JOURNAL OF EXPERIMENTAL ZOOLOGY 284:586–594 (1999)

Plasma Steroid Hormone Profiles and Reproductive Biology of the Epaulette Shark, *Hemiscyllium ocellatum*

M.R. HEUPEL,* J.M. WHITTIER, AND M.B. BENNETT
Department of Anatomical Sciences, University of Queensland, St. Lucia, Queensland, Australia 4072

ABSTRACT Examination of the reproductive biology of the oviparous epaulette shark, *Hemiscyllium ocellatum*, was conducted on a wild population. Male sharks were found to reach maturity at between 55–60 cm total length (TL) and female sharks mature around 55 cm TL. Blood samples collected from mature male and female sharks were analyzed for sex steroid hormones to examine seasonal hormone patterns. Plasma samples were analyzed via radioimmunoassay techniques with female samples measured for estradiol, progesterone, and androgen concentrations, and male samples measured for androgen concentrations. Male androgen concentrations showed a single broad peak from July to October with maximum hormone concentrations (60 ng/ml) occurring in August. Male androgen concentrations were lowest in December–February (<20 ng/ml), and appeared to correlate with reproductive activity and water temperature. Female androgen concentrations were an order of magnitude lower than those for males and showed peaks in June (6 ng/ml) and December (8 ng/ml). Estradiol concentrations in females peaked during the months of September–November (0.5 ng/ml) coinciding with the egg laying period. Progesterone concentrations ranged up to 0.5 ng/ml prior to the mating season. Observations of ova size and egg production showed eggs develop in pairs and ova are ovulated at a size of 25–27 mm. Females lay eggs from August to January. Males were observed with swollen claspers from July through December, with the highest amount of sperm storage in the epididymis occurring between August through November. Our observations indicate that epaulette sharks in the waters near Heron Island mate from July through December. *J. Exp. Zool. 284:586–594, 1999.*

The epaulette shark, *Hemiscyllium ocellatum*, is a small benthic shark commonly found in shallow water on coral reefs in northern Australia and New Guinea (Last and Stevens, '94). Previous research on aquarium-held individuals showed that this species bred year-round in a captive environment, producing up to 50 eggs per year (West and Carter, '90), but nothing is known of the reproductive biology of this species in the wild.

Defining the breeding cycle of a wild population is an integral component to understanding the biology of a species. Some elasmobranch species, such as the lesser spotted dogfish, *Scyliorhinus canicula*, and the black dogfish, *Centroscyllium fabricii*, appear to have a continuous breeding season (Sumpter and Dodd, '79; Yano, '95), whereas many other species including the bonnethead shark, *Sphyrna tiburo*, (Manire et al., '95), the Australian sharpnose shark, *Rhizoprionodon taylori*, (Simpfendorfer, '92) and the blacktip shark, *Carcharhinus limbatus,* (Castro, '96) have distinct seasonal breeding cycles.

Reproductive hormone dynamics have been studied in a range of sharks and rays in order to characterize the reproductive patterns of these fishes (e.g., Sumpter and Dodd, '79; Koob et al., '86; Rasmussen and Gruber, '90, '93; Callard et al., '91, '93, '95; Rasmussen and Murru, '92; Manire et al., '95; Manire and Rasmussen, '97). Callard et al. ('91) described the hormone cycles for oviparous and viviparous reproductive strategies as either synchronous or asynchronous. The oviparous strategy was defined as a synchronous cycle in which estradiol and progesterone concentrations peak at the same time. Oviparous species were assumed to have a short cycle in which both hormones increase during the follicular growth phase and decline during the luteal phase. An asynchronous pattern was used to define the

Grant sponsors: The Australian Coral Reef Society; Great Barrier Reef Marine Parks Authority; University of Queensland, Australia Postgraduate Research Scholarship.

Work was conducted under QFMA permit numbers 6435 and PRM000801 and GBRMPA permit numbers G95/454 and G95/595.

*Correspondence to: M.R. Heupel, Mote Marine Lab, 1600 Ken Thompson Parkway, Sarasota, FL 34236-1096.

viviparous reproductive strategy with estradiol being dominant and peaking in the follicular growth phase and progesterone dominant and peaking in the luteal phase which occurs later in the cycle.

Our research into the reproductive biology of the epaulette shark was designed to examine the reproductive biology of this species and the seasonality of reproductive activity in the wild on a tropical reef off Heron Island, Queensland, Australia. Components of the study involved anatomical observation, histological analysis and sex steroid hormone analysis to determine if there was a defined breeding season in this species.

MATERIALS AND METHODS

Hemiscyllium ocellatum were captured during low tides by hand netting over the reef flat area on Heron Island Reef. This large platform reef surrounds Heron Island, a coral cay situated at 23°27'S and 151°55'E. Approximately 500 sharks were examined during tagging for a mark recapture study. The reproductive condition (i.e., gravid, carrying egg purses) and any obvious evidence of mating activities of mature females were noted. The size (inner clasper length) and condition of claspers of mature males were recorded and calcification of claspers was examined to estimate size of maturation.

