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 chondrichthyan 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 chondrichthyan 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.

Casper calcification is the most common external method used to assess sexual maturity in male chondrichthyans (Clark and Von Schmidt, 1965). However, not all species (eg: 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 chondrichthyans, 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.
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 2.21) 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).

\[
P = \frac{e^{(x+bx)}}{1 + e^{(x+bx)}} \quad \text{Equation 3.1}
\]

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:

$$D = B_0 + B_1X_1 + B_2X_2 + \ldots + B_nX_n$$  \hspace{1cm} \textit{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 $P(Gi/D)$ was estimated by:

$$P(Gi/D) = \frac{P(D/Gi)P(Gi)}{\sum_{i=1}^{n} P(D/Gi)P(Gi)}$$  \hspace{1cm} \textit{Equation 3.3}

Where $P(Gi)$ 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 $P(D/Gi)$ 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 E₂, (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, $r^2=0.80$, $n=609$), and 761 mm TL (95% confidence interval = 754.98 – 789.60, $r^2=0.84$, $n=462$) 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, χ² = 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:

\[ D = -6.919 + 0.008*TL + 0.90*T + 0.22*E₂ \]

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.
Table 3.1: Standardized discriminant function coefficients and correlations of the discriminant linear function for draughtboard shark females. Total 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>Total length</td>
<td>0.61</td>
<td>0.90</td>
</tr>
<tr>
<td>Testosterone</td>
<td>0.42</td>
<td>0.80</td>
</tr>
<tr>
<td>Estradiol</td>
<td>0.40</td>
<td>0.71</td>
</tr>
<tr>
<td>Progesterone</td>
<td>0.16</td>
<td>0.58</td>
</tr>
</tbody>
</table>

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:

\[
D = -4.239 + 0.070\times CL + 0.234\times T
\]
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.
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>

**Multi-dimensional scaling (MDS)**

**FEMALES**

A combination of T and E₂ successfully separated the reproductive stages of female sharks. Based on the discriminant function result of the contribution of P₄ into the separation of both groups, P₄ 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).
Males

Based on the results from the discriminant function analysis, where CL and T played a major role in the separation between juveniles and adults, E₂ and P₄ 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).
Figure 3.4: Multi-dimensional scaling (MDS) of juvenile (white triangles) and adult (black triangles) draughtboard shark males of known maturity, using clasper length and testosterone. The vertical dashed line represents the “95% cut off” line whereby 95% of adults were to the left of this line.

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>95% Confidence interval TL (mm)</th>
<th>95% Confidence interval n</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Females</strong> (macroscopic)</td>
<td>814</td>
<td>-</td>
<td>798 – 830</td>
<td>118</td>
</tr>
<tr>
<td><strong>Females</strong> (LDPM analysis)</td>
<td>823</td>
<td>1.10</td>
<td>812 – 832</td>
<td>118</td>
</tr>
<tr>
<td><strong>Females</strong> (MDS analysis)</td>
<td>829</td>
<td>1.84</td>
<td>811 – 848</td>
<td>118</td>
</tr>
<tr>
<td><strong>Males</strong> (macroscopic)</td>
<td>779</td>
<td>-</td>
<td>762 – 790</td>
<td>111</td>
</tr>
<tr>
<td><strong>Males</strong> (LDPM analysis)</td>
<td>776</td>
<td>-0.38</td>
<td>768 – 783</td>
<td>111</td>
</tr>
<tr>
<td><strong>Males</strong> (MDS analysis)</td>
<td>782</td>
<td>0.25</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.

![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-url)

**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.
Figure 3.6: MDS ordination of draughtboard shark males (known and unknown maturity) using clasper length and testosterone. J (juveniles): white triangle, A (adults): black triangle, U (unknown): grey circles. The vertical dashed line represents the “95% cut off” line whereby 95% of adults were to the left of this line.

Size at maturity

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>Method</th>
<th>TL (mm)</th>
<th>$r^2$</th>
<th>$a$ and $b$ values</th>
<th>95% Confidence interval</th>
<th>n</th>
</tr>
</thead>
<tbody>
<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 3.5:** Proportion of mature draughtboard sharks caught in lobster traps from a marine reserve and from the rest of Tasmania.

<table>
<thead>
<tr>
<th>Location</th>
<th>Proportion adult animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Females</td>
</tr>
<tr>
<td>Marine Reserve</td>
<td>0.29</td>
</tr>
<tr>
<td>Rest of Tasmania</td>
<td>0.38</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 E$_2$, or P$_4$ 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 (O) 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 claspsers 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 chondrichthyan 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
eqlanteria (Rasmussen et al., 1999) and Dasyatis 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 (ie. 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 chondrichthynes, 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 through 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.