Reproduction in Elasmobranchs

(Chapter from Module Seven of the e-quarist course)

This chapter has been supplied by my good friend and colleague Dr Jon Daly. Since 2004, Jon and I have been working on developing Assisted Reproduction Technologies (ART) to assist with captive elasmobranch reproduction. This came about because in 2002, the Australian Federal Government banned the collection from the wild by public aquariums of Grey Nurse (or Sand Tiger) sharks (*Carcharias taurus*).

This chapter has been adapted from Jon's PhD "Development of Assisted Reproductive Technologies in captive fish", which was completed in 2008. You will see that there are many references (as it was from his PhD). They are not included in the reference list of this module. However, if any student wishes to follow up on any aspect, please contact The Aquarium Vet and we can provide details of the specific reference.

Jon and I have also co-authored a chapter for the second Elasmobranch Husbandry Manual which is currently in print. "The use of reproductive technologies in breeding programs for elasmobranchs in aquaria" will be made available later in 2016.

Male reproductive anatomy and spermatogenesis

The reproductive system of male elasmobranchs is made up of several key structures that are involved in the development of sperm and delivery of sperm to the female reproductive tract. Some of these structures are homologous to mammalian structures, while others are unique to elasmobranchs.

Testes

The testes are paired, elongated organs suspended from the dorsal wall at the anterior end of the body cavity by the mesorchium (Hamlett 1999). See Figure 81. The testes in all male elasmobranchs are embedded in the epigonal organ, which is composed of lymphomyeloid tissue (Engel and Callard 2005). Spermatogenesis and steroidogenesis are the two functions of the testes, but there are notable differences in structure and function compared to mammalian testes. The main difference is that elasmobranch testes do not have seminiferous tubules.

Spermatogenesis occurs within discrete membrane-bound units called spermatocysts, which contain sertoli cells and a cohort of synchronously developing germ cells (Jones and Jones 1982; Pratt 1988; Engel and Callard 2005). Spermatocyst formation begins at the germinal zone with the association of a single sertoli cell with a single germ cell to form a unit termed a "spermatoblast" (Parsons and Grier 1992; Hamlett 1999). Following proliferation of sertoli and germ cells, the spermatocyst eventually consists of up to 500 sertoli cells each with a complement of closely associated developing germ cells. There is a maturational progression of spermatocysts radiating from the germinal zone towards the efferent ductules, as existing spermatocysts are displaced by the formation of new ones (Stanley 1966; Pratt 1988; Parsons and Grier 1992; Hamlett 1999).

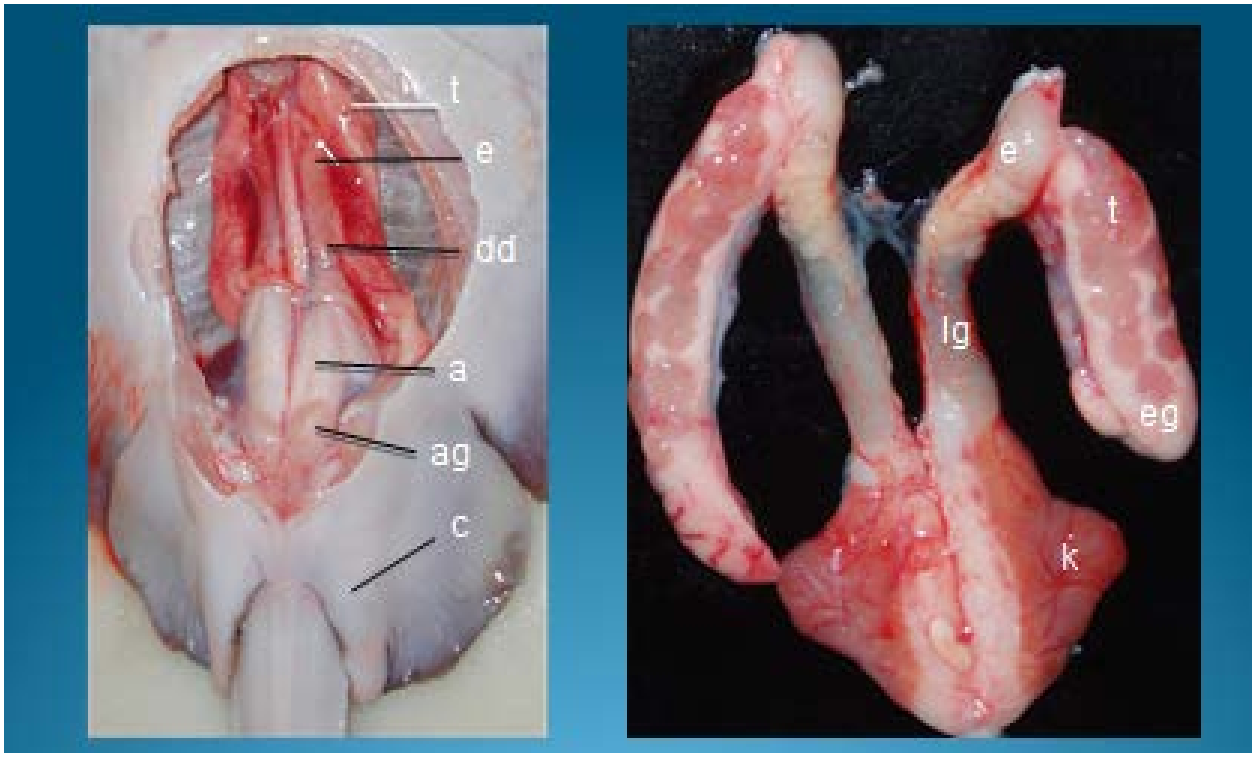

Figure 1 - Male reproductive anatomy of a sparsely spotted stingaree (*Urolophus paucimaculatus*) (courtesy of Jon Daly). KEY: ampulla of ductus deferens (a), alkaline gland (ag), clasper (c), ductus deferens (dd), epididymis (e), epigonal organ (eg), kidney (k), leydig gland (lg), testis (t).

The location of the germinal zone within the testis and the direction of spermatocyst maturation vary between elasmobranch species. Pratt (1988) was the first to define the three different testis types in elasmobranches (see Figure 82). They are:

- diametric
- radial
- compound

In **diametric testes**, the germ cells are located in a strip on one side of the testis, and developing spermatocysts progress across the width of the testis to the efferent ductules located on the opposite side. This testis type is the most common among elasmobranchs. It

is found in the carcharhinid sharks e.g. blue shark (*Prionace glauca*) and bronze whaler (*Carcharhinus brachyurus*) (Pratt 1988)), and is also observed in the Port Jackson shark (*Heterodontus portusjacksoni*) (Jones and Jones 1982), piked dogfish (*Squalus acanthias*) (Dobson and Dodd 1977b; Callard *et al.* 1985; Dubois and Callard 1989) and draughtboard shark (*Cephaloscyllium laticeps*) (Awruch *et al.* 2008b).

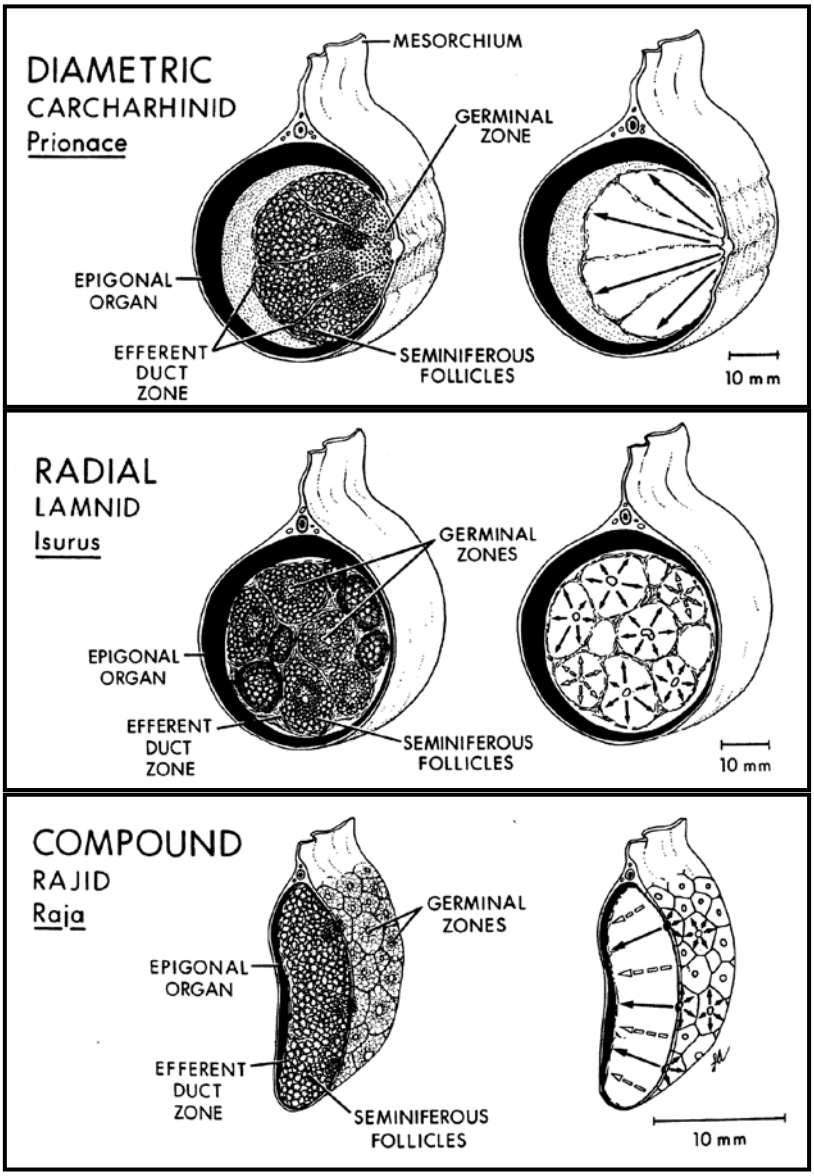

Figure 2 - The three types of elasmobranch testes (from Pratt 1988)

In **radial testes**, the germinal zone is located at the centre of multiple lobular structures in the testis that are separated by a duct system. Developing spermatocysts progress in a radial pattern toward the outer margin of the lobe and are collected by the duct system after spermiation. Species with a radial testis type include the lamnoid sharks such as the shortfin

mako (*Isurus oxyrinchus*), white shark (*Carcharodon carcharias*), and thresher shark (*Alopias vulpinus*) (Pratt 1988).

Compound testes are a combination of both radial and diametric types. Spermatocysts develop within multiple lobular structures that are not separated by a duct system. The germinal zone is located at the centre of each lobule, with spermatocysts developing first towards the outer edge and then across the diameter of the testis to the efferent ductules located on the opposite surface. This testis type has only been reported in a few species of skate and ray, including the Atlantic stingray (*Dasyatis sabina*) (Maruska *et al.* 1996), freshwater stingray (*Himantura signifer*) (Chatchavalvanich *et al.* 2005), little skate (*Raja erinacea*), and smooth butterfly ray (*Gymnura micrura*) (Pratt 1988). The compound testis is presumed to be the testis type common to all skate (Rajid) species (Pratt 1988; Hamlett 1999; Engel and Callard 2005).