Mature females from all months except May, June, July, and December and mature males from all months except February, May, June, and July were collected for reproductive organ examination. These specimens (32 female and 12 male) were used in morphometric as well as histological examination. Measurements of oviducal glands (length, width, thickness) and ova diameter were taken and oviducal gland measurements were multiplied to obtain a volume estimate. Lengths and widths of testes were measured. These measures were compared to the size of the animal, time of year, and condition of organs (e.g., active, inactive, regressing). Samples of testes were immersion fixed in 4% formaldehyde for subsequent examination. These samples were processed for histology (Shannon Citadel 2000) and embedded in paraffin wax. Sections (7 μm) were cut and mounted on glass slides before staining with Masson's trichrome. Stained sections were examined and photographed under light microscopy (Zeiss Axiophot, Germany). Testes were cut in cross section and the number of spermatocysts in each stage were counted, measured, and expressed as a percentage of total spermatocysts. Individual stages of spermatogenesis were categorized into seven stages as described by Maruska et al. ('96): stage 1, primary or germinal zone; stage 2, early spermatocysts; stage 3, spermatocytes; stage 4, spermatids; stage 5, immature sperm; stage 6, mature spermatocysts; and stage 7, degeneration zone.

Blood samples for plasma hormone analysis were taken from five mature male and five mature female sharks for each calendar month. A heparinized syringe was used to take a 1 ml blood sample from the caudal vessel of sharks. Blood was transferred to an eppendorf tube, stored on ice for up to 2 hr after which samples were centrifuged, the plasma pipetted off into clean eppendorf tubes, and stored frozen at –70°C. Plasma samples from male sharks were assayed for androgens and samples from females were analyzed for estradiol, progesterone and androgens. Samples were analyzed using coated-tube radioimmunoassay kits for estradiol, progesterone, and androgens (ICN Diagnostics, Costa Mesa, CA) and counted using a gamma counter (Beckman, Fullerton, CA). Coated tube assay kits were validated by comparing results of a pooled sample including five female samples with results for the same pooled sample analyzed by traditional radioimmunoassay methods. Due to practical considerations the assays were run in two separate batches. Samples from January, May, and August formed the second batch along with additional samples from other months. Due to a change in the assay (by the manufacturer) the values for progesterone and estradiol in the second assay were significantly lower than in the original assay and therefore these data were not used in this analysis.

Hormone concentrations were analyzed statistically using Sigmastat (Jandel Scientific, San Raphael, CA). Non-parametric Kruskal-Wallace one-way ANOVA on ranks followed by an all pairwise multiple comparison (Dunn's method) were conducted using a critical probability value of 0.05. Mean hormone concentrations were correlated with mean monthly maximum water temperatures obtained from records held at Heron Island Research Station (Table 1).

RESULTS

Males

Clasper elongation and calcification in male sharks generally occurred when sharks were between 55–60 cm total length (TL) (Fig. 1) with the smallest mature male 54 cm TL and the largest immature male 61 cm TL. Inner length measurements of fully calcified claspers were consistently about 7% of the total body length of the shark.

TABLE 1. Average monthly water temperatures from Heron Island Reef recorded by research station staff[1]

Month	Average maximum temperature (°C)
January	27.9
February	27.9
March	27.1
April	25.1
May	24.2
June	22.8
July	21.7
August	21.8
September	23.2
October	24.5
November	26.7
December	27.2

[1]Water temperature was measured daily from the jetty adjacent to the study site at a depth of 1 m at 8:30 am. Note: the thermometer is attached to a float to maintain a depth of 1 m at all times.

Histological examination of sections of testes showed the various stages of sperm production throughout the year (Table 2). In April sharks had begun sperm production for the mating season. Stages 1–6 were present and 50–75% of spermatocytes of individual testes were in stages 3 or 4. In August all stages of sperm production were present, with a limited portion of the testis devoted to stages 1 and 7. The epididymis contained sperm during August–November, with fullness appearing to be greatest in November. Testes in this condition were observed to be enlarged compared to previous months, and contained about 50% of spermatocysts in stages 5 or 6.

Measurements of spermatocysts in various stages of spermatogenesis showed the expansion of spermatocysts from stages 1–5 (Fig. 2). Stage 1 cells were generally about 1.0 μm in diameter. Stage 7 spermatocysts were not measured due to their degenerative state. Sertoli cell size was consistent throughout the year and ranged from 0.7–1.0 μm in diameter. When comparing the size of spermatogenic stages by month it was noted that on average November samples had the largest spermatocysts (i.e., stage 5: 34 mm). Samples from September and December were of similar sizes (stage 5: 30 mm in both), but specimens from February were considerably smaller (stage 5: 16 mm).

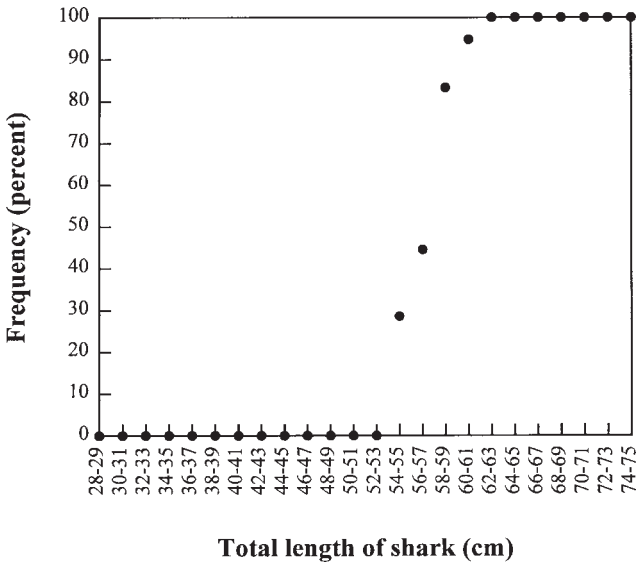

Fig. 1. Percentage of 249 male *Hemiscyllium ocellatum* with fully calcified claspers as a function of total length.

