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Research article

The use of a gonadotropin releasing hormone antagonist in captive sand tiger sharks, *Carcharias taurus*, and the serum levels of the antagonist and reproductive steroid hormones

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Abstract
Sand tiger sharks, *Carcharias taurus*, are commonly maintained in large public aquaria throughout the world, but limited success has been achieved in captive reproduction. Successful captive reproduction in this species has only been documented in four institutions to date. In this investigation, steroid hormone assays in male sand tiger sharks were compared before and after dosing with a GnRH antagonist (Antide; Bachem, Inc., Torrance, CA). Three male sand tiger sharks were sampled serially for 12 months. All three sharks received two microsphere-encapsulated doses of Antide (0.185 and 0.225 mg·kg^{-1}), 69 days apart. Serum samples were assayed for concentrations of the steroid hormones 17β-oestradiol, progesterone, testosterone, 5α-dihydrotestosterone, corticosterone, and the GnRH antagonist Antide via radioimmunoassay. The results obtained demonstrate cyclical patterns in the steroid profiles, and that mean Antide level peaked 33 and 27 days following each injection, respectively. Circulating Antide was detectable eight months after the final treatment. Contrary to the anticipated effect, however, concentrations of all five steroids increased immediately following initial administration of the antagonist. Although the lack of control subjects precludes solid conclusions regarding the effects of the antagonist upon steroid hormone levels, notable declines in the levels were observed concurrent with peak circulating Antide levels. Further, although we cannot attribute it to the antagonist, mean androgen levels declined more sharply following antagonist administration than those observed in a previous investigation in this group of male sharks. This is the first documented use of a GnRH antagonist in any chondrichthyan. Further research, including control subjects, is suggested to investigate the effects of the antagonist upon steroid hormones, reproductive cycles and behaviour.

Introduction

The sand tiger shark, *Carcharias taurus*, occurs worldwide in a scattered distribution in coastal warm temperate to tropical waters, but is absent from the central and eastern Pacific Ocean (Compagno 2001; Castro 2011; Ebert et al. 2013). Despite its occurrence in scattered coastal areas, information from molecular genetics and tagging studies indicate little if any mixing between populations (Stow et al. 2006; Ahonen et al. 2009). As a consequence, several populations have been reported to have declined by as much as 90% and are listed from vulnerable to critically endangered by the IUCN (Pollard and Smith 2000, 2005; Pollard et al. 2003; Chiaramonte et al. 2007). In the northwest Atlantic Ocean, the sand tiger shark is recognised as a species of concern due to reported declines in abundance, although a recent status update reported only a small decline and urged its retention on the species of concern list as a precautionary measure (Carlson et al. 2009). Wise management in wild and captive situations is therefore warranted.

Sand tiger sharks are commonly maintained in large aquaria due to their hardiness and large size, but reproductive success resulting in viable young has been limited (Henningsen et al. 2004). Reproductive behaviours in captive sand tiger sharks have

been documented (Gordon 1993; Henningsen et al. 2004; Schneble 2014). Investigations that focus on reproductive physiology and its relationship to captive groups, such as Henningsen et al. (2008) and the current investigation, provide key information for successful captive management and reproduction.

Gonadal hormones, principally androgens, are known to affect sexual behaviour in both sexes across vertebrate taxa, although they are also strongly influenced by social context or status (Nelson 2000; Wallen 2001; Taves et al. 2008). In bony fishes, the androgens testosterone (T) and 11-ketotestosterone (11KT) have been shown to act as mediators of sexual aggression and dominance in male sexual conflicts (Desjardins et al. 2008a, b; Taves et al. 2008). Several steroid hormones have been linked to various stages of the reproductive cycle in both sexes of elasmobranchs (Gelsleichter 2004). The principal steroid hormones in male elasmobranchs are T and 5α-dihydrotestosterone (DHT) (Garnier et al. 1999; Manire et al. 1999; Henningsen et al. 2008), and levels of T and DHT have been linked to sexual conflicts in male sand tiger sharks (Henningsen et al. 2008). The synthesis and release of gonadal steroids is regulated by pituitary gonadotropins and ultimately by the hypothalamic tropic hormone gonadotropin releasing hormone (GnRH).

Several forms of GnRH occur across animal taxa, including seven that have been identified in the brains of some elasmobranchs (Sherwood and Lovejoy 1993; Wright and Demski 1993; Forlano et al. 2000). GnRH is produced by the hypothalamus and reaches the pituitary via the systemic circulation (Demski 1990a, b; Sherwood and Lovejoy 1993; Wright and Demski 1993). In chondrichthyan fishes, gonadotropin (GTH) producing cells are located in an anatomically separate lobe of the pituitary (ventral lobe in elasmobranchs, buccal lobe in holocephalans). Two vertebrate type GTHs (I and II) have been identified in elasmobranchs, and the β subunits are similar to the β subunits of FSH and LH GTHs, respectively. The GTHs in turn act upon gonadal and extragonadal receptors to mediate the production of steroids (Querat et al. 2001; Engel and Callard 2005; Lutton et al. 2005).

GnRH agonists and antagonists have been used to regulate, promote and inhibit reproduction and sexual conflicts in a variety of vertebrate taxa (e.g., Atkinson et al. 1998; Felberbaum et al. 2000). Another potential use of these GnRH analogues is to regulate the timing of endocrine events associated with reproduction. In other vertebrates investigated, GnRH antagonists cause a rapid decline in gonadotropin secretion through competitive binding of GnRH receptors (e.g. Hiurne et al. 2004). Antide is a third-generation GnRH antagonist that has the advantage of inducing minimal histamine release (Leal et al. 1991). The pharmacokinetics of Antide have been examined in other vertebrate models, and in some species, such as the cynomolgus monkey, *Macaca fascicularis*, it is effective at suppressing steroid and gonadotropin levels for extended periods from a single low dose (Danforth et al. 1990, 1991). Although the use of a GnRH agonist or antagonist has been suggested, there are no previous published accounts of its use in an elasmobranch (Henningsen et al. 2004).

The glucocorticoid corticosterone (CS) was also assayed as it is found in measurable levels in elasmobranchs. Corticosterone is often assayed in elasmobranchs, as cortisol is absent in this group, and the principal glucocorticoid, 1α-hydroxycorticosterone (1α-OHB), unique to elasmobranchs, is difficult to measure (Idler and Truscott 1966, 1967; Truscott and Idler 1968; Hazon and Balmont 1998; Gelseichter 2004; Skomal and Bernal 2010). Being involved in the primary stress response, 1α-OHB is also involved in energy metabolism and ion regulation (Hazon and Balmont 1998). Corticosterone was found to be involved in reproduction, and to vary across different stages of the reproductive cycle in the bonnethead shark, *Sphyrna tiburo*, and the Atlantic stingray, *Dasyatis sabina* (Manire et al. 2007). Unlike bony fishes, elasmobranchs possess a distinct interrenal gland, analogous to the adrenal gland in mammals, and part of the hypothalamic–pituitary interrenal system. Glucocorticoids are synthesised and released in response to the pituitary adrenocortotropic hormone (ACTH), and ultimately in response to hypothalamic corticotropin releasing hormone (CRH) (Hazon and Balmont 1998; Gelsleichter 2004; Skomal and Bernal 2010).