Spermatogenesis

Spermatogenesis begins with the association of a single gonocyte with a single primitive sertoli cell (Hamlett 1999; Engel and Callard 2005). In the initial stages of spermatocyst formation, both cells undergo co-ordinated mitosis, maintaining the 1:1 ratio of germs cells: sertoli cells (Stanley 1966; Callard *et al.* 1988). In the early stages of development, the spermatocyst has a central lumen, with germ cells located around the periphery of the spermatocyst and sertoli cells at the lumen (Parsons and Grier 1992; Maruska *et al.* 1996; Engel and Callard 2005; Awruch *et al.* 2008b).

At the end of sertoli cell proliferation there are between 250 and 500 sertoli cells per spermatocyst, with some variation between species (Stanley 1966; Jones and Jones 1982; Parsons and Grier 1992; Hamlett 1999). The germ cells undergo four further mitoses, changing the ratio of germ cells:sertoli cells to 16:1 (Callard *et al.* 1988; Engel and Callard 2005). Towards the end of the mitotic stage, sertoli cells begin migrating toward the periphery of the spermatocyst and take up a position adjacent to the basement membrane (Callard *et al.* 1988; Maruska *et al.* 1996; Engel and Callard 2005; Awruch *et al.* 2008b).

The spermatocysts increase in diameter prior to the first meiotic division due to an increase in size of both the primary spermatocyte nucleus and sertoli cell cytoplasm (Stanley 1966; Callard *et al.* 1988; Parsons and Grier 1992; Engel and Callard 2005). By the end of the primary spermatocyte stage, migration of sertoli cells to the periphery of the spermatocyst is complete (Sourdaine and Jegou 1989; Parsons and Grier 1992; Maruska *et al.* 1996; Awruch *et al.* 2008b). The sertoli cells lining the basement membrane of the spermatocyst become closely associated and gap junctions form between adjacent cells, creating a barrier between the spermatocyst contents and the testicular stroma (Engel and Callard 2005). The secondary spermatocyte stage, prior to the second meiotic division, is brief (Sourdaine and Jegou 1989; Parsons and Grier 1992; Maruska *et al.* 1996; Engel and Callard 2005) and characterised in

some species by spermatocytes with a small, condensed nucleus (Dobson and Dodd 1977b; Parsons and Grier 1992; Hamlett 1999).

At the completion of the second meiosis, 64 spermatids are associated with each sertoli cell and completely fill the interior of the spermatocyst (Jones and Jones 1982; Callard *et al.* 1988; Parsons and Grier 1992; Hamlett 1999). Early spermatids have an elliptical nucleus (Parsons and Grier 1992; Maruska *et al.* 1996; Hamlett 1999; Awruch *et al.* 2008b), and at this stage fluid spaces begin to form between the spermatids and the relationship between the sertoli cell and its cohort of germ cells starts to become apparent (Callard *et al.* 1988; Engel and Callard 2005).

Spermiogenesis involves condensation and elongation of nuclear chromatin, reduction of the cytoplasm, and formation of the acrosome, midpiece and flagellum (Stanley 1966; Callard *et al.* 1988; Sourdaine and Jegou 1989; Parsons and Grier 1992; Maruska *et al.* 1996; Hamlett 1999; Engel and Callard 2005; Awruch *et al.* 2008b). Elongated spermatids gradually become laterally aligned at the head to form bundles, which are closely associated anteriorly with the sertoli cells (Stanley 1966; Callard et al. 1988; Sourdaine and Jegou 1989; Parsons and Grier 1992; Maruska et al. 1996; Engel and Callard 2005). See Figure 83.

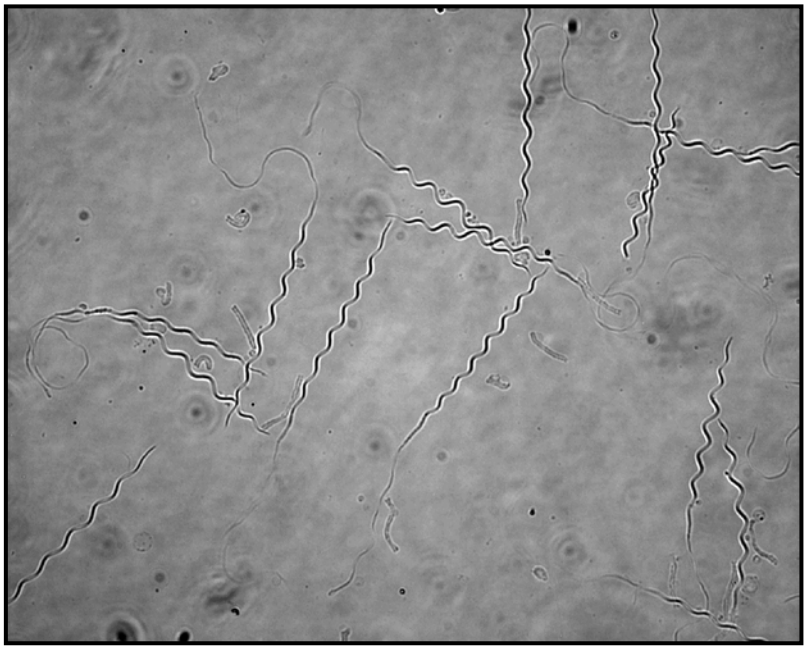

Figure 3 - Semen sample from a sparsely spotted stingaree (*Urolophus paucimaculatus*) (courtesy of Jon Daly).

Spermatids in mature spermatocysts have the helical nucleus characteristic of elasmobranch spermatozoa and form tightly packed bundles embedded in the sertoli cell cytoplasm (Sourdaine and Jegou 1989; Parsons and Grier 1992; Maruska *et al.* 1996; Hamlett 1999). At spermiation, the sertoli cells rupture to release the spermatids, which flow out of the spermatocyst (along with some sertoli cell remnants) into the collecting duct system (Stanley

1966; Callard *et al.* 1988; Maruska *et al.* 1996). The remains of the spermatocyst and sertoli cells left behind gradually break down and are resorbed (Stanley 1966).

In many elasmobranch species, the testes undergo annual seasonal changes in spermatogenesis (Wourms 1977; Parsons and Grier 1992). This is often marked by changes in gonadosomatic index (GSI) (Parsons and Grier 1992; Maruska *et al.* 1996; Lucifora *et al.* 2005) and in some species the presence of a degenerate zone in the testes (Simpson and Wardle 1967; Jones and Jones 1982). In some species, seasonal changes in GSI correspond with the annual breeding season, while others breed all year round irrespective of GSI, or have a defined mating season but no change in GSI (Parsons and Grier 1992). Changes in testicular function and the presence of a degenerate zone in the testes are believed to be linked to seasonal cycles in pituitary hormone levels.

Studies indicate that the ventral lobe of the pituitary gland is the primary source of gonadotropins in elasmobranchs (Dobson and Dodd 1977a), and that seasonal changes in gonadotropin levels are probably related to water temperature (Dobson and Dodd 1977c).

Spermatozoa morphology

As in mammals, the sperm of elasmobranchs is made up of a head, midpiece, and tail (see Figure 84). The head of all chondrichthyan sperm is long (> 30 µm) and helical in structure, with the nucleus following the same course (Jamieson 2005). Stanley (1971a) suggested that the sperm of *Squalus suckleyi* (= *Squalus acanthias*) derives its helical head shape from a series of intranuclear fibres, which join together during spermatid elongation to form fibrillar nuclear sheets.

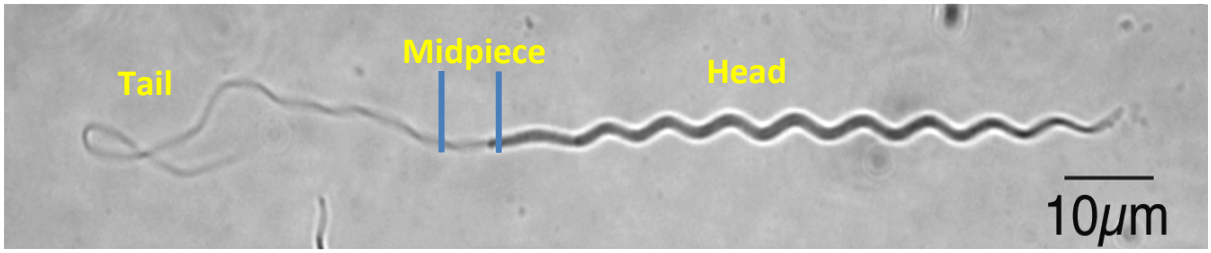

Figure 4 - Sperm from a sparsely spotted stingaree (*Urolophus paucimaculatus*) (courtesy of Jon Daly).

The midpiece of elasmobranch sperm consists of an axial midpiece rod around which the mitochondria are arranged (Stanley 1971a; 1971b),and these in turn are surrounded by a fibrous sheath (Jamieson 2005). The axial midpiece rod is a structure unique to the class

Chondrichthyes, and takes the place of the nine coarse fibres that make up the centre of the mammalian sperm midpiece (Stanley 1971a; Jamieson 2005).

The flagellum (or tail) is comprised of two key structures, the central axoneme and the longitudinal columns. As the central axoneme rotates on its longitudinal axis along the length of the flagellum, the longitudinal columns remain fixed at doublet positions 3 and 8 to create a double helix structure (Stanley 1971b; 1983; Jamieson 1991).

Hypothalamic-pituitary-gonadal axis and steroidogenesis

As in mammalian species, the testes in elasmobranchs are the main sites of androgen production (Hamlett 1999; Gelsleichter 2004; Engel and Callard 2005). The presence of a hypothalamic-pituitary-gonadal axis in elasmobranchs has been suggested, although many aspects of this axis remain undefined (Engel and Callard 2005). The presence of several forms of gonadotropin-releasing hormone (GnRH) in the hypothalamus of elasmobranchs is well established (Lovejoy *et al.* 1992; Calvin *et al.* 1993; Gelsleichter 2004). Some of these forms are unique to elasmobranchs, while others are similar to GnRH forms found in chicken, salmon, and lamprey (Lovejoy *et al.* 1992; Calvin *et al.* 1993). Stimulation of the pituitary by GnRH is suggested to occur via the general circulation, as there is no direct neural or vascular link between the hypothalamus and pituitary in elasmobranchs (Gelsleichter 2004).

Gonadotropins are produced in the ventral lobe of the pituitary gland in elasmobranchs (Dobson and Dodd 1977a; Sumpter *et al.* 1978; Querat *et al.* 2001). Querat *et al.* (2001) determined that three glycoprotein hormone subunits (α, β1, and β2) similar to those that make up mammalian gonadotropins were present in the ventral lobe of the pituitary gland in *S. canicula*. They found that the amino acid sequences of the β1 and β2 subunits were orthologous to the β subunits of mammalian follicle stimulating hormone (FSH) and luteinising hormone (LH) respectively.