A distinct annual cycle in androgen concentrations was observed in male sharks (Fig. 3). Differences in androgen concentrations between months were statistically significant (Kruskal-Wallace = 48.5, $P < 0.01$). Hormone concentrations were significantly lower ($P < 0.05$) from December through February (southern hemisphere summer) with concentrations of <20 ng/ml observed. Concentrations rose gradually and peaked in July–October at about 60 ng/ml before starting to decline in November. There was an inverse correlation between androgen concentrations and water temperature ($r^2 = 0.93$). The highest concentrations of androgen coincided with observations of males with red and swollen claspers. Males in this condition were frequently found between July to December and were assumed to be mating.

Females

Females were found to mature at approximately 55 cm TL. Females less than 55 cm had thin strap-like ovaries and only small non-yolky ova present. Females above this size had well developed ovaries with yolky ova present.

Measurements of 32 oviducal glands from mature females showed a change in size through the year (Fig. 4). Oviducal glands were smallest in January–April. Subsequently, glands showed an increase in width during August–November when sharks were reproductively active.

Sizes of vitellogenic ova were variable throughout the year with a range of 3–27 mm (Fig. 5). Small ova (3–6 mm) were present in all females examined. Females sampled in January had few large ova present, and in February–March females had few ova that appeared to be undergoing resorption. By April there were small numbers (10–15 per individual) of yolky ova that were 3–5 mm in diameter. In August females had at least five pairs of large, yolky ova of varying sizes. The larg-

TABLE 2. Testicular activity of mature male Hemiscyllium ocellatum *throughout the year indicating presence of sperm and stages of spermatogenesis present*

Month	Stages present	Sperm in epididymis	Description
Jan–Mar	1–5	N	Period of least activity
April	1–6	N	Sperm production has begun
Aug–Nov	1–7	Y	High sperm production
December	3–7	Y	Decrease in sperm production

est observed ova were about 25–27 mm with all subsequent pairs smaller. The presence of egg pairs at this stage was observed throughout the remainder of the breeding season (September–November). No samples from ovaries were obtained in December, but pairs of egg capsules were collected from females during August, October, November, December, and January. Examination of females during tagging excursions revealed gravid or pregnant females from August through early January. Females were also noted to have red, irritated tissue around the cloaca during the months of July and August. This was probably a result of mating activities. It was presumed that ova were ovulated at a size of 25–27 mm since this was the largest size of ova present in any ovary.

Egg capsules were produced in pairs with at least half of the egg capsule formed before ovulation. One female collected for dissection in August had partially developed egg capsules within her uterus. The egg capsules were half formed, but no ovum had been ovulated. There were several pairs of large yolky ova present in the ovary suggesting the female was capable of ovulation. Egg capsules at deposition were approximately 90 × 35 mm, green-brown in color with fine hair-like clumps of tendrils that covered the entire surface.

Androgen concentrations measured in females were about an order of magnitude lower than those in males (2–8 ng/ml) (Fig. 6a). Androgen concentrations were not significantly different between months (Kruskal-Wallace = 11.8, $P = 0.38$) and there was no correlation between water temperature and hormone concentrations ($r^2 < 0.01$).

Estradiol and progesterone appeared to have seasonal patterns (Fig. 6b, c). Estradiol concentrations were low during the southern autumn and winter (March–Aug.) with concentrations of 0.05–0.2 ng/ml. Concentrations rose to a peak (0.5 ng/ml) in spring and early summer (September–November) before declining again in December–February. Estradiol concentrations were significantly different between months (Kruskal-Wallace = 33.1, $P < 0.01$) but sample size was not large enough to distinguish where differences occurred. There was a weak inverse correlation between water temperature and estradiol concentration ($r^2 = 0.19$). Progesterone concentrations showed a different cycle by peaking in autumn and winter months (June–July) at concentrations up to 0.5 ng/ml. Concentrations decreased slightly in September–October and con-

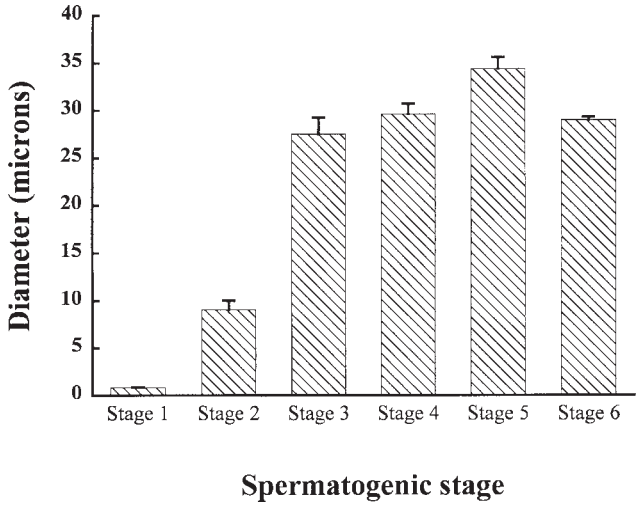

Fig. 2. Average diameter of spermatocysts in stages 1–6 measured from male *H. ocellatum* in the month of November. Bars indicate standard error.