The goals of the current investigation were to measure the levels of circulating steroid hormones in captive mature male sand tiger sharks and to evaluate the GnRH antagonist.

Methods

Animals and aquarium system

During the study period, three mature male and two mature female sand tiger sharks were maintained in a closed recirculating aquarium system as previously described by Sabalones (1995). Water temperature was maintained at 25.0 ± 0.02 SEM °C with no seasonal variation. The photoperiod varied seasonally, from 11.5:12.5 light:dark from December to mid-January, to 13.5:10.5 light:dark from June to mid-July.

The dominance hierarchy in this group of males has been determined based on behavioural responses, and the sharks have been designated as α, β and γ for the dominant to most subordinate male, respectively, and sexual conflicts were documented as described by Henningsen et al. (2004, 2008) and Schneble (2014).

Sampling and treatment of sharks

The three male sharks in the investigation were sampled monthly beginning in August 2006 and continuing through August 2007. Two additional samples were collected 7 days after each of the two treatments, for a total of 15 samples per shark. For each venipuncture, the sharks were isolated one at a time in the shallow back-up portion of the exhibit and carefully restrained inside a vinyl stretcher. Blood was collected either via caudal venipuncture or from the dorsal post-cardinal sinus (Rasmussen and Murru 1992; Stoskopf 1993). Following venipuncture, the sharks were weighed and returned to the exhibit. The blood was allowed to clot at room temperature and then centrifuged at 3400 rpm (1744 x g) for 5 min. The resulting serum was placed into aliquots and stored at -80° C until analysed.

The antagonist used in the investigation was research grade Antide (D-2-Nal-p-chloro-D-Phe-b-(3-pyridyl)-D-Ala-Ser-Lys(nicotinoyl)-D-Lys(nicotinoyl)-Leu-Lys(isopropyl)-Pro-D-Ala-NH_2; Bachem, Inc., Torrance, CA). Antide was incorporated into polymer-based microspheres as described by Holland et al. (1998). The microspheres were made for a 90-day sustained release at a target dosage of 0.25 $mg \cdot kg^{-1}$ body weight of each shark, although the exact release kinetics of the microspheres in sharks at a temperature of 25° C are unknown. The microspheres were injected intramuscularly into the epaxial musculature of each of the three male sharks using a 16-gauge needle. The sharks received two Antide injections, the first on September 27, 2006 (Bachem Lots 2500073 and 0568846), and the second on December 5, 2006 (Bachem Lot 2501585). The applied dose was less than the target dose due to losses incurred during resuspension of the microspheres, and was 0.185 and 0.225 $mg \cdot kg^{-1}$ for the first and second treatment, respectively. All blood sample collection and treatment protocols were reviewed and approved by the National Aquarium's Biological Programs Research Committee.

Steroid radioimmunoassay (RIA)

Serum samples were assayed for 17β-oestradiol (E_2), progesterone (P_4), T, and DHT via standard RIA following chromatographic separation. Corticosterone was measured following extraction

with diethyl ether by the Endocrine Service Laboratory) of the Oregon National Primate Research Center, Oregon Health and Science University. For details of this method, see Rasmussen and Gruber (1990) and Manire et al. (1995).

Antide assay

Serum levels of Antide were determined via RIA. The assay was performed using a homologous RIA developed for human serum (Woods Assay, Inc., Portland, OR). Bound and free tracer were separated by standard second antibody; the standard was authentic Antide (Lot 00DE01, maintained at -80° C). Results were expressed in pg·20 μl^{-1} but were converted to pg·ml^{-1} for comparison to the original dosage and steroid level.

Statistical analysis

All statistical analyses were conducted using SigmaStat 3.1 (Systat Software, Inc., Point Richmond, CA, USA) at the level of significance of $P < 0.05$. Unless otherwise noted, results are given as mean ± SEM. Steroid data obtained from the serial samples were analysed by repeated measures one-way analysis of variance (RM-ANOVA) with time of measurement (month) as the variable. Data that were not normally distributed were analysed using the non-parametric Kruskall-Wallis one-way ANOVA. Multiple comparison (post-hoc) tests were used to determine the source of the variation.

Results

Steroid and Antide levels

An annual cycle was observed in the mean levels of all steroids monitored (Figures 1–3). Significant elevations ($P < 0.05$) in T were observed on October 4, 2006 and July 31, 2007 and in DHT on October 4 and 30, 2006 and June 27, 2007 (Figure 1a, b).

The elevations in E2 were noted to be significant on October 4 ($P < 0.001$) and October 30, 2006 ($P < 0.05$), and on August 28, 2007 ($P < 0.001$) (Figure 2a). Significant elevations in P4 were observed on October 30 and December 5, 2006 ($P < 0.05$), and a significant depression observed on June 27, 2007 (Figure 2b).

Significant elevations in CS were observed on October 4 and 30, 2006 (Figure 3).

The mean levels of all steroids rose significantly following the first Antide treatment on September 27, 2006, and then declined. The levels then further declined or stayed at depressed levels following the second Antide treatment on December 5, 2006. The mean levels of E_2, P_4, and CS declined more gradually than the androgens T and DHT, or from October through December. The mean level of P_4, however, plateaued before and after the second treatment prior to declining (Figure 2b). Mean levels for four of the steroids remained low from January through May 2007, while CS increased beginning in March 2007.