The androgens **testosterone** and **dihydrotestosterone** (DHT) have both been detected in the serum of male elasmobranchs (Manire and Rasmussen 1997; Snelson *et al.* 1997; Tricas *et al.* 2000). In the mammalian testis, testosterone is produced by Leydig cells in the testicular interstitium in response to LH stimulation, and converted to DHT by the sertoli cells (Johnson and Everitt 2000). In elasmobranchs, there is debate as to the presence and function of Leydig cells, and the sertoli cells are generally considered to be the primary site of androgenesis (Gelsleichter 2004; Engel and Callard 2005). Elasmobranch sertoli cells possess many of the structural characteristics of steroidogenic cells, including smooth endoplasmic reticulum, lipid droplets, and mitochondria with tubulovesicular cristae (Pudney and Callard 1984a; Prisco *et al.* 2002). Enzymes involved in androgenesis, including 13β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase, have also been detected in elasmobranch sertoli cells (Prisco *et al.* 2008).

Sertoli cells in the elasmobranch testis undergo a cyclical pattern of proliferation and degeneration concurrent with spermatogenesis, including mitosis during the early stages of spermatocyst development (Engel and Callard 2005). At the spermatid stage, there is a large increase in volume of the smooth endoplasmic reticulum in the sertoli cell (Pudney and Callard 1984a). This corresponds to an increase in the concentrations of testosterone and DHT in the serum, which reportedly rise during the mid to late stages of spermatogenesis (Manire and Rasmussen 1997; Snelson *et al.* 1997; Tricas *et al.* 2000). These observations have lead to the suggestion that sertoli cells in post-meiotic spermatocysts are the primary source of testicular androgens in elasmobranchs (Gelsleichter 2004).

A similar role has been suggested for **oestrogen** in the elasmobranch testis (Gelsleichter 2004). Several authors have reported seasonal changes in oestrogen concentration in the serum of male elasmobranchs (Snelson *et al.* 1997; Tricas *et al.* 2000; Henningsen *et al.* 2008). Oestrogen levels are reportedly highest during spermatocyte meiosis in some species (Callard *et al.* 1985; Cuevas *et al.* 1992), while oestrogen receptors are located primarily in pre-meiotic spermatocysts (Callard *et al.* 1985). This has lead to the suggestion that oestrogen may be involved in controlling growth of early spermatocysts via negative feedback (Engel and Callard 2005).

Progesterone levels in the serum follow the same post-meiotic rise as testosterone and DHT, except that progesterone levels begin to rise later and last longer than the androgens (Manire and Rasmussen 1997; Snelson *et al.* 1997; Tricas *et al.* 2000). Both progesterone production (Callard *et al.* 1985; Sourdaine *et al.* 1990; Sourdaine and Garnier 1993) and the distribution of progesterone receptors (Cuevas and Callard 1992) are highest in post-meiotic spermatocysts, suggesting that progesterone may act in concert with testosterone in maturation of spermatids and spermiation.

Genital ducts

The genital ducts in male elasmobranchs are made up of several key structures: the efferent ductules, epididymides, ductus deferentes, and ampullae of the ductus deferentes (refer back to Figure 81). These structures are essentially homologous to those in the mammalian reproductive tract, but with notable differences in function. At spermiation, the spermatocyst breaks down to release the spermatozoa, which enter the epididymis along with remnants of the degenerate sertoli cells via the efferent ductules (Stanley 1966; Callard *et al.* 1988; Jones *et al.* 2005).

From the efferent ductules, spermatozoa travel through the highly convoluted epididymis. The epididymis is closely associated with the ventral surface of an accessory organ called the Leydig gland. The Leydig gland is the modified anterior portion of the pre-pubertal kidney, and secretes material contributing to the seminal matrix in the epididymis via numerous small

tubules (Pratt 1979; Jones and Jones 1982; Callard *et al.* 1988; Jones and Lin 1993; Hamlett 1999; Jones *et al.* 2005).

As in mammals, elasmobranch spermatozoa undergo a period of maturation in the epididymis (Jones *et al.* 1984; Jones and Lin 1993). This is associated with protein secretion from the Leydig gland and epididymis (Jones *et al.* 1984; Jones *et al.* 2005), changes in the sperm membrane (Rojas and Esponda 2001), and an increase in the capacity for motility (Jones *et al.* 1984; Minamikawa and Morisawa 1996).

Following maturation in the epididymis, sperm travel through the ductus deferens, which gradually widens to form the ampulla of the ductus deferens. The ductus deferens is associated with the beginnings of sperm bundle (spermatophore) formation (Jones and Jones 1982; Hamlett 1999; Jones *et al.* 2005).

Copulatory appendages

Male elasmobranch copulatory appendages consist of the paired claspers, which extend posteriorly from the pelvic fins (Gilbert and Heath 1972; Hamlett 1999). See Figure 85. Clasper morphology shows considerable species variation, but all contain a series of cartilaginous supports, consisting of a basal cartilage connected to the pelvic fin (basipterygium), axial and marginal cartilages making up the clasper shaft, and a series of moveable terminal cartilages that often have sharp external spurs (Gilbert and Heath 1972; Compagno 1999a). The cartilaginous supports, together with the associated musculature, aid in movement and flexion of the claspers during copulation (Gilbert and Heath 1972; Hamlett 1999).

The claspers in immature males are relatively small and the cartilaginous supports soft, but at puberty, the claspers undergo a rapid increase in size and the cartilage becomes calcified (Gilbert and Heath 1972; Walker 2005). Calcification of the claspers has been used as an indicator of male maturity in several shark species (Pratt 1979; Castro *et al.* 1988; Heupel *et al.* 1999; Walker 2005). During copulation, the male inserts one clasper into the females' cloaca and flares the terminal cartilages to ensure that the clasper stays in position so sperm can be delivered (Gilbert and Heath 1972; Jones *et al.* 2005; Pratt and Carrier 2005).

Claspers do not have a lumen like the mammalian penis, but instead have a groove running longitudinally on the dorsal side of the claspers to aid the passage of semen (Hamlett 1999; Jones *et al.* 2005). The groove is either completely or partially enclosed by the axial and marginal cartilages making up the clasper shaft (Compagno 1999a).

Figure 5 - Claspers of a male broad-nose seven-gill shark (*Notorynchus cepedianus*)

Elasmobranchs also have accessory glands or sacs that are associated with the claspers. Sharks have paired, muscular “siphon sacs” which lie subcutaneously on either side of the ventral midline in the pelvic region of the shark (Gilbert and Heath 1972). See Figure 86. The siphon sacs empty through the clasper groove via an apopyle located at the posterior end of the sac (Gilbert and Heath 1972). The siphon sacs are thought to be filled with seawater by flexion and crossing of the claspers, and this fluid can be ejected from the siphon sacs by contraction of the muscular lining to aid in delivery of sperm (Gilbert and Heath 1972). In many cases, seawater is required to activate spermatozoa.

Siphon sac walls contain glandular tissue which produces serotonin (5-hydroxytryptamine). Serotonin is a powerful stimulator of smooth muscle contraction. Following copulation it is proposed that the smooth muscle contractions assist with the transfer of sperm through the female oviducts and hence fertilization.

Male elasmobranchs will often take seawater in leading up to and during the mating season. They may suddenly appear quite swollen and uncomfortable (see Figure 87). I have seen this on many occasions and it always resolves spontaneously and does not need intervention.

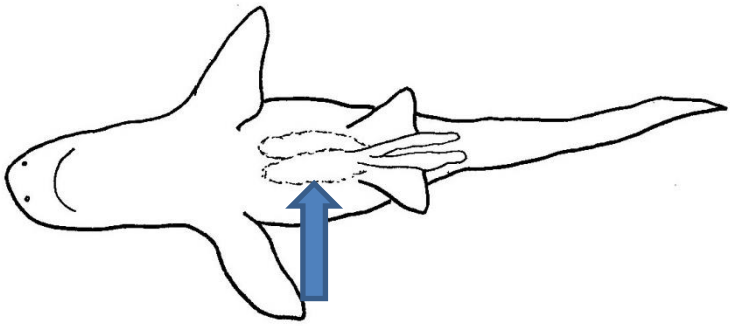

Figure 6 - Position of the siphon sacs (arrow) in male elasmobranchs that have them

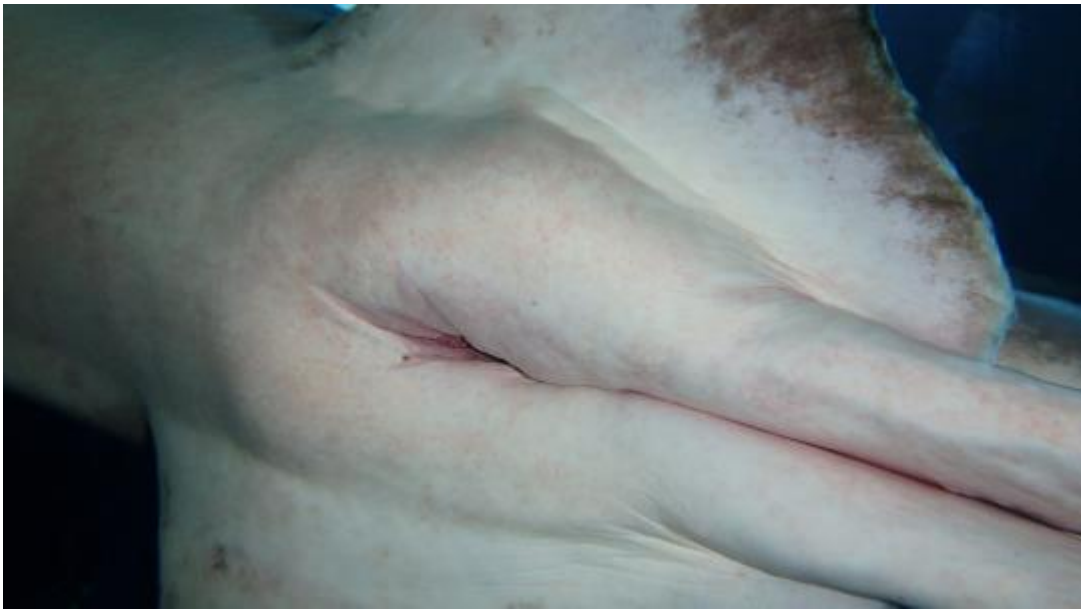

Figure 7 - Swollen siphon sacs in a male sand tiger shark (*Carcharias taurus*).