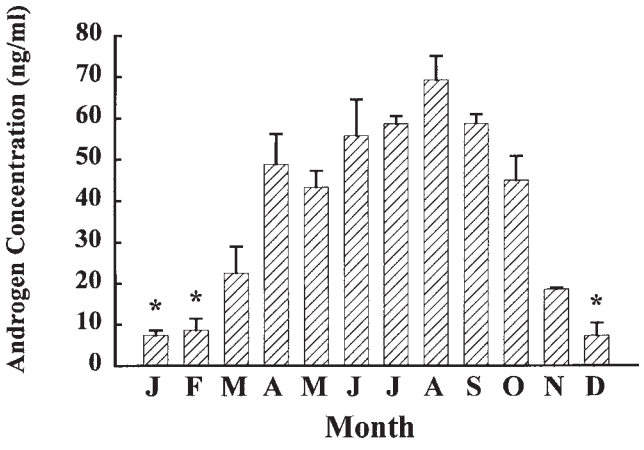

Fig. 3. Distribution of average monthly androgen concentrations (ng/ml) with standard errors for male *H. ocellatum* sampled on Heron Island Reef. Asterisks indicate months with significantly lower ($P < 0.05$) androgen values.

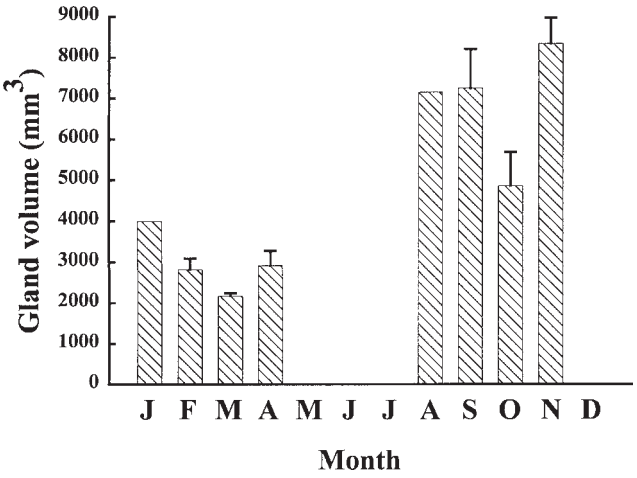

Fig. 4. Yearly volume distribution of oviducal glands from 32 mature female *H. ocellatum* excluding the months of May, June, July, and December.

tinued this trend through the remainder of the year. Progesterone concentrations were also significantly different between months (Kruskal-Wallace = 17.6, $P = 0.03$) but differences could not be statistically determined due to restricted sample sizes. There was a slightly stronger inverse correlation ($r^2 = 0.37$) to water temperature than that for estradiol.

DISCUSSION

Information pertaining to the life history of hemiscyllid sharks in Australian waters is limited. Although these species are commonly observed and are generally known to be oviparous (Compagno, '84; Last and Stevens, '94), their reproductive timing and periodicity are unknown. The limited research available on *H. ocellatum* includes one study on sharks maintained in a controlled aquarium environment (West and Carter, '90). There are no data available concerning the reproductive activities of *H. ocellatum* in its natural coral reef habitat.

Male and female *H. ocellatum* were determined to be reproductively mature at similar sizes. This was based on male clasper calcification and examination of female reproductive tracts. Using calcification of claspers to determine sexual maturity in male sharks has been used in many studies on elasmobranch species, including the Atlantic sharpnose shark, *Rhizoprionodon terraenovae* (Parsons, '83), the blue shark, *Prionace glauca* (Pratt, '79), the chain dogfish, *Scyliorhinus retifer* (Castro et al., '88) the sandbar shark, *Carcharhinus plumbeus* (Joung and Chen, '95) and the