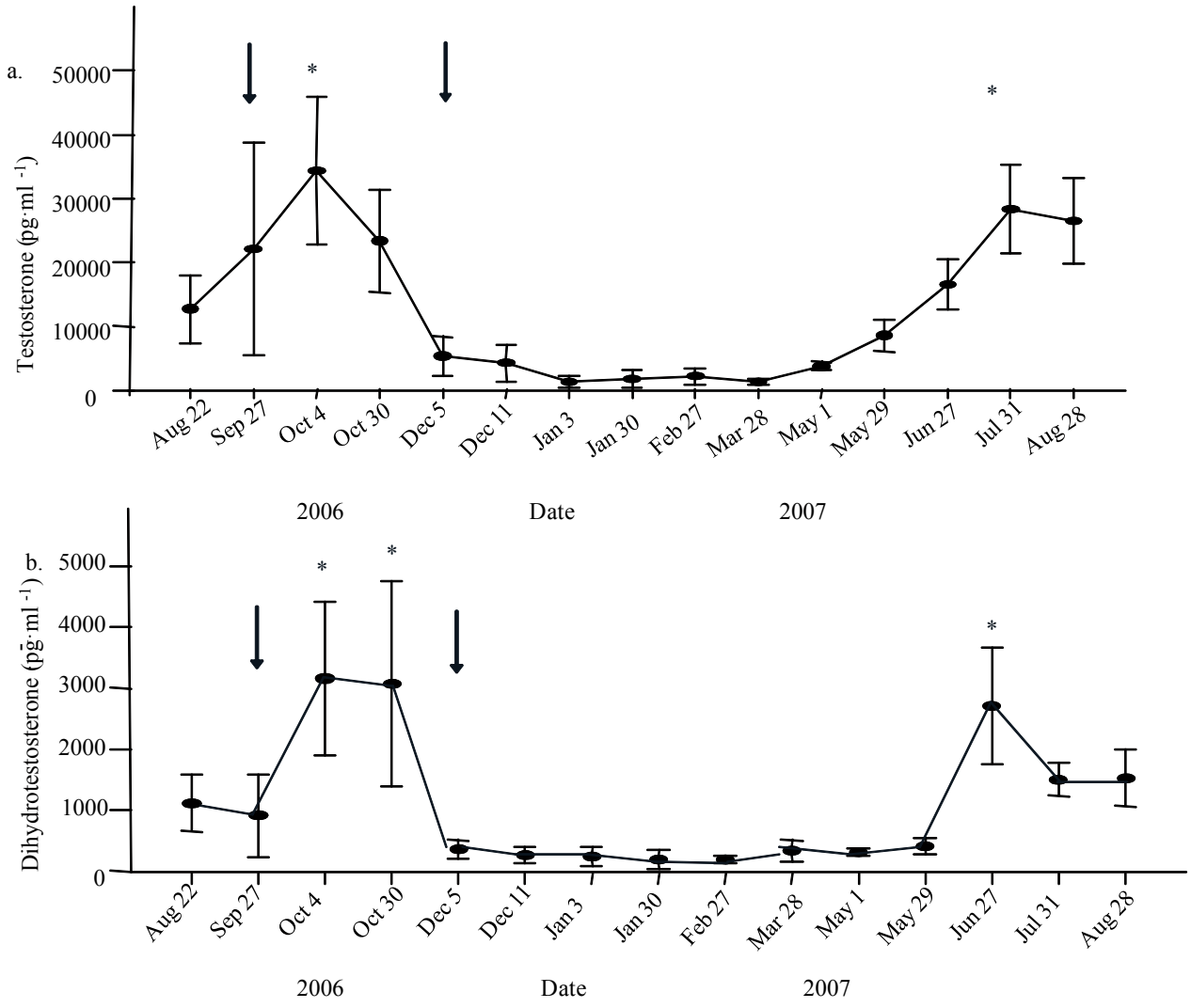

Figure 1. a. Testosterone and b. dihydrotestosterone levels in pg·ml^{-1} in the male sand tiger sharks from August 2006 to August 2007. Values are mean ± SEM. Arrows indicate dates of Antide treatments. Asterisk (*) indicates significance at $P < 0.05$ and ** indicates significance at $P < 0.001$.

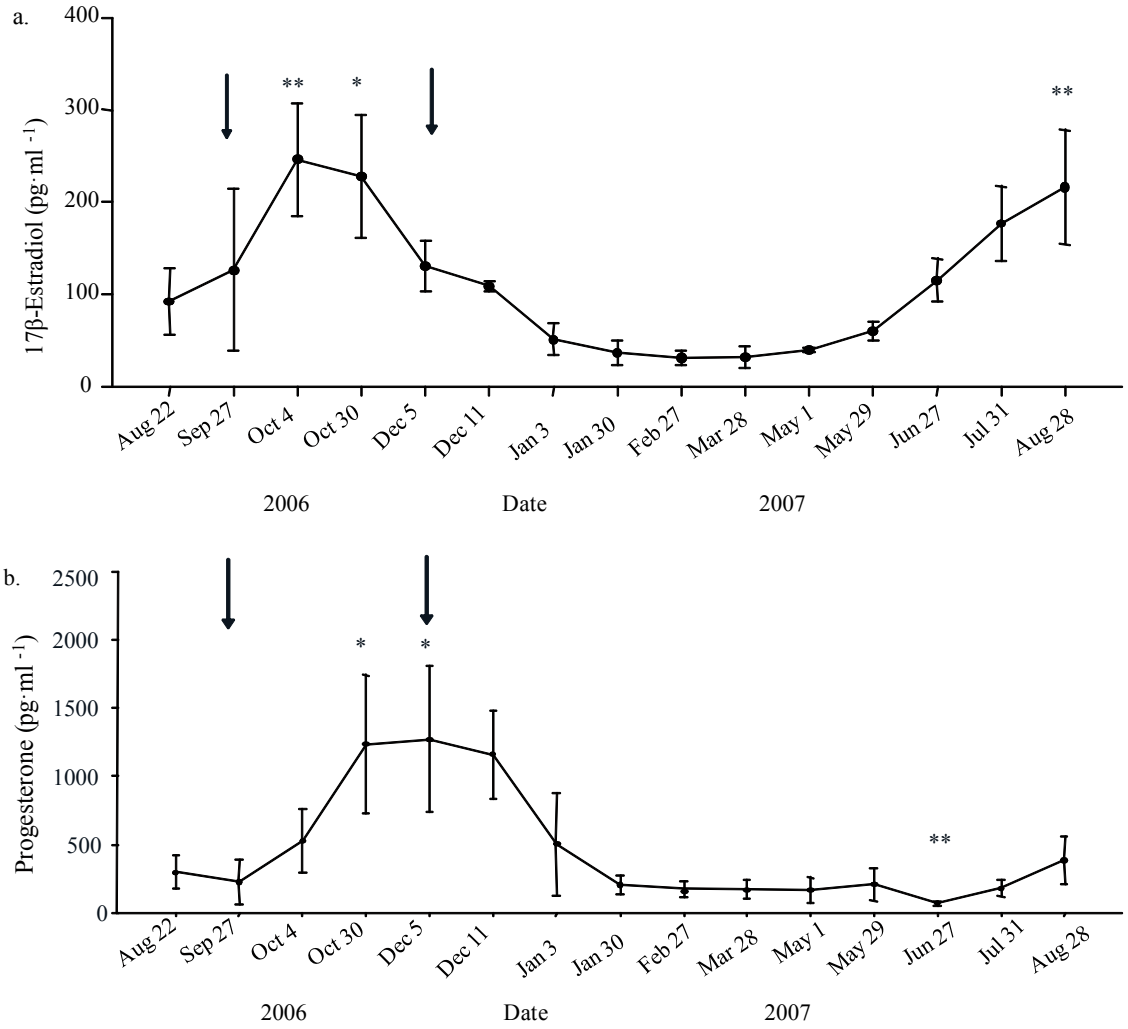

Figure 2. a. 17β-Oestradiol and b. progesterone levels in pg·ml^{-1} in the male sand tiger sharks from August 2006 to August 2007. Values are mean ± SEM. Arrows indicate dates of Antide treatments. Asterisk (*) indicates significance at $P < 0.05$ and ** indicates significance at $P < 0.001$.

