Hormones and migration in tiger sharks *(Galeocerdo cuvier)*: can they be related?

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Abstract
Behaviors associated with reproduction, territorial defense and hierarchical position within a population are strongly shaped by the endocrine system and may influence breeding, feeding, and migratory patterns. This study examined the link between endocrine reproductive parameters [plasma Testosterone (T), Progesterone (P_4) and 17β-estradiol (E_2)], total cholesterol and movement behavior of three juvenile males of similar size tagged with a satellite transmitter off Fernando de Noronha Archipelago (FEN), Brazil. The results suggested a possible link between the parameters and the movement pattern observed. This preliminary analysis indicates that hormonal profile might thus be, at least partially, related to the habitat shift observed, being consistent to the regulating role of the endocrine system as an evolutionary adaptation molded to e.g. avoid interspecific competition or to optimize foraging efficiency in species which move through wide-ranging home-ranges such as the tiger shark.

Zusammenfassung
Die Verhaltensweisen, die mit Fortpflanzung, Revierverteidigung und der Rangstellung in einer Population verbunden sind, werden stark vom Endokrinsystem bestimmt und können die Brut-, Nahrungs- und Wanderverhaltensweisen beeinflussen. Die vorliegende Arbeit untersucht die Verknüpfung zwischen endokrinen Reproduktions-Parametern [Plasma-Testosteron (T), Progesteron (P_4) und 17β-Estradiol (E_2)], Gesamt-Cholesterol und Bewegungsverhalten von drei jungen Männchen ähnlicher Größe, die mit einem Satelliten-Sender vor dem Fernando-de-Noronha-Archipel (