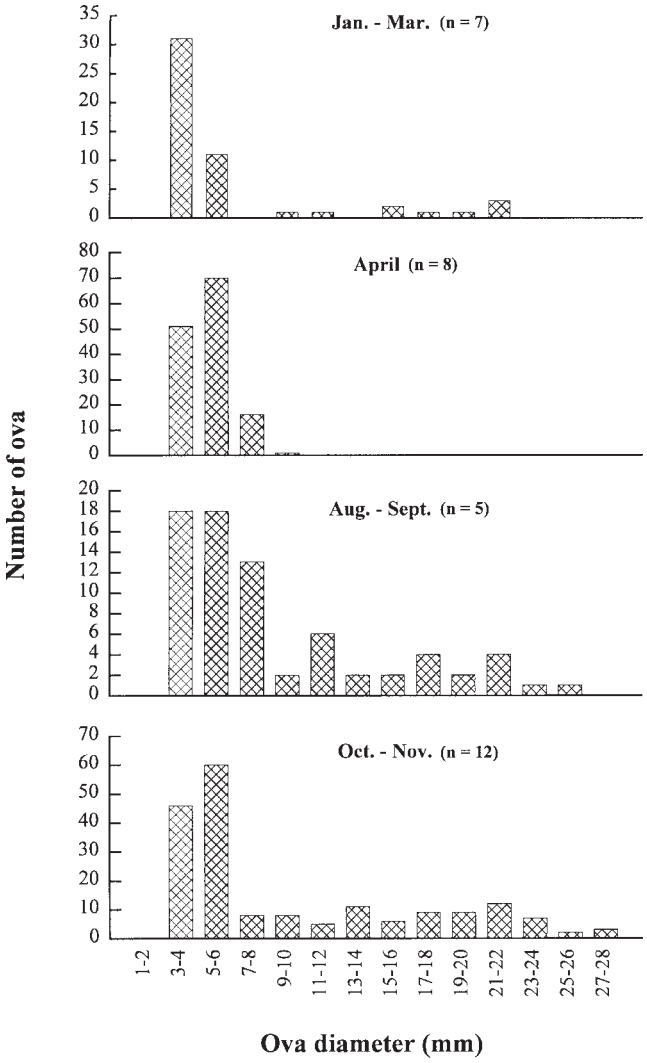

Fig. 5. Measurements of ova diameter from 32 mature female *H. ocellatum* throughout the year. Graphs depict the progression of ovum size, number, and development through the year.

bonnethead shark, *S. tiburo* (Manire and Rasmussen, '97). The claspers of mature *R. terraenovae* were found to be about 7–8% of total body length (Parsons, '83), similar to the measures found for *H. ocellatum* in this study.

Histological examination of testes showed that sperm production had a seasonal cycle. Testes were found to be inactive during the months of January–March, a period when androgen concentrations were at their lowest. Sperm production began in April and continued to increase through the months of August–November with all stages of sperm production present. Sperm production increased as androgen concentrations also began to increase. The epididymis contained the larg-

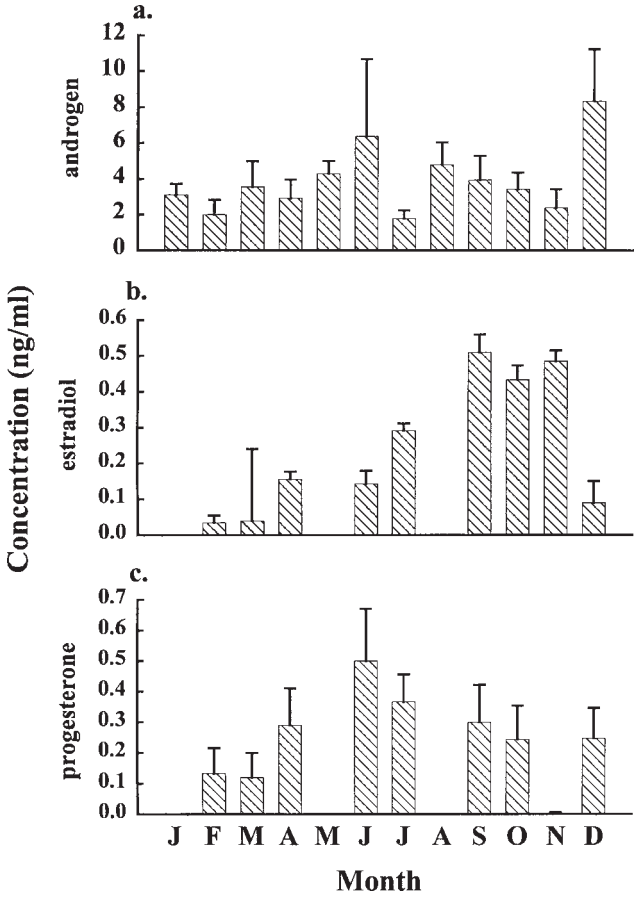

Fig. 6. Distribution of average monthly (**a**) androgen, (**b**) estradiol, and (**c**) progesterone concentrations (ng/ml) with standard errors for mature female *H. ocellatum* sampled on Heron Island Reef.

est amount of sperm during November and in December sperm production dropped off as the mating season ended. This suggests that male epaulette sharks are producing sperm for mating during the second half of the year. Males were observed to have red, swollen claspers from July–November. These observations support the hormonal data where androgen concentrations were highest from June to October and suggest that males generally mate between July and November. Parsons and Grier ('92) defined a seven-stage process of spermatogenesis for *S. tiburo* and stated that not all spermatogenic stages were present throughout the year. Based on their study Parsons and Grier ('92) concluded that many shark species may undergo an annual testicular cycle of regression and recrudescence, while fewer species may have spermatogenic stages present throughout the year.