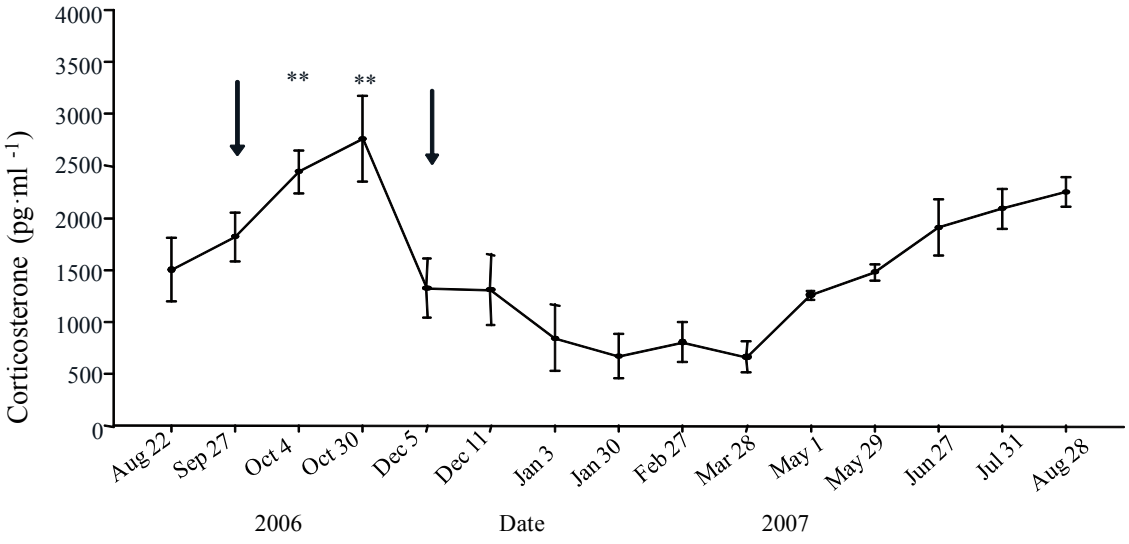

Figure 3. Corticosterone levels in pg·ml^{-1} in the male sand tiger sharks from August 2006 to August 2007. Values are mean ± SEM. Arrows indicate dates of Antide treatments. Asterisk (*) indicates significance at $P < 0.05$ and ** indicates significance at $P < 0.001$.

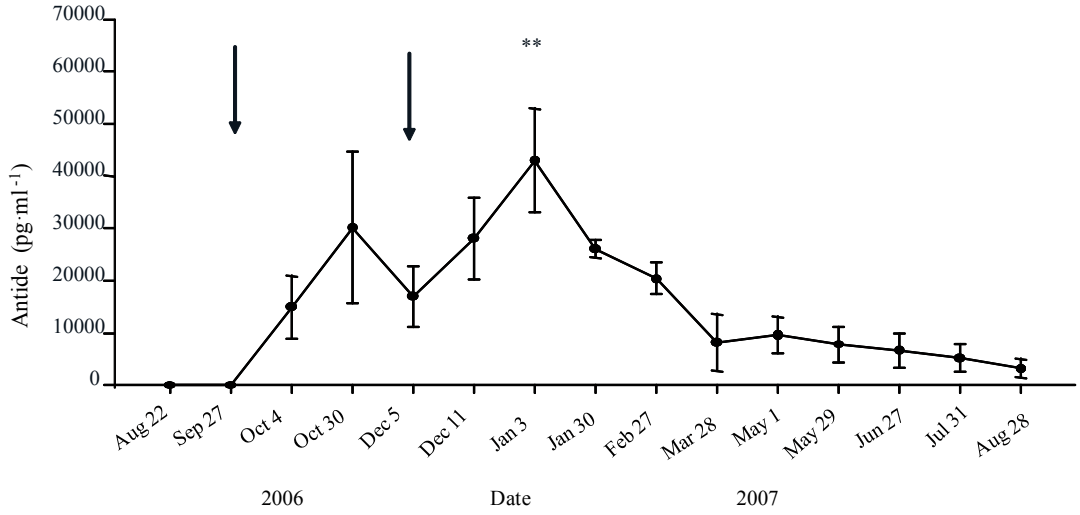

Figure 4. Mean Antide levels in pg·ml^{-1} in the male sand tiger sharks from August 2006 to August 2008. Values are mean ±SEM. Arrows indicate dates of Antide treatments. Asterisk (*) indicates significance at $P < 0.05$ and ** indicates significance at $P < 0.001$.

Mean Antide levels increased following each treatment, peaking 29 and 33 days post-treatment, with a significant elevation of 43,017 pg·ml^{-1} on January 3, 2007 ($P < 0.001$). Mean serum Antide levels declined thereafter throughout the remainder of the study period to a mean concentration of 3,216 pg·ml^{-1} on August 28, 2007 (Figure 4).

The levels of steroids and Antide in individual sharks from different than those described by the mean values. The

