier et al., 1999; Gelsleichter, 2004). Studies on Squalus acantbias (Simpson et al., 1963; Simpson et al., 1964; Callard, 1991a) have found that P₄ may primarily be the substrate for the production of T and other androgens.

The endocrinology of oviparous elasmobranchs has been reported in only a few species such as Scyliorhinus canicula (Sumpter and Dodd, 1979), Leucoraja erinacea (Koob et al., 1986), Raja eglanteria (Rasmussen et al., 1999), Hemiscyllium ocellatum (Heupel et al., 1999) and Leucoraja ocellata (Sulikowski et al., 2004). Of these species, only S. canicula belongs to the family Scyliorhinidae.
The aim of the present study was to investigate the reproductive biology of the draughtboard shark, *Cephaloscylium laticeps*, a common species in southeast Australia. The draughtboard shark belongs to the family Scyliorhinidae (Springer, 1979) and the only information available states that the species is oviparous and that males reach maturity at approximately 820 mm total length (Last and Stevens, 1994).

This study explores the anatomy and histology of the gonads, correlations of changes in gonad condition with plasma levels of gonadal steroids, and assessment of the seasonality of the reproductive cycle. The majority of the data were collected from sharks immediately after capture from the wild, however some sharks were also kept in captivity to address questions associated with embryo development and periodicity of egg laying.
Chapter two - Reproduction

2.2 MATERIAL AND METHODS

2.2.1 SOURCE OF SAMPLES AND DATA COLLECTION

Draughtboard sharks were obtained from two different sources:

1) Commercial and research surveys

A total of 636 females and 468 males were collected throughout Tasmanian coastal waters as bycatch from rock lobster trap, gillnet and hook fisheries between June 2002 and April 2004 (Fig. 1.1 and 1.2, Table 2.1).

Table 2.1: Number of female and male draughtboard sharks sampled, sorted by date and location of capture in Tasmania.

<table>
<thead>
<tr>
<th>Date</th>
<th>Locations</th>
<th>Derwent Estuary</th>
<th>East Coast</th>
<th>South West Coast</th>
<th>North West Coast</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Date</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>Jun 2002</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Jul 2002</td>
<td>5</td>
<td>25</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Aug 2002</td>
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<td>-</td>
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<td>-</td>
<td>16</td>
<td>18</td>
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<td>-</td>
<td>-</td>
<td>55</td>
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<td>Dec 2002</td>
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<td>34</td>
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<td>March 2003</td>
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<td>2</td>
<td>39</td>
<td>46</td>
<td>25</td>
</tr>
<tr>
<td>April 2003</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>May 2003</td>
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<tr>
<td>Jun 2003</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>22</td>
</tr>
<tr>
<td>Jul 2003</td>
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<td>2</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Nov 2003</td>
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<tr>
<td>Dec 2003</td>
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<td>-</td>
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<tr>
<td>Jan 2004</td>
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</tr>
<tr>
<td>Feb 2004</td>
<td>6</td>
<td>40</td>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>March 2004</td>
<td>9</td>
<td>-</td>
<td>10</td>
<td>-</td>
<td>12</td>
</tr>
<tr>
<td>April 2004</td>
<td>1</td>
<td>-</td>
<td>33</td>
<td>27</td>
<td>19</td>
</tr>
</tbody>
</table>
After capture sharks were euthanased by immersion in a benzocaine bath consisting of 0.5 l of benzocaine solution (40 g benzocaine, 1 ethanol) in 8 l of seawater, and the following measurements were taken from both males and females: total length (mm TL), total body weight (g TBW), liver weight (g), and stomach weight (g).

In addition, the following data were also recorded for males: calcification, rotation, and length of the clasper (mm) (from the distal end of the metapterigyum to the tip), testes weight (g) and weights (g) of the seminal vesicles: prior to and after expression of any sperm. Ovary weight (g), oviducal gland width (mm) (at the widest part) and weight (g), presence, condition and length of egg cases were recorded from females. For the first 20 female sharks, the diameter (mm) of all follicles was measured, after which only the largest 20 follicles were measured.

The following indices were calculated:

\[
\text{Gonadosomatic Index (GS)} = \left( \frac{\text{Gonadal (testes or ovary) weight}}{\text{Total weight}} \right) \times 100
\]

\[
\text{Hepatosomatic Index (HS)} = \left( \frac{\text{Liver weight}}{\text{Total weight}} \right) \times 100
\]

Where total weight = Total body weight – (Gonadal weight + Liver weight + Stomach weight)

\[
\text{Proportion of sperm within seminal vesicle (PS) = } \left( \frac{\text{weight of seminal vesicle – weight of seminal vesicle after expression of any sperm}}{\text{weight of seminal vesicle}} \right) \times 100
\]

**Histology**

For histological analysis, ovaries, testes and seminal vesicles were fixed in Bouin’s solution, embedded in paraffin, sectioned at 7µm and stained with Haematoxylin-eosin. Stained sections were examined and photographed under a light microscope (Leica DMLB2 microscope with a Leica DFC 320 camera). Spermatogenic stages in the testis
were classified according to the descriptions of (Callard, 1991b) and (Parsons and Grier, 1992).

**BLOOD SAMPLING**

To correlate hormone levels with reproductive condition, blood samples (~3 ml) from 118 females and 113 males, were collected by caudal venipuncture using pre-heparinized syringes fitted with 22G needles. After extraction, blood samples were placed on ice for 3-6 h and then centrifuged for 5 minutes at 8000 rpm. The plasma was collected and stored at -15°C until thawed for analysis.

**2) Captive sharks**

Four female and three male sharks caught off Bruny Island (Southern Tasmania) during January 2003 were held in captivity until December 2004 at Woodbridge Marine Discovery Centre (southern Tasmania). A female shark caught at Bicheno (east coast of Tasmania) in April 2004 was held in captivity until July 2005 at the Bicheno Aquarium on the east coast of Tasmania. At Woodbridge, females and males were placed in a tank of 7 m length, 3 m width and 1.2 m depth, supplied with water at ambient temperature (10-11°C winter and 16-17°C summer) pumped from the sea. At Bicheno, the female was held in a tank of 2.2 m length, 2.2 m width and 0.8 m depth, under static holding conditions with a complete change of water once a month. The water temperature ranged from 11-12°C in winter to 21-22°C in summer. Captive sharks were monitored for the presence of egg laying and development of the embryos.
2.2.2 STEROID HORMONE MEASUREMENT

Levels of 17β-estradiol (E2), Progesterone (P4), Testosterone (T) for both females and males, and 11-Ketotestosterone (11-KT) in males were measured by radioimmunoassay (RIA). Plasma samples (200 µl) were extracted twice with ethyl acetate (1 ml) and 100 µl aliquots were transferred to assay tubes for evaporation prior to addition of an assay buffer. Assay reagents for E2, T and 11-KT were used as described by Pankhurst and Carragher (1992). Progesterone was measured using [1,2,6,7-³H] Progesterone supplied by Amerhsam Biosciences UK Ltd. The antibody is a polyclonal full serum antibody raised in sheep and was donated by Dr Ken McNatty, Wallaceville Animal Research Station, Upper Hutt, New Zealand. The assay protocol used was as described by Pankhurst and Carragher (1992). Steroid assays were validated by assessment of the slope of serial dilutions of extracted plasma against assay standards. All samples diluted parallel to standard curves. Extraction efficiency was determined from recovery of ³H– labelled steroid added to pooled aliquots of plasma. Extraction efficiencies were 86, 74, 86 and 88% for T, E2, P4 and 11-KT respectively. Each sample was analysed in duplicate and the assay values were corrected accordingly to account for the extraction efficiency. The detection limit for all assays was 0.15 ng.ml⁻¹ plasma. Interassay variability was determined by repeat measurement of a pooled internal standard and was 13 (9), 11 (9), and 9 (7) (%CV (n)) for T, E2 and P4 respectively. 11-Ketotestosterone data were measured in a single assay.
2.2.3 Classification of Reproductive Stage of the Sharks

Females

In this study the term “follicle” refers to the oocyte and surrounding theca and granulosa layers prior to ovulation, and the term “ovum” refers to the oocyte after ovulation.

The ovarian follicles were classified into four different stages based on follicle size, colour, and histological characteristics as follows: previtellogenic (PV), early vitellogenic (EV), vitellogenic (V), and mature (M) (Table 2.2, Fig. 2.1).

Based on follicle classification, oviducal gland condition and the presence of egg cases in the uterus, females were then classified into five different reproductive stages as shown in Table 2.3 and Fig. 2.2.

Males

Sperm was present in the seminal vesicles of individuals with uncalcified claspers (Figure 3.4). As calcified claspers are required for copulation (Clark and Von Schmidt, 1965), the presence of sperm could not be used as a reliable indicator of functional maturity. Therefore, clasper condition (determined by assessing the rigidity of the clasper by hand) was used to decide the sexual stage of males. Males were classified as juvenile, sub-adult and adult according to Table 2.4, and Fig. 2.5.
Table 2.2: Classification of ovarian follicles based on size, colour and histological characteristics.

<table>
<thead>
<tr>
<th>Follicle type (See Fig. 2.1)</th>
<th>Maximum Follicle Diameter (MFD)</th>
<th>Colour</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Follicular epithelium</td>
</tr>
<tr>
<td>Previtellogenic (PV)</td>
<td>MFD &lt; 7 mm</td>
<td>Very white</td>
<td>Single row of columnar cells</td>
</tr>
<tr>
<td>Early vitellogenic (EV)</td>
<td>7 mm ≤ MFD ≤ 10 mm</td>
<td>Slightly yellow</td>
<td>Pseudostratified</td>
</tr>
<tr>
<td>Vitellogenic (V)</td>
<td>10 mm &lt; MFD &lt; 30 mm</td>
<td>Yellow</td>
<td>Single layer of tall columnar cells, slightly losing the pseudostratified appearance</td>
</tr>
<tr>
<td>Mature (M) *</td>
<td>MFD ≥ 30 mm</td>
<td>Yellow</td>
<td>No histology due to size of follicle</td>
</tr>
</tbody>
</table>

* 30 mm follicular diameter was the smallest follicle found within the initial egg encapsulation stage in pregnant animals.
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Figure 2.1: Examples of follicle development (cross-sections stained with haematoxylin-eosin).

a) Presence of yolk inside the follicle. No yolk is present in PV follicle. Yolk started to be distinguished in EV follicle, and follicle is filled with yolk in V follicle. b) Detail of the follicle walls.


Table 2.3: Classification of female sexual stages based on maximum follicular diameter (MFD), oviducal gland characteristics and presence of egg cases. PV: Previtellogenic, EV: Early vitellogenic, V: Vitellogenic, M: Mature.

<table>
<thead>
<tr>
<th>Female stages</th>
<th>Type of MFD (Refer Table 2.2)</th>
<th>Oviducal gland</th>
<th>Colour</th>
<th>Width (mm) ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Juvenile (J)</td>
<td>PV</td>
<td>Translucent</td>
<td>9 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>Sub-adult (Sa)</td>
<td>EV</td>
<td>Pink</td>
<td>25 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>Adult Stage 1 (As1)</td>
<td>V</td>
<td>Light red</td>
<td>33 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>Adult Stage 2 (As2)</td>
<td>V or M</td>
<td>Dark red</td>
<td>&gt; 35</td>
<td></td>
</tr>
<tr>
<td>Adult pregnant (Ap) **</td>
<td>M</td>
<td>Dark red</td>
<td>&gt; 35</td>
<td></td>
</tr>
</tbody>
</table>

** Pregnant animals were defined by the presence of either partially formed egg cases in the oviducal gland or fully developed egg cases in the uterus.
For pregnant females, four stages of egg case development were identified (Fig. 2.3)

**Stage 1:** Posterior coiled tendrils of the egg case are developed and protrude from the posterior part of the oviducal gland.

**Stage 2:** Posterior half of the egg capsule is developed and protrudes from the oviducal gland.

**Stage 3:** The entire egg capsule is complete, but only the anterior coiled tendrils are still contained within the oviducal gland. An ovum can be distinguished inside the egg case.

**Stage 4:** The egg case is free of the oviducal gland. An ovum can be distinguished inside the egg case.
Table 2.4: Classification of male sexual stages based on clasper calcification.

<table>
<thead>
<tr>
<th>Male stages</th>
<th>Clasper condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Juvenile (J)</td>
<td>Non-calcified</td>
</tr>
<tr>
<td>Sub-adult (Sa)</td>
<td>Partially calcified</td>
</tr>
<tr>
<td>Adult (A)</td>
<td>Fully calcified</td>
</tr>
</tbody>
</table>

Figure 2.4: Seminal vesicle of juvenile draughtboard shark (cross-section stained with haematoxylin-eosin).

Figure 2.5: Macroscopic view of the development of the testes in juvenile, sub-adult and adult draughtboard sharks. Criteria for classification as given in Table 2.4
2.2.4 **Data Analysis**

Plasma hormone level comparisons were analysed by one-way ANOVA and subsequent Tukey’s multiple comparison tests (Quinn and Keough, 2002). For this and all subsequent ANOVAS, residual plots were undertaken to assess the equality of variances, and data was transformed (square root or logarithmic) where necessary. To determine the relationship between adult and pregnant females a regression analysis was made using SPSS. Assessment of reproductive seasonality of oviducal gland weight, MFD, sperm accumulated in seminal vesicle and GS$_i$ in males, was made using one-way ANOVA and Tukey’s multiple comparison tests. Unless otherwise noted, all data were analysed using SPSS (SPSS® Base 10.0).

For the analyses of monthly variations in GS$_i$ and oviducal gland for females, the sample size in the period March and April was considerably larger (n >120) than in the other months. To ensure that sample sizes were not affecting the statistical results, the ANOVA was repeated 15 times, randomly discarding a number of 50 animals each time.

The significance level was set at $P=0.05$ for all analysis.
2.3 RESULTS

2.3.1 REPRODUCTIVE DEVELOPMENT

Description of reproductive system

FEMALES

The reproductive system of *Cephaloscyllium laticeps* consisted of a single external ovary, a single ostium, a pair of oviducts, oviducal glands and uteri (Fig. 2.6). The ovary, embedded in the epigonal organ, was attached beneath the vertebral column to the anterior-dorsal body by thin connective tissue. The smallest ovaries contained follicles of less than 7 mm in diameter with non-discernible yolk. As the follicles grew by acquisition of yolk, a group of 4-6 yellow follicles of similar size began to differentiate. Follicles began vitellogenesis at about 10 mm diameter and reached the size for ovulation at around 30 mm diameter. Follicles were ovulated in pairs and each ovum enters a separate oviduct.

Macroscopic analysis was unable to separate atretic follicles from corpora lutea although either or both were present in the ovary.

While the MFD of previtellogenic, early vitellogenic and vitellogenic follicles generally correlated with juvenile, sub-adult, and adult stage 1 respectively; a small proportion of vitellogenic follicles were also present in adult stage 2. Mature follicles were present in both adult stage 2 and adult pregnant stages (Table 2.3 and Fig 2.7).

The relationship between maximum follicular diameter (MFD) and oviducal gland width was sigmoideal (Fig. 2.8). The oviducal gland initially increased to about 30 mm with small changes in MFD. Between 30-45 mm oviducal gland width, there was a substantial change in MFD. The MFD remained high for the latter parts of the oviducal gland growth (Fig. 2.8).
Figure 2.6: Reproductive system of female draughtboard shark. General view of reproductive organs. a) *In situ*, b) Dissected to show complete reproductive system, c) Cross-section of ovary from a juvenile shark (stained with haematoxylin-eosin).
Figure 2.7: Size distribution of follicles in the different maturation stages of the draughtboard shark.
MALES

A pair of equally developed testes and genital ducts (epididymis, efferent ducts, and seminal vesicles) constituted the macroscopic structure of the male reproductive system. Testes, cylindrical in shape, were enveloped in a thin layer of epigonal organ (Fig. 2.9). The proportion of epigonal organ attached to the testes decreased from juvenile to adult (Fig. 2.10). Histological sections revealed a diametric spread of spermatocyst development from the germinal zone to the efferent duct zone (Fig. 2.10). Seven stages of spermatogenesis were distinguished (Fig. 2.11). Each of the three male categories (juvenile, sub-adult, and adult) contained at least some spermatocysts with

Figure 2.8: Relationship between maximum follicular diameter and oviducal gland width in the draughtboard shark.
spermatozoa although the proportion of spermatocysts containing spermatozoa substantially increased from juveniles to adults.

Histological examination showed an inverse relationship between the different spermatogenesis stages and male sexual categories. The proportion of early stages of spermatogenesis (stage 3 and 4) decreased and the proportion of late stages (stages 6 and 7) increased with the transition from juvenile to adult categories (Table 2.5).

Figure 2.9: Reproductive system of male draughtboard shark.
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Figure 2.10: Histological sections of testes of draughtboard shark stained with haematoxylin-eosin. 
- a: Juvenile, b: Sub-adult, c: Adult, d: detail of germinal zone, e: detail of efferent duct zone.

Table 2.5: Percentage of spermatogenesis stages in male draughtboard shark.

<table>
<thead>
<tr>
<th>Male sexual stages</th>
<th>Proportion of spermatogenesis stages</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stage 1+2+3</td>
</tr>
<tr>
<td>Juvenile</td>
<td>0.76</td>
</tr>
<tr>
<td>Sub-adult</td>
<td>0.53</td>
</tr>
<tr>
<td>Adult</td>
<td>0.44</td>
</tr>
</tbody>
</table>
Figure 2.11: Male testes - microscopic view. Cross-section of testis stained with haematoxylin-eosin.

1: Stage 1, spermatocysts containing spermatoblasts with spermatogonia. Sertoli cells can be seen in the interior of spermatocysts. 2: Stage 2, Sertoli cells (nuclei) are seen migrating from the interior to the periphery of spermatocysts. 3: Stage 3, spermatocysts contain primary spermatocytes. Sertoli cells have completed the migration to the basement membrane. 4: Stage 4, spermatocysts contain secondary spermatocytes. 5: Stage 5, spermatocysts contain spermatids with elliptical nucleus. 6: Stage 6, spermatozoa are developed. 7: Stage 7, head of spermatozoa tightly packed forming a spiral shape. A: detail of 1, B: detail of 2, C: detail of 3.

Female and male tissue indices

In females, GS$_{1}$ remained the same for juveniles and sub-adults, but increased significantly in adult females. In contrast, HS$_{1}$ reached its peak in sub-adult animals (Fig. 2.12). The increase in GS$_{1}$ was most marked between adult stage 1 (As$_{1}$) and adult stage 2 (As$_{2}$), where there was a four-fold increase. The HS$_{1}$ steadily increased from juvenile to As$_{1}$, after which it significantly declined (ANOVA, P < 0.001). In males, GS$_{1}$ showed a linear and significant increase from juveniles to adults (ANOVA, P < 0.001), and HS$_{1}$ was not significantly different at any of the male sexual stages (Fig. 2.12).