In *H. ocellatum*, male androgen concentrations rose prior to the breeding season and remained high throughout the remainder of the egg laying season. Testosterone concentrations in the lemon shark, *Negaprion brevirostris*, and other carcharhinids were high during the breeding season, but concentrations vary widely among species. *Negaprion brevirostris* had a range of 75–110 ng/ml testosterone, while a study of several species of carcharhinid sharks reported a range of 0.85–358 ng/ml. Our results from male *H. ocellatum* fall slightly below concentrations for *N. brevirostris*, but were within the range of those found for other carcharhinid species. Based on these results, we propose that testosterone may be important in sexual behaviors, reproductive functions, or may serve as a precursor for other unidentified steroids (Rasmussen and Gruber, '90, '93). Reproductive activity in male sharks may also be related to water temperature based on the inverse correlation between water temperature and androgen concentrations. The end of the mating season and decrease in androgens coincide with water temperature increases during summer months. Whether water temperature plays any role as a reproductive cue for male *H. ocellatum* is unknown, but should be investigated further.

Estradiol concentrations vary among species as well as throughout the reproductive cycle. Studies on various carcharhinid sharks report estradiol concentrations ranging from 0.4–4.5 ng/ml and 0.6–2.0 ng/ml (Rasmussen and Gruber, '90; Rasmussen and Murru, '92). *Raja erinacea* had estradiol concentrations between 0.2–2.0 ng/ml depending on the reproductive status of the female (Koob et al., '86). Estradiol concentrations of the bonnethead shark, *S. tiburo*, were analyzed throughout the reproductive cycle. Concentrations were lowest during early pregnancy (mean = 0.20 ng/ml) but increased at mating (mean = 8.98 ng/ml) and peaked prior to ovulation (mean = 25.03 ng/ml) (Manire et al., '95). Although estradiol concentrations measured in *H. ocellatum* appeared low with a peak of 0.5 ng/ml, these results are similar to other oviparous species (e.g., Koob et al., '86; Callard et al., '91). Estradiol concentrations in *H. ocellatum* increased to their peak during the period of egg laying while decreasing and remaining low during the period of regression and prior to mating.

Increases in estradiol concentrations during the follicular growth phase are common and have been linked to follicle size in the skate, *R. erinacea,* with estradiol concentrations increasing in parallel with follicle size (Koob et al., '86). Further re-

search on *R. erinacea* and *Squalus acanthias* supported these data and showed that increases in both estradiol and testosterone characterized the follicular phase (Callard et al., '93). Research on carcharhinid sharks also showed an increase in estradiol just prior to mating as oocytes were maturing (Rasmussen and Gruber, '90, '93; Rasmussen and Murru, '92). This increase is thought to set ovulatory events in motion or may regulate the reproductive cycle (Rasmussen and Murru, '92; Rasmussen and Gruber, '93). Estradiol concentrations in female *H. ocellatum* were highest in the second half of the year and would coincide with maximum ova sizes, ovulation and egg laying. Fluctuations in estradiol concentrations were correlated with changes in water temperature, but because the relationship was weak it is unlikely that water temperature plays a role in the timing of these changes.

Androgen concentrations in females of other species are generally found to parallel estradiol concentrations. Several species have been analyzed and showed an increase in testosterone concentrations (along with estradiol) prior to and during mating (Callard et al., '91; Rasmussen and Murru, '92; Rasmussen and Gruber, '93; Manire et al., '95). Koob et al. ('86) found testosterone fluctuated with estradiol, but was present in higher concentrations. Testosterone concentrations decrease after mating and remain low throughout the rest of the reproductive cycle of many elasmobranch species (Rasmussen and Murru, '92; Rasmussen and Gruber, '93). Testosterone may be important in initiating some sequential ovulatory events and may have a role in courtship. Due to its lower concentrations throughout the rest of the cycle, it does not appear to be important during pregnancy in viviparous species (Rasmussen and Murru, '92; Rasmussen and Gruber, '93). Androgen concentrations in female *H. ocellatum* did not vary significantly throughout the reproductive season. Although it is possible that androgens are important in initiating changes in the reproductive tract, no supporting evidence was found from androgen levels in *H. ocellatum*.

Progesterone concentrations in female *H. ocellatum* were usually low except for a peak from April–July prior to the egg laying period. As with estradiol, there was a weak correlation between water temperature and hormone concentrations, but water temperature probably does not play a major role in progesterone activity. Peaks in progesterone concentrations are thought to help prepare the reproductive tract for the egg production season. Manire et al. ('95) reported progesterone increased during preovulation (mean = 8.9 ng/ml) and ovulation (mean = 16.6 ng/ml) prior to a peak after ovulation (mean = 26.6 ng/ml) in *S. tiburo.* This result is similar to that described for the dogfish *S. acanthias* (Callard et al., '93). However, both *S. tiburo* and *S. acanthias* are viviparous species and show a different pattern from the one described for the oviparous skate *R. erinacea*. Koob et al. ('86) reported an elevation in progesterone for a restricted two day period before encapsulation with a sharp drop on the day of encapsulation and low concentrations throughout the rest of the year. Callard et al. ('93) reported elevated concentrations of progesterone pre- and peri-ovulation in *R. erinacea*. Serial samples examined from one captive female *H. ocellatum* showed a peak in progesterone the morning the eggs had been laid (Heupel, unpublished data). Concentrations previous to and after this point were essentially undetectable, suggesting that progesterone is most active at oviposition in *H. ocellatum*. Progesterone is thought to regulate events associated with ovulation, encapsulation, and egg retention in oviparous species and may have specific triggering roles in viviparous species. Progesterone may also inhibit activities such as vitellogenesis (Koob et al., '86; Rasmussen and Murru, '92; Callard et al., '93; Manire et al., '95).