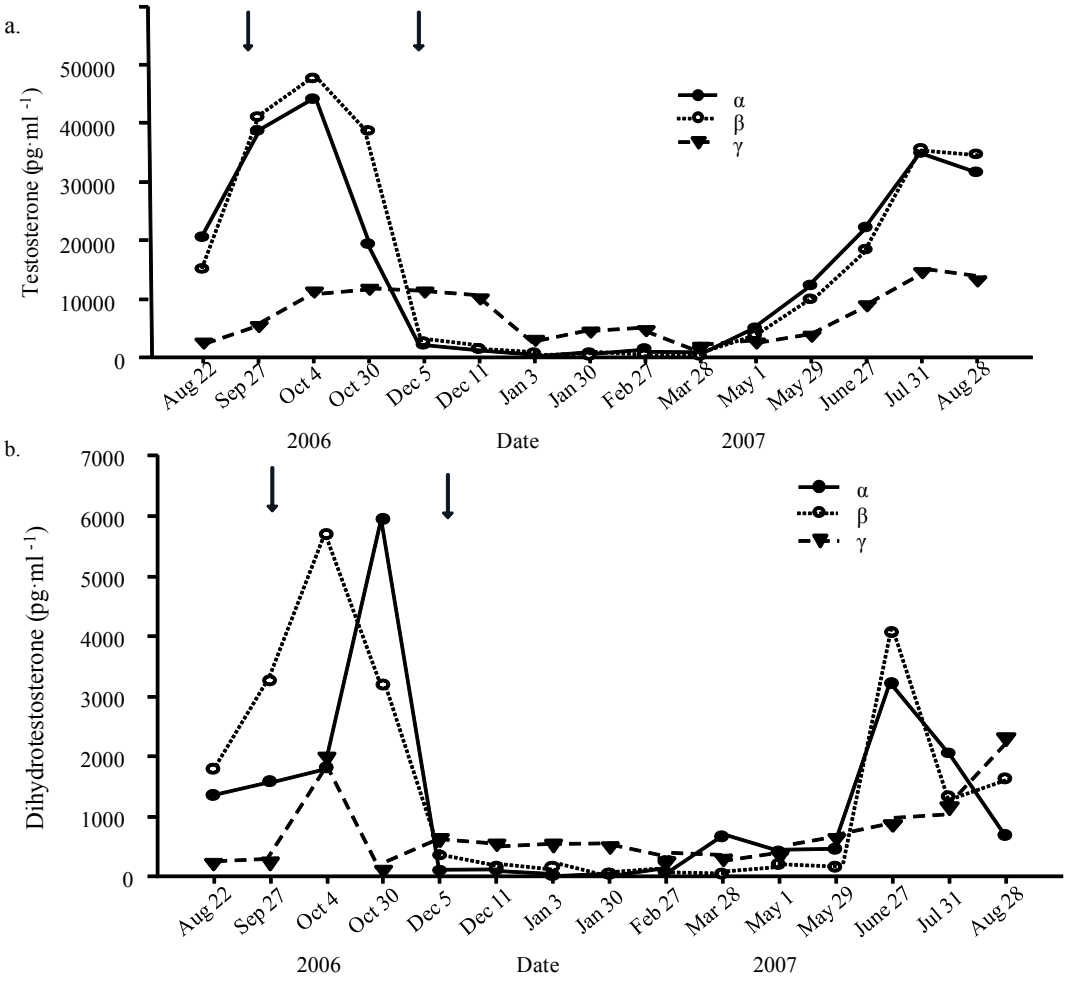

Figure 5. a. Testosterone and b. dihydrotestosterone levels in pg·ml^{-1} in the individual male sand tiger sharks from August 2006 to August 2007. α, β, and γ represent hierarchal status from dominant to subordinate. Arrows indicate dates of Antide treatments.

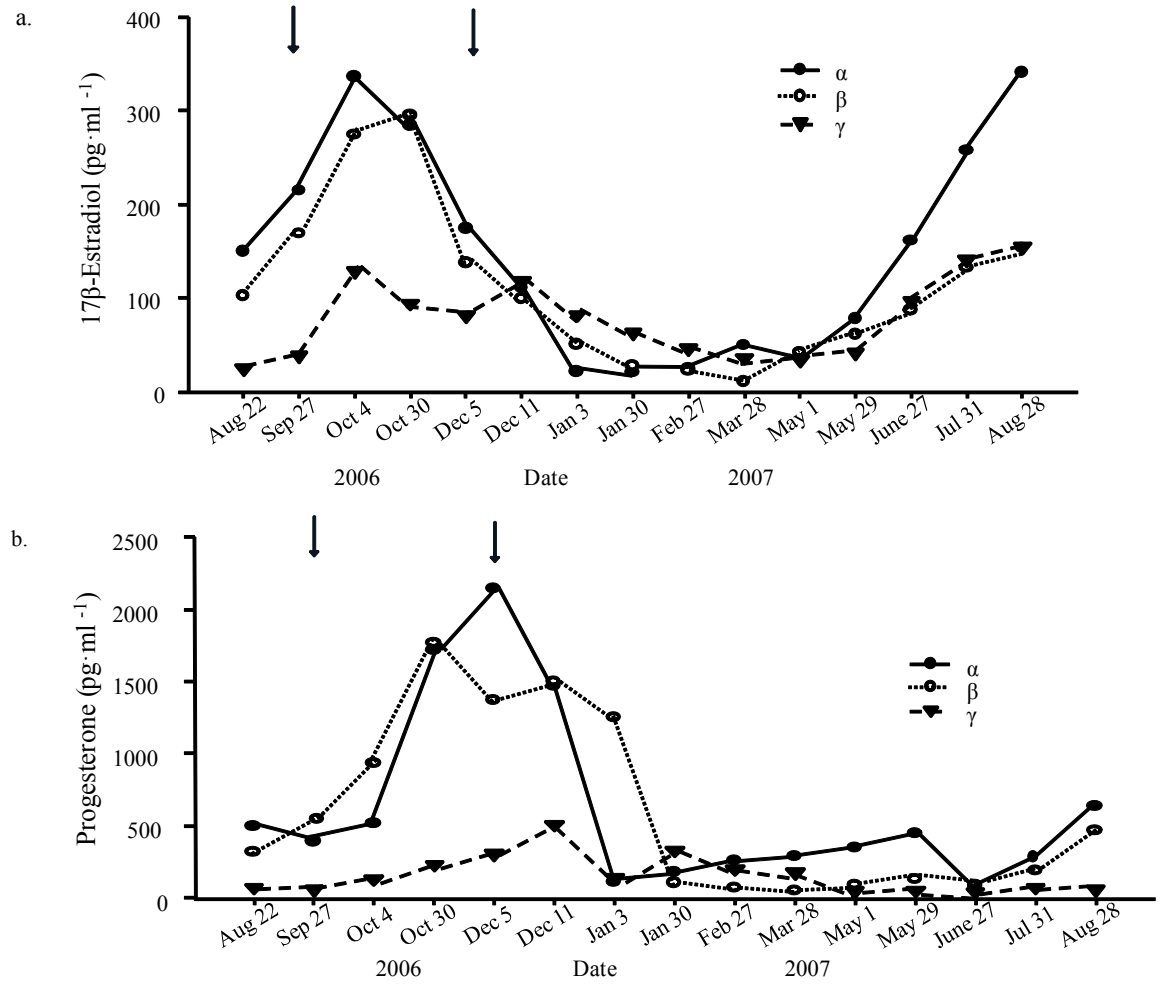

Figure 6. a. 17β-oestradiol and b. progesterone levels in pg·ml^{-1} in the individual male sand tiger sharks from August 2006 to August 2007. α, β, and γ represent hierarchal status from dominant to subordinate. Arrows indicate dates of Antide treatments.

concentrations of T, DHT, E2, and P4 were much higher in the α and β males than in the γ male (Figures 5 and 6). The trend in CS concentration in the γ male was more similar to those of the α and β males, and declined at a slower rate following the second treatment (Figure 7).

Antide levels in individual sharks reached the highest maximum concentrations on January 3, 2007 of 36,600 pg·ml^{-1}, 29,950 pg·ml^{-1}, and 62,500 pg·ml^{-1} in the α, β, and γ male, respectively (Figure 8). Serum levels of Antide declined following the January 3, 2007, peak in the α and γ males. In the β male, however, an elevation in Antide level from 250 pg·ml^{-1} to 15,750 pg·ml^{-1} was observed from March 28 to May 1, 2007.

Discussion

In the current investigation, an annual cycle in steroid concentration was observed in the three males at both the group (mean) and individual levels. This corroborates the results given in Henningsen et al. (2008), which involved the same three male sand tiger sharks during 2001 and 2002. The levels at the beginning of the current study, however, were noticeably higher in the pretreatment samples, August and September 2006, than reported for the same months in 2001–2002 (Henningsen et al. 2008). Sexual conflicts were also initiated earlier in 2006 and 2007, peaking in October and November (personal observation). In previous years, the peak occurred from February to April (Henningsen et al. 2008; Schneble 2014). It is suspected that a long-term elevation in water temperature in the early summer of 2005 shifted the reproductive cycle in the males to an earlier time. Temperature and photoperiod are major factors affecting the reproductive cycle (Demski 1990 a,b). Steroid levels reported in Henningsen et al. (2008) and in the current study support this theory. Of note, however, the sequential elevations in steroid levels in the males according to dominance hierarchy as reported by Henningsen et al. (2008) and Schneble (2014) were not obvious in the current investigation, perhaps due to a combination of the temporal shift in reproductive cycles and their manipulation by the application of Antide. Indeed, based upon the steroid levels of the individual males, the Antide treatments may have led the α and β males to be more synchronous.