Skates and rays do not possess siphon sacs, instead they have paired clasper and alkaline glands. Clasper glands are located subcutaneously in the base of each clasper and are thought to be homologous to the siphon sacs in sharks (Lacy 2005). The clasper glands are made up of glandular and secretory tissue, and open into the clasper groove via an apopyle (Lacy 2005). The glands contain a viscous secretion that coagulates in contact with seawater (LaMarca 1964). Although the exact functions of the clasper gland and its secretions are unknown, it has been suggested that they are involved in lubrication of the clasper or maintaining sperm motility (Lacy 2005). The alkaline glands are actually sacs containing a clear fluid, located within the body cavity of skates and rays, and lie on the ventral surface of the kidney or the ampulla of the ductus deferens (Lacy 2005). As the name suggests, the glands contain a highly alkaline fluid (pH 8.9 – 9.2), although the exact function of this fluid is unknown (Lacy 2005). A duct from the alkaline gland opens into the cloaca in close

proximity to the ductus deferens, so it has been suggested that fluid from the gland is involved in propulsion of semen at ejaculation, or maintaining sperm motility (Lacy 2005).

Female Reproductive Anatomy

The reproductive anatomy of female elasmobranchs consists of several key structures adapted for the production of numerous ova, internal fertilization, and formation of an egg case. These structures consist of the ovaries, ostium, anterior oviducts, oviducal glands, isthmuses, and uteri. The paired reproductive tracts (from anterior oviducts to uteri) lie on the dorsal surface of the body cavity on either side of the vertebral column.

Ovarian Structure and Function

The ovaries occupy a similar position in the body cavity to the testes in male elasmobranchs, located dorsally within the body cavity and closely associated with the epigonal organ (Pratt 1988; Hamlett and Koob 1999). Ovaries in mature elasmobranchs contain oocytes, yolky follicles, atretic follicles, and corpora lutea (Hamlett and Koob 1999; Lutton *et al.* 2005). Pratt (1988) described two types of ovary in female elasmobranchs, those that are located within the epigonal organ (internal), and those that are attached to but outside the epigonal organ (external). See Figure 88. It has also been suggested by other authors that some of the apparent species differences may in fact be due to the stage of the reproductive cycle the shark is in at the time of examination, rather than actual morphological differences between species (Lutton *et al.* 2005).

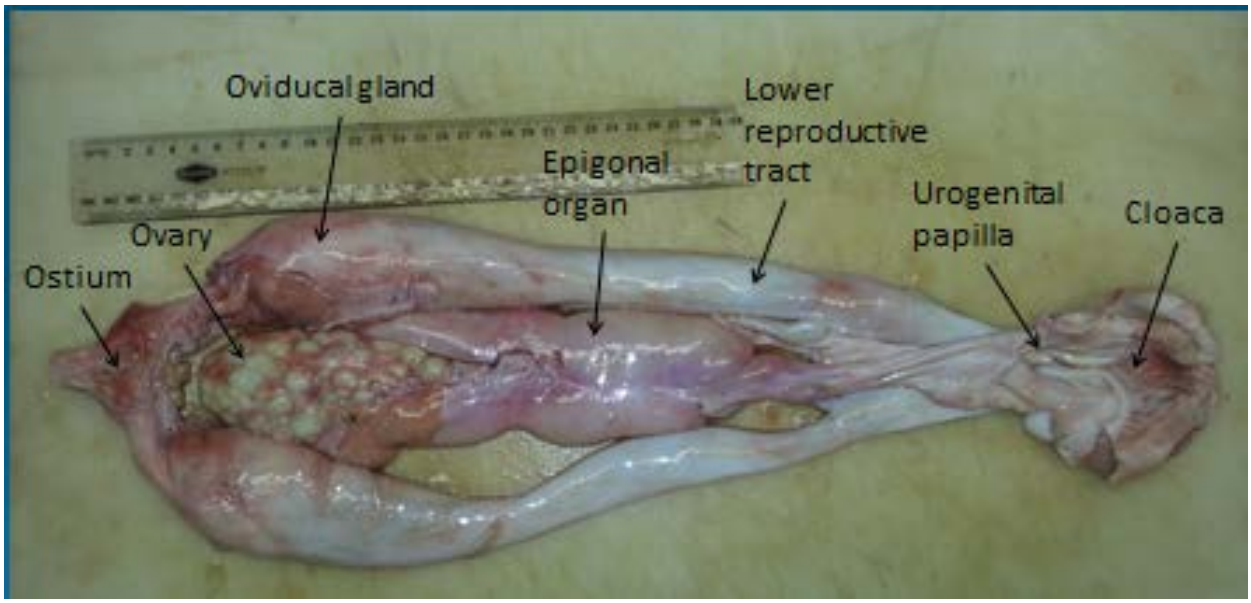

Figure 8 - Reproductive tract of a female leopard shark (*Stegostoma fasciatum*) (courtesy Jon Daly).

Ovarian function varies between species. In most skate species, both ovaries are functional and contribute to the production of follicles and steroid hormones (Koob *et al.* 1986). In most ray species, both ovaries develop follicles but only one ovary ovulates and the other is thought to be mainly steroidogenic (Hamlett and Koob 1999; Lutton *et al.* 2005). In many shark species, including the viviparous carcharhinids and lamnoids (Pratt 1988; Gilmore 1993; Hamlett and Koob 1999), and oviparous Australian swell shark (*Cephaloscyllium laticeps*) (Awruch *et al.* 2008b) a single functional ovary is present. As an example, in sand tiger sharks (*C. taurus*) the right ovary is functional (see Figure 89). In other shark species, including *S. acanthias*, both ovaries are reportedly functional (Hamlett and Koob 1999; Lutton *et al.* 2005). Seasonal cycles in follicular growth are observed in all elasmobranch species. Follicular growth occurs over a period of months leading up to the breeding season, at which point ovulation and fertilization occur.

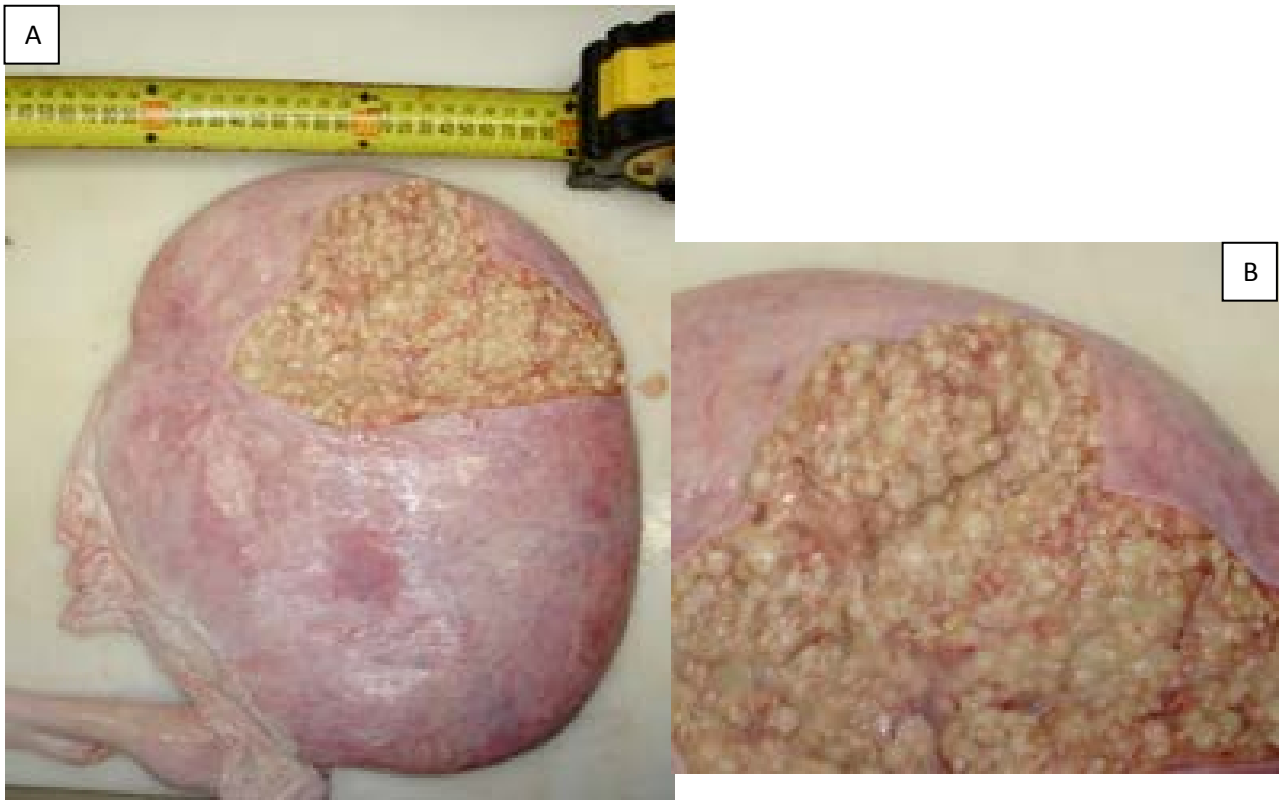

Figure 9 – A = Ovary of a sand tiger shark (*Carcharias taurus*) - approximately 25 to 30 centimetres in diameter; B = close up showing ova (up to 10 millimetres in diameter)

Ovarian activity during the reproductive cycle varies between species, and can be considered as either continuous or punctuated (Koob and Callard 1999). In species with a continuous cycle, follicular growth occurs during pregnancy so that females are ready to mate soon after parturition (Hamlett and Koob 1999; Koob and Callard 1999). In species with a punctuated cycle, ovarian activity ceases during pregnancy and resumes in the following year leading up to the breeding season (Hamlett and Koob 1999; Koob and Callard 1999). Species that utilise oophagy as a source of embryo nutriment continue to produce oocytes and ovulate through pregnancy until around two months prior to parturition (Gilmore *et al.* 1983; Gilmore 1993; Hamlett and Koob 1999). At this point ovulation ceases and the ovary atrophies, with

folliculogenesis resuming in the following year leading up to the next breeding season (Gilmore *et al.* 1983; Gilmore 1993; Hamlett and Koob 1999).

Vitellogenesis and ovulation

During folliculogenesis, oocytes in the elasmobranch ovary accumulate yolk through a process known as **vitellogenesis**. The yolk precursor **vitellogenin** is produced in the liver and travels to the ovary through the systemic blood circulation, and can be detected in the blood serum of female sharks during the period of follicular growth (Craik 1978; Callard *et al.* 1988). There is evidence that vitellogenin production by the liver is controlled by oestrogenic hormones, as shown by administration of exogenous hormones (Callard *et al.* 1988).

In elasmobranchs, accumulation of yolk takes place over several months and in some species can take twelve months (e.g. *N. cepedianus*) (Ebert 1989; 1996) or longer (e.g. *S. acanthias*) (Hamlett and Koob 1999). See Figures 90 and 91. There is great variation in oocyte size at ovulation between species, from as small as 1mm in the spadenose shark *Scoliodon sorrakowah* (= *Scoliodon laticaudus*) to 100 mm or larger in nurse sharks (*Ginglymostoma* species) (Wourms 1977; Callard *et al.* 1988). The mature oocyte is spherical in shape with a blastodisc containing the oocyte nucleus located at one pole (Wourms 1977). The majority of the oocyte is filled with yolk, and there is only a small amount of cytoplasm associated with the blastodisc (Wourms 1977). The yolk accumulated in the oocyte is the initial, and in some species the only, source of foetal nutriment in all elasmobranch species.