The female ovulatory cycle

A general pattern was observed in follicle development in the ovary of female draughtboard sharks. When the follicle size increased beyond 7 mm diameter, a group of 4-6 follicles started to differentiate. These developed until they reached approximately 30 mm diameter when they were ovulated. As these follicles developed, a second, third and fourth group of 4-6 follicles also started to differentiate. Each of these size class groups shared the same diameter, and the differences in diameter between subsequent groups were about 4.5 mm. All pregnant females showed follicles of all size classes indicating follicular development continued throughout the ovulatory, egg retention and oviposition cycle (Fig. 2.7).

Observations from captive sharks demonstrated that eggs were released in pairs with 12-24 h intervals between each egg of the same pair. One female, maintained in a tank by itself for 15 months, laid eggs at routine intervals of approximately 28 days. All the eggs laid by this shark showed embryonic development, confirming that female draughtboard sharks stored sperm for at least 15 months.
Figure 2.12: GS\textsubscript{I} and HS\textsubscript{I} for female and male stages in draughtboard shark. Values are mean + SE. For each index, different letters show significant differences between sexual stages. Juvenile (n= 317, 156) Sub-adult (n= 69, 36) for females and males respectively. Adult males (n=249), Adult stage 1 (n= 41), Adult stage 2 (n=103), Adult pregnant (n=92). GS\textsubscript{I}: Gonadosomatic Index, HS\textsubscript{I}: Hepatosomatic index.
2.3.2 EMBRYO DEVELOPMENT

Observations from a single captive shark at Bicheno, showed that the female swam in circles just before and during oviposition to enmesh the tendrils around any object protruding from the substratum. Egg cases found by divers in the field (south and east coast of Tasmania) have always been found attached to objects protruding from the substratum. Observations from 20 eggs laid at Bicheno, showed that at early stages of development, the embryo - connected through the yolk stalk to the external yolk sac of 30 mm diameter - did not show any movement. When the embryo was about two months old, and had grown to approximately 20 mm in TL, external gills were visible. At this stage the embryo swam vigorously. The external gills reached their greatest development at about four months, when the embryo was approximately 50-60 mm TL. At about five months old, the embryo skin developed pigmentation, the external gills were reabsorbed, and the internal gills became functional. At this stage mouth movements started. At about six months of age, there was a substantial increase in total length (to about 120 mm), gill movements increased and the adult shape was evident. At this stage, the external yolk sac became markedly reduced in size. At about nine-ten months of age, the yolk stalk disappeared and the external vitelline sac was about 3-5 mm diameter. Hatching in captivity occurred when the embryo was about 11-12 months old and had reached 160-180 mm in TL (Fig. 2.13). One of the females kept in captivity at Woodbridge, laid two eggs that were successfully hatched 13 months later. The size of these two juveniles was 160 mm and 177 mm TL respectively.
Figure 2.13: Embryo development of draughtboard shark. A: Recently laid egg. B, C, D, E, F and G: 1, 2, 4, 5, 6 and 10 months after oviposition. H: Recently hatched embryo. SVE: External vitelline sac. Photos D, E and G by Bryan Hughes.
2.3.3 **Endocrine correlates**

**Females**

Hormone levels varied among fish with different follicle types. Testosterone began to increase in female sharks with vitellogenic follicles, prior to reaching its highest levels in fish with mature follicles. Estradiol levels increased steadily with follicle development reaching a peak in fish with vitellogenic follicles. The lowest levels of $P_4$ were found in early vitellogenic females and the highest levels in sharks with mature follicles. Similar plasma levels of $P_4$ were found in fish with previtellogenic and vitellogenic follicles (Fig. 2.14a). Assessment of steroid levels in adult animals showed that both $T$ and $P_4$ levels were significantly higher in As2 and Ap (ANOVA, $P<0.001$), whereas $E_2$ did not show a significant difference between any of the adult stages (Fig. 2.14b). To assess the possible role of steroids in pregnancy, plasma steroid concentrations of pregnant females were plotted against the different egg case stages. Non-pregnant animals that contained MFD $\geq 30$ mm (type M) were classified as stage 0 and were included in the plot to compare the hormone levels prior to egg case development. Due to the low number of pregnant females encountered during the study, stages 1 and 2, and stages 3 and 4 were combined. Only $P_4$ displayed a significant change, reaching a peak of 8 ng.ml$^{-1}$ during the early stages of egg case development (ANOVA, $P<0.001$) (Fig. 2.14c).

Plasma $T$ levels were low in fish with oviducal glands of width $<50$ mm and a significant increase occurred as the oviducal gland reached its maximum size (ANOVA, $P<0.001$). Estradiol increased along with oviducal gland growth. Progesterone showed a small increase in levels in the latter stages of oviducal gland growth before substantially increasing in pregnant animals (Fig. 2.15).
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Figure 2.14: Plasma levels of T, E$_2$ and P$_4$ in draughtboard shark for follicle types (a), for adult sexual stages (b), and for egg case development (c). Values are mean ± SE for (a) and (b), and ± SE for (c). For each hormone, different letters show significant differences between follicles, sexual stages and egg cases respectively. PV: Follicle previtellogenic (n=61), EV: Follicle early vitellogenic (n=10), V: Follicle vitellogenic (n=16), M: Follicle mature (n=27). As1: Adult stage 1 (n=12), As2: Adult stage 2 (n=11), Ap: Adult pregnant (n=20). Stage 0: non-pregnant animals with MFD type M (n=12). Stage 1 and 2 represent the development of the first half of the egg case (n=6) and stage 3 and 4 the completion of egg case development (n=14). T: testosterone, E$_2$: 17β-estradiol, P$_4$: progesterone.
A diagrammatic summary of the ovulatory and hormonal cycle of the draughtboard shark is presented in Fig. 2.16. Follicular development occurred in parallel with ovulation, egg retention and oviposition. Testosterone remained low during the early stages of follicle development and started to increase in sharks with late vitellogenic follicles, reaching a maximum in sharks with mature follicles. Estradiol steadily increased with follicle development up to the late vitellogenic stage. During the last stages of follicle maturation $E_2$ declined slightly, after which it remained relatively high. Progesterone remained low until just prior to ovulation, when it rapidly increased. After ovulation, $P_4$ declined and then remained at a medium level.
Figure 2.16: A summary of ovulatory and endocrine cycles in female draughtboard sharks. **PV:** Follicle previtellogenic, **EV:** Follicle early vitellogenic, **V:** Follicle vitellogenic, **M:** Follicle mature. **T:** Testosterone, **E₂:** 17β-estradiol, **P₄:** Progesterone.
**Males**

Plasma levels of 11-KT were undetectable (< 0.15 ng.ml\(^{-1}\)) in all sharks. Testosterone showed a significant increase from juveniles to adults (ANOVA, P< 0.001). Estradiol and P\(_4\) were detectable but displayed only minor and non-significant differences between sexual stages (Fig. 2.17).

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**Figure 2.17:** Relationship between plasma steroid levels and male sexual categories. Values are means ± SE. Different letters show significant differences between sexual stages. Juvenile (n=54), Sub-adult (n=6), Adult (n=53). **T:** testosterone, **E\(_2\)**: 17\(^\beta\)-estradiol, **P\(_4\)**: progesterone.
2.3.4 SEASONALITY OF REPRODUCTION

*Females*

Females of adult stage 1 formed a greater proportion of the catch in the later part of the year, while pregnant females were more common in the first half of the year. There was no seasonal trend in the intermediate stage (As2) (Fig. 2.18). A weak inverse relationship was found between adult stage 1 and pregnant females ($r^2 = 0.54$).

Concurrent with the apparent decline in pregnant females, and the increase in adult stage 1 females throughout the year, there was a decline in both the MFD and oviducal gland width (Fig. 2.19). Due to the small number of adult females present during most surveys, samples were combined into bimonthly groups.

The low number of blood samples collected each month resulted in the data being aggregated into periods of two or three months. In general, T and P₄ showed a similar trend to oviducal gland width and MFD with highest levels from the beginning to the middle of the year and the lowest levels at the end of the year. E₂ was highest at the start of the year prior to falling to the lowest level in the March-April period (Fig. 2.20).
Figure 2.18: Percentage of adult female sexual stages per month in the draughtboard shark. No adult animals were captured in September. N values are the total numbers of sharks of all stages captured during that month. **Ap**: Adult pregnant, **As2**: Adult stage 2, **As1**: Adult stage 1.
Figure 2.19: Monthly variations of maximum follicular diameter (MFD) and oviducal gland weight in adult draughtboard shark females. Values are means ± SE. For each variable, different letters show significant difference. Numbers are sample sizes. Because of the low sample size for oviducal gland weight for Sep-Oct period, this period was excluded from the statistical analysis.
Figure 2.20: Mean grouped monthly plasma steroid levels for adult female draughtboard sharks. Values are mean ± SE. Different letters show significant differences between sample times. Numbers are sample sizes, T: testosterone, E$_2$: 17β-estradiol, P$_4$: progesterone.
**Males**

No significant monthly difference was found in the proportion of sperm in the seminal vesicle (PS) and in the GS\textsubscript{i} throughout the year (ANOVA, P< 0.001). Due to the small sample sizes, May and June, August to October, and November and December samples were grouped (Fig. 2.21).

Average T values in males tended to decline throughout the year although the change was not significant. Due to small monthly sample sizes, the samples were grouped into periods of four months (Fig. 2.22).

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**Figure 2.21:** Monthly variations of gonadosomatic index and proportion of sperm in the seminal vesicle of adult draughtboard shark males. Values are means ± SE. There were no significant differences (P > 0.05) between months. Numbers are sample sizes. GS\textsubscript{i}: Gonadosomatic index, PS: proportion of sperm in seminal vesicle.
Figure 2.22: Monthly plasma testosterone (T) levels for adult male draughtboard sharks. Values are mean ± SE. Numbers are sample sizes. There were no significant differences (P > 0.05) between sampling times.
2.4 DISCUSSION

FEMALES

The entire family Scyliorhinidae displays an external type of ovary (Pratt, 1988) although the position of the ovary in the coelomic cavity varies among species. *Cephaloscyllium laticeps* showed a single ovary located in the middle of the body cavity. In contrast, several species (*Apristurus brunneus, Halaelurus canescens* and *Scyliorhinus canicula*) have two ovaries present with only one being functional, and others (e.g. *Cephalurus cephalus*) have two ovaries that are equally developed (Springer, 1979; Dodd, 1983; Cross, 1988; Balart *et al.*, 2000). Although, the genus *Cephaloscyllium* is considered to occupy the most primitive position within the family and *Cephalurus* is the most advanced group (Nakaya, 1975; Springer, 1979; Dulvy and Reynolds, 1997), no correlations appear to exist between reproductive mode and ovarian symmetry (Hamlett and Koob, 1999).

Vitellogenesis commenced in *C. laticeps* when follicles reach about 10 mm in diameter. Similar to other elasmobranch species (Dodd, 1983; Storrie, 2004), the follicular epithelium in *C. laticeps* began as a simple structure but as the follicle grew and becomes vitellogenic, the epithelium became slightly pseudostratified. The differentiation of the theca into internal and external layers in *C. laticeps* was also reported in other elasmobranch species (Dodd, 1983; Prisco *et al.*, 2002; Storrie, 2004). However, in some other species such as *Urolophus jamaicensis* (Hamlett and Koob, 1999) the theca remains undifferentiated.

Female draughtboard sharks were reproductively active all year round, showing a constant overlap between follicular recruitment and development, and egg laying. As a result, the ovary of adult sharks exhibited the full range of developing follicles in addition to atretic follicles and *corpora lutea* from previously ovulated follicles. Hormone levels were therefore a composite of different stages of follicle development. Testosterone, $E_2$ and $P_4$ were found in all the different sexual stages although their
concentrations varied between these stages. Testosterone, along with E₂, were found to be the main steroids present during follicular development and both declined, although not to baseline levels, during encapsulation and oviposition. In contrast, P₄ was only found in its highest concentrations during the ovulatory period.

Plasma E₂ levels increased during follicle development of *C. laticeps*, reaching highest concentrations (mean 4 ng.ml⁻¹) in sharks with vitellogenic follicles, prior to declining (mean 2.5 ng.ml⁻¹) as follicles reached their maximum sizes. These results were also correlated with the decrease in the HS₂ in the later stages of maturity. The substantial increase in GS₁ between stages As₁ and As₂ and the corresponding decline in HS₁ suggested that liver resources were being diverted to gonad development at this time.

The increase in E₂ concentrations with the progression from previtellogenic to vitellogenic follicles was related to the increase of steroidogenic activity, and stimulated the liver to synthesize and release vitellogenin for its uptake by the developing follicle (Dodd and Sumpter, 1984; Ho, 1987). Similar increases in E₂ have been reported for both oviparous and viviparous sharks (Manire *et al.*, 1995; Heupel *et al.*, 1999; Tricas *et al.*, 2002; Sulikowski *et al.*, 2004). *In vitro* studies on oviparous sharks also found the major production of E₂ to be correlated with intermediate sized vitellogenic follicles (Tsang and Callard, 1982; Koob and Callard, 1991; Callard *et al.*, 1993).

Estradiol levels were found to decline in *C. laticeps* as follicles reached their maximum size prior to ovulation, after which E₂ levels rose to approximately equivalent levels of mature follicles prior to egg case development (3-3.5 ng.ml⁻¹). Although a decrease in E₂ levels before ovulation was also reported in *Lencoraja erinacea*, the plasma steroid concentrations in that species decreased to baseline levels during oviposition (Koob *et al.*, 1986). The present results also contrasted with seasonal oviparous sharks where E₂ was at its highest concentration prior to ovulation (Sumpter and Dodd, 1979; Heupel *et al.*, 1999; Rasmussen *et al.*, 1999). High levels of E₂ before ovulation were also observed in viviparous sharks (Manire *et al.*, 1995; Snelson *et al.*, 1997; Koob and Callard, 1999;
Tricas et al., 2000). The decline in E₂ as follicles reach maximum size in C. laticeps may be associated with changes in plasma levels of T and P₄. Plasma T levels increased (mean 1.5 ng.ml⁻¹) during the latter stages of maturation of the follicles of C. laticeps, suggesting a down regulation of P₄ aromatase activity (regulating conversion of T to E₂), leading to accumulation of T rather than its onward conversion to E₂. Similar observations in T levels have been found in several other species of shark (Dodd et al., 1983; Callard et al., 1993; Manire et al., 1995). Moreover, in teleost fishes, most authors have found maximum T concentrations just prior to ovulation or during final oocyte maturation (reviewed in Pankhurst, 2006). Current results found that T levels remained high (mean 1.5 ng.ml⁻¹) after ovulation in C. laticeps. Similar findings were reported for Leucoraja ocellata and Raja eglanteria (Rasmussen et al., 1999; Sulikowski et al., 2004), but contrast with the results of (Sumpter and Dodd, 1979; Koob et al., 1986) where T was only elevated during follicle growth in Leucoraja erinacea and Scyliorhinus canicula. Both the increase in E₂ and the maintenance of high T concentrations after ovulation observed in the present study, may have resulted from the continual recruitment of follicles into vitellogenesis that occurred during the reproductive process. This is similar to teleost fishes with asynchronous gamete development where there is often no fall in plasma steroids after ovulation, as there are further clutches of follicles undergoing vitellogenesis (Pankhurst et al., 1999).

Several studies have also suggested additional roles that estradiol and androgens may perform during the reproductive cycle of elasmobranchs. Estradiol has been suggested to play a role in development and function of the reproductive tract (particularly the oviducal gland). Gilmore (1983) and Koob and Callard (1991) suggested that endocrine factors may induce egg capsule secretion, and Reese and Callard (1991) identified an E₂ receptor in the oviduct of Leucoraja erinacea. As C. laticeps reproduced throughout the year, the role E₂ plays in the growth of the oviducal gland was difficult to determine with E₂ levels also being associated with recruitment of the next batch of maturing
follicles. A possible role of E₂ in the storage of spermatozoa by the oviducal gland was suggested by (Gelsleichter, 2004). This last author reported that females of *Sphyrna tiburo* from populations exhibiting high rates of infertility, showed a reduced peak in preovulatory E₂ related with an apparent decline in the viability of stored spermatozoa by the oviducal gland (Gelsleichter, 2004). As *C. latioeps* was able to store viable spermatozoa, the high levels of E₂ found in draughtboard sharks after ovulation may indicate that E₂ has also a role in the maintenance of spermatozoa. Estradiol has also been associated with the expression of relaxin, the hormone required to enlarge the cervix to allow egg passage during oviposition. Although relaxin has been identified in the ovaries of several sharks and its effects are considered to be estrogen dependent (Tsang and Callard, 1983; Callard *et al.*, 1988; Koob and Callard, 1991), this hormone and its interactions with E₂ were not investigated in this study. There were, however, no signs of a change in E₂ in females in the present study with fully encapsulated ova that would support this hypothesis.

Androgens may also be associated in the regulation of sperm storage by the oviducal gland. Studies on *Sphyrna tiburo* females showed that T levels were highest for the 4-5 months between the mating and the ovulatory period suggesting that T is involved in the regulation of sperm storage by the oviducal gland (Manire *et al.*, 1995). As E₂ was correlated with oviducal gland function, it is possible that the oviducal gland’s ability to store sperm is regulated by a combination of both E₂ and T. Androgens might play a role in encapsulation and oviposition, as levels of T increased by the onset of breeding activity and remained high during egg laying in *Raja eglanteria* (Rasmussen *et al.*, 1999), and elevated T levels were found during egg case formation and oviposition in *Leucoraja ocellata* (Sulikowski *et al.*, 2004). This hypothesis is also supported with the finding in the present study, where T levels remained high in *C. latioeps*. Further assessment of the possible roles of both E₂ and T in *C. latioeps* will require manipulative experiments.
Plasma levels of P₄ remained low (mean < 1.5 ng.ml⁻¹) during the follicular phase in *C. laticeps*, but showed a marked peak (mean 8 ng.ml⁻¹) in animals carrying partially formed egg cases without an ovum, indicating that ovulation had not yet taken place (Fitz and Daiber, 1963). A similar peak in P₄ levels just before ovulation was observed in other oviparous species (Koob *et al*., 1986; Heupel *et al*., 1999; Rasmussen *et al*., 1999). After ovulation, P₄ levels in *C. laticeps* fell, but still remained elevated during egg encapsulation and oviposition.