Glucocorticoids are steroid hormones involved in the response to acute and chronic stressors. 1α-hydroxycorticosterone (1α-OHB) is the principal glucocorticoid in elasmobranchs, but there is no antiserum available for the assay and so most studies evaluate corticosterone (Rasmussen and Gruber 1990; Manire et al. 2007). The current investigation supports the observations by Manire et al. (2007) in male bonnethead sharks and male Atlantic stingrays of elevated CS around the time of mating and of variation in CS

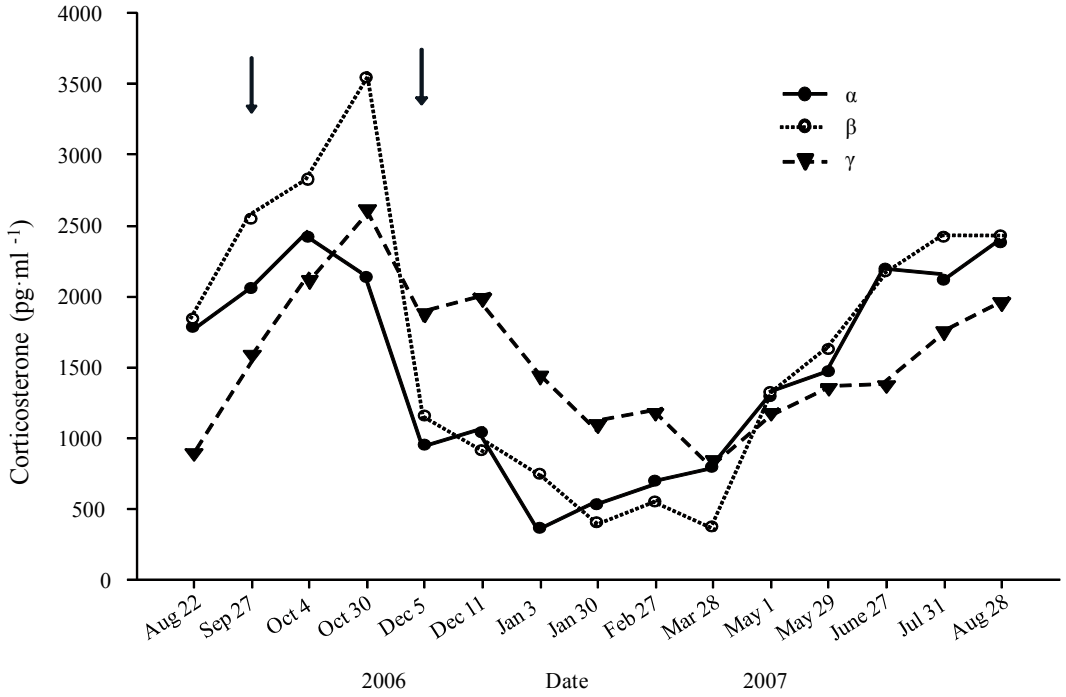

Figure 7. Corticosterone levels in pg·ml^{-1} in the individual male sand tiger sharks from August 2006 to August 2007. α, β, and γ represent hierarchal status from dominant to subordinate. Arrows indicate dates of Antide treatments

concentrations over the course of the reproductive cycle. As noted by Manire et al. (2007), small changes in CS may be reflective of larger changes in 1α-OHB. The prolonged elevations of CS in the γ male in the current investigation, despite Antide treatment, may be indicative of stress associated with being the most subordinate male.

While all three sharks in the current investigation received the same dosage of Antide and were of similar weight, noticeable differences were observed in the circulating Antide levels. Due to the variable time involved in capturing, sampling, and dosing the sharks, the time delay between subjects may have reduced Antide bioavailability in the microspheres following resuspension, as it is best applied within a one- to two-hour period following resuspension (Kight, personal observation). In the current investigation, the maximum time from suspension to treatment was 3.5 hours. The observed concentrations, however, do not fully explain the differences in Antide level due to treatment order. Of note was the observation of a substance leaking from

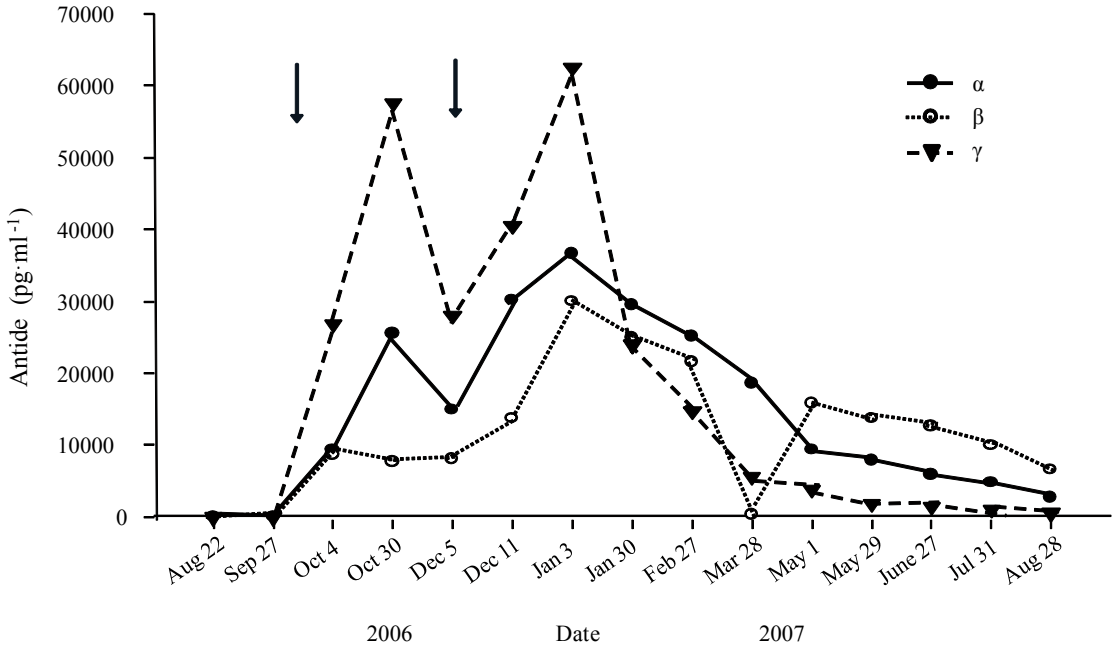

Figure 8. Antide levels in pg·ml^{-1} in the individual male sand tiger sharks from August 2006 to August 2007. α, β, and γ represent hierarchal status from dominant to subordinate. Arrows indicate dates of Antide treatments.

one of the injection sites in the γ male in early January 2007. This male also showed a 62% decline in Antide level from January 3 to January 30, 2007, samples compared to the 20% and 17% declines observed for the α and β males, respectively. This decline may not be completely attributable to a purported leak of microspheres, as slightly smaller declines were observed following the first peak on October 30, 2006, in both the α (42%) and γ (51%) males, while a 7% increase in Antide level occurred from October 30 to December 5, 2006, in the β male. Interestingly, a dramatic decrease in food intake was observed in the γ male from December through mid-January 2007, concurrent with the peak Antide level. The return to normal interest in food was temporally linked to a rapid decline in the Antide concentration. Although there were transient small-scale elevations in steroids associated with this change in the γ male, these cannot be positively associated with the decline in Antide.