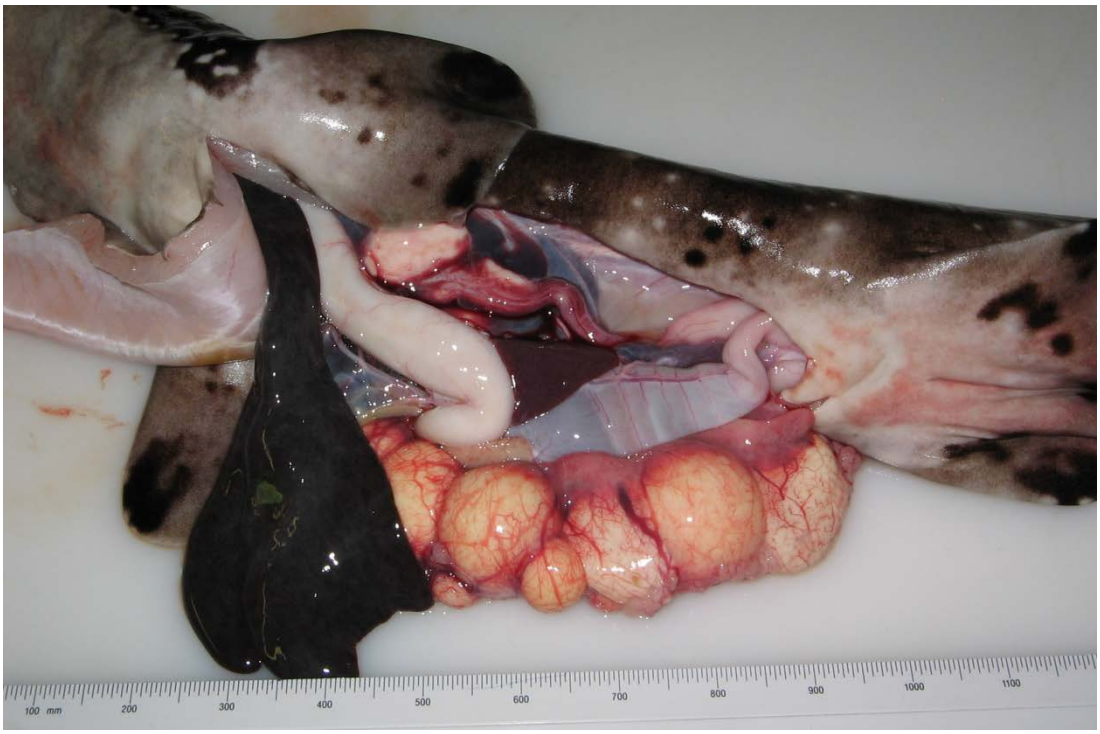

Figure 10 – Necropsy of a mature female collared catshark (*Parascyllium collare*) displaying many well-developed ova (up to 3 centimetres in diameter)

At ovulation, the outer germinal epithelium of the follicle bursts open, and the oocyte is released into the body cavity where it is collected by the ostium in sharks and transported through the oviducts (Lutton *et al.* 2005). In *S. canicula*, cilia within the peritoneum aid in the passage of the oocyte to the ostium (Metten 1939). Although the yolky oocyte is quite large, passage through the oviduct and oviducal gland is apparently simple. This is likely due to the effects of oestrogenic hormones, which are elevated during vitellogenesis (Craik 1978), on the female reproductive tract.

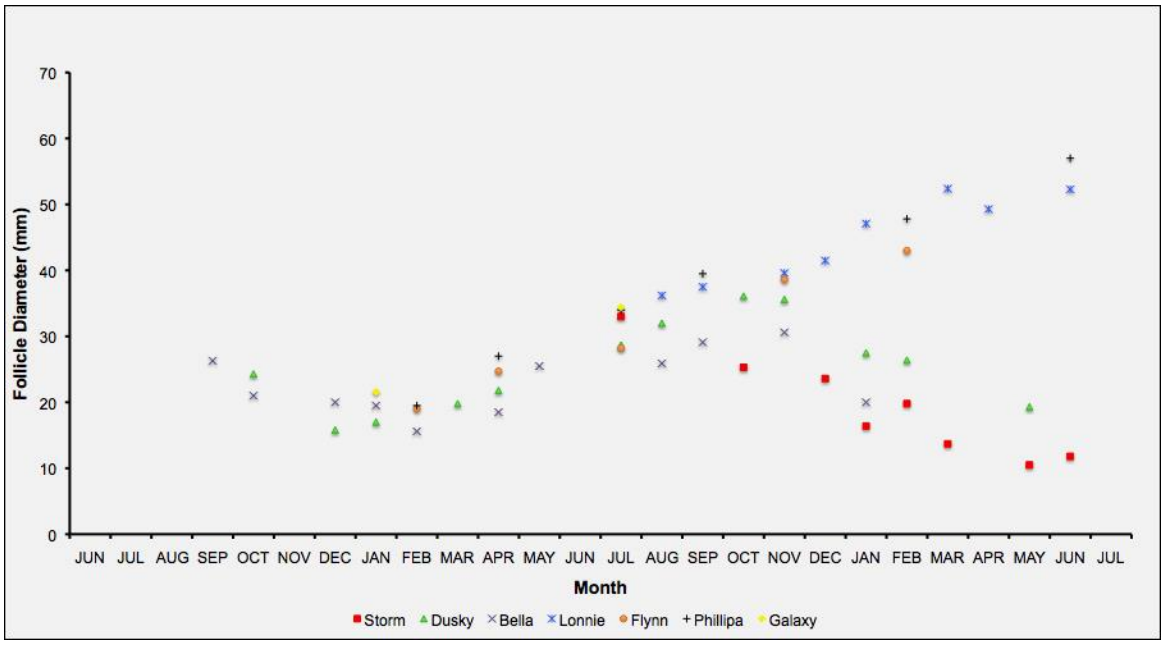

Figure 11 - Graph showing ovarian development in broad-nose seven-gill sharks (*Notorynchus cepedianus*) at Melbourne Aquarium (courtesy of Jon Daly).

Oestradiol is known to cause an increase in size of the oviducal gland, as well as influencing the expression of relaxin, which is believed to be involved in enlarging parts of the tract during parturition (Koob *et al.* 1984; Callard *et al.* 1988). A combination of these effects could aid in the passage of the large, yolky oocyte through the anterior oviduct to the oviducal gland.

Steroidogenesis

The ovaries are also responsible for the steroidogenesis of the female sex hormones. Testosterone and oestradiol are known to be important in follicular development in both ovoviviparous and viviparous shark species (Koob and Callard 1999), and ovarian oestrogens are also thought to be involved in controlling vitellogenin production by the liver (Hamlett and Koob 1999).

Progesterone in female viviparous elasmobranchs is primarily produced by **corpora lutea** in the ovary (Tsang and Callard 1987a; Hamlett and Koob 1999). In the oviparous little skate (*Raja erinacea*) and viviparous bonnethead shark (*Sphyrna tiburo*), there is a serum progesterone peak just prior to ovulation (Manire and Rasmussen 1997; Koob and Callard 1999). In *S. acanthias* and *S. tiburo*, serum progesterone is elevated during the first half of pregnancy, but then drops back to relatively low levels near term (Tsang and Callard 1987b; Manire and Rasmussen 1997).

Ostium and anterior oviduct

The elasmobranch ostium is analogous to the mammalian fimbria or avian infundibula in form and function, and is a funnel shaped opening at the beginning of the anterior oviduct and is lined by ciliated columnar epithelial cells (Metten 1939; Wourms 1977; Hamlett and Koob 1999; Callard *et al.* 2005). The oviduct can be separated into two sections: the anterior or upper oviduct, located between the ostium and oviducal gland, and the isthmus, which connects the oviducal gland to the uterus (see Figure 92).

After collection by the ostium, oocytes travel through the anterior oviducts toward the oviducal gland. The oviducts lack smooth muscle and instead have cilia to aid in the transport of oocytes to the oviducal glands (Metten 1939; Hamlett and Koob 1999; Callard *et al.* 2005).

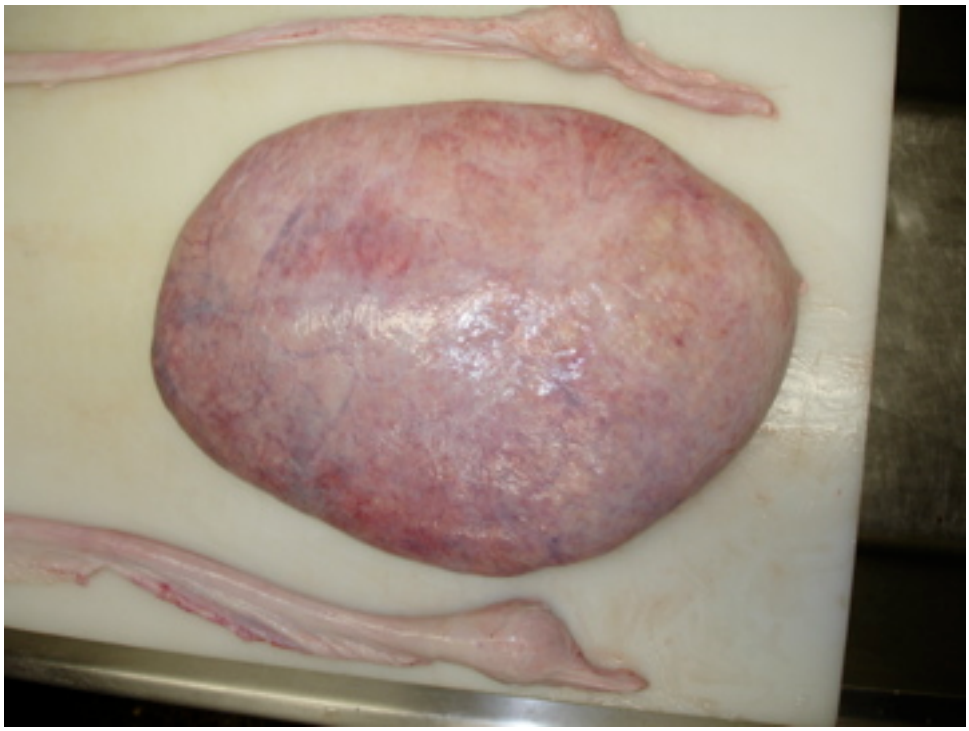

Figure 12 - Ostium and oviducts of a female sand tiger shark (*Carcharias taurus*)

Oviducal Glands

The oviducal glands, also known as the shell or nidamental glands, are paired glands that perform two main roles in the function of the female reproductive system: production of the egg case, and sperm storage after mating (Hamlett and Koob 1999; Hamlett *et al.* 2005b).