Although studies on chondrichthyan endocrinology have advanced in the last few years, there is still insufficient information to present a unified pattern of endocrine control that encompasses all the oviparous species. Several studies have shown that the same steroids hormones appear to behave differently in different oviparous species. For example, while T concentrations were elevated during egg capsule formation and oviposition in *Raja eglanteria* (Rasmussen *et al*., 1999) and *Leucoraja ocellata* (Sulikowski *et al*., 2004), in *Leucoraja erinacea* (Koob *et al*., 1986) T production was reported to be very low during the egg case production and oviposition. Although there is no explanation for the differences in endocrine patterns among different species, it is important to note that while some studies have been undertaken in the wild (Sulikowski *et al*., 2004; Sulikowski *et al*., 2005a), others were done in captivity (Koob *et al*., 1986). Furthermore, several authors have reported differences in the reproductive cycle between sharks held in captivity in aquariums and sharks captured from the wild (Carrier *et al*., 1994; Heupel *et al*., 1999). Heupel (1999) found *Hemyscillum ocellatum* to adjust its reproductive cycles from a seasonal cycle in the wild to an annual cycle in captivity. Different factors such as stress of capture or husbandry could produce differences in hormone levels or patterns of secretion (Wardle, 1981; Cliff and Thurman, 1984). A decrease in steroid hormone levels in response to confinement or acute stress were reported for the teleost fish *Onchorynchus nerka* and *Acanthopagrus butcheri* (Haddy and Pankhurst, 1999; Kubokawa *et al*., 1999). Sampling strategies, particularly the time of sampling in relation to the
reproductive cycle, could also account for differences in the reported roles of these hormones. However, to date there is insufficient information to understand if the differences in steroid hormone behaviour for oviparous species are a result of the sampling methodology or are related to differences between species. Caution should be applied when comparisons or generalization between oviparous species are made.

**MALES**

Male *C. laticeps* had two equally developed testes and reproductive ducts. The origin and propagation of spermatoocytes within the testes was characterised by a diametric development, and was consistent with other species of carcharhiniformes that have been studied (Pratt, 1988). In *C. laticeps*, the proportion of mature spermatoocytes and plasma T concentrations increased with sexual maturation. Similar results have been found for both oviparous and viviparous elasmobranchs (Rasmussen and Gruber, 1993; Heupel *et al*., 1999; Manire *et al*., 1999; Tricas *et al*., 2000; Gelsleichter *et al*., 2002; Sulikowski *et al*., 2004). The results of the present study support the view of (Callard *et al*., 1985; Sourdaine *et al*., 1990; Sourdaine and Garnier, 1993) that T plays a major role in the regulation of testis development. In contrast, detectable levels of 11-KT were not found in *C. laticeps*. Although, 11-KT is the main androgen reported for teleost fishes (Pankhurst, 2006), and despite 11-KT being reported in both *Sphynx tiburo* (< 2.21 ng.ml<sup>-1</sup>) (Manire *et al*., 1999) and *Scylliorhinus canicula* (< 0.27 ng.ml<sup>-1</sup>) (Garnier *et al*., 1999), this hormone appears to play no role in *C. laticeps*.

In *C. laticeps*, clasper length and testis weight, along with the proportion of spermatoocytes containing spermatozoa, increased with increasing T concentrations. Similar results have been reported for other elasmobranch species (Callard, 1991a; Rasmussen and Murru, 1992; Sulikowski *et al*., 2005b). No androgen receptors have so far been identified in male reproductive tracts (Callard, 1991b; Conrath and Musick,
2002), however, it is unlikely that androgens would not be involved in the development
of male reproductive organs. As androgens have such widespread actions as anabolic
agents in a wide range of vertebrate tissues (Eckert, 1988), it is likely that they would
exert similar effects on chondrichthians. It also cannot be excluded that other
unmeasured androgens might also play a role in male reproductive development. As
both clasper length and testis weight increased simultaneously in *C. laticeps* with the
increase in T levels, it is suggested that T regulated both events.

Various authors (Heupel *et al.*, 1999; Tricas *et al.*, 2000) have associated T
concentrations with different stages of spermatogenesis suggesting the possible role of
T in regulating the final stages of sperm maturation. Plasma T concentrations increased
during the middle to late stages of spermatogenesis in some species such as *Hemiscyllium
ocellatum* (Heupel *et al.*, 1999), *Leucoraja ocellata* (Sulikowski *et al.*, 2004) and *Dasyatis sabina*
(Tricas *et al.*, 2000). Cuevas and Callard (1992) reported that androgen receptors were
primarily localized in the early stages of spermatogenesis in the testis of *Squalus acantbias*,
suggesting that androgens may regulate the development of spermatogonia. Although
the proportion of spermatocysts containing spermatozoa increased from juvenile to
adult animals in parallel with the increase in T concentrations in *C. laticeps*, more
experimental studies need to be done to understand the role of T and possibly other
androgens, in regulating spermatogenesis.

Estradiol was present at very low plasma concentrations in male *C. laticeps* (< 0.20
ng.ml⁻¹), similar to levels in *S. canicula* where plasma concentrations did not exceed 0.05
ng.ml⁻¹ (Garnier *et al.*, 1999). In males of the elasmobranch species *Dasyatis sabina* and
*Sphyra tiburo*, relative changes in E₂ levels have been reported throughout the year, but
the absolute value of E₂ concentrations (< 0.27 and < 0.072 ng.ml⁻¹ respectively) were
similar to, or lower than for *C. laticeps* (Manire and Rasmussen, 1997; Tricas *et al.*, 2000).
Several studies have shown elevated E₂ levels to be associated with the early to middle
stages of spermatogenesis (Manire and Rasmussen, 1997; Snelson *et al.*, 1997; Tricas *et
Furthermore, on the basis that estrogen and androgen receptors were found to be higher in the regions of premeiotic stages of spermatogenesis, (Callard, 1991a) suggested that intratesticular estrogens and androgens may cooperate in regulating the early stages of spermatogenesis. Estradiol is also implicated in spermatogonial proliferation in teleosts (Pankhurst, 2006). Gelsleichter (2004) proposed that circulating levels of \( E_2 \) might not reflect its rate of production or function in testis, if the effects of this hormone were mainly paracrine. Furthermore, studies in \( S.\ canicula \) showed that plasma \( E_2 \) reaches maximum values of 0.05 ng.ml\(^{-1}\), while the maximum value in testicular \( E_2 \) was 0.50 ng.ml\(^{-1}\) (Garnier et al., 1999). It is not known whether \( E_2 \) plays a role in modulating spermatogenesis in \( C.\ laticeps \); however, any such function is not reflected in changing plasma levels of \( E_2 \).

Detectable levels of \( P_4 \) (~ 1 ng.ml\(^{-1}\)) were found in male \( C.\ laticeps \) at all sexual stages; however, there were no marked changes with changing sexual stage. This contrasts with \( Sphyrna\ tiburo \) and \( Negaprion\ brevirostris \) where \( P_4 \) increased with testicular development (Rasmussen and Gruber, 1993; Manire and Rasmussen, 1997). Because \( P_4 \) receptors were found to be higher in the post meiotic stage of spermatogenesis, (Cuevas and Callard, 1992) suggested that \( P_4 \) is primarily associated with spermiogenesis and spermiation. Several authors (Manire and Rasmussen, 1997; Gelsleichter, 2004) have identified \( P_4 \) as a possible precursor of androgen synthesis, while others reported that \( P_4 \) peaks independently of \( T \) (Snelson et al., 1997; Garnier et al., 1999; Gelsleichter, 2004). The role that \( P_4 \) plays in male \( C.\ laticeps \) remains unclear. As there was no increase in \( P_4 \) with sexual development, it is unlikely that plasma \( P_4 \) was involved in spermatogenesis, with levels of \( P_4 \) instead reflecting the rate at which it was being converted to downstream steroid metabolites such as \( T \).
SEASONALITY OF REPRODUCTION AND EGG LAYING BEHAVIOUR

The positive relationship between oviducal gland width and MFD suggests that the draughtboard shark displayed a continuous breeding cycle, although within this cycle there were peaks in both MFD and the oviducal gland weight between January and June indicating that this was a preferred period for egg deposition. Elevated values of T and P4 also coincided with this period. A greater proportion of adult stage 1 females were found towards the end of the year and these females contained ovarian follicles in the advanced stages of vitellogenesis with corresponding elevated levels of E2. This suggests that C. laticeps was reproductively active throughout the year with an increase in mature egg production in the first half of the year (austral summer and autumn).

This type of reproductive strategy, where animals are reproductively active throughout the year but tend to exhibit one or two peaks in activity (Wourms, 1977), has also been reported in other oviparous elasmobranchs including some scyliorhinid sharks (Sumpter and Dodd, 1979; Cross, 1988; Richardson et al., 2000; Sulikowski et al., 2004). In contrast, other oviparous species have well defined annual reproductive cycles with reproduction restricted to a shorter period of the year. For example, in Hemiscyllium ocellatum, females were found to lay eggs between August and January (Heupel et al., 1999), and in Raja eglanteria between January and August (Rasmussen et al., 1999).

On the basis of the limited information available, there appear to be two basic reproductive strategies in oviparous chondrichthians. One group has a short incubation period (less than 6 months), a limited time of sperm storage (at least 8 months), and a shorter time between ovulation of each successive pair of eggs (less than about 1 week). This group displays a seasonal reproductive cycle and tends to be found at lower latitudes. In contrast, higher latitude species tend to be able to reproduce all year round, have longer incubation periods (longer than 6 months), are able to store sperm for
longer period (at least two years) and oviposition of successive pairs of eggs occurs at longer intervals (longer than one week) (Table 2.6). Similar results were found by (McLaughlin and O’Gower, 1971) in their study on the genus Heterodontus, suggesting that the breeding season is longer among cool water species. However, in chondrichthyan males, there is no clear trend between the reproductive cycles and fish from low or high latitudes. For *C. laticeps* males, the lack of marked annual changes in GS, proportion of spermatozoa in the seminal vesicles, or T levels would suggest that males are able to produce spermatozoa all year round. Other high latitude oviparous species such as *Leucoraja ocellata* and *Amblyraja radiata*, also continuously produce mature spermatocysts throughout the year (Sulikowski et al., 2004; Sulikowski et al., 2005a). In contrast, for morphological and hormone data some species from high and low latitudes such as *Hemiscyllium ocellatum* and *Scyliorhinus canicula* show clear seasonality in their reproductive cycles (Garnier et al., 1999; Heupel et al., 1999). Males of *H. ocellatum* were reported to have red and swollen claspers (indicating the mating season) from July to November, with a peak in androgen concentrations from July to October (Heupel et al., 1999). In *S. canicula* males, both gonadal activity and T concentration were found to peak in winter (Garnier et al., 1999; Henderson and Casey, 2001).

Among teleosts, fish at higher latitudes tend to have a markedly seasonal and synchronised reproductive cycle within the population, while species at lower latitudes are likely to display multiple spawning and less population synchrony (review in (Pankhurst, 2006)). Teleost fishes tend to produce large numbers of eggs and larvae that are dependent on environmentally driven phytoplankton and zooplankton production cycles to survive. These cycles are more seasonal in higher latitudes. In contrast, oviparous chondrichthyan young hatch as small juveniles and do not undertake a larval phase that is dependent on seasonal planktonic production cycles. The fact that high latitude elasmobranchs do not show seasonality suggests that the large size at hatching uncouples juveniles from dependence on seasonal production cycles.
There are still significant gaps in understanding the process by which the environmental signals are transmitted into the endocrine process that control reproduction. However, in teleost fishes, there is evidence that the main factor driving high latitude species is photoperiod, followed by temperature and social interactions, while in low latitudes species this hierarchy may be inverted (Pankhurst and Porter, 2003).

In chondrichthyans, one possible explanation for seasonality among low latitude species relates to sperm storage. In oviparous species found in warm waters the period of sperm storage is usually coincident with the winter months (Rasmussen et al., 1999) (Table 2.6). Furthermore, studies on androgen concentration and sperm production by males from warm water species, showed that there is an inverse relationship between androgen concentrations and temperature (Garnier et al., 1999; Heupel et al., 1999), and that sperm production and its accumulation by the seminal vesicle is higher during the winter months (Heupel et al., 1999). It could be hypothesised that the short periods of sperm storage reported for elasmobranch females in lower latitudes may reflect the inability of sperm to survive for long periods at elevated temperatures. An effect of this type would truncate the period of reproduction among low latitudes species.

It is important to note that other selective forces such as juvenile mortality, growth rate, size and age at maturity, offspring size, fecundity and longevity will influence the differences in life history strategies of chondrichthyan species between high and low latitudes (Stevens, 1999; Frisk et al., 2001; Cortés, 2004). However, the discussion of these parameters is beyond the scope of the present study.

The relationship between female and male reproductive cycles can vary. While both sexes of several species have a synchronized reproductive cycle (Heupel et al., 1999; Kyne and Bennett, 2002; Sulikowski et al., 2004), there is an un-coupling of reproductive activity in others (Ellis and Shackley, 1997; Henderson and Casey, 2001). In Scliorhinus canicula, the cycle of females and males was not synchronised with GS1 peaking during
May in females, and November and December in males (Henderson and Casey, 2001). In *Hemyscillum ocellatum*, females lay eggs from August to January and males have the highest volume of sperm in the epididymis during August to November, indicating a synchronous cycle (Heupel *et al.*, 1999). For females that are able to store sperm (Pratt, 1993), there is no necessity to have a synchronous reproductive cycle between both sexes. In the case of *C. laticeps*, females and males presented unsynchronised cycles. Although females were able to reproduce all year round, a peak in egg deposition was found between January to June. However, males did not show a peak in sperm production at any time of the year.

Observation of *C. laticeps* held in captivity for 15 months, showed that females were able to store sperm for this extended period. Although, migratory behaviour, peaks in sexual aggregations and the benefit of different copulation and fertilization times have all been hypothesized to explain sperm storage in vertebrates (Dodd *et al.*, 1983; Birkhead and Moller, 1993; Pratt, 1993; Conrath and Musick, 2002), there is limited biological information available for *C. laticeps* to support any of these theories. However, the presence of mature adult females and males throughout the year would suggest that sperm storage was not associated with disaggregation of the sexes.

Studies on the mating behaviour of scyliorhinid sharks show that the male initially bites the tail and then the pectoral axilla of the female (Castro *et al.*, 1988). No mating scars have been distinguished in any of the female *C. laticeps* sampled. However, the skin of the draughtboard shark is very thick and this could prevent the damage that gives rise to mating scars in other species. On the base of high levels of androgen during the mating season, T has been associated with copulatory activity in elasmobranch species (Rasmussen and Gruber, 1993; Heupel *et al.*, 1999; Tricas *et al.*, 2000). However, Crews (1984) and Parsons and Grier (1992) suggest that a peak in testicular development or circulating levels of gonadal hormones may not necessarily coincide with the peak in the mating season. In species such as *Mustelus grisens* and *Mustelus manazo* there is a 6 month
delay between the peak in GS₁ and the mating season (Parsons and Grier, 1992). Currently the mating season (if there is one) in *C. laticeps* is not known.

**INCUBATION PERIOD**

The different stages of embryonic development in *C. laticeps* were similar to those reported for other scyliorhinids (Mellinger *et al.*, 1986; Castro *et al.*, 1988). In *C. laticeps*, the incubation period in captivity was similar to the incubation time suggested by Castro (1998) for *Scyllorhinus retifer* in the wild. In species from temperate waters such as *Leucoraja erinacea* (Richards *et al.*, 1963) and *Raja clavata* (Ellis and Shackely, 1995), embryo development takes between six months to one year; while in species from warm waters such as *Raja eglanteria* (Luer and Gilbert, 1985) and *Hemiscyllium ocellatum* (West and Carter, 1990) the incubation period takes around four-five months (Table 2.6). However, incubation time in captive *Scyllorhinus canicula* varied according to the temperature of the aquarium, being shorter (180 days) for egg deposited in warm water compared to those deposited in cold waters (285 days) (Capapé, 1977). For this species, the increased water temperature was considered to increase the metabolic rate of development of the embryo. For sharks with incubation times of less than 12 months, the amount of time the egg is exposed to warmer (summer) or cooler (winter) temperatures may account for variability in incubation times. For *C. laticeps*, with a incubation time of approximately 12 months, temperature-generated variation in incubation period was unlikely as each egg would appear to experience the same total exposure to winter and summer temperatures, irrespective of the time of oviposition. This assumes that temperature-dependent effects on development do not change with stage of development.
In summary, female *C. laticeps* presented an external type ovary with follicles starting vitellogenesis at 10 mm diameter and maturing at 30 mm. Testosterone and E$_2$ played a major role during the follicular phase, while P$_4$ peaked during the ovulatory phase. Females were reproductively active all year round with a seasonal period between January to June where a greater proportion of eggs were laid. Deposition of the eggs occurred once a month, and the incubation period is about 12 months. Male *C. laticeps* presented diametric type testes. Testosterone played a major role changing according to the sexual stage. Males were able to produce sperm all year round. The mating season for this species (if there is one) remains to be determined.
Table 2.6: Reproductive information on oviparous species. Data was not included in the table when it was considered unreliable due to sampling strategies or small sample size.

*Roja eglanteria* lives either in both high and low latitudes.