This investigation provides the first documented use of a GnRH antagonist in any chondrichthyan species. In addition, it is the first investigation to show the release kinetics of a GnRH analog in a chondrichthyan. At this point, we may only speculate on the actions of the antagonist Antide on this group of male sand tiger sharks, primarily due to a lack of control subjects. Although in the current study, the sexual conflicts did not continue to the extent described previously in this group of sharks by Henningsen et al. (2008) and Schneble (2014), we could not determine the role of Antide in this difference. In addition, the mean levels of steroids, especially the androgens, declined more sharply than that observed by Henningsen et al. (2008) following Antide administration, but without a control, this cannot be attributed to the antagonist. The small number of male sand tiger sharks in the current investigation limited the use of concurrent controls, but earlier endocrine assays demonstrated cyclical patterns associated with the reproductive cycle in these captive sand tiger sharks. Comparisons of the timing of the seasonal elevations in steroids from previous results and this investigation may indicate some effect from the antagonist, but without a control this remains speculative. While this limits conclusions, this initial study has shown that the GnRH antagonist Antide can provide sustained release for about one month, and persists in circulation for at least eight months. Future investigations using larger sample sizes and control subjects may prove useful in managing reproductive cycles and sexual conflicts in captive elasmobranchs.

Acknowledgments

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References

Ahonen H., Harcourt R. G., Stow A. J. (2009) Nuclear and mitochondrial DNA reveals isolation of imperiled grey nurse shark populations, *Carcharias taurus*. *Molecular Ecology* 18: 4409–4421.

Atkinson S., Ragen T.J., Gilmartin W.G., Becker B.L., Johanos T.C.(1998) Use of a GnRH agonist to suppress testosterone in wild male Hawaiian monk seals (*Monachus schauinslandi*). *General and Comparative Endocrinology* 112: 178–182.

Carlson J.K., McCandless C.T., Cortés E., Grubbs R.D., Andrews M., MacNeil A., Musick J.A. (2009) *An Update on the Status of the Sand Tiger Shark,* Carcharias taurus*, in the Northwest Atlantic Ocean*. NOAA Technical Memorandum NMFS-SEFSC-585.

Castro J.I. (2011) *The Sharks of North America.* New York: Oxford University Press.

Chiaramonte G., Domingo A., Soto J. (2007) *Carcharias taurus* (Southwest Atlantic Subpopulation). In: 2008 IUCN Red List of Threatened Species. www.iucnredlist.org (downloaded on 14 May 2009).

Compagno L.J.V. (2001) *Sharks of the World. An Annotated and Illustrated Catalogue of Shark Species Known to Date. Volume 2. Bullhead, Mackerel and Carpet Sharks (Heterodontiformes, Lamniformes and Orectolobiformes).* FAO Species Catalogue for Fishery Purposes. No. 1, Vol. 2. Rome: FAO.

Danforth D.R., Gordon K., Leal J.A., Williams R.F., Hodgen G.D. (1990) Extended presence of antide (Nal-Lys GnRH in circulation: prolonged duration of gonadotropin inhibition may derive from antide binding to serum proteins. *Journal of Clinical Endocrinology and Metabolism* 70: 554–556.

Danforth D.R., Williams R.F., Gordon K., Leal J.A., Hodgen G.D. (1991) Inhibition of pituitary gonadotropin secretion by the gonadotropin-releasing hormone antagonist Antide. II. Development of an *in vitro* bioassay for characterization of pharmacokinetics and pharmacodynamics of Antide in circulation. *Endocrinology* 128: 2041–2044.

Demski L.S. (1990a) Neuroendocrine mechanism controlling sexual development and behavior of sharks and rays. *Journal of Aquarium and Aquatic Science* 5: 53–67.

Demski L.S. (1990b) Elasmobranch reproductive biology: implications for captive breeding. *Journal of Aquarium and Aquatic Science* 5: 84–95.

Desjardins J.K., Stiver K.A., Fitzpatrick J.L., Balshine S. (2008a) Differential responses to territory intrusions in a cooperatively breeding fish. *Animal Behaviour* 75: 595–604.

Desjardins J.K., Stiver K.A., Fitzpatrick J.L., Milligan N., Van Der Kraak G.J., Balshine S. (2008b) Sex and status in a cooperative breeding fish: behavior and androgens. *Behavioral Ecology and Sociobiology* 68: 785–794.

Ebert D.A., Fowler S., Compagno L. (2013) *Sharks of the World*. Plymouth: Wild Nature Press.

Engel K.B., Callard G.V, (2005) The testis and spermatogenesis. In: Hamlett W.C. (ed.). *Reproductive Biology and Phylogeny of Chondrichthyes: Sharks, Batoids, and Chimaeras.* Enfield, NH: Science Publishers, Inc., 171–200.

Felberbaum R.E., Ludwig M., Diedrich K. (2000) Clinical applications of GnRH-antagonists. *Molecular and Cellular Endocrinology* 166: 9–14.

Forlano P.M., Maruska K.P., Sower S.A., King J.A., Tricas T.C. (2000) Differential distribution of gonadotropin-releasing hormone immunoreactive neurons in the stingray brain: functional and evolutionary considerations. *General and Comparative Endocrinology* 118: 226–248.

Garnier D.H., Sourdaine P., Jegou B. (1999) Seasonal variations in sex steroids and male sexual characteristics in *Sclyliorhinus canicula*. *General and Comparative Endocrinology* 116: 281–290.

Gelsleichter J. (2004) Hormonal regulation of elasmobranch physiology. In: Carrier J.C., Musick J.A., Heithaus M.R. (eds). *Biology of Sharks and Their Relatives*. Boca Raton, FL: CRC Press, 287–323.

Gordon I. (1993) Pre-copulatory behavior of captive sand tiger sharks, *Carcharias taurus*. *Environmental Biology of Fishes* 38: 159–164.

Hazon N., Balmont R.J. (1998) Endocrinology. In: Evans D.E. (ed.). *The Physiology of Fishes*, 2nd edn Boca Raton, FL: CRC Press, 441–464..

Henningsen A.D., Smale M., Garner R., Gordon I., Marin-Osorno R., Kinnunen N. (2004) Captive breeding and sexual conflict. In: Smith M., Warmolts D., Thoney D., Hueter R. (eds). *Elasmobranch Husbandry Manual*. Columbus, Ohio: Ohio Biological Survey, 239–250.