Oviducal gland morphology varies between species, but is essentially composed of four sections, each with a different role in the production of the egg case (Hamlett *et al.* 1998; Hamlett and Koob 1999; Hamlett *et al.* 2005b). The first two sections (at the anterior end of the oviducal gland), the proximal club zone and papillary zone, produce jelly-like coats that cover the oocytes (Hamlett *et al.* 1998; Hamlett and Koob 1999; Hamlett *et al.* 2005b). The baffle zone is associated with secretory glands and spinnerets that produce the factors comprising the egg case (Hamlett *et al.* 1998; Hamlett and Koob 1999; Hamlett *et al.* 2005b).

In oviparous species, the egg case is a tough, shell-like coating that provides protection for the embryo within (see Figure 93). In viviparous species, the egg case is more of a membranous cover than a shell, allowing the developing foetuses to hatch out into the uterus (Hamlett and Koob 1999).

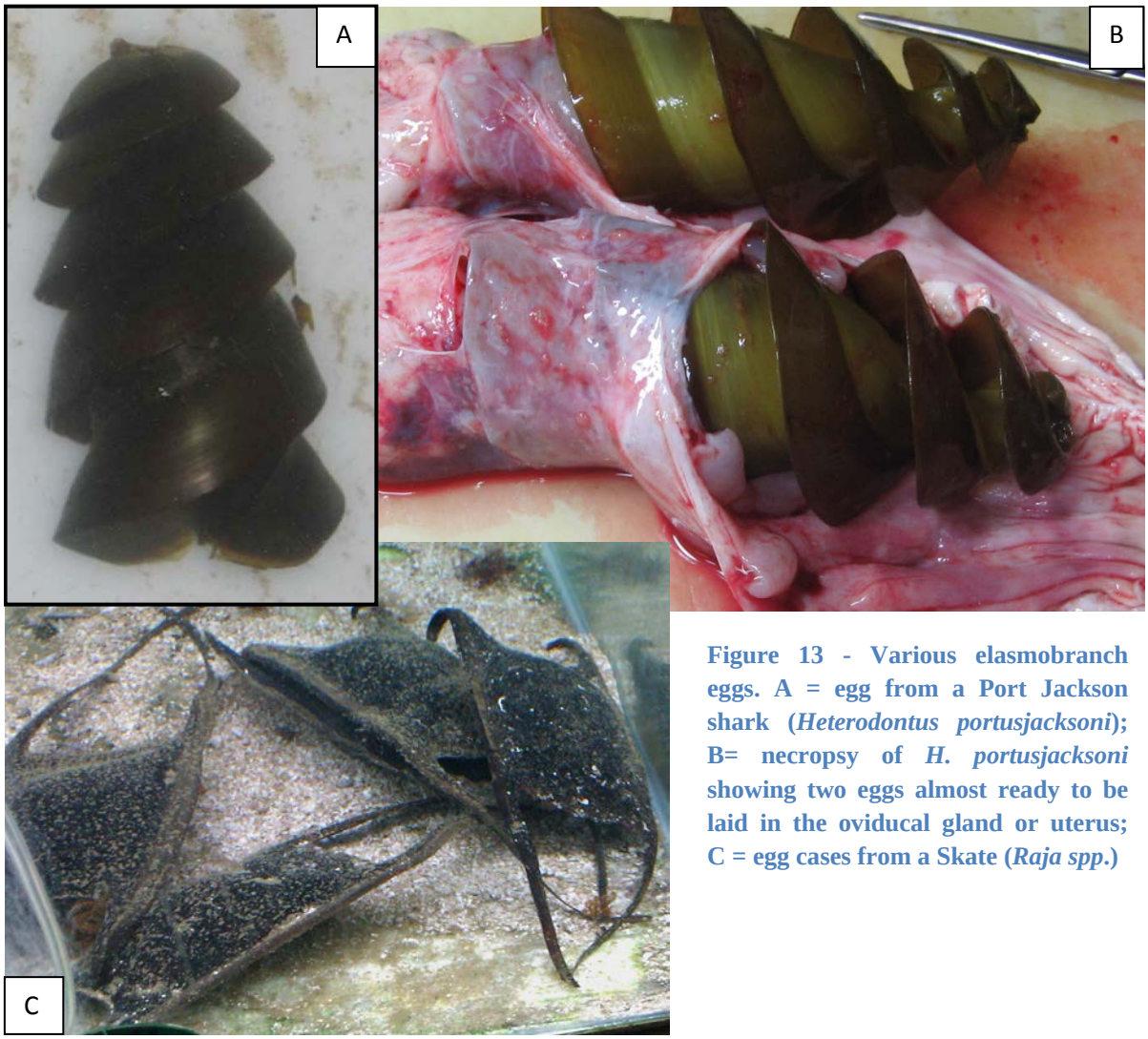

Figure 13 - Various elasmobranch eggs. A = egg from a Port Jackson shark (*Heterodontus portusjacksoni*); B= necropsy of *H. portusjacksoni* showing two eggs almost ready to be laid in the oviducal gland or uterus; C = egg cases from a Skate (*Raja spp*.)

The terminal zone has cells that secrete mucus, which may aid in the passage of the egg case to the uterus (Hamlett *et al.* 1998; Hamlett *et al.* 2005b). Sperm storage after mating occurs in the terminal zone of the oviducal gland (Metten 1939; Pratt 1993; Hamlett *et al.* 2002a; Smith *et al.* 2004; Hamlett *et al.* 2005b). It has also been suggested that the terminal zone is an immunologically privileged region that prevents sperm from being attacked by maternal antigens during storage (Hamlett *et al.* 2005b).

The exact timing of fertilization is not clear, as the descriptions given in the above literature suggest that the oocyte is encapsulated prior to reaching the region of sperm storage. Oviducal gland function is thought to be hormonally controlled, although the specific actions of the various hormones are not known (Callard *et al.* 2005). Oestrogen is thought to be involved in oviducal gland growth in *R. erinacea* and *S. canicula* (Koob *et al.* 1986; Callard *et al.* 1988; Callard *et al.* 2005), and it has been suggested that the secretions produced by the oviducal gland also require hormonal input (Hamlett and Koob 1999; Callard *et al.* 2005).

Uteri

Encapsulated and fertilised oocytes travel through the isthmuses to the uteri on each side of the tract. The left and right uteri are formed by the rapid widening of the reproductive tract on the respective sides, and remain as separate uterine horns along their entire length. Structure and function of the elasmobranch uterus varies considerably between reproductive modes.

In oviparous species, the uterus is not involved in housing or nutrition of the developing embryos, but plays an important role in preparing the assembled egg case for oviposition (Hamlett and Koob 1999). In these species, the egg case is hardened in the uterus by a quinone tanning mechanism (Koob and Hamlett 1998).

In viviparous elasmobranchs the structure and function of the uterus varies between species depending on the role it plays in development and nourishment of the embryos. The uterus of viviparous species is more vascularised than in oviparous species (Hamlett and Hysell 1998) to provide for gas and nutrient exchange (see Figure 94). In some species, the uterus becomes compartmentalised to accommodate individual foetuses during gestation (Hamlett *et al.* 1993), and trophonemata (uterine villi) develop in ray species to produce histotroph (uterine milk), a substance that provides nutrition to developing foetuses (Hamlett *et al.* 1993; Hamlett and Hysell 1998).

In placental species, such as the Atlantic sharpnose shark, *Rhizoprionodon terraenovae*, the uterus adapts to provide a vascularised site for delivery of nutrients to the placenta (Hamlett *et al.* 1993). The fluid within the uterus varies between species and reproductive modes, but

generally contains factors that are necessary for development of embryos (Lombardi *et al.* 1993).

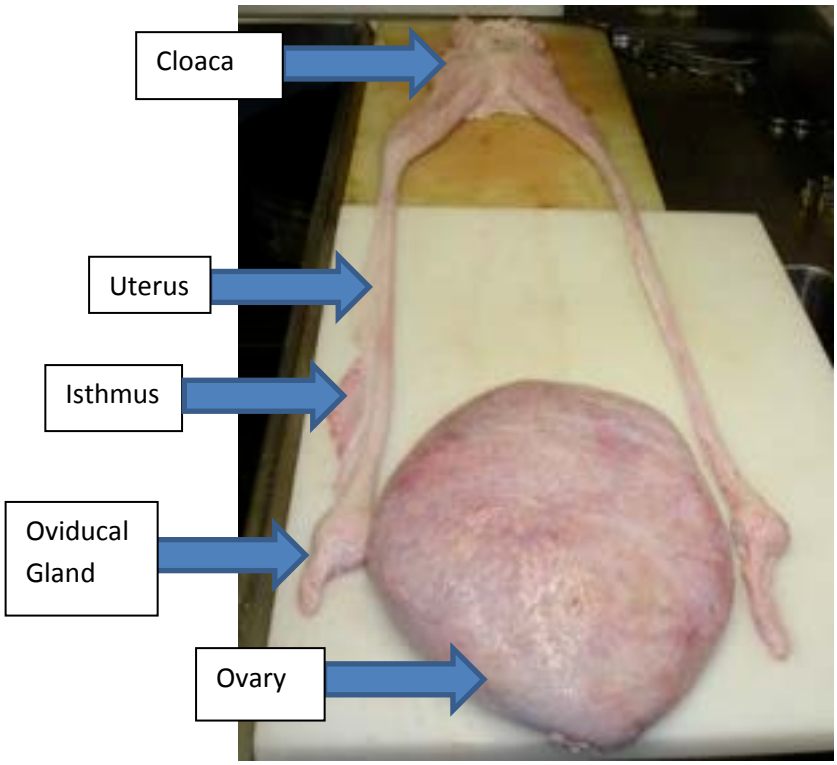

Figure 14 - Reproductive tract of a female sand tiger shark (*Carcharias taurus*).

The posterior end of the uterus is constricted by a muscular cervix (Hamlett and Koob 1999; Callard *et al.* 2005), which may play an important role in maintaining the uterine environment during pregnancy. At parturition and during oviposition, the cervix must dilate considerably in order to allow passage of the foetus or egg capsule, which presumably happens under hormonal influence (Callard *et al.* 2005). In the female elasmobranch, relaxin production is regulated by oestradiol (Callard *et al.* 1988). As oestradiol levels are known to rise gradually towards the end of pregnancy (Hamlett and Koob 1999), it is suggested that a combination of these hormones is responsible for relaxation of the cervix to accommodate parturition (Callard *et al.* 2005).

Reproductive Modes

Elasmobranchs have two basic modes of reproduction: oviparity (egg-laying) and viviparity (live birth) (Pratt and Castro 1990; Gilmore 1993; Hamlett and Koob 1999).

Oviparity

In oviparous species, oocytes are fertilised and enclosed in an egg case that is deposited on the ocean floor (oviposition) (Hamlett and Koob 1999). Development occurs entirely within the egg case with the foetus gaining nutrition from an external yolk sac within the case (lecithotrophy) (Hamlett and Koob 1999). All skates and around 40% of shark species are oviparous (Hamlett and Koob 1999). Although females may select the site of oviposition, there is no further parental care before or after hatching (Wourms 1977; Hamlett and Koob 1999). See Figure 95.