** No data on water temperature was available; but most of the studies by Dodd were in captivity in cold water.

<table>
<thead>
<tr>
<th>Species name</th>
<th>Reproductive activity Females</th>
<th>Sperm storage</th>
<th>Time between laying</th>
<th>Time between each egg</th>
<th>Incubation time</th>
<th>Latitude</th>
<th>Location</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. laticeps</td>
<td>Year round; peak in Jan - June</td>
<td>At least 15 months</td>
<td>20-28 days</td>
<td>12-24 hrs</td>
<td>12 months</td>
<td>High</td>
<td>Tasmania</td>
<td>(This study)</td>
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<tr>
<td>R. eglanteria</td>
<td>Year round; peak in Jan-Aug</td>
<td>At least 3 months</td>
<td>4-5 days</td>
<td>Min-hours</td>
<td>3 months</td>
<td>Low</td>
<td>Captivity (20-22°C)</td>
<td>(Luer and Gilbert, 1985)</td>
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<tr>
<td>R. clavata</td>
<td>Year round; peak in spring</td>
<td>No data</td>
<td>No data</td>
<td>No data</td>
<td>3 months</td>
<td>High</td>
<td>Delaware Bay, USA (9°C)</td>
<td>(Fitz and Daiber, 1963)</td>
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<tr>
<td>S. retifer</td>
<td>No data</td>
<td>At least 15 weeks</td>
<td>0-2 days</td>
<td>No data</td>
<td>19 weeks</td>
<td>High</td>
<td>Captivity (14.9°C)</td>
<td>(Ellis and Shackely, 1995)</td>
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<td>A. brunneus</td>
<td>No data</td>
<td>No data</td>
<td>48 hrs</td>
<td>24 hrs</td>
<td>No data</td>
<td>High</td>
<td>Captivity (11-16°C)</td>
<td>(Holden et al., 1971)</td>
</tr>
<tr>
<td>P. xaniurus</td>
<td>No data</td>
<td>At least 843 days</td>
<td>15 days</td>
<td>From minutes up to 8 days</td>
<td>8.5 months</td>
<td>High</td>
<td>Captivity (11.7-12.8°C)</td>
<td>(Castro et al., 1988)</td>
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<tr>
<td>H. regani</td>
<td>Year round; peak in Dec-May</td>
<td>No data</td>
<td>No data</td>
<td>No data</td>
<td>14 months (10°C)</td>
<td>High</td>
<td>British Columbia, Canada</td>
<td>(Jones and Geen, 1977)</td>
</tr>
<tr>
<td></td>
<td>Year round</td>
<td>No data</td>
<td>No data</td>
<td>No data</td>
<td>No data</td>
<td>High</td>
<td>California, USA</td>
<td>(Cross, 1988)</td>
</tr>
<tr>
<td></td>
<td>Year round</td>
<td>No data</td>
<td>No data</td>
<td>No data</td>
<td>No data</td>
<td>High</td>
<td>Cape Town (98-515m)</td>
<td>(Richards on et al., 2000)</td>
</tr>
<tr>
<td>Species</td>
<td>Life Cycle</td>
<td>Temperature</td>
<td>Location</td>
<td>References</td>
<td></td>
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<tr>
<td><em>S. canicula</em></td>
<td>Year round; peak in May</td>
<td>High</td>
<td>Tunisia (Capapé, 1977)</td>
<td>(Capapé, 1977)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>177-180 days (19-24°C)</td>
<td>(Henderson and Casey, 2001)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No data</td>
<td>No data</td>
<td>West coast of Ireland</td>
<td>(Dodd et al., 1983)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No data</td>
<td>No data</td>
<td>No data but probably cold waters**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No data</td>
<td>No data</td>
<td>Captivity (14°C)</td>
<td>(Mellinger, 1983)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Year round; peak in spring and summer</td>
<td>High</td>
<td>Plymouth, England</td>
<td>(Ford, 1921)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 months; peak in June-July</td>
<td>No data</td>
<td>British waters</td>
<td>(Ellis and Shackley, 1997)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>No data</td>
<td>No data</td>
<td>Captivity (16°C)</td>
<td>(Mellinger et al., 1986)</td>
<td></td>
<td></td>
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<tr>
<td><em>L. erinacea</em></td>
<td>Year round; peak in Nov-Jan and June-Jul</td>
<td>High</td>
<td>Connecticut and Rhode Island</td>
<td>(Richards et al., 1963)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Year round; peak in spring and fall</td>
<td>No data</td>
<td>Delaware Bay, USA (°C)</td>
<td>(Fitz and Daiber, 1963)</td>
<td></td>
<td></td>
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<tr>
<td><em>H. ocellatum</em></td>
<td>Aug-Jan</td>
<td>Low</td>
<td>Heron Island, Australia (21-28°C)</td>
<td>(Heupel et al., 1999)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Year round</td>
<td>No data</td>
<td>Captivity (25°C)</td>
<td>(West and Carter, 1990)</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td><em>A. radiata</em></td>
<td>Year round; peak in September</td>
<td>High</td>
<td>Western Maine, USA (°C)</td>
<td>(Sulikowski et al., 2005a)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><em>L. ocellata</em></td>
<td>Year round peak in</td>
<td>High</td>
<td>Western Maine, USA (°C)</td>
<td>(Sulikowski et al., 2004)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Species</td>
<td>Activity Period</td>
<td>Genera</td>
<td>Months</td>
<td>Temperature</td>
<td>Location</td>
<td>Reference</td>
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</tr>
<tr>
<td>G. melastomus</td>
<td>Year round, peak in summer and winter</td>
<td>No data</td>
<td>No data</td>
<td>No data</td>
<td>High</td>
<td>South of Portugal</td>
<td>(Costa et al., 2005)</td>
<td></td>
</tr>
<tr>
<td>P. extenda</td>
<td>Year round; peak in summer</td>
<td>No data</td>
<td>No data</td>
<td>No data</td>
<td>High</td>
<td>Puerto Quequén, Argentina</td>
<td>(Braccini and Chiaramonte, 2002)</td>
<td></td>
</tr>
<tr>
<td>R. maculata</td>
<td>Year round</td>
<td>No data</td>
<td>No data</td>
<td>No data</td>
<td>5 months (13-18°C)</td>
<td>High</td>
<td>Plymouth, England</td>
<td>(Clark, 1922)</td>
</tr>
<tr>
<td>R. brachyura</td>
<td>Year round</td>
<td>5-6 weeks</td>
<td>No data</td>
<td>No data</td>
<td>4 months (9-18°C)</td>
<td>High</td>
<td>Plymouth, England</td>
<td>(Clark, 1922)</td>
</tr>
<tr>
<td>R. microcellata</td>
<td>No data</td>
<td>No data</td>
<td>No data</td>
<td>No data</td>
<td>5-7 months</td>
<td>High</td>
<td>Captivity (14.2-16.3°C)</td>
<td>(Koop, 2005)</td>
</tr>
<tr>
<td>H. portusjacksoni</td>
<td>Aug and Sep.</td>
<td>No data</td>
<td>Approx 13 days</td>
<td>No data</td>
<td>No data</td>
<td>Low</td>
<td>Central coast of New South Wales, Australia</td>
<td>(McLaughlin and O’Gower, 1971)</td>
</tr>
<tr>
<td>C. plagiosum</td>
<td>No data</td>
<td>No data</td>
<td>No data</td>
<td>No data</td>
<td>4 months</td>
<td>Low</td>
<td>Captivity (24-26°C)</td>
<td>(Tullis and Peterson, 2000)</td>
</tr>
</tbody>
</table>
CHAPTER THREE:

Non-lethal assessment of reproductive parameters: draughtboard shark - a case study
3.1 Introduction

The reduction and collapse of global fish stocks due to over exploitation is increasing, with several species nearing extinction (Dulvy et al., 2003; Cortés, 2004; Mullon et al., 2005). These declines have called for conservation strategies to be developed for marine resources such as; implementing fisheries management policies, establishing a global system of marine protected areas (MPAs) where fisheries are restricted, or declaring some species as threatened or endangered where their capture is prohibited. Currently, due to the potential for chondrichthians to be strongly susceptible to overfishing, the impact on fishing chondrichthyan species around the world is the focus of considerable international concern (Stevens et al., 2000).

Chondrichthyan populations are harvested by commercial, artisanal, and recreational fisheries (Bonfil, 1994; Walker, 1998) and while some species are the direct target of the fishery others are taken as bycatch. It is commonly accepted that chondrichthians have slow growth, long life span, late sexual maturity, a low fecundity, long gestation period and low natural mortality compared to teleost fish (Cortés, 2000; Stevens et al., 2000). These life history strategies make this group very vulnerable to high levels of fishing pressure and have led to a number of conservation and management strategies in an attempt to protect chondrichthyan populations from decline (Simpendorfer and Donohue, 1998; Stevens et al., 2000; Musick, 2004).

In order to manage chondrichthyan species, it is necessary to develop demographic models that address their vulnerability to exploitation. Understanding their life history strategies, particularly their reproductive cycles, is fundamental if species are to be managed so that they reproduce to maintain appropriate population levels. Knowledge of the size at which animals mature, is required to ensure that the species has sufficient time to replace the stock prior to being harvested or impacted upon by fishing, and the spatial and temporal timing of reproduction is therefore essential for sustainable
fisheries management to ensure that fishery activities are minimised during reproductive periods.

Clasper calcification is the most common external method used to assess sexual maturity in male chondrichthians (Clark and Von Schmidt, 1965). However, not all species (e.g. seven gill sharks) alter the degree of calcification in their claspers as they reach maturity, therefore the sacrifice of these males is necessary. In females, as macroscopic examination of the ovaries from dissected animals is the only method to assess sexual maturity, the sacrifice of females is always required. However, there are many circumstances where killing the animal is inappropriate as in the case of endangered or protected species, or species residing in MPAs. Similarly it may be inappropriate to sacrifice bycatch species that would normally be returned to the water alive. For studies on the reproductive biology and management of these species there is a need to obtain data on reproduction without the requirement to kill the animal. Furthermore, any investigation of the temporal and spatial timing of reproduction, for both sexes, currently requires the examination of gonadal condition after dissection of the animal.

Gonadal steroids, obtained from blood samples, could be used as endocrine markers to determine the reproductive status of sharks without the need to kill and dissect the shark. Only a few studies have compared the levels of plasma steroid hormones between juvenile and adult chondrichthians, and all of these suggest that hormones could be used as an indicator of maturation status (Rasmussen and Gruber, 1990; Rasmussen and Murru, 1992; Rasmussen and Gruber, 1993; Gelsleichter et al., 2002). However, despite these results, only one study has linked plasma steroid hormones to histological and morphological studies of the gonads to address size at onset of sexual maturity (Sulikowski et al., 2005b).
This study has demonstrated that changes in plasma levels of reproductive hormones are associated with maturation for both sexes in *C. laticeps*, and that reproductive hormones reflect the temporal timing of reproduction (see chapter 3). This chapter examines whether the endocrine markers, testosterone (T), 17β-estradiol (E₂) and progesterone (P₄) could be used as an unambiguous indicator of sexual maturity in both males (where gonadal sexual maturity might occur in advance of clasper calcification) and females (where there are no external morphological markers of maturation), and therefore eliminate the need for sacrificing sharks for subsequent macroscopic examination of the gonads. The results from the assessment were then applied to draughtboard sharks sourced from a marine protected area where only non-destructive sampling methods are appropriate.
Chapter three - Non-lethal assessment of reproductive parameters

3.2 MATERIALS AND METHODS

3.2.1 SOURCE OF SAMPLES AND DATA COLLECTION

Draughtboard sharks were obtained from two different sources:

1) Commercial and research surveys: Animals from these surveys (see chapter 3, section 3.2.1) were used to calculate size at maturity and to validate plasma steroid levels against macroscopic examination of the gonads.

2) Surveys at a marine reserve: Eighty-two females and 54 males were caught between May 2002 and May 2003 using rock lobster traps in the Crayfish Point Reserve in southern Tasmania (Fig. 1.2a). Total length and total weight for each sex and clasper length for males were recorded. Blood samples (as described in Chapter 2, section 2.2.1) were taken prior to releasing the sharks.

For all sharks, steroid hormones were measured as described in section 2.2.2.

3.2.2 DATA ANALYSIS

To determine the maturity of sharks, from the marine reserve of unknown maturation stage, that were released immediately after taking blood, plasma hormone concentrations were compared with the hormone concentrations from sharks of known maturity stages.
Size at maturity of sharks dissected

Reproductive stages of the sharks were described in section 2.2.3. For this chapter adult females (As1, As2 and Ap) were combined into a single adult group. For both sexes, juveniles and sub-adults were combined into a single group called juveniles.

To establish size at maturity of all sharks sampled in this study, oviducal gland width (for females) and clasper length (for males) were compared to total length. Oviducal gland width and clasper length were chosen as they were morphological parameters that progressively grew with maturity, and were independent of the reproductive cycle. In contrast, gonadal weight varied within mature animals depending on the cyclic gametogenesis stage of the ovary or testis.

To determine the size at which 50% of the sharks were mature, animals were grouped as either juvenile or adults. Sharks were grouped into 25 mm length-classes ranging from 170 to 1020 mm. For dissected sharks, clasper calcification (males) and macroscopic examination of the gonads (females) were used to distinguish between juveniles and adults. A logistic regression was applied to each sex separately. The proportion of adult animals ($P$) at 25 mm length class was obtained using the following equation (Neter et al., 1990).

$$P = \frac{e^{(a+bx)}}{1 + e^{(a+bx)}}$$  \hspace{1cm} Equation 3.1

Where $a$ and $b$ are constants and $x$ is the medium value of the length-class. Confidence intervals around the logistic model were obtained by conducting 1000 simulations in a bootstrapping routine where data were randomly sampled with replacement for each of the 25 mm length classes (Turner et al., 2002). The middle 95% of the bootstrap replicates constituted the confidence intervals. Values of $P$ and the 95% confidence limits were obtained from equation 3.1 using Excel (Microsoft® Excel 2000).
**Sharks of known maturation stage**

**LINEAR DISCRIMINANT PREDICTIVE MODEL (LDPM)**

For both sexes, weighted averages of the predictive variables: total length (TL), testosterone (T), 17β-estradiol (E₂), and progesterone (P₄) (for females) and clasper length (CL), T, E₂, and P₄ (for males), were used to obtain discriminant function scores (D) to distinguish juveniles from adult sharks. Discriminant function scores (D) were calculated as follows:

\[ D = B_0 + B_1X_1 + B_2X_2 + \ldots + B_nX_n \]  

*Equation 3.2*

Where \( X_i \) is the value of each independent variable (i) and \( B_i \) is the coefficient estimated from the data. From the discriminant scores it was possible to obtain the probability that a shark was either a juvenile or adult. This probability \( P(G_i/D) \) was estimated by:

\[ P(G_i/D) = \frac{P(D/G_i)P(G_i)}{\sum_{i=1}^{j} P(D/G_i)P(G_i)} \]  

*Equation 3.3*

Where \( P(G_i) \) is the prior probability and is an estimate of the likelihood that a shark belongs to a particular group (juveniles or adults). The prior probability was calculated as the observed proportion of sharks in each group. The conditional probability \( P(D/G_i) \) is the probability of obtaining a particular discriminant function value of \( (D) \) if the shark belongs to a specific group. To calculate this probability, normal probability theory (the \( D \) scores are normally distributed for each group) was assumed. Each shark was known to belong to a particular group, and the conditional probability of the observed \( (D) \) score given membership in the group was calculated.
The predictive function was built using Excel and SPSS (SPSS® Base 10.0).

**MULTI-DIMENSIONAL SCALING (MDS)**

For both sexes, a multidimensional scaling (MDS) ordination based on the variables \( T \) and \( E_2 \), (for females), and \( T \) and \( CL \) (for males) was used to separate juveniles and adults using normalized Euclidean distances. Data were transformed when necessary. To test the null hypothesis that there were no assemblage differences between groups (juveniles and adults) in the spatial matrix, a one-way analysis of similarities (ANOSIM) and a Pairwise test were performed. The MDS and ANOSIM were performed using the Primer® software package (Clarke and Gorley, 2001). Adults were separated using a 95% cut off line. The line was calculated as the position on the MDS ordination where 95% of adults were correctly classified.

The significance level was set at \( P=0.05 \) for all data analyses.

**Size at maturity**

To determine the size at which 50% of the sharks were mature, animals were grouped as either juvenile or adult using LDPM and MDS analysis. Sizes at maturity estimates were calculated for each method using equation 3.1.
Sharks of unknown maturation stage

To determine the size at maturity, sharks were classified as either juvenile or adult based on their \( D \) scores using LDPM or on their MDS ordination. Size at 50% maturity was calculated using equation 3.1 for both methods.

Reproductive cycle

For both sexes, differences in the proportion of adult sharks that came from the marine reserve and from the rest of Tasmania were compared using a Chi-Square test (Quinn and Keough, 2002).

Hormone comparisons were analysed by one-way ANOVA and Tukey’s multiple comparison tests (Quinn and Keough, 2002). Residual plots were undertaken to assess the equality of variances and data were transformed where necessary.

All data were analysed using SPSS and the significance level was set at \( P=0.05 \) for all data analyses.
3.3 RESULTS

3.3.1 SIZE AT MATURITY OF ALL SHARKS DISSECTED

In females, oviducal gland width increased exponentially between 750-850 mm TL (Fig. 3.1a). The largest juvenile female found was 850 mm TL and the smallest adult was 730 mm TL. For males, clasper length showed a steady increase as the animal grew until 715 mm TL (Fig 3.1b). From 700-780 mm TL, clasper length rapidly increased (Fig. 3.1b). The largest juvenile male recorded was 830 mm TL and the smallest adult male was 725 mm TL. Size at 50% maturity of females was estimated at 815 mm TL (95% confidence interval = 812.58 – 842.79, r²=0.80, n=609), and 761 mm TL (95% confidence interval = 754.98 – 789.60, r²=0.84, n=462) for males (Fig. 3.1c).
Figure 3.1: Changes in (a) oviducal gland width (females) and (b) clasper length (males) with total length. Male claspers were classified as non-, partially and fully calcified for juveniles, sub-adults and adults respectively. Males mature at smaller sizes than females (c).
3.3.2 SHARKS OF KNOWN MATURITY STAGE (BLOOD TAKEN BEFORE DISSECTED)

**Linear discriminant predictive model (LDPM)**

**FEMALES**

Discriminant function analysis using TL, T, E₂ and P₄ showed significant differences between juvenile and adult sharks (Wilk’s Lambda, \( \chi^2 = 121.697, P < 0.001 \)). Both the standardized coefficient and the correlation of each variable with the discriminant function showed that total length was the main variable to contribute to the divergence between juveniles and adults. Testosterone and Estradiol contributed in similar proportion while P₄ did not explain any additional separation between groups (Table 3.2). Progesterone was found to only play a major role in draughtboard sharks during the ovulatory cycle (see chapter 2), and because its level only varied within adult animals this hormone was unlikely to contribute to the separation between the two groups. Therefore the model was rerun excluding P₄. Discriminant function scores \((D)\) generated using TL, T and E₂ were substituted into equation 3.2 as follows:

\[
D = -6.919 + 0.008 \times TL + 0.90 \times T + 0.22 \times E_2
\]

Conditional probabilities under the discriminant scores \((D)\) were generated for both groups. The prior probability of any shark to be juvenile was 0.63 and to be adult was 0.37. The group to which a case belongs is based on its largest posterior probability. From 118 females, 92% of cases were correctly classified.
Males

Clasper calcification is traditionally used to determine maturity in male sharks, however, the differences between partially and fully calcified claspers can be very subjective. As the calcification of the clasper was related to clasper length (CL) (Fig. 3.2), clasper length was included to separate maturity stages in male sharks. Discriminant function analysis combining CL, T, E₂ and P₄ showed significant differences between juveniles and adults (Wilk’s Lambda, \( \chi^2 = 41.377, P < 0.001 \)). Clasper length and T were the main contributors to the separation of juveniles and adults. Both E₂ and P₄ played a minor role in the divergence of the two groups and were excluded from the analysis (Table 3.2). Discriminant function scores (\( D \)) were generated using the following equation:

\[
D = -4.239 + 0.070 \times \text{CL} + 0.234 \times \text{T}
\]
Conditional probabilities under the discriminant scores were generated for juveniles and adults. The prior probability was estimated as 0.55 and 0.45 for juveniles and adults respectively. The group to which a case belongs was based on its largest posterior probability. From 111 males, 99% of cases were correctly classified.