It has been suggested that hormone concentrations in females of oviparous species peak more than once during a season (Koob et al., '86; Callard et al., '91, '93, '95). However, this was not the case in our studies of *H. ocellatum*, and was not seen in several other studies on oviparous species. Research by Sumpter and Dodd ('79) examined the hormone cycles of the lesser spotted dogfish, *S. canicula*. This species is oviparous and has an extended, if not continuous, breeding season. Despite the extended reproductive cycle of this species, estradiol and testosterone concentrations displayed a distinct annual cycle. Both hormones fluctuated together, rising as the ovary recrudesced and falling as the rate of egg laying decreased. Although this study did not include progesterone analysis it clearly defined one estradiol peak rather than several throughout the season. This pattern of one single peak in hormone concentrations per year is similar to that observed for *H. ocellatum* sampled on Heron Island Reef.

Most oviparous elasmobranchs produce eggs in pairs (Luer and Gilbert, '85; Castro et al., '88; Ellis and Shackley, '95; Yano, '95). Epaulette sharks also

produce eggs in pairs and appear to ovulate ova into egg capsules after they are at least half formed. Studies of at least two other oviparous elasmobranchs, the dogfish *S. canicula,* and the clearnose skate, *R. eglanteria,* have shown similar patterns of ovulation. Ova were not present in egg capsules less than three-fourths formed in *S. canicula* (Metten, '39) and *R. eglanteria* formed two-thirds of the egg capsule prior to ovulation and fertilization (Luer and Gilbert, '85). Metten ('39) also described one pair of egg capsules that were fully formed but smaller than normal and without ova. No explanation was given for eggs in this condition, and no explanation is obvious for the same condition observed in *H. ocellatum* in this study.

The egg laying behavior of dogfishes has been well documented with detailed descriptions of attachment of the long tendrils of the egg capsule to a vertical structure and the use of this structure to pull the egg capsule from the oviduct (Castro et al., '88). However, due to the difference in tendrils found on egg capsules of *H. ocellatum,* it is unlikely that they use this type of strategy. The long hair-like tendrils would appear to be more suited for egg laying similar to that described for the clearnose skate, *R. eglanteria*. The egg laying behavior of *R. eglanteria* described by Luer and Gilbert ('85) included the female settling quietly on the sediment before contracting the pelvic fins ventrally, shaking the pelvis from side to side, and swimming away leaving a single egg capsule on the sediment. This activity was violent enough to leave the egg capsule covered in sediment from the bottom of the tank. This type of egg laying behavior would appear to be effective for depositing eggs under and among coral, and because the egg capsules of *H. ocellatum* lack long tendrils, it is likely that this type of method would be used to attach egg capsules to coral. One female *H. ocellatum* was held in a large aquarium with a number of different types of shelter and coral including one small piece of *Acropora* coral. Although there were several other types of coral present, the shark placed both egg capsules on the one piece of *Acropora*. We were unable to remove the egg from the coral by gently pulling the two apart. Although no egg capsules have been discovered on the reef flat at Heron Island Reef, the habits of this species suggest eggs are deposited under coral heads. The presence of very small juvenile sharks in *Acropora* beds suggest they may have hatched in that environment.

The length of time between laying of successive egg pairs is variable among oviparous elasmobranch species. The thornback ray, *Raja clavata,* can produce a pair of eggs from 0–2 days after the previous pair (Ellis and Shackley, '95). The clearnose skate, *R. eglanteria,* takes slightly longer with 4.5 ± 2.2 days between egg pairs (Luer and Gilbert, '85) and the chain dogfish, *S. retifer,* requires 14–16 days between laying egg pairs (Castro et al., '88). The period between egg pair production for *H. ocellatum* was not determined in the present study. Observation of captive (wild caught) females at Heron Island Reef showed that none produced more than one pair of eggs (Heupel, unpublished data). This may have resulted from females being kept in isolation when found to be gravid. One female kept isolated from male sharks produced a pair of empty egg capsules. Whether this was the result of not having a male present in the tank, or was due to some other influence, is unknown. However, the presence of red, swollen claspers and sperm production from July to December suggests that males are capable of mating throughout the egg laying season.

Wourms ('77) defined three types of reproductive cycle in elasmobranchs: (1) breeding throughout the year; (2) partially defined annual cycle with one or two peaks during the year; and (3) a well defined annual or biennial cycle. Although epaulette sharks held in a captive aquarium environment fell into the first category of Wourms' description (West and Carter, '90), animals sampled in the natural environment fell into the last category. The differences in results between aquarium-held sharks and wild-caught sharks may be due to a lack of seasonal temperature variation in the aquarium environment. As shown by correlation of water temperature and male testosterone concentrations, seasonal temperature changes may be a cue for commencement and conclusion of the mating period. Removal of this cue may result in continuous mating activities. Further examination of the effects of water temperature should be conducted.