The egg case has structures that aid in anchoring them to the ocean substrate, and the external structure and appearance of the egg case varies considerably between species (Hamlett and Koob 1999). These structures include tendrils that wrap around seagrass (e.g. *S. canicula*), spiral flanges that give the egg case a spiral appearance and allow it to wedge in rock crevices (e.g. heterodontid species), and lateral flanges that give the egg case a concave shape that allows it to stick to the sand by suction (e.g. holocephalans) (Hamlett and Koob 1999; Hamlett *et al.* 2005b).

Figure 15 - Elephant fish, also known as an Australian ghostshark (*Callorhinchus milii*) which is a chimaera, laying eggs.

Egg cases usually have small holes at the corners or ends that are plugged by egg jelly (Hamlett and Koob 1999). Approximately one third of the way through gestation the egg jelly dissolves and the egg case becomes permeable to water, and the foetus spends the remainder of gestation bathed in seawater (Hamlett et al. 1993; Hamlett and Koob 1999). See Figure 96.

Figure 16 - Using a torch to examine a brown-banded bamboo shark (*Chiloscyllium punctatum*) egg case that is almost full term. If doing this do not lift the egg out of the water

Viviparity

In viviparous shark species, the egg case in which the fertilised oocyte is encased is a thin membranous sheath termed a "candle case" (Hamlett and Koob 1999; Hamlett *et al.* 2005b) (see Figure 97). Initial development takes place within the candle case, after which the embryo hatches out and continues development within the maternal uterus. The distinction between embryo and foetus is not well defined in elasmobranchs, with many authors referring to developing young as "embryos" throughout gestation (Gilmore *et al.* 1983; Hamlett and Koob 1999; Gilmore *et al.* 2005; Hamlett *et al.* 2005a; Hamlett *et al.* 2005c). For clarity, young will be referred to as "embryos" during development within the candle case, and "foetuses" during the post-hatch stage in the uterus.

Viviparous elasmobranch species have evolved a range of methods for providing nutriment to the developing foetus (Hamlett and Koob 1999; Hamlett *et al.* 2005b). This has led to the characterisation of reproductive modes based on the type of maternal investment. These reproductive modes were defined by Hamlett *et al.* (2005b) as lecithotrophy, and various forms of matrotrophy including histotrophy, ovatrophy, adelphotrophy, and placentatrophy. Developing foetuses in all viviparous species gain initial nutriment from lecithotrophy (Wourms 1977; Hamlett *et al.* 2005c). This involves absorption of yolk from the external yolk sac directly into the stomach via the ductus vitellointestinalis (also known as the yolk stalk) (Hamlett and Koob 1999). In lecithotrophic viviparous species (e.g. *N. cepedianus* (Ebert 1989), yolk in the external yolk sac is the sole source of embryo nutriment, although it has been suggested that even these species gain some nutriment from histotrophy (Hamlett *et*

al. 2005c). Lecithotrophic viviparity is the most common form of embryo nutriment in viviparous sharks (Hamlett *et al.* 2005c).

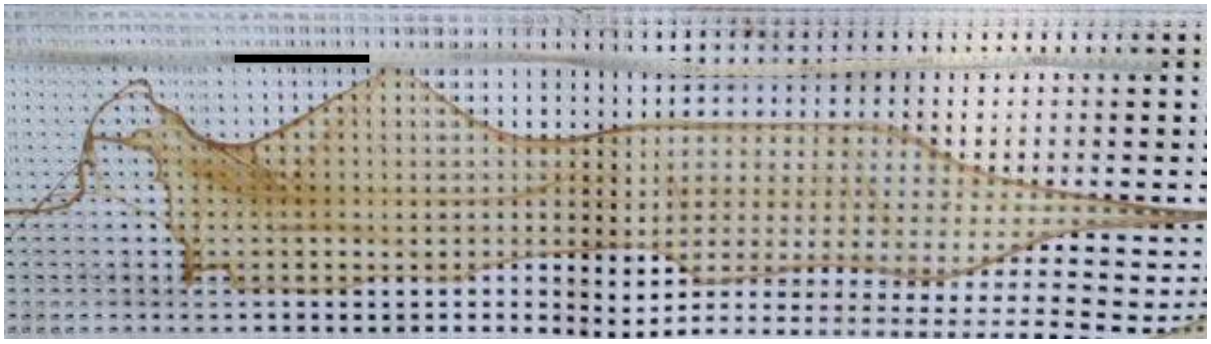

Figure 17 - One of several candle cases released by a female lemon shark (*Negaprion brevirostris*). Black bar is 10 centimetres in length

Ovatrophy and adelphotrophy

Ovatrophy (also known as oophagy) is restricted to the lamnoid sharks, with all species from this group examined so far exhibiting this form of embryo nutrition (Springer 1948; Gilmore 1993; Gilmore *et al.* 2005). In these species, the female continues to ovulate yolky ova throughout pregnancy, which are consumed by the developing foetuses (Gilmore *et al.* 1983; Gilmore 1993). This form of foetal nutrition is also associated with the precocious development of dentition, enabling foetuses to consume the ova (Gilmore 1993).

Adelphotrophy (uterine cannibalism) has only been confirmed in one species, *C. taurus*, but has been suggested to occur in thresher sharks (*Alopias* species) due to low numbers of developing young in the uterine horns (Gilmore 1993; Gilmore *et al.* 2005). Foetal development, ovatrophy, and adelphotrophy in *C. taurus* have been well documented by Gilmore *et al.* (1983) and the following is a summary of these events. Multiple embryos (up to seven) at different developmental stages are present in each of the uterine horns during early gestation. Hatching of the most developed embryo from the candle case into the uterus occurs at around 60 mm in length.

After using up yolk supplies in the external yolk sac, the foetus actively predates and consumes its less developed littermates until only one foetus survives in each uterine horn. The foetus then feeds on yolky, unfertilised ova until around two months prior to parturition when ovulation ceases. At this point, the foetus has a distended stomach filled with yolk, and this is the sole source of nutriment for the remainder of gestation. In *C. taurus*, a maximum of two pups are born at 90-120 centimetres in length after a gestation of nine to twelve months (Gilmore *et al.* 1983).

Histotrophy and placentatrophy

All ray species exhibit histotrophy (Hamlett and Koob 1999; Hamlett *et al.* 2005c). Following the lecithotrophic stage, villous structures called **trophonemata** develop from the uterine lining. Trophonemata are highly vascularised and secrete a **histotroph** that is rich in proteins and lipids (Hamlett and Hysell 1998; Hamlett *et al.* 2005c). During early development the histotroph is absorbed by the foetus via external gill filaments, but later in development it is ingested (Hamlett *et al.* 2005a). Trophonemata proliferate and produce progressively more concentrated histotroph as pregnancy progresses, and it has been suggested that oestrogen may play a role in this proliferation (Hamlett *et al.* 2005c). Trophonemata may also be involved in gas exchange in the uterus (Hamlett and Koob 1999; Hamlett *et al.* 2005c).

Species utilising placentatrophy develop a maternal-foetal connection within the uterus (Hamlett *et al.* 1993; Hamlett and Hysell 1998; Hamlett and Koob 1999). In placental species, foetuses develop within separate uterine compartments that are created by folding of the ventral and dorsal uterine walls (Hamlett *et al.* 2005a). After the initial lecithotrophic stage the foetus gains nutriment via histotroph, and in the later stages of gestation the distal region of the empty yolk sac becomes vascularised and closely associated with the uterine wall and functions as a placenta (Hamlett *et al.* 1993; Hamlett and Koob 1999; Hamlett *et al.* 2005a). The region of the uterus in contact with the yolk sac placenta becomes highly vascularised for nutrient and gas exchange, and attachment sites are observed as uterine scars post-partum (Hamlett *et al.* 2005a).

Pre-Copulatory and Mating Behaviour

The behaviour of male and female elasmobranchs around the time of mating can be complex. In the wild, segregation by size and sex is known to occur in many elasmobranch species including the nurse shark, *Ginglymostoma cirratum* (Pratt and Carrier 2001), *S. acanthias* (Callard *et al.* 1988), the blue shark (*Prionace glauca*) (Pratt 1979), and *C. taurus* (Pollard *et al.* 1996). Sexual segregation occurs as part of the migration pattern of these sharks, which is often linked to water temperature (Pratt and Carrier 2005) (see Figure 98). Pregnant females are also known to segregate from the rest of the population (Pratt 1979; Pratt and Carrier 2005). Males and females of some species return to the same breeding and pupping areas each year (Callard *et al.* 1988; Ebert 1989; Pratt and Carrier 2001; Saville *et al.* 2002; Pratt and Carrier 2005).

Much of what is known about the pre-copulatory and mating behaviour of wild and captive elasmobranchs comes from observations on the nurse shark (*Ginglymostoma cirratum*) (Pratt and Carrier 2001) and *C. taurus* (Gordon 1993) respectively. A range of pre-copulatory behaviours has been described in elasmobranchs, with most exhibited by males (Carrier *et al.*

2004; Pratt and Carrier 2005). Male behaviours include following of the female (either from behind or side by side) (see Figure 99), biting of the female, and in some species male competition for a female (Gordon 1993; Pratt and Carrier 2001; Chapman et al. 2003; Carrier et al. 2004; Pratt and Carrier 2005). Male biting of the female can be quite extensive in some species, and female *P. glauca* and sandbar sharks (*Carcharhinus plumbeus*) have considerably thicker skin than males to prevent significant injury during mating (Pratt 1979; Pratt and Carrier 2001).

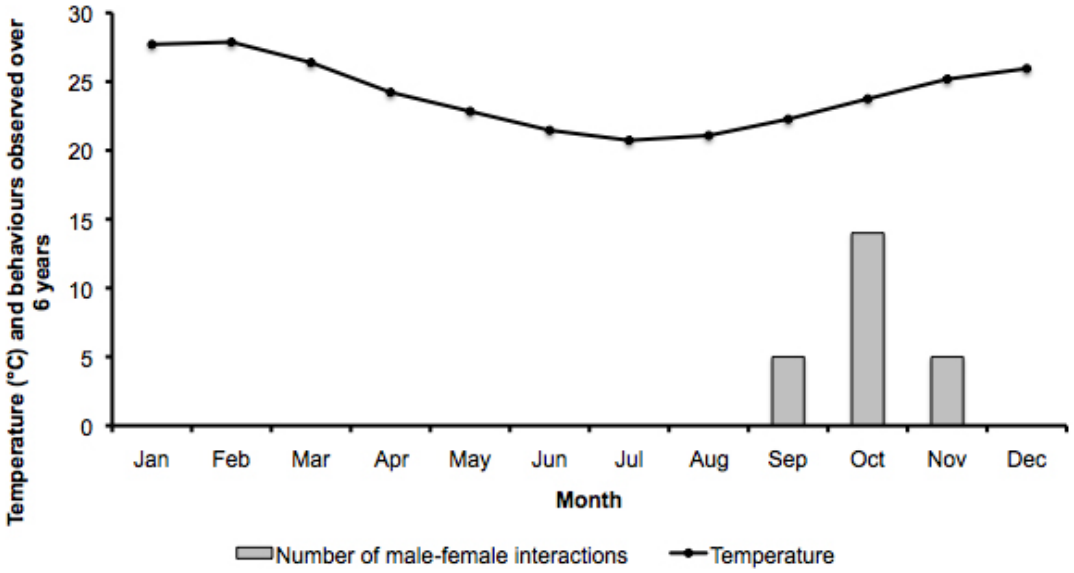

Figure 18 - Mating interactions in sand tiger sharks (*Carcharias taurus*) in an Australian aquarium compared with water temperature

Figure 19 - Male sand tiger shark (*Carcharias taurus*) tailing a female.