![Figure 3.2: Relationship between clasper calcification and clasper length for draughtboard shark males.](image-url)
**Multi-dimensional scaling (MDS)**

**FEMALES**

A combination of T and E$_2$ successfully separated the reproductive stages of female sharks. Based on the discriminant function result of the contribution of P$_4$ into the separation of both groups, P$_4$ was not included in the MDS analysis. The majority of adult animals were on the left side of the ordination and the juveniles on the right side (Stress=0) (Fig. 3.3). ANOSIM analysis showed that there were significant differences between the reproductive stages (Global R=0.61, P< 0.001). A ‘95% cut off’ line for adults resulted in 90% of the females correctly classified; eight juveniles were classified as adults and five adults as juveniles (Fig. 3.3).

Table 3.2: Standardized discriminant function coefficients and correlations of the discriminant linear function for draughtboard shark males. Clasper length showed the highest standardized coefficient and the highest relationship with the discriminant function.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Standardized coefficient</th>
<th>Correlation with discriminant function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clasper length</td>
<td>0.89</td>
<td>0.96</td>
</tr>
<tr>
<td>Testosterone</td>
<td>0.59</td>
<td>0.78</td>
</tr>
<tr>
<td>Estradiol</td>
<td>0.16</td>
<td>0.12</td>
</tr>
<tr>
<td>Progesterone</td>
<td>0.07</td>
<td>0.27</td>
</tr>
</tbody>
</table>
Based on the results from the discriminant function analysis, where CL and T played a major role in the separation between juveniles and adults, E$_2$ and P$_4$ were excluded from the MDS analysis. A combination of CL and T separated adult male sharks from most of the juveniles (Stress=0.01) and ANOSIM analysis demonstrated that there were significant differences between the reproductive stages (Global R=0.70, P< 0.001) (Fig. 3.4). Based on a ‘95% cut off’ lines of adults, 97% of the 111 males sampled were correctly classified (Fig. 3.4).
Size at maturity

Hormone analysis was undertaken on 229 sharks that were also dissected. Size at maturity was calculated for these sharks using equation 3.1 based on macroscopic examination of the gonads (destructive sampling) and after classification of the sharks into juveniles or adults using either LDPM or MDS analysis (non-destructive sampling). For the MDS method, sharks on the left of the ‘95% cut off’ line were classified as adults and sharks on the right of the ‘95% cut off’ line were classified as juveniles. All three analyses resulted in a similar size at 50% maturity for both sexes, with females within 1.8% and males within 0.4% of the estimated values from macroscopic examination (Table 3.3).
Table 3.3. Comparison of the size at 50\% maturity between destructive (visual examination) and non-destructive (LDPM and MDS) methods for female and male draughtboard sharks. Percentage differences in the size at maturity using the non-destructive method were compared with the destructive method. LDPM: linear predictive discriminant model, MDS: Multi-dimensional scaling.

<table>
<thead>
<tr>
<th></th>
<th>50% maturity TL (mm)</th>
<th>Percentage difference</th>
<th>$r^2$</th>
<th>$a$ and $b$ values</th>
<th>95% Confidence interval TL (mm)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females (macroscopic)</td>
<td>814</td>
<td>-</td>
<td>0.80</td>
<td>$a = -32.16$, $b = 0.04$</td>
<td>798 – 830</td>
<td>118</td>
</tr>
<tr>
<td>Females (LDPM analysis)</td>
<td>823</td>
<td>1.10</td>
<td>0.77</td>
<td>$a = -59.55$, $b = 0.07$</td>
<td>812 – 832</td>
<td>118</td>
</tr>
<tr>
<td>Females (MDS analysis)</td>
<td>829</td>
<td>1.84</td>
<td>0.75</td>
<td>$a = -28.54$, $b = 0.03$</td>
<td>811 – 848</td>
<td>118</td>
</tr>
<tr>
<td>Males (macroscopic)</td>
<td>779</td>
<td>-</td>
<td>0.80</td>
<td>$a = -47.70$, $b = 0.06$</td>
<td>762 – 790</td>
<td>111</td>
</tr>
<tr>
<td>Males (LDPM analysis)</td>
<td>776</td>
<td>-0.38</td>
<td>0.80</td>
<td>$a = -63.08$, $b = 0.08$</td>
<td>768 – 783</td>
<td>111</td>
</tr>
<tr>
<td>Males (MDS analysis)</td>
<td>782</td>
<td>0.25</td>
<td>0.82</td>
<td>$a = -34.23$, $b = 0.04$</td>
<td>760 – 802</td>
<td>111</td>
</tr>
</tbody>
</table>
3.3.3 **Sharks of Unknown Maturity**

Sharks from the marine reserve were categorized as juveniles or adults based on their posterior probabilities for the LDPM analysis. For the MDS ordination, the unknown sharks were overlaid on the MDS plots for sharks of known maturity (Fig. 3.5 and 3.6). Sharks that fell to the left of the “95% cut off” line for adults were classified as adults and those on the right as juveniles.

![Fig. 3.5: MDS ordination of draughtboard shark females (known and unknown maturity) using testosterone and 17β-estradiol. J (juveniles): white circles, A (adults): black circles, U (unknown): grey triangles. The vertical dashed line represents the “95% cut off” line whereby 95% of adults were to the left of this line.](image)
For both sexes, the LDPM and MDS analysis resulted in a similar size at 50% maturity (Table 3.4). There was no difference between the estimates of size at maturity for female and male draughtboard sharks caught in the marine reserve compared to those caught from the rest of Tasmania. The LDPM estimates of size at maturity were closer to the macroscopic estimates for all values except males in the marine reserve. Similarly, the confidence limits for the LDPM were narrower than the corresponding MDS for all analyses except for males in the marine reserve. The techniques were sensitive to sample sizes with the smaller sample sizes from the reserve population resulting in larger increasing the 95% confidence limits (Fig. 3.7).
Table 3.4. Size at 50% maturity for female and male draughtboard sharks based on the hormone results of the linear discriminant predictive model (LDPM) and multi-dimensional scaling (MDS) analysis.

<table>
<thead>
<tr>
<th></th>
<th>50 % maturity</th>
<th></th>
<th>a and b values</th>
<th>95% Confidence interval</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ogive</td>
<td>$r^2$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TL (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females (LDPM analysis)</td>
<td>818</td>
<td>0.74</td>
<td>$a = -72.09, b = 0.09$</td>
<td>803 – 855</td>
<td>82</td>
</tr>
<tr>
<td>Females (MDS analysis)</td>
<td>828</td>
<td>0.76</td>
<td>$a = -9.44, b = 0.01$</td>
<td>775 – 870</td>
<td>82</td>
</tr>
<tr>
<td>Males (LDPM analysis)</td>
<td>768</td>
<td>0.83</td>
<td>$a = -43.84, b = 0.06$</td>
<td>745 – 803</td>
<td>54</td>
</tr>
<tr>
<td>Males (MDS analysis)</td>
<td>757</td>
<td>0.84</td>
<td>$a = -25.73, b = 0.03$</td>
<td>740 – 795</td>
<td>54</td>
</tr>
</tbody>
</table>

Method

Figure 3.7: Comparison of 50% size at maturity and 95% confidence limits for female (circles) and male (triangles) draughtboard sharks caught in a marine reserve and from the rest of Tasmania using linear discriminant analysis (LDPM) and multi-dimensional scaling ordination (MDS).
Reproductive Seasonality

For both sexes the proportion of adult animals found in the marine reserve was only slightly less than those found from the rest of Tasmania, although this difference was non significant (Table 3.5).

<table>
<thead>
<tr>
<th>Location</th>
<th>Proportion adult animals</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Females</td>
<td>Males</td>
</tr>
<tr>
<td>Marine Reserve</td>
<td>0.29</td>
<td>0.48</td>
</tr>
<tr>
<td>Rest of Tasmania</td>
<td>0.38</td>
<td>0.54</td>
</tr>
</tbody>
</table>

To compare monthly variations of hormones between sharks obtained from the marine reserve and the rest of Tasmania, sharks were grouped into three periods due to the small sample sizes. For females, there were no significant differences between E₂, or P₄ in sharks from the marine reserve compared with those from the rest of Tasmania. In contrast, T levels were lower in sharks from the marine reserve than from the rest of Tasmania in the March-May period (ANOVA, P< 0.001) (Fig. 3.8). The levels of T were significantly lower for males in the marine reserve for the March-May period (ANOVA, P< 0.001), although the gradual decline in T levels from January to December was consistent in both the marine reserve and the rest of Tasmania (Fig. 3.9).
Figure 3.8: Seasonal variations in testosterone (T), 17β-estradiol (E₂) and progesterone (P₄) for adult female draughtboard sharks caught in a marine reserve (○) and the rest of Tasmania (●). Values are mean ± SE. Numbers are sample sizes. * Values are significant different.
Figure 3.9: Seasonal variations in testosterone for adult male draughtboard sharks caught in a marine reserve (●) and the rest of Tasmania (▲). Values are mean ± SE. Numbers are sample sizes. * Values are significant different.
3.4 Discussion

Size at maturity obtained from blood samples was within 2% of the size at maturity obtained from macroscopic examinations of gonads. For both sexes in *C. laticeps*, the combination of external features (e.g., total length in females and clasper length in males) and gonadal steroids can be used to obtain reproductive information for management of sharks without having to sacrifice the animal.

From macroscopic examination of dissected animals it was clear that for draughtboard sharks, maturity is strongly size dependent. Sharks larger than 860 and 870 mm TL (females and males respectively) were all adults and sharks below 750 and 710 mm TL (females and males respectively) were all juveniles.

For *C. laticeps*, both the linear discriminant predictive function and the multi-dimensional scaling analysis provided objective methods to classify sharks as juveniles or adults, and therefore address size at maturity and reproductive seasonality. Furthermore, steroid hormones could determine the stage of maturity in the intermediate length size classes where sharks could be either juveniles or adults. For these sharks neither total length or clasper calcification could be used to determine the reproductive stage of the animals, therefore hormones provided a mechanism for determining the reproductive status of these sharks. The LDPM had narrower confidence limits and was, in general, closer to the macroscopic estimates giving a more precise and accurate method than the MDS, although no significant differences were found between values. The sample sizes would suggest that for *C. laticeps*, it is necessary to have approximately 100 sharks with a significant proportion in the critical region between 100% adults and 100% juveniles to obtain an accurate estimate of size at maturity.

When selecting the hormones to use to separate juvenile or adult sharks, understanding the role that each of the gonadal steroids play in shark reproduction is important. Hormone analysis is relatively costly, thus knowledge of which hormones
contributed to the separation of the reproductive stages should enable costs to be minimized. For draughtboard sharks there was a need to measure only two hormones, T (for both sexes) and E₂ (for females), to separate juveniles from adults. Testosterone and E₂ were found to be the principal hormones during the follicular phase of females, while elevated plasma P₄ was found primarily in the ovulatory phase (see chapter 2). As P₄ only varied in adult females and was dependent on the female ovulatory phase, it was possible to find adult females with low or high levels of P₄, whereas juvenile females always had low levels of P₄. Therefore, P₄ was not a reliable discriminant factor for separating juvenile and adult females. In males, only T showed a significant increase from juvenile to adult animals (see chapter 2) and thus was the main contributor to the separation. In *C. laticeps* males, clasper length and T contributed to the separation of juveniles from adults. As the degree of calcification and size of claspers were external features that could be readily assessed, clasper length and calcification will be the most cost-effective method of identifying the size at sexual maturity of *C. laticeps* males.

Steroid hormones were also important in providing data on seasonality of reproduction. While clasper calcification can be used to address size at maturity in several chondrichthians species, dissection of these males is still required to understand seasonality. Although *C. laticeps* was not found to have a defined seasonal reproductive pattern (see chapter 2), variations in hormone levels followed similar trends in reproductive activity obtained from macroscopic examination of the gonads. In seasonally reproductive species such as *Hemiscyllium ocellatum* (Heupel *et al.*, 1999), *Raja eglanteria* (Rasmussen *et al.*, 1999) and *Dasylatis sabina* (Tricas *et al.*, 2000), strong correlations in hormones and reproductive seasons have been reported.

A concern could be that the lower steroid plasma levels in seasonally reproductive sharks captured during their non-reproductively active period could confound the estimates of size at maturity as they could be classified as juveniles (i.e. if the hormone
values fall to values equivalent of juveniles). To obtain size at maturity estimates it is essential to sample animals during the reproductive period.

The population of *C. laticeps* from the Crayfish Point Reserve showed that the proportion of adult sharks (for both sexes) was similar to the proportion in the rest of Tasmania, suggesting that although this reserve would offer protection to adult animals, this protection was not preferential. The similar seasonal trends in reproductive hormones for females and males between the reserve population and the rest of Tasmania is expected, as tagging studies (see chapter 4) demonstrated that this species can move substantial distances and mix between regions. Although the seasonal sample sizes were small, the similarity in trends between the dominant hormones and macroscopic examination of the gonads in each sex demonstrated the potential of hormones to define spatial and temporal variability in reproduction without the need to sacrifice the sharks.

Different methods, such as ultrasonography and endoscopy, have been used to assess gestation period and reproductive condition in females without killing the animal (Carrier *et al.*, 2003) (J. Daly, Melbourne aquarium, Melbourne. pers. comm.). To date no estimates of size at sexual maturity or seasonality of reproduction have been reported using these techniques. Ultrasonography or endoscopy also require substantial handling and manipulation of the sharks, which could affect both the shark and its embryos (Carrier *et al.*, 2003). Obtaining a blood sample from the draughtboard shark for estimating hormone concentration, involved a minimal handling time (2-3 minutes) before the shark was returned to the water. The sample could be taken at sea in exposed and rough conditions, making hormones a less invasive, quick technique.

Endocrine markers provide a non-destructive way to obtain information on somatic, temporal and spatial reproductive parameters for management of sharks. Non-destructive techniques are essential for sampling marine species on the world’s threatened and endangered species lists, of which there are many chondrichthyans.
(IUCN, 2006). Understanding the impact of fishing operations on bycatch is also required for industry accreditation and meeting ecosystem based fishery management objectives (Hall et al., 2000). In circumstances where the bycatch is not retained, sacrificing the shark to obtain information on reproductive status would no longer be required.

A general trend or common pattern in the MDS ordination or the \(D\) score values of the LDPM analyses may also be found to distinguish juvenile and adult sharks for the different reproductive modes (oviparity and viviparity). In this case, it would no longer be necessary to sacrifice sharks that are not a target or by-product of fishing operations. If the relationship between steroid hormones and reproduction is reproductive mode specific or generic to all chondrichthyans, then validation for different species would not be required and future reproductive needs (size at maturity, seasonal reproductive activity) for management could be addressed non-destructively though blood sampling. As hormones can also provide information on the seasonality of reproduction, they have the potential to provide necessary information required for the conservation and management of shark populations without the need to sacrifice the animal.
CHAPTER FOUR:

Movements, activity patterns and habitat utilisation
4.1 INTRODUCTION

Efforts to study the movement of fish at either the population or individual levels have been in progress for over a century (Casey and Taniuchi, 1990; Kohler and Turner, 2001). Tagging is the most used method for studying fish movements, and provides important information on life history and population dynamics (Hilborn, 1990; Eiler, 2000). Recently, the importance of incorporating fish behaviour and habitat utilisation as components of fish movement studies has been recognized for marine management and conservation programs (Shumway, 1999; Koehn, 2000).

Historically, migrations, movement patterns and habitat preferences of fishes were determined by fishery dependent mark and recapture or visual (in situ) observations (Gunn, 2000; Stevens, 2000; Lowe et al., 2003). Initially, mark-recapture studies used conventional tags which are defined as those that can be identified visually without the use of detection equipment (Kohler and Turner, 2001). Conventional tagging experiments of cartilaginous fishes were first reported in the 1930s (McFarlane et al., 1990; Kohler and Turner, 2001; Latour, 2004), and have subsequently continued to be an important source of information for understanding chondrichthyan populations (McFarlane et al., 1990; Hurst et al., 1999; Stevens, 2000).

Conventional mark-recapture studies rely on recaptures of the tag by a variety of sampling gears used by either researchers or fishers. Bias associated with conventional mark-recapture studies can occur when selectivity of sampling gears varies with habitat, or habitat-specific movements alter catch rates, or fleet dynamics alter the probability of recapture (Kohler and Turner, 2001; Simpendorfer and Heupel, 2004; Bolle et al., 2005).

The development of acoustic tracking technology in the 1960’s enabled detection of the animal independent of the need to be recaptured. By either the use of hand held detectors (active tracking) or moored ‘listening receivers’ (passive tracking) detailed information on animal behaviour and movement can be obtained. Passive acoustic
monitoring technology allows movement patterns of multiple individuals tagged with acoustic transmitters to be determined (Heupel and Hueter, 2001; Voegeli et al., 2001; Heupel and Hueter, 2002). Hydrophone (listening) stations (receivers) record the date, time and identity of an aquatic animal fitted with an acoustic transmitter swimming within the detection range of the receiver (Voegeli et al., 2001). One of the most promising current applications of acoustic tags to fishery management is elucidation of home range area (Kramer and Chapman, 1999), and habitat utilisation (Sibert and Nielsen, 2000). While there are many studies applying acoustic technology in sharks and rays (Holland et al., 1999; Heupel and Hueter, 2001; Klimley et al., 2002; Nakano et al., 2003; Garla et al., 2006)), only a few have addressed home range or habitat utilisation (Morrissey and Gruber, 1993a; Morrissey and Gruber, 1993b; Heithaus et al., 2002; Heupel et al., 2004; Duncan and Holland, 2006; Heupel et al., 2006b).

Although *Cephaloscyllium laticeps* is primarily caught as bycatch in rock lobster traps and other inshore hook and gill net fisheries, there is concern that the small amount of byproduct that is currently caught has the potential to expand (J. Lyle, TAFI Marine research Laboratories, Hobart. pers. comm.). As a precautionary measure, Tasmania has implemented a possession limit of two draughtboard sharks per person, or five sharks per boat per day to constrain future catches.

Walker (2005) reported a 54% decline in draughtboard sharks caught in Bass Strait, southern Australia between 1973-76 and 1999-2001. Although the cause for this decline is uncertain, Walker suggested that it might be due to a change in fishing patterns in an attempt to minimise bycatch of this species rather than a true decline in abundance due to fishing.

Prior to considering any increased utilisation of this species it was important to understand the mixing of populations between regions. Small-scale movement patterns and habitat utilisation was also considered important to establish if sharks were more vulnerable to capture at certain times of the day and on certain substrates. Knowledge
of the behaviour of draughtboard sharks can therefore be used to both increase exploitation through targeted fishing or to minimise bycatch by avoidance.