Henningsen A.D., Murru F.L., Rasmussen L.E.L., Whitaker B.R., Violetta G.C. (2008) Serum levels of reproductive steroid hormones in captive sand tiger sharks, *Carcharias taurus* (Rafinesque), and comments on their relation to sexual conflicts. *Fish Physiology and Biochemistry* 34: 437–446.

Hiurne J.A.F., van Loenen A.C.D., Schats R., McDonnell J., Hompes P.G.A., Schoemaker J., Homburg R., Lambalk C.B. (2004) Dose finding study of daily gonadotropin-releasing hormone (GnRH) antagonist for the prevention of premature luteinizing hormone surges in IVF/ICSI patients: antide and hormone levels. *Human Reproduction* 19: 2206–2215.

Holland M.C.H., Hassin S., Zohar Y. (1998) Effects of long-term testosterone,

gonadotropin-releasing hormone agonist, and pimozide treatments on gonadotropin II levels and ovarian development in juvenile female striped bass (*Morone saxatilis*). *Biology of Reproduction* 59: 1153–1162.

Idler D.R., Truscott B. (1966) 1α-hydroxycorticosterone from cartilaginous fish: a new adrenal steroid in blood. *Journal of the Fisheries Research Board of Canada* 23: 615–619.

Idler D.R., Truscott B. (1967) 1α-hydroxycorticosterone: synthesis in vitro and properties of an interrenal steroid in the blood of cartilaginous fish (Genus *Raja*). *Steroids* 9: 457–477.

Leal J.A., Gordon K., Danforth D.R., Williams R.F., Hodgen G.D. (1991) Antide: a "third-generation" GnRH antagonist with minimal histamine release. *Drugs of the Future* 16: 529–537.

Lutton B.V., St. George J., Murrin C.R., Fileti L.A., Callard I.P. (2005) The elasmobranch ovary. In: Hamlett W.C. (ed.). *Reproductive Biology and Phylogeny of Chondrichthyes: Sharks, Batoids, and Chimaeras*. Enfield, NH: Science Publishers, Inc., 237–281.

Manire C.A., Rasmussen L.E.L., Hess D.L., Hueter R.E. (1995) Serum steroid hormones and the reproductive cycle of the female bonnethead shark, *Sphyrna tiburo*. *General and Comparative Endocrinology* 97: 366–376.

Manire C.A., Rasmussen L.E.L., Gross T.S. (1999) Serum steroid hormones including 11-ketotestosterone, 11-ketoandrostenedione and dihydroprogesterone in juvenile and adult bonnethead sharks, *Sphyrna tiburo*. *Journal of Experimental Zoology* 284: 595–603.

Manire C.A., Rasmussen L.E.L., Maruska K.P., Tricas T.C. (2007) Sex, seasonal, and stress-related variations in elasmobranch corticosterone concentrations. *Comparative Biochemistry and Physiology Part A* 148: 926–935.

Nelson R.J. (ed.) (2000) *An Introduction to Behavioral Endocrinology.* Sunderland: Sinauer.

Pollard D., Smith A. (2000) *Carcharias taurus*. In: IUCN 2008. 2008 IUCN Red List of Threatened Species. www.iucnredlist.org (downloaded on 14 May 2009).

Pollard D., Gordon I., Williams S., Flaherty A., McAuley R. (2003) *Carcharias taurus* (East coast of Australia subpopulation). In: IUCN 2008. 2008 IUCN Red List of Threatened Species. . www.iucnredlist.org (downloaded on 14 May 2009)..

Pollard D., Smith A. (2005) Species status reports. In: Fowler S.L., Cavanagh R.D., Camhi M., Burgess G.H., Cailliet G.M., Fordham S.V., Simpfendorfer C.A., Musick J.A. (eds.). *Sharks, Rays, and Chimaeras: The Status of Chondrichthyan Fishes. Status Survey.* Gland, Switzerland and Cambridge, U.K.: IUCN/SSC Shark Specialist Group, 245–248.

Querat B., Tonnerre-Doncarli C., Genies F., Salmon C. (2001) Duality of gonadotropin in gnathostomes. *General and Comparative Endocrinology* 124: 308–314.

Rasmussen L.E.L., Gruber S.H. (1990) Serum levels of circulating steroid hormones in free-ranging carcharhinoid sharks. In: Pratt H.L. Jr., Gruber S.H., Taniuchi T. (eds). *Elasmobranchs as Living Resources: Advances in the Biology, Ecology, Systematics, and the Status of the Fisheries*. NOAA Technical Report NMFS 90. U.S. Department of Commerce, 143–155.

Rasmussen L.E.L., Murru F.L. (1992) Long-term studies of serum concentrations of reproductively related steroid hormones in individual captive carcharhinids. *Australian Journal of Marine and Freshwater Research* 43: 273–281.

Sabalones J. (1995) Considerations on the husbandry of sharks for display purposes. *International Zoo Yearbook* 34: 77–87.

Schneble E.A. (2014) *A Review of Reproductive Behavior of the Sand Tiger Shark (*Carcharias taurus*) with Special Emphasis on Captive Sharks at the National Aquarium Baltimore.* M.S. thesis. Dania Beach, FL.: Oceanographic Center, Nova Southeastern University.

Sherwood N.M., Lovejoy D.A. (1993) Gonadotropin-releasing hormone in cartilaginous fishes: structure, location, and transport. *Environmental Biology of Fishes* 38: 197–208.

Skomal G., Bernal D. (2010) Physiological response to stress in sharks. In: Carrier J.C., Musick J.A., Heithaus M.R. (eds). *Sharks and Their Relatives II: Biodiversity, Adaptive Physiology, and Conservation.* Boca Raton, FL: CRC Press, Taylor and Francis Group, LLC, 459–490

Stoskopf M.K. (1993) Shark pharmacology and toxicology. In: Stoskopf M.K. (ed.). *Fish Medicine.* Philadelphia: W.B. Saunders Inc., 809–816.

Stow A., Zenger K., Briscoe D., Gillings M., Peddemors V., Otway N., Harcourt R. (2006) Isolation and genetic diversity of endangered grey nurse shark (*Carcharias taurus*) populations. *Biology Letters* 2: 308–311.

Taves M.D., Desjardins J.K., Mishra S., Balshine S. (2008) Androgens and dominance: sex specific patterns in a highly social fish (*Neolamprologus pulcher*), *General and Comparative Endocrinology* 161: 202–207.

Truscott B., Idler D.R. (1968) The widespread occurrence of a corticosteroid 1α-hydroxylase in the interrenals of Elasmobranchii. *Journal of Endocrinology* 40: 515–526.

Wallen K. (2001) Sex and context: hormones and primate sexual motivation. *Hormones and Behavior* 40: 339–357.

Wright D.E., Demski L.S. (1993) Gonadotropin-releasing hormone (GnRH) pathways and reproductive control in elasmobranchs. *Environmental Biology of Fishes* 38: 209–218.