Mating scars have been observed in several species including *P. glauca* (Stevens 1974; Pratt 1979), *N. cepedianus* (Ebert 1989), *C. Taurus* (Gilmore *et al.* 1983; Gilmore 1993), the lemon shark (*Negaprion brevirostris*) (Feldheim *et al.* 2002), and the porbeagle shark (*Lamna nasus*) (Jensen *et al.* 2002), and are considered to be an indicator of mating activity in shark species. Biting is required for the male to gain hold of the female during mating (Wourms 1977; Pratt 1979; Carrier *et al.* 2004), but may also be used to signal the male's intent to mate or bring about female submission (Carrier *et al.* 2004). In captive *C. taurus*, the male will display clasper movements such as flexing, splaying, and crossing, which are often accompanied by an altered swim pattern, involving increased speed and swimming in the upper water section (Gordon 1993).

In some species, males patrol or stalk the area where the females are located, and this can lead to chasing of the female, e.g. *G. cirratum* (Pratt and Carrier 2001), or following and nosing (where the male positions his snout close to the females' cloaca) e.g. *C. taurus* (Gordon 1993). Male competition has been reported in *G. cirratum* (Carrier *et al.* 1994; Pratt and Carrier 2001) and the white-tip reef shark (*Triaenodon obesus*) (Pratt and Carrier 2005), and in southern stingrays (*Dasyatis americana*) multiple males have been observed following a single female (Chapman *et al.* 2003). Male-male aggression has been observed in captive *C. taurus* (Gordon 1993), and Henningsen *et al.* (2008) recently reported that this was linked with a dominance hierarchy in this species.

Female behaviour is mainly restricted to avoidance or acceptance of mating attempts. Avoidance behaviours include the female moving close to the substrate, such as *C. taurus* (Gordon 1993), or into shallow water, such as *G. cirratum* (Pratt and Carrier 2001), to prevent the male from approaching her cloaca. If a male *G. cirratum* grasps the pectoral fin of a female before she is ready to mate, she will twist out of his grasp and quickly move away (Pratt and Carrier 2001). When a captive female *C. taurus* is interested in mating she will exhibit movements of her pelvic fins, such as cupping and flaring, which are displayed when a mature male is in close proximity (Gordon 1993). When she is ready to mate, she will begin to swim slowly within a small area with her cloaca exposed. Wild female *G. cirratum* have not been observed showing specific body movements, but rather will cease resisting the male's grasp or will not seek refuge in shallow water when a male approaches (Pratt and Carrier 2001).

Mating in elasmobranchs can be an aggressive act, with males biting at the body and pectoral fins of a female until they can gain a hold (see Figures 100 and 101). In sharks, once the male has gained a sufficient hold on the female he inserts a clasper into the females' cloaca (Pratt and Carrier 2001; 2005). In smaller species, the male wraps his body around the female to facilitate insertion (Gilbert and Heath 1972; Castro *et al.* 1988; Pratt and Carrier 2001; 2005), but in larger species, the male remains alongside the female (Tricas and Le Feuvre 1985; Gordon 1993; Carrier *et al.* 1994; Ebert 1996; Pratt and Carrier 2005).

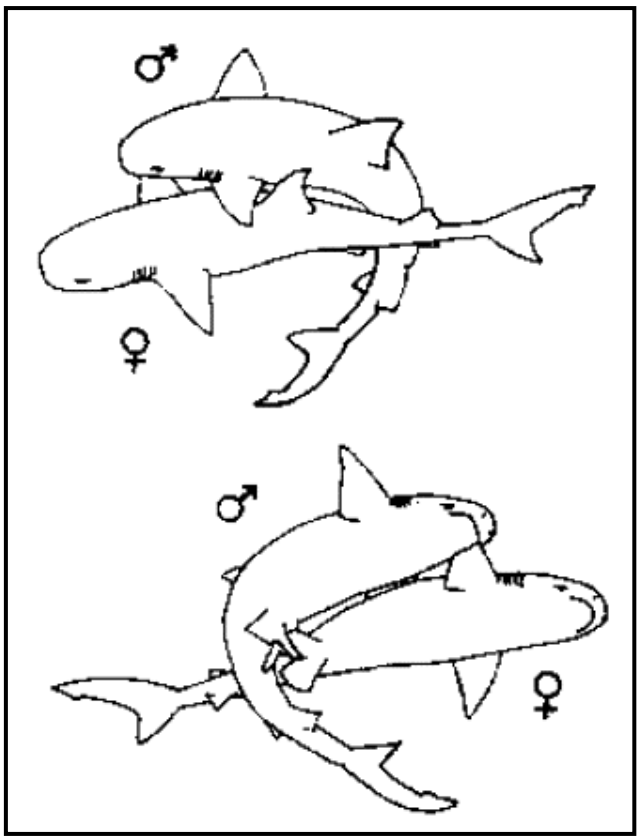

Figure 20 - Diagram depicting copulation in sharks (from Pratt and Carrier 2001)

Males generally use the clasper on the opposite side to the female (i.e. the left clasper, if the female is on his right hand side and vice versa) so that the base of the clasper closes over the male cloaca and directs the flow of semen (Pratt 1979; Castro *et al.* 1988; Gordon 1993; Carrier *et al.* 1994). During ejaculation, semen from both ampullae is released from the urogenital papilla and washed along the grooved claspers into the female reproductive tract with the aid of fluid ejected from the siphon sacs (Gilbert and Heath 1972; Pratt and Carrier 2005). See Figures 86 and 87. In some stingray species, copulation occurs with the male on his side or back so that the posterior regions of the male and female ventral surfaces are opposed (Yano *et al.* 1999; Chapman *et al.* 2003; Pratt and Carrier 2005). In others the male still approaches from below but his dorsal surface remains uppermost (Tricas 1980; Pratt and Carrier 2005). In most elasmobranch species, mating is seasonal and coincides with peak sperm production in males and the presence of mature follicles in the ovaries of females (Parsons and Grier 1992; Hamlett and Koob 1999). Some species have been reported to mate all year round with or without seasonal changes in male GSI (Parsons and Grier 1992), and in other species mating begins several months before the mature follicles are present in the ovary (Maruska *et al.* 1996).

Figure 21 - Copulation in epaulette sharks (*Hemiscyllium ocellatum*). The male is on top and has right clasper is inside the female. The arrow points to his left clasper

Polyandry has been reported and suspected in some elasmobranch species. Feldheim *et al.* (2002) used microsatellite markers to identify multiple paternity in *N. brevirostris*, and showed that up to four males contributed to a single litter. Pratt and Carrier (2001) reported that individual female *G. cirratum* were mated by multiple males, and recent work by Saville *et al.* (2002) showed that multiple paternity occurs in this species. Female *D. americana* (Chapman *et al.* 2003), have also been observed copulating with multiple males, but it is not known whether multiple paternity resulted from these matings.

Multiple paternity, was recently described in grey nurse sharks (*Carcharias tarus*), held and bred in captivity in Australia (Townsend *et al.* 2015). This was also the first record of this occurrence in captivity in any elasmobranch species.

Reproductive technologies

Assessing reproductive cycles

A large proportion of the information available on the reproductive biology of elasmobranchs comes from surveys of animals that have been caught as part of a fishery or specifically for research. This usually involves necropsy measurement of reproductive parameters (e.g. gonad condition and pregnancy) from multiple individuals to determine the timing of

reproductive events such as maturity, mating, and pregnancy (Walker 2005). This is useful for describing the biology of a particular species and/or population, but does not allow repeated assessment of individual animals.

One way that this can be achieved is by measuring circulating hormones in serum or plasma by radioimmunoassay (RIA). Several reproductive hormones are present in the blood of elasmobranchs, and these can be associated with reproductive parameters in live sharks. Early work on developing RIA for use in elasmobranchs involved collecting blood from sharks maintained for a short period in captivity, and comparing the concentrations of steroid hormones with the condition of reproductive organs following dissection (Sumpter and Dodd 1979; Koob *et al.* 1986). Since then, RIA has been used to compare circulating steroid hormone levels with ovarian and testicular cycles in several elasmobranch species from wild populations, either with or without dissection for assessment of reproductive organs (Rasmussen and Gruber 1993; Heupel *et al.* 1999; Tricas *et al.* 2000; Sulikowski *et al.* 2004; Awruch *et al.* 2008a; Awruch *et al.* 2008b). RIA has also been used to monitor circulating hormone levels in captive elasmobranchs to assess the reproductive status individuals from breeding populations (Rasmussen and Murru 1992; Henningsen *et al.* 2008).

Another method for assessing the reproductive status of elasmobranchs in a captive population is with ultrasonography. Although there are only two reports on ultrasound of sharks in the literature (please note this was in 2006), the results obtained by these studies demonstrate the potential of this technique in elasmobranch species. Walsh *et al.* (1992) tested the use of ultrasonography in eleven sharks from three species (*G. cirratum*, *N. brevirostris*, and *S. tiburo*) with the aim of assessing the potential diagnostic applications of this technique. They were able to easily perform ultrasound by sedating the shark and turning it onto its back whilst retaining it in water.

The use of ultrasonography in shark species was further expanded by Carrier *et al.* (2003) in a three year study on *G. cirratum* involving ultrasound and endoscopy. In this study, the investigators captured female nurse sharks that were observed to mate in the wild and followed their progress in captivity throughout gestation. There is more information regarding ultrasonography, including pictures, in Chapter 7 on Diagnostic Procedures.

Sperm activation and motility

There is relatively little information available on the activation and motility of elasmobranch sperm. Jones *et al.* (1984) reported that mature sperm from *H. portusjacksoni* were activated by a phosphate-buffered elasmobranch ringer solution based on the ionic composition of elasmobranch blood. Similarly, work by Minamikawa and Morisawa (1996) showed that sperm from the banded houndshark, *Triakis scyllia,* could maintain activity in solutions that replicate blood or uterine conditions.

Glucose was found to have a positive effect on the duration of motility, and the investigators concluded that hexoses were likely to be important for maintaining sperm motility in elasmobranchs. This is supported by observations in a recent study by Luer *et al.* (2007), who reported that sperm from the clearnose skate (*Raja eglanteria*) maintained high motility in an elasmobranch-modified poultry semen extender containing fructose.

Although hexoses appear to have an influence on maintaining motility, the specific factors controlling activation and immobilization (sperm storage) of elasmobranch sperm are still not clear.