Previous studies in other scyliorhinids showed that sharks are characterised as slow swimmers (Springer, 1979; Compagno, 1984) and are often found resting in caves either alone or in aggregations (Nelson and Johnson, 1970; Sims et al., 2005). Nelson and Johnson (1970) reported nocturnal activity patterns for the scyliorhinids *Heterodontus francisci* and *Cephaloscyllium ventriosus* and Sims (2001) found differences in the day night activity between males and females of *Scyllorhinus canicula*.

This study investigated the movement behaviour of the draughtboard shark using conventional and acoustic tagging. Conventional tags were used to identify longer-term movement (> 6 months) over larger geographic regions, while acoustic tagging evaluated short-term movements (< 6 months) and habitat utilisation.
4.2 MATERIAL AND METHODS

4.2.1 ACOUSTIC TAGGING

*Study site, acoustic receivers and transmitters*

An array of 82 VR2 automated acoustic receivers (Vemco Ltd., Nova Scotia) were deployed in October 2002 and retrieved in July 2003 in southeast Tasmania, Australia (Fig. 4.1a). The sea floor in these areas consisted of sand, silt, seagrass and low profile reef (Barrett *et al.*, 2001; Jordan *et al.*, 2001). Each receiver was secured to a vertical steel post on a concrete mooring, approximately 1 m above the sea floor.

An extensive array of receivers was established as a series of acoustic ‘curtains’ separating the main bays and channels in southeast Tasmania (Fig. 4.1b). The depth of receiver placement varied from 2 to 55 m. The distance between receivers was chosen to ensure that detection distances had substantial overlap and varied from 720 to 930 m depending on the habitat type. Receivers were positioned at the entrances of bays and channels to ensure that no shark could move into or out of these areas without being detected.

Within the extensive array, an intensive array was established at the Crayfish Point Reserve (total area= 800 m$^2$) and the adjacent areas of Alum Cliff and Taroona High (Fig. 4.1 c). In the Crayfish Point Reserve, the sea floor includes a complex mix of sand, silt, low and high profile reef (Barrett *et al.*, 2001; Jordan *et al.*, 2001). The complexity of this habitat resulted in a reduction of the detection range for the acoustic receivers to a minimum of 60 m (Semmens, unpublished data). Thus, the receivers were placed approximately 100 m apart to provide sufficient overlap for determining position from detection at multiple receivers. The receivers were placed in depths from 2 to 11 m. The receivers that formed a small ‘curtain’ perpendicular to the shore at Alum Cliff and Taroona High were 400-450 m apart.
The transmitters (V8SC-2H: Vemco, Nova Scotia), were cylindrical in shape, 30 mm in length, 9 mm in diameter and weigh 3.1 g in water. The transmitters emit a 69 kHz frequency “ping” code repeated after a random delay of 20 to 60 s. The battery life was set at 180 days.

Figure 4.1: Acoustic receiver positions. a: Map of Tasmania showing out the southwest area. b: Receiver positions in the southwest area. Extensive curtains are labelled as: B: Lower mid-channel, C: Upper mid-channel, D: Upper channel, E: Upper Derwent, F: Lower Derwent, G: Storm Bay, H: Frederick Henry Bay, I: Norfolk Bay, J: Dunally, K: Eaglehawk Neck. c: Receivers position in Crayfish Point Reserve and adjacent areas. d: Receiver positions at the intensive array area established by the Crayfish Point Reserve, Alum Cliff and Taroona High.
**Sampling methodology**

Between January and March 2003, 25 (15 females, 9 males, 1 no sex recorded) draughtboard sharks were caught in rock lobster traps. Fifteen sharks were sourced from the Crayfish Point Reserve and 10 sharks from the east coast of Tasmania (42-43°S, 147-148°E) (Fig. 2.1). All sharks were released in the Crayfish Point Reserve. Prior to release, total length, total weight, and clasper length (for males) was recorded. Sharks were fitted with the acoustic transmitters and injected with 25mg/kg of the antibiotic tetracycline dissolved to saturation in seawater.

Initially, two sharks were internally tagged by inserting the tag into the body cavity. After capture, these sharks were injected with a localised anaesthetic (Xylocaine 0.5%, 25 mg in 5 ml), and a 3-4 cm incision was made in the ventro-lateral region (the ventral region was considered unsuitable as the sharks rest on the sea floor) towards the rear of the stomach cavity. The transmitters were coated in 100% paraffin to prevent transmitter rejection and to cover any sharp protrusion on the transmitter surface that might irritate the shark (Heupel and Hueter, 2001). The transmitter was inserted and the cavity closed using surgical glue (Indermil® Loctite Corporation, Dublin) and a disposable skin stapler (Royal 35W, United State Surgical Corporation, Ltd). The sharks were then held in captivity for one week prior to being released in the Crayfish Point Reserve. However, the tagging wound was observed to re-open in several sharks, probably due to the frenetic movements following release. Because of the uncertainty associated with a partially open wound, the remaining 23 sharks were tagged externally.

For the external tagging, two 1.10 mm x 38 mm surgical needles were joined to the distal end of the transmitters. The transmitter was attached to the base of the first dorsal fin by the needles piercing through the fin. The needles passed through buttons on the opposite side of the fin and were then crimped and the excess needle length removed (Fig. 4.2).
Analysis of the data

Raw data collected by the receivers, including transmitter number, and time and date of detection was downloaded (in March-April and July 2003) using the VR2 data processing software (Vemco Ltd). Data from both arrays was analysed using ArcView 3.2 (ESRI 1999) with the Animal Movement Analyst Extension (AMAE) tool (Hooge and Eichenlaub, 2000) and Microsoft Excel.

Performance of the receivers within the Crayfish Point Reserve

Receiver performance could be affected by the interference of acoustic noise (e.g. signals from motoring small vessels) in addition to obstruction and ‘bounce’ of acoustic signals in association with different substrate types (e.g. sand, high profile reef) and vegetation (e.g. density of seaweeds such as kelp). To evaluate the performance of the receivers, data from sharks detected in the inner arrays of the Crayfish Point Reserve and arrays outside the reserve (i.e. Alum Cliff or Taroona High) were compared. The data were examined to determine if sharks detected inside, and subsequently outside the reserve (or vice versa) were also detected by the outer ring of the Crayfish Point Reserve receivers (Fig. 4.3). The performances of the receivers were then assessed by counting.
the times that the outer receivers detected the movement of 3 (1 internally and 2 externally tagged) sharks that moved, on 232 occasions, in and out of the inner area of the Crayfish Point Reserve. Detection on the outer ring receivers indicated progress of sharks from inner to outer regions and suggested good receiver performance.

![Diagram of Crayfish Point Reserve](image-url)

**Figure 4.3:** The Crayfish Point Reserve was subdivided into four outside areas (1-4) and 1 inside area (inner). Sharks moving from the inner region of the reserve to either Alum Cliff or Taroona high (or vice versa) had to be detected by the outer ring receivers (areas 1, 2, 3 and 4) of the reserve.

**DEFINITION OF MOVEMENT**

For the intensive array, it was possible to obtain large datasets if the sharks remained for periods of time within the reserve and adjacent areas. Due to the complexity of the habitat, sharks could move by swimming between adjacent high profile reefs (e.g. in narrow channels within the reef structure), over higher profile reefs (e.g. elevated position in the water column) and over low profile reefs and other
habitats. Thus, the detection distance of the receivers changed as the habitat increases in complexity (Fig. 4.4). Rocks and differing densities of macroalgae decreased the detection distance and small movements of a shark, particularly vertically within the water column, could result in detections by different receivers suggesting different locations. Thus, movement in this study was defined as either movement from one area to another non-adjacent area of the reserve (eg. from area 1 to 3 and from 2 to 4, or non-adjacent joint areas from 1,2 to 3,4 and 4,1 to 2,3) or a consistent pattern of a new set of receivers detecting a transmitter (see Fig. 4.3). For the extensive array, Alum Cliff and Taroona High, movement was defined as when more than two non-adjacent receivers of the curtain detected a shark.

**Figure 4.4:** Generalised representation of the reef habitat. The receiver (VR2) reception is permanently blocked when there are rocks between the sharks and the receivers, resulting in no detection (x). Intermittent blocking of the receiver reception (✓√) occurs when there are algae between the sharks and the receivers. A receiver will also intermittently detect a shark, when the shark moves around a rock situated between the shark and the receiver (✓√). When there is not obstruction between the shark and the receiver, sharks will be continuously detected (✓).
**Definition of stationary or minor movement periods**

When the shark was detected by the same receiver or set of receivers for at least 60 minutes at intervals of 1 minute or less (60 + detections in an hour) the shark was considered to be stationary. Often during this time it was possible for an additional receiver to detect the shark, but it was unknown if the shark had moved slightly or the detection was associated with movement of the habitat (e.g. kelp) between the shark and the receiver. In any case, stationary or minor movements indicated that the shark is not actively swimming.

Differences in the number of sharks with stationary periods were tested by Student t-test (Quinn and Keough, 2002). To determine if there was a difference with size, sharks were classified as juveniles and adults according to 50% length at maturity (see chapter 2). Sharks smaller or larger than 815 mm (for females) and 760 mm TL (for males) were considered as juveniles and adults respectively.

**Definition of unaccountable time**

There were times where an individual draughtboard shark could not be accounted during tracking period. There were two possible reasons why the VR2 system did not record the shark positions: 1) sharks were moving to areas outside the detection range of the receivers, 2) sharks remained in areas were the VR2 system was obstructed by external noise (e.g. motor vessel) or marine substrate.

**Definition of presence**

As there were a large number of periods in the data set when individual sharks could not be accounted for, the presence of a shark in any given area was defined as when one or a group of receivers recorded at least 10 detections. This minimises the
number of false detections (single detection of any transmitter code) caused by external noises, producing false readings of the transmitter identification number (tag) by the receivers.

HABITAT UTILISATION

Habitat utilisation was determined as the 95% probability of a draughtboard shark being found within a certain area calculated as the 95% kernel utilisation distribution (KUD) (Worton, 1987) using the AMAE tool in Arcview. The spatial use of the habitat through time was evaluated by examining the 95% KUD estimates for each shark per month and by combining all months together. A student t-test was used to compare the total number of animals that had stationary periods (see definition above) found in each of the 5 areas of the Crayfish Point Reserve and the overlap areas (Fig 4.3) to determine if sharks preferred specific areas of the Reserve.

HABITAT PREFERENCE

To evaluate the preferred habitats that sharks used to either move or have stationary periods it was necessary to use separate approaches for movement and stationary periods:

1. To determine if sharks preferred to move in any specific habitat, the proportion of movements in each area was calculated, for each shark, as the number of movements within an area divided by the total number of movements for that shark. Differences in the proportion of movements in different areas were tested using a Chi-Square test (Quinn and Keough, 2002).
To determine if there was a preference for certain boundary regions of the Crayfish Point Reserve for access into and away from the reserve, the proportion of movements in each area was compared. For this part of the analysis, area 1 of the reserve includes the overlap between areas 1 and 4 (area 4,1), area 2 includes the overlap between areas 1,2, area 3 includes the overlap between areas 2,3, and area 4 includes the overlaps between areas 3,4.

To determine if sharks preferred to spend stationary periods in any specific area, the proportion of the hours that sharks were stationary in each area was calculated as the number of hours that all sharks were stationary in each area, divided by the total number of hours that sharks were stationary. Differences in these proportions were tested using Chi-square. As this analysis was based on time, the same analysis was undertaken based solely on the occurrence of a stationary period irrespective of the length of the stationary period (providing it was greater than one hour). Thus, a single stationary period of 20 hours provided a count of 1 whereas 3 separate one-hour stationary periods separated by periods of movement provided a count of 3.

2. Habitat preference for both movement and stationary periods was determined as the 50% kernel utilisation distribution (KUD) using the AMAE tool in Arcview. The 50% contour was chosen to indicate the areas of greatest use.

**Day-night activity**

Day-night habitat utilisation and preferences were calculated using both the 95% and 50% KUDs respectively.
SITE FIDELITY FOR THE CRAYFISH POINT RESERVE

To determine if sharks remained in, or dispersed away from the Crayfish Point Reserve region, the number of days that each shark was detected in the Crayfish Point Reserve was compared for each month after release and differences tested by a one way ANOVA and Tukey’s multiple comparison tests (Quinn and Keough, 2002).

4.2.2 CONVENTIONAL TAGGING

Study site and sampling methodology

Between January 2000 and January 2005, sharks were tagged during routine fishery dependent and independent rock lobster catch sampling trips around southwestern and eastern Tasmania and in the Crayfish Point Reserve (Fig. 1.2a). Each shark was tagged with a 35 mm yellow standard Rototag (Daltons, Henly-on-Thames, England) externally attached to the second dorsal fin. For each shark, sex, total length and clasper length (males) were recorded.

Analysis of the data

Differences in the proportion of sexes, sizes and time at liberty were tested by Chi-square. The time at liberty was subdivided into 7 periods: 1) 0 to 6 months, 2) 7 to 11 months, 3) 1-2 years, 4) 2-3 years, 5) 3-4 years, 6) 4-5 years and 7) > 5 years. Size frequency distributions were compared between sexes of released and tagged sharks from different areas, using a randomisation procedure with the Kolmogorov-Smirnov test statistic \((D)\) with data pooled across sexes. Size frequency data from the two distributions being compared were pooled and randomly reallocated to each original distribution and the test statistic \((D)\) recalculated. The procedure was repeated 1000
times and the test of significant difference between the two distributions made by comparing the value of the observed test statistic to the distribution of \( D \) values obtained by the randomisation procedure. Significant differences were identified when less than 20 of the \( D \) values obtained from the randomisation procedure exceeded the value of \( D \) from the original distributions (Haddon, 2001).

To calculate short and long term site fidelity for the Crayfish Point Reserve, data was standardized to account for differing effort (number of trap lifts) undertaken in the different surveys, by the following equation:

\[
P_{ij} = C_j \left[ \frac{T_i \left( \frac{S_{ri}}{S_{ri}} \right)}{S_{ij}} \right]
\]

Where \( P_{ij} \) is the proportion of sharks recapture in trip \( i \) that where tagged in trip \( j \), where \( j > i \). \( C_j \) is the catch rate (number of sharks/trap) of sharks tagged during \( j \), and was calculated as \( \frac{S_j}{T_j} \), where \( S_j \) is the number of sharks caught and tagged in trip \( j \) and \( T_j \) is the total number of traps set to capture the sharks in the trip \( j \). The value \( T_i \) is the total number of traps set to capture the sharks in trip \( i \), \( S_{ri} \) is the number of sharks recaptured in trip \( i \) that were tagged in trip \( j \), and \( S_s \) is the total number of sharks caught in trip \( i \).

To calculate the expected catchability the following assumptions were made:

1. Catch rate was a function of effort
2. The Crayfish Point Reserve had no finite carrying capacity
3. Tagged sharks were distributed randomly within the population
Differences in the proportion of sharks recaptured either per month or per year in the Crayfish Point Reserve were tested using Chi-square test.

All statistical analyses, for both acoustic and conventional tagging, were carried out using SPSS (SPSS® Base 10.0). The significance level was set at $P=0.05$ for all data analysis.
4.3 Results

4.3.1 Acoustic Tagging

Transmitter performances

Of the 25 sharks that were acoustically tagged, six transmitters did not start working until one month after attachment (they were incorrectly set to start one month after the battery was connected), one transmitter (#143) was never recorded and one transmitter (#156), attached to a shark that was caught on the east coast of Tasmania, was recorded only twice on the day of tagging. Transmitters #143 and #156 were excluded from the analysis.

Performances of the receivers within the Crayfish Point Reserve

The performances of the receivers was considered to be high as 97% (± 0.43 SE) and 86% (± 4.44 SE) of sharks that moved between the inner region of the Crayfish Point Reserve and Alum Cliff or Taroona High respectively were detected (Table 4.1).

Table 4.1: Movements of three draughtboard sharks between the inner areas of the Crayfish Point Reserve (CPR) and Alum Cliff or Taroona High.

<table>
<thead>
<tr>
<th>Number of sharks</th>
<th>Inner CPR-Alum Cliff</th>
<th>Inner CPR-Taroona High</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total number of movements</td>
<td>Percentage of movement detected by the outer CPR receivers</td>
</tr>
<tr>
<td>2 sharks (externally tagged)</td>
<td>92</td>
<td>97% (n=89)</td>
</tr>
<tr>
<td>1 shark (internally tagged)</td>
<td>49</td>
<td>96% (n=47)</td>
</tr>
<tr>
<td>Total</td>
<td>141</td>
<td>97% (n=136)</td>
</tr>
</tbody>
</table>
**Movement patterns**

For the 17 sharks where transmitters were working on release, 10 were recorded at the marine reserve on the same day of released, six within the first week of being released and one more than a week after release (Fig. 4.5). Presence of sharks was detected in the entire intensive array and some areas of the extensive array (the River Derwent, the Upper Channel and Storm Bay (Fig. 4.6)).

![Diagram](image.png)

**Figure 4.5:** Number of draughtboard sharks recorded at the Crayfish Point Reserve (CPR) soon after being released.
Sharks showed two typical patterns of movement. The majority of the sharks (n=20) were never recorded beyond the Derwent River during the survey period (Fig. 4.7a). However, three females moved away from the intensive array and beyond the Derwent River (Fig. 4.7b).
The minimum time that sharks were recorded before leaving the extensive array was 11 days and the maximum overall time was 188 days. The last records for the 23 sharks were evenly split between the intensive array (n=11) and the extensive array (n=12) (Table 4.2). Although the sharks that were translocated from the east coast of Tasmania tended to leave the reserve earlier than those captured from the Crayfish Point Reserve (Fig. 4.8), all of the nine sharks sourced from the east coast remained within the Derwent River region during the study period.

Neither of the two sharks that were internally tagged demonstrated any behaviour that would suggest an impact of tagging different to the fin-tagged sharks. One of the sharks moved away from the Crayfish Point Reserve and out of Storm Bay whereas the other was last located, at the end of the study, within the reserve.
Table 4.2: Summary data for draughtboard sharks tracked in the southeast region of Tasmania. All sharks were tagged and released at the Crayfish Point Reserve. Ten sharks were caught on the east coast of Tasmania and translocated to the Crayfish Point Reserve (CPR), the other 15 were caught at the CPR. Two sharks were internally tagged, these sharks are indicated with a single asterisk (*). One transmitter did not work (shark ID 143). AC: Alum Cliff, B: Lower mid channel, E: Upper Derwent River, EC: East coast of Tasmania, F: Lower Derwent River, G: Storm Bay, TH: Taroona High. (**) For 6 sharks, transmitters started working one month after insertion; the date of tagging was used as the date of the first record. Sharks with less than two hits (#156, #143) were excluded from the analysis.

<table>
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<th>Shark ID</th>
<th>Sex</th>
<th>Size</th>
<th>Source</th>
<th>Date of tagging</th>
<th>Date of last record</th>
<th>Number of hits</th>
<th>Zone of last record</th>
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<td>920</td>
<td>EC</td>
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<td>29/03/03</td>
<td>313</td>
<td>F</td>
</tr>
<tr>
<td>163 **</td>
<td>F</td>
<td>760</td>
<td>EC</td>
<td>26/04/03</td>
<td>4/05/03</td>
<td>7</td>
<td>AC</td>
</tr>
<tr>
<td>162 **</td>
<td>M</td>
<td>760</td>
<td>EC</td>
<td>26/04/03</td>
<td>29/04/03</td>
<td>20</td>
<td>TH</td>
</tr>
<tr>
<td>161 **</td>
<td>M</td>
<td>880</td>
<td>EC</td>
<td>26/04/03</td>
<td>28/05/03</td>
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<td>F</td>
</tr>
<tr>
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<td>-</td>
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<tr>
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<td>146 **</td>
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<td>660</td>
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<td>16/01/03</td>
<td>22/07/03</td>
<td>1313</td>
<td>CPR</td>
</tr>
</tbody>
</table>
Movement behaviour

Two types of movement behaviour were recorded; sharks that only showed movement and sharks that alternated movement with stationary periods. Nine sharks showed continuous movement and 12 sharks alternated between movement and stationary periods (Fig. 4.9, Table 4.3). Movement behaviour could not be determined for two sharks due to a low number of detections. All sharks were out of the range of detection for several days (Table 4.3). Within the total number of days between the first record and the end of the study period, the percentage of days that sharks were detected averaged 17% (± 4.11% SE) with 1% and 69% as the minimum and maximum (Table 4.3). The average time spent by sharks having stationary periods was eight hours per day (± 1 hr SE) with one shark spending a continuous period of five consecutive days stationary. There was no difference in the average time spent in stationary periods for
either sex (seven females and five males) or between juveniles (n=7) and adults (n=5).

No correlation existed between the movements of the draughtboard sharks and lunar phases (Fig. 4.9).

**Figure 4.9:** Examples of movement behaviour of draughtboard sharks. Nine sharks showed only movement records (eg: shark #146), while 12 sharks alternated between movements and stationary periods (eg: shark #147 and #140). The figure indicates both movement and stationary behaviour simultaneously as these behaviours could occur on the same day” was added in the figure legend. Dashed lines represent the initial and the last record of the sharks. Black and grey regions represent movement and stationary periods, respectively. Lunar phases are shown at the top of the graph.
Table 4.3: Type of movement of draughtboard sharks. The total number of records were classified as movement, stationary period, or uncertain. Sharks showed days when they were not recorded. *The percentage of days that had shark records was calculated assuming that all sharks were alive at the end of the study period.

<table>
<thead>
<tr>
<th>Shark ID</th>
<th>Number of detections</th>
<th>Movement</th>
<th>Stationary</th>
<th>Uncertain</th>
<th>Number of days between first and last record</th>
<th>Number of days recorded between first and last record</th>
<th>Number of days between last record and the end of the study period</th>
<th>Percentage of days within the study period that shark was recorded *</th>
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<td>164</td>
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</tbody>
</table>
**Habitat utilisation**

To determine habitat utilisation, the analysis was restricted to sharks with $\geq 20$ movements. Sharks ($n=12$) with less than 20 detections, had insufficient information to demonstrate movement behaviour (Fig. 4.10)

![Figure 4.10: The distribution of movements for the 23 acoustically tagged draughtboard sharks.](image)

For the 11 sharks with $\geq 20$ movements, movements were recorded in all areas of the intensive array, the Upper and Lower Derwent River and the Upper Channel (Fig. 4.11a and Fig. 4.11b). The majority of the sharks remained within the Derwent River region (Fig. 4.11a). Nine of the 11 draughtboard sharks had $\geq 20$ movements in the Crayfish Point Reserve. These sharks utilised all regions of the reserve, with a minimum
of five sharks being recorded in each region and a maximum of nine sharks being recorded in region three. No region was visited by all 11 sharks (Fig. 4.11a).

![Number of sharks detected](image1)

**Figure 4.11**: a) Number of draughtboard sharks detected in the different study regions estimated by 95% KUD contours. Sharks (n=11) with at least 20 recorded movements were used to calculate habitat utilisation of movement. CPR: Crayfish Point Reserve. b) Examples of habitat utilisation distribution for draughtboard sharks estimated by 95% KUD contours.

The majority of the sharks (n ≥ 8) were detected as having stationary periods in the Crayfish Point Reserve and in the Derwent River array, while less sharks (n ≤ 3) were found to have minimal movements around Alum Cliff and Taroona High (Fig. 4.12a and Fig. 4.12b). As the Derwent River, Alum Cliff and Taroona High have substrates of sand with small areas of reef (Table 4.4), it is uncertain if these sharks were in stationary...
periods on the sand which surrounds these receiver arrays, were in stationary periods on the small reef areas, or are utilising the cement tyre that was used to support the listening stations. In comparison to the broad areas of the Crayfish Point Reserve moved over by the draughtboard sharks, sharks showed a preference for areas within the reserve to have stationary periods. The majority of the sharks were detected in area 1 and its overlap with area 2, followed by a significant decline (t-test, P<0.001) in the other areas of the reserve used for stationary periods (Fig. 4.12a).

Table 4.4: Type of marine substrate for the intensive and extensive array. CPR: Crayfish Point Reserve.

<table>
<thead>
<tr>
<th>Area</th>
<th>Substrate type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area 1 of CPR</td>
<td>Mostly high profile reef and some sand and hard sand</td>
</tr>
<tr>
<td>Area 2 of CPR</td>
<td>Mostly silty sand and some patchy reef</td>
</tr>
<tr>
<td>Area 3 of CPR</td>
<td>Mostly different profile sands and some patchy reef</td>
</tr>
<tr>
<td>Area 4 of CPR</td>
<td>Different profile reefs (low, patchy and high)</td>
</tr>
<tr>
<td>Inner area of CPR</td>
<td>Reefs and some sand and hard sand</td>
</tr>
<tr>
<td>Alum Cliff</td>
<td>Sand and small areas of reef</td>
</tr>
<tr>
<td>Taroona High</td>
<td>Sand, silty sand, and small areas of reef</td>
</tr>
<tr>
<td>Derwent</td>
<td>Silty sand, sand and small patchy reef</td>
</tr>
</tbody>
</table>
Based on both movement and stationary periods, all monitored sharks showed similar habitat utilisation either by month or when all months were combined (Fig. 4.13 a and b).
Figure 4.13: Examples of individual and combined monthly habitat utilisation for (a) movements and (b) stationary periods of draughtboard sharks using 95% KUD contours. CPR: Crayfish Point Reserve.
**Habitat preference**

Of the total shark movements (n= 2594), 6% occurred when sharks moved between the outside and the inside of Crayfish Point Reserve. When moving between Alum Cliff and Crayfish Point Reserve, draughtboard sharks used the region of the reserve (area 1) adjacent to Alum Cliff on 80% of occasions. In contrast, sharks that moved between Taroona High and Crayfish Point Reserve used the closest area of the reserve (area 4) equally (43%) to the furthest area of the Crayfish Point Reserve (area 1). Sharks moving between the Derwent River and Crayfish Point Reserve used the closest area of the reserve (area 3) and the adjacent area (area 1) in equal proportions (38%). Area 1 was the preferred region for sharks to enter or leave the Crayfish Point Reserve (Fig. 4.14).

Despite the higher number of receivers in the Crayfish Point Reserve, the greatest number of movements were recorded by the six receivers at the Alum Cliff. These receivers recorded a significantly greater number of detections than all the other sites (Chi-square, P< 0.001) (Fig 4.15a). Although not significantly different, the two receivers at the Taroona High also had a higher number of movements compared to the Crayfish Point Reserve. The draughtboard sharks tended to move along the shore rather than spend time moving within the reserve. To determine if there was a preference for sharks to move around the Alum Cliff region compared to the Taroona High region, the proportion of detections at Taroona High were compared with the proportion of detections at the two inner (closer to shore) receivers of the six at Alum Cliff. These two receivers are at approximately the same distance from the Crayfish Point Reserve and cover the same detection radius as the Taroona High receivers. The two receivers at the Alum Cliff detected a significantly greater proportion of shark movements (0.30 ± 0.12
SE) than those at Taroona High (0.10 ± 0.05 SE) (Chi-square, P < 0.001), indicating that tagged sharks dispersed out of the Derwent River rather than further up the river.

Within the Crayfish Point Reserve the two sides of the reserve closest to the Alum Cliff (areas 1 and 2) detected a greater proportion of movements than those facing Taroona High (Fig. 4.15b). At a finer scale, sharks showed a higher proportion of movements in shallower regions (the overlaps areas 3,4 and 4,1) rather than the deeper region (the overlaps areas 1,2 and 2,3) (Chi-square, P < 0.001). Different areas of the Crayfish Point Reserve overlapped within a 50% KUD for each shark, two sharks were detected only in area 1, two sharks used the composite region of areas 1, 2 and the inner region of the reserve, two shark used only area 1 and 3, and three sharks used the entire reserve (Table 4.5 and Fig. 4.16). All nine sharks monitored were detected in area 1 during this study.
Figure 4.15: Area preferences for draughtboard sharks. a) Intensive and extensive array. b) Areas of the Crayfish Point Reserve (CPR). Values are mean + SE of the proportion of movements of each shark within specific regions. AC: Alum Cliff, TH: Taroona High, D: Upper and Lower Derwent, UC: Upper Channel. Different letter shows significant differences between movements and different areas.
Table 4.5: Areas within the Crayfish Point Reserve (CPR) that overlapped with the estimated 50% KUD of individual draughtboard sharks.

<table>
<thead>
<tr>
<th>Areas</th>
<th>Number of sharks whose 50% KUD overlapped in each area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area 1 and the joint area of 2 and 4</td>
<td>2</td>
</tr>
<tr>
<td>Areas 1, 2 and inner area</td>
<td>2</td>
</tr>
<tr>
<td>Area 1 and 3</td>
<td>2</td>
</tr>
<tr>
<td>The whole CPR</td>
<td>3</td>
</tr>
</tbody>
</table>

The whole CPR: Crayfish Point Reserve.

Figure 4.16: Example of habitat utilisation for draughtboard sharks determined by 50% KUD contours. CPR: Crayfish Point Reserve.

In contrast to shark movements within the Crayfish Point Reserve, the areas that overlapped within the 50% KUD contours for each shark indicated that sharks had stronger preferences for areas in which to have stationary periods (Table 4.6, Fig. 4.17). Area 1 and its overlap with area 4 has 66% of the 33 recorded stationary periods and 92% of the 674 hours spent stationary were recorded in these two areas (Fig. 4.18a and b). These areas are the only two areas characterised by a high profile reef (Table 4.4), indicating preferences for this reef type. Although areas 2 and the overlapping area 3,4 had a high proportion of movements they recorded a low number of stationary periods indicating that they were only transit zones to other areas of the Crayfish Point Reserve.
However, areas 1 and the overlapping area 1,4 were preferred for both visiting and having stationary periods.

Table 4.6: Areas within the Crayfish Point Reserve (CPR) that overlapped with the estimated 50% KUD of individual draughtboard sharks during their stationary periods.

<table>
<thead>
<tr>
<th>Areas</th>
<th>Number of sharks whose 50% KUD overlapped in each area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area 1 and the joint area of 2 and 4</td>
<td>7</td>
</tr>
<tr>
<td>Areas 1, 2 and inner area</td>
<td></td>
</tr>
<tr>
<td>Area 1 and 3</td>
<td></td>
</tr>
<tr>
<td>Inner area</td>
<td>2</td>
</tr>
<tr>
<td>The whole CPR</td>
<td></td>
</tr>
</tbody>
</table>

Figure 4.17: Example of habitat utilisation for draughtboard shark stationary periods determined by 50% KUD contours. **CPR**: Crayfish Point Reserve.
Figure 4.18: Habitat preferences for draughtboard shark stationary periods. a) Proportion of hours that draughtboard sharks spent in stationary periods in each area. b) Number of stationary periods spent in each area.
Day-night activity, habitat utilisation, and preference

Twelve draughtboard sharks were excluded from the day-night activity analysis because they had less than 20 movements (see Fig. 4.10). Of the remaining 12 animals, nine moved mostly at night, while the other three moved predominantly during the day (Fig. 4.19). Similar patterns were found irrespective of whether the sharks were inside or outside of the Crayfish Point Reserve. No sharks had an even distribution of movements over a 24 hr period.

The majority of the sharks (n=8) showed similar 95% and 50% KUD distributions between day and night suggesting no change in diel activity patterns either for habitat utilisation and preferences (Fig. 4.20 a and c). The remaining four sharks showed distinct diel patterns, although the areas used still overlapped (Fig. 4.20 b and d). Three sharks increased their 50% KUDs during the night moving out of the Crayfish Point Reserve and one shark increased its 50% KUD during the day.
Figure 4.19: Example of daily movement activity of draughtboard sharks. Two distinctive movement patterns were found, 3 sharks moved more during the day (shark 147) and 9 sharks moved more at night (shark 116).
Sharks tended to leave the Crayfish Point Reserve within the first month of being released. The average number of days spent at the reserve significantly decreased from 6 days (± 2 days), just after being released, to 1 day (± 1 day) after 4 to 5 months (t-test, P< 0.001) (Fig. 4.21).
4.3.2 CONVENTIONAL TAGGING

Between January 2000 and January 2005, 1234 draughtboard sharks were tagged in southwest and eastern Tasmania and the Crayfish Point Reserve. The Crayfish Point Reserve showed the highest recapture rate, 36% of 364 sharks tagged, followed by eastern and southwestern areas where the recapture rate was 9% (sharks tagged n=398) and 3% (sharks tagged n=472) respectively (Table 4.7). However, the fishing effort by researchers in the reserve was higher than for southwest and eastern Tasmania. The maximum time at liberty ranged from one month to up to five years in the Crayfish Point Reserve, from one month up to four years in both eastern and southern Tasmania.

Figure 4.21: Average number of days spent at the Crayfish Point Reserve for the draughtboard sharks. Values are means (± SE). Different letters show significant differences.
There were no significant differences in the proportion of recaptures (for either sex or size) with time (chi-square, P< 0.001).

Table 4.7: Summary of draughtboard sharks conventional tagging. Sharks were tagged in southwestern and eastern Tasmania and in the Crayfish Point Reserve.

<table>
<thead>
<tr>
<th>Area</th>
<th>Tagged (n)</th>
<th>Recaptures (n)</th>
<th>Recaptures (%)</th>
<th>Maximum time at liberty (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crayfish Point Reserve</td>
<td>364</td>
<td>132</td>
<td>36.3</td>
<td>5</td>
</tr>
<tr>
<td>Southwestern of Tasman</td>
<td>a</td>
<td>472</td>
<td>17</td>
<td>3.6</td>
</tr>
<tr>
<td>Eastern of Tasmania</td>
<td>398</td>
<td>37</td>
<td>9.3</td>
<td>4</td>
</tr>
</tbody>
</table>

**Distances travelled**

The majority of the draughtboard sharks were recaptured in the vicinity of where they were released (Table 4.8). The majority of the draughtboard sharks travelled a maximum distance of up to 10 km over the study period. However, for 4%, 7% and 18% of the sharks tagged in the Crayfish Point Reserve and on the east and southwest coast, the maximum distances travelled were 75, 250, and 300 km respectively (Fig. 4.22). No relationship between time at liberty and distances travelled was found.

Table 4.8: Recapture areas for conventionally tagged draughtboard sharks.

<table>
<thead>
<tr>
<th>Area</th>
<th>Recaptures in same area (n)</th>
<th>Recaptures in different areas (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crayfish Point Reserve</td>
<td>118</td>
<td>14</td>
</tr>
<tr>
<td>Southwestern of Tasman</td>
<td>a</td>
<td>15</td>
</tr>
<tr>
<td>Eastern of Tasmania</td>
<td>34</td>
<td>3</td>
</tr>
</tbody>
</table>
Figure 4.22: Examples of maximum reported distances travelled for draughtboard sharks. --- Tagged in southwestern Tasmania. ---- Tagged in eastern Tasmania. ----- Tagged in Crayfish Point Reserve. The lines represent the shortest possible route between the release and the recapture position.
Length-frequency composition of tagged and recaptured sharks

The size composition of female and male tagged draughtboard sharks was similar in the southwest area and the Crayfish Point Reserve, but was significantly different from eastern Tasmania which had a larger number of smaller males (Kolmogorov-Smirnov, P< 0.005) (Fig. 4.23). There were insufficient recaptures to compare the size of the sharks that were recaptured for the southwest and east coast populations. For the Crayfish Point Reserve, the size of recaptured sharks was not significantly different from the initial population that was tagged (Fig. 4.23).

Figure 4.23: Length-frequency composition of tagged and recaptured female (black bars) and male (grey bars) draughtboard sharks in different regions of Tasmania.
**Short and long term site fidelity for the Crayfish Point Reserve**

Sharks were found up to five years after release at the Crayfish Point Reserve. Around 2% (proportion 0.02) of sharks were subsequently recaptured each month during the first 11 months of release (Fig. 4.24). Sharks showed a gradual dispersion away from the Crayfish Point Reserve over the six years since tagging began (Fig. 4.25).

![Figure 4.24](image)

**Figure 4.24**: Monthly proportion of recaptured draughtboard sharks at the Crayfish Point Reserve. Numbers are mean + SE. Numbers above the bars are total number of recaptures. No significant differences were found between months (Chi-square test).
Figure 4.25: Yearly proportion of recaptured draughtboard sharks at the Crayfish Point Reserve. Numbers are mean ± SE. Numbers above the bars are total number of recaptures. No significant differences between years were found (Chi-square test).
4.4 DISCUSSION

A few studies have been reported using passive acoustic receivers to understand shark movements in complex reef habitats (Chapman et al., 2005; Garla et al., 2006). However, this was the first study using passive acoustic technology to address bottom dwelling sharks moving within a complex environment. In addition to external noise (produced by shipping traffic, waves, currents and vegetation) that can provide mixed signals to the receivers (Clements et al., 2005; Heupel et al., 2006a), the physical nature of the habitat (rocky reef) could also reduce the distance over which signals can be reliably detected. Draughtboard sharks were routinely observed by divers to rest in depressions in the reef and under ledges, which could result in missing or uncertain records. Consequently, it was difficult to know if sharks were resting in the reef area without being detected or had left the area. In this study, the combination of a complex habitat and the movement dynamics of the bottom dwelling draughtboard shark created uncertainty in positioning an animal. Despite these shortcomings a greater understanding of behaviour of this species was developed, including small-scale reef utilisation preferences. By determining the accuracy of the outer ring of receivers in the Crayfish Point Reserve and finding that the probability of a shark being detected was high (86 to 97%), there was greater confidence in correlating detections to behaviour.

In this study, only two sharks were internally tagged with acoustic transmitters. Initial trials with surgical implantation in aquaria studies were problematic with the sharks opening the insertion wound when they flexed as they were released back into the water. The attachment of external transmitters required less handling of the shark and did not involve local anaesthesia, surgery and recovery. However, external tags were susceptible to fouling which can lead to a reaction at the attachment site. Skin damage caused by abrasion of a fouled conventional tag was occasionally observed in draughtboard sharks. Moreover, sharks could rub the external transmitters against the reef or other sharks,
and the tags could also increase the probability of entanglement in nets and lines. McKibben and Nelson (1986) suggested that the behaviour of three grey reef sharks, *Carcharhinus amblyrhynchos*, was altered by the continual irritation of the dorsal fin where the transmitter was attached. As no draughtboard sharks have been resighted carrying the external transmitters, the impact of the external acoustic tag on the sharks remains uncertain. The two sharks with internally placed tags did not appear to behave any differently to the externally tagged sharks during the period of the study. Similar conclusions on the behaviour of externally and internally tagged sharks were reported for other species such as: *Galeocerdo cuvier* (Holland et al., 1999), *Negaprion brevirostris* (Gruber et al., 1988) and *Scyliorhinus canicula* (Sims et al., 2001). From this study it could be suggested that although the external tagging procedure was quicker, the uncertainty of possible damage on draughtboard sharks due to the external tags is an important factor that could affect future shark behaviour. Further research is required to determine the benefits of either internally or externally tagging draughtboard sharks.

While it was difficult to determine the impact of *in situ* tagging on draughtboard sharks, 41% of individuals were not recorded in the Crayfish Point Reserve on the day of release. This suggests that they moved to regions of the reef where they were not detected. As all sharks were released towards the middle of the reserve it was unlikely that they could have swam beyond the detection limits of the outer Crayfish Point Reserve receivers before transmitting their first signal (ie. Maximum time between ‘pings’ was 60 seconds). It was also unlikely that all these sharks they would have swam through the outer ring of receivers without being detected given the greater than 86% chance of detection. It was possible that tagging affected the initial behaviour of the sharks causing them to rest and recover from capture and tagging in regions of the reef (eg. caves) where signals could not be detected.

Sharks tagged in this study were either, captured and released in Crayfish Point Reserve or captured on the east coast of Tasmania and released in the Crayfish Point
Chapter four - Movement

Reserve. The majority of the draughtboard sharks that were translocated from the east coast did not stay within the release area for longer than one month, after which they left the intensive array to be finally recorded around the lower section of the Derwent River. While no significant difference could be detected with the small number of sharks tagged, there were indications that sharks did show some site fidelity as the sharks sourced from the Crayfish Point Reserve were sighted back in the reserve on more occasions and tended to disperse less widely than those sourced from the east coast.

The rapid decrease in acoustically tagged sharks recorded in the Crayfish Point Reserve was similar to the conventional tag data from the reserve where the majority of sharks were not seen after tagging. The acoustic data suggests that these sharks were still in the general vicinity of the Derwent River but only revisited the Crayfish Point Reserve at decreasing intervals as time increased. As with the large distances recorded for conventional tag recaptures, two of the acoustically tagged sharks moved out of the Derwent River and associated Storm Bay. Similarly, (McLaughlin and O’Gower, 1971) found that the demersal shark *Heterodontus portusjacksoni* undertook both short movements around its reef habitats and occasional long (hundred of kilometres) movements. Only short-term movements were reported in the scyliorhinidae *Scyliorhinus canicula* (Rodriguez-Cabello *et al.*, 1998; Sims *et al.*, 2001).

Draughtboard sharks of both sexes and all sizes used the complete habitat within the boundaries formed by the intensive array, the Derwent River and the Upper Channel. Within the Derwent region, the Crayfish Point Reserve appeared to be towards the limit of draughtboard shark habitat as a greater proportion of sharks moved between the Reserve and the mouth of the Derwent rather than moving in the other direction. Within the small region occupied by the Crayfish Point Reserve, draughtboard sharks did not use the reserve in a random manner. The great number of movements detected on the Derwent mouth side of the Reserve (area 1) would be expected as a result of the greater movement in this direction (as noted above). However, the high use of area 1 by
sharks entering and exiting the reserve from the Taroona High site suggest that sharks were actively using this area as a region to enter and exit the Crayfish Point Reserve. The main difference between area 1 and the rest of the Crayfish Point Reserve was the increased presence of higher profile reef. As higher profile reef would be expected to interfere with acoustic signal transmission, detections in this region were possibly under represented.

Cooper (1978) and Simpendorfer and Heupel (2004) recommended that the temporal pattern of spatial occupation is crucial for determining whether an animal randomly visits habitat or the habitat is the area usually occupied by it (home range). In species such as neonate blacktip sharks *Carcharhinus limbatus* (Heupel *et al.*, 2004), the sixgill shark *Hexanchus griseus* (Dunbrack and Zielinski, 2003), and the temperate rocky reef teleost *Cheilodactylus fuscus* (Lowry and Suthers, 1998) changes over time of the home range or seasonal variations in habitat use were reported. These seasonal movements were related to survival strategies, feeding activity and reproductive behaviour. In contrast, and similar to other species such as juveniles of *Carcharhinus perezi* (Garla *et al.*, 2006), the coral reef fish *Plectomorus leopardus* (Zeller, 1997) and the snapper *Pagrus auratus* (Parsons *et al.*, 2003), draughtboard sharks showed no temporal patterns of habitat utilisation throughout the study period.

Draughtboard sharks showed a preference for crepuscular and night time activity in comparison to moving during the day. Similarly, theses same activity periods have been reported for other bottom dwelling shark species in their natural environment, such as the angel shark *Squatina californica* (Standora and Nelson, 1977), the horn shark *Heterodontus francisci* (Nelson and Johnson, 1970), the scyliorhinids *Scyliorhinus canicula* (Sims *et al.*, 2001), and *Cephaloscyllium ventriosum* (Nelson and Johnson, 1970). Movements in draughtboard sharks were probably associated with feeding activity, as the main dietary items are nocturnally active animals such as octopus (*Octopus maorum*), squids, southern rock lobster (*Jasus edwardsii*) and crabs (Awruch, personal observation).
Although night time activity was most common, several sharks also moved during the day. This has also been observed for other bottom dwelling species such as *Heterodontus portujacksoni* (McLaughlin and O’Gower, 1971), *Heterodontus francisci* and *Cephaloscyllium ventriosum* (Nelson and Johnson, 1970) which were all found to feed mainly at night with a small number of observations of day time feeding. Although day/night differences in habitat utilisation are common among chondrichthyans (Gruber *et al.*, 1988; Holland *et al.*, 1993; Sims *et al.*, 2001; West and Stevens, 2001; Sims, 2003) for the majority of the draughtboard sharks there were limited differences between the areas utilised during the day and night. This suggests that the sharks had established feeding areas or recognised certain habitat types (eg: high profile reef) as a more productive region to locate food.

Within the Crayfish Point Reserve, draughtboard sharks preferred high profile reef habitat to have stationary periods. In addition, divers have reported draughtboard sharks resting in rocky crevices by themselves or in groups. Similar activity has been reported for *H. portujacksoni* (McLaughlin and O’Gower, 1971) and other species of scyliorhinids such as *C. ventriosum, S. canicula, S. stellaris* and *C. ventriosum* (Nelson and Johnson, 1970; Sims *et al.*, 2001; Sims *et al.*, 2005). Although periods of inactivity have been reported in other species, such as *H. francisci, C. ventriosum, S. stellaris* and *S. canicula* (Nelson and Johnson, 1970; Sims *et al.*, 2001; Sims *et al.*, 2005), this was the first time that a continuous period of five days in stationary behaviour has been documented for any species. Avoidance of predators, thermoregulation, sexual behaviour and digestion have all been suggested as reasons for periods of inactivity among benthic sharks, especially within the Scyliorhinids (Economakis and Lobel, 1998; Sims *et al.*, 2001; Sims, 2003; Sims *et al.*, 2005). (Sims *et al.*, 2001) reported different sexual aggregation behaviours in *S. canicula*, where the resting periods in males occur on gravel substratum in deep water, while females rest in caves or under rocks in shallow water. In other species such as *S. stellaris*, no sexual segregation was reported with both sexes found to rest in a rocky
habitat (Sims et al., 2005). In this study, there was no correlation between periods of inactivity and either physical parameters such as lunar phase, diel cycle, months or tides or between biological parameters such as sex, reproductive condition, or size. It is therefore most likely that the reason for extended periods of inactivity was due to digestion of prey. Awruch (unplub. data) found that large prey items (e.g. 4 kg octopus) were often present in the stomach of the sharks and these would be expected to take a considerable period to digest. Observations in captivity found that draughtboard sharks swallowed rather than chewed food items, and recreational fishers report that a problem with catching draughtboard sharks is that they swallow the baited hooks. It is postulated that the long stationary periods that were found in this study were associated with sharks digesting large prey items.

The recapture of the majority of the draughtboard sharks in the vicinity of where they were released was most likely a function of the research design as few (16.2%) were returned by non-researchers. Research surveys in the Crayfish Point Reserve and the east and southwest coasts revisited the same sites. Thus it is reasonable to expect that the majority of the recaptures would come from these surveys. The higher number of research recaptures in the Crayfish Point Reserve (89%) could be associated with the increased and more frequent sampling undertaken in this region. In contrast, surveys in southwest and eastern Tasmania occurred once a year at similar periods and for the same duration.

The lack of tag reporting by fishers using traps or nets clearly highlights the problems associated with gathering data on by-catch species. Although there is greater recognition that fisheries are to be managed under the principles of ecosystem based fisheries management, and that recording and reporting of by-catch is important, the reporting of recaptured tagged animals that are returned to the sea (i.e. no commercial value) remains problematic. During this study, the tagging program was published in fishing industry magazines and explained through talks given to both gillnet and trap fishers. Fishers
were familiar with reporting tags as many of the Tasmanian target species have been the subject of tagging studies. Despite this lack of reporting, conventional tag returns have indicated a degree of mixing over larger ranges. Large distance movements were recorded between eastern and western Tasmania and between southern and northern Tasmania. The conventional tag returns have also demonstrated longer-term site affinities with several sharks being recaptured in the same location up to five years after tagging. Similarly, long-term site fidelity or philopatric behaviour (animals returning to a specific location) has been recorded for other species of sharks. The horn shark *H. portujacsoni*, the dogfish *S. canicula* and the hammerhead shark *Sphyma tiburo* were reported to return to a specific location after periods of absence that can be measured in months or years (Rodriguez-Cabello *et al.*, 1998; Sims *et al.*, 2001; Heupel *et al.*, 2006b).

In summary, although short-term, the acoustic tag data has revealed information on specific habitat preferences, movements, activity and stationary patterns about this species that were previously unknown. Together with the conventional tagging data, there was confirmation that while mixing between broad regions does occur, the general pattern of movement was of limited dispersion within Tasmania’s major coastal regions.
General conclusions
The aim of the present study was to investigate the biology and ecology of the draughtboard shark, Cephaloscyllium laticeps, a common predator of rocky reef ecosystems in southeastern Australia. This thesis has focused on two important components: reproduction, including endocrine control, and the study of movement, activity patterns and habitat utilisation.

In chapter two, the reproductive biology of Cephaloscyllium laticeps was described in detail. As reproduction is one of the most important events in the life cycle of any living organism, being the primary requirement for successful propagation, understanding the reproductive process was considered the first necessary component for scientific investigation in this species. Sexually mature draughtboard shark females were found throughout the year with a slightly higher proportion of pregnant females in the first six months of the year. Sperm production in males was also higher early in the year with a subsequent slight decline later in the year, evident from both macroscopic examination and steroid hormones levels. Females laid two eggs at monthly intervals and embryo development took approximately one year. Together with the results of other studies, two basic reproductive strategies in oviparous chondrichthyans are apparent. Species from higher latitudes, such as the draughtboard shark, tend to reproduce all year round and have longer periods of egg incubation, sperm storage and time between oviposition of successive pairs of eggs. In contrast, species from lower latitudes tend to have distinctive reproductive seasons and have relatively shorter periods for incubation, sperm storage and oviposition.

In addition to macroscopic examination of gonadal stages, the role of steroid hormones in sexual maturation and egg development was explored, in view of their actions as triggers or regulators of all aspects of reproduction. In the present study, the steroid hormones testosterone (T) and 17β-estradiol (E₂) played a major role during the
follicular phase of draughtboard shark females, while progesterone (P₄) was primarily involved with the ovulatory phase. These results follow the general pattern in T and E₂ during follicle maturation reported in other oviparous species. However, there appears to be no constant pattern during the latter stages of the reproductive cycle, with a diverse behaviour of these hormones in different oviparous species (Sumpter and Dodd, 1979; Koob et al., 1986; Heupel et al., 1999; Sulikowski et al., 2004). In contrast, and in accordance with previous studies (Koob et al., 1986; Heupel et al., 1999; Koob and Callard, 1999), the results of this work showed a clear role of P₄ during ovulation and oviposition. In draughtboard males, T was the main steroid hormone produced during sexual development. The results of the present study supported the view that T played a major role in the regulation of testis development and in the final stages of sperm maturation, as was suggested by various authors (Callard et al., 1985; Sourdaine et al., 1990; Sourdaine and Garnier, 1993; Heupel et al., 1999; Tricas et al., 2000). Although studies on chondrichthyan endocrinology have advanced in the last few years (reviewed in Gelsleichter, 2004), the information is still very limited and insufficient to completely understand the endocrine control of reproduction in all the oviparous species in this group of fish. However, results from the present study provided new information and an improved understanding of endocrine control of reproduction and reproductive strategies in oviparous chondrichthyans.

Based on the positive correlation between sexual maturity and steroid hormone levels described in chapter two, chapter three explored the potential of steroid hormone measurements as a non-destructive technique to assess reproduction for applied fisheries research. Hormone measurements were found to produce almost identical results in estimating size at maturity and elucidating the reproductive cycle as macroscopic examination of gonadal stages from dissected sharks. Although only validated for *Cephaloscyllium laticeps*, it is possible that this technique will be widely
applicable for describing size at maturity and reproductive seasonality in different chondrichthyan species. Positive correlations found by other authors between macroscopic observations and steroid hormones for different species and different reproductive modes (Rasmussen and Gruber, 1990; Tricas et al., 2000; Sulikowski et al., 2004), suggest that there is the potential to apply these methods to all chondrichthians. While validation of the steroid hormone levels for different chondrichthians will require sacrificing a small number of individuals, the hormone levels may be sufficiently consistent between species or reproductive groups to minimise the need for validating each species.

Whether studying chondrichthians bycatch for ecosystem based fisheries management or managing vulnerable and endangered species, the need for reproductive data to ensure that populations contribute to future generations is essential (Hall et al., 2000; Walker et al., 2005). Therefore, non-lethal sampling for biological assessment will be increasingly important in the management of vulnerable chondrichthyan populations. These results were the first to demonstrate the potential use of steroid hormones for applied fisheries management and open the way for hormone measurements to become a significant scientific tool for non-destructive sampling in chondrichthians.

In chapter four, a combination of conventional and acoustic tagging studies were used to understand the movements, activity patterns, and habitat used by draughtboard sharks. As a bottom dwelling species it was not surprising to find that sharks alternated between swimming and stationary periods, although the finding that a shark could be stationary for up to five continuous days has never been previously reported. Although the majority of the sharks tended to move at night (probably related to movements of their main prey items), they also make opportunistic movements at other times.

Sharks were found to use all regions of the Crayfish Point Reserve during the six month study, with preferred regions for stationary periods as well as entering and exiting
the reserve. The preference for sharks having stationary periods in high profile reef areas suggested that the sharks may seek caves as refuge area for predator avoidance. Although information on draughtboard shark predators has not been reported, this species has been found in the stomach of seven-gill sharks, *Notorynchus cepedianus* (Last, P., CSIRO Marine and Atmospheric Research, Hobart. pers. comm.). Both the conventional and acoustic tagging data showed a preference for sharks to remain in the general vicinity of tagging. Gradual dispersion rather than established migratory routes appeared to be the general movement pattern, however, recaptures from conventionally tagged sharks did demonstrate that this species is capable of travelling relatively long distances.

The results of this work have increased the understanding of the behaviour of bottom dwelling sharks, particularly within the Scyliorhinidae family. Although, a few studies have described the movement patterns and habitat utilisation of scyliorhinids (Nelson and Johnson, 1970; Sims *et al.*, 1993; Sims *et al.*, 2001; Sims *et al.*, 2005), no information on longer-term movements (>6 months) using acoustic technology have been previously reported. In addition, this was the first work using listening stations to understand movement behaviour in bottom dwelling sharks in complex reef habitats, and it was the first study using passive tracking within the scyliorhinids.

With the move to ecosystem based fisheries management it is important to consider the sustainability of catches of major bycatch species. Fundamental to management of bycatch will be the need to ensure that populations can be sustained through adequate reproduction and available habitat. The results of the present study provided important information on the reproductive cycle, movements, habitat requirements, and activity patterns of draughtboard sharks. This will allow development of management plans that consider the requirement of this temperate, rocky reef predator.
Marine protected areas (MPA’s) or fishery closures have been reported as an effective spatial tool for fisheries management (Jamieson and Levings, 2001; Stevens, 2002; Baelde, 2005; Blyth-Skyrme et al., 2006). Although sharks are usually highly mobile animals which often have an extensive distribution (Stevens, 2002), MPA’s can still play a useful role in their management and conservation; as closed areas effectively reduce fishing mortality protecting parts of the population. However, as draughtboard sharks showed no indication of distinctive reproductive seasons or areas, and no strong site fidelity; the implementation of shark refuge areas is unlikely to be particularly effective in protecting draughtboard sharks. Instead, a minimum legal size above the 50% size at maturity that enables sharks to reproduce for several years before being harvested should be implemented. Limiting catches between January and June, the time of peak egg deposition, should also be considered.

In summary, this study has markedly increased the knowledge of the biology and ecology of the draughtboard shark. With new requirements to address bycatch in integrated ecosystem based management programs, together with the ecosystem consequences of removing upper trophic level predators, it is important to conserve draughtboard sharks to have a healthy southern Australian reef ecosystem. As well as providing important life-history information on this species, this work has been some of the first to investigate hormonal control of reproduction in chondrichthyans in the wild. This study has also pioneered the use of hormone measurements as a non-lethal sampling tool for elasmobranch reproductive studies, which has important conservation implications for protected and endangered species.
REFERENCES