ACKNOWLEDGMENTS

We thank the staff at Heron Island Research Station for their help throughout this research; A. Chan for technical assistance, K. Townsend, T. Turner, and S. Bennett for field assistance. We also thank Dr. C. Manire and two anonymous reviewers for their advice and comments. This work was undertaken while the primary author was in receipt of an Overseas Postgraduate Research Scholarship at the University of Queensland, Australia.

LITERATURE CITED

Callard IP, Etheridge K, Giannoukos G, Lamb T, Perez L. 1991. The role of steroids in reproduction in female elasmobranchs and reptiles. Ster Biochem Mol Biol 40:571–575.

Callard IP, Fileti LA, Koob TJ. 1993. Ovarian steroid synthesis and the hormonal control of the elasmobranch reproductive tract. Envir Biol Fishes 38:175–185.

Callard IP, Putz O, Paolucci M, Koob TJ. 1995. Elasmobranch reproductive life histories: endocrine correlates and evolution. In: Goetz FW, Thomas P, editors. Proceedings of the Fifth International Symposium on the Reproductive Biology of Fish. Austin, TX, 2–8 July. p 204–208.

Castro JI. 1996. Biology of the blacktip shark, *Carcharhinus limbatus*, off the southeastern United States. Bull Mar Sci 59:508–522.

Castro JI, Bubucis PM, Overstrom NA. 1988. The reproductive biology of the chain dogfish *Scyliorhinus retifer*. Copeia 1988:740–746.

Compagno LJV. 1984. FAO species catalogue, volume 4. Sharks of the world: an annotated and illustrated catalogue of shark species known to date. Part 1. Hexanchiformes to Lamniformes. FAO Fisheries Synopsis No. 125, Rome. p 188–199.

Ellis JR, Shackley SE. 1995. Observations on egg-laying in the thornback ray. J Fish Biol 46:903–904.

Joung SJ, Chen CT. 1995. Reproduction in the sandbar shark, *Carcharhinus plumbeus*, in the waters off northeastern Taiwan. Copeia 1995:659–665.

Koob TJ, Tsang P, Callard IP. 1986. Plasma estradiol, testosterone, and progesterone concentrations during the ovulatory cycle of the skate (*Raja erinacea*). Biol Reprod 35:267–275.

Last PR, Stevens JD. 1994. Sharks and rays of Australia. Melbourne: CSIRO. p 513.

Luer CA, Gilbert PW. 1985. Mating behavior, egg deposition, incubation period, and hatching in the clearnose skate, *Raja eglanteria*. Env Biol Fishes 13:161–171.

Manire CA, Rasmussen LEL. 1997. Serum concentrations of steroid hormones in the mature male bonnethead shark, *Sphyrna tiburo.* Gen Comp Endocrinol 107:414–420.

Manire CA, Rasmussen LEL, Hess DL, Hueter RE. 1995. Serum steroid hormones and the reproductive cycle of the female bonnethead shark, *Sphyrna tiburo.* Gen Comp Endocrinol 97:366–376.

Maruska KP, Cowie EG, Tricas TC. 1996. Periodic gonadal activity and protracted mating in elasmobranch fishes. J Exp Zool 276:219–232.

Metten H. 1939. Studies on the reproduction of the dogfish. Phil Trans Roy Soc Ser B 230:217–241.

Parsons GR. 1983. The reproductive biology of the Atlantic sharpnose shark *Rhizoprionodon terraenovae* (Richardson). Fishery Bull 81:61–73.

Parsons GR, Grier HJ. 1992. Seasonal changes in shark testicular structure and spermatogenesis. J Exp Zool 261: 173–184.

Pratt HL. 1979. Reproduction in the blue shark, *Prionace glauca*. Fishery Bull 77:445–470.

Rasmussen LEL, Gruber SH. 1990. Serum concentrations of circulating steroid hormones in free-ranging carcharhinoid sharks. NOAA Tech Rep 90:143–155.

Rasmussen LEL, Murru FL. 1992. Long-term studies of serum concentrations of reproductively related steroid hormones in individual captive carcharhinids. Aust J Mar Freshwater Res 43:273–281.

Rasmussen LEL, Gruber SH. 1993. Serum concentrations of reproductively-related circulating steroid hormones in the free-ranging lemon shark, *Negaprion brevirostris.* Env Biol Fishes 38:167–174.

Simpfendorfer CA. 1992. Reproductive strategy of the Australian sharpnose shark, *Rhizoprionodon taylori* (Elasmobranchii: Carcharhinidae), from Cleveland Bay, northern Queensland. Aust J Mar Freshwater Res 43:67–75.

Sumpter JP, Dodd JM. 1979. The annual reproductive cycle of the female lesser spotted dogfish, *Scyliorhinus canicula* L., and its endocrine control. J Fish Biol 15:687–695.

West JG, Carter S. 1990. Observations on the development and growth of the epaulette shark *Hemiscyllium ocellatum* (Bonnaterre) in captivity. J Aquariculture Aquat Sci 5:111–117.

Wourms JP. 1977. Reproduction and development in chondrichthyan fishes. Am Zool 17:379–410.

Yano K. 1995. Reproductive biology of the black dogfish *Centroscyllium fabricii*, collected from waters of western Greenland. J Mar Biol Assoc UK 75:285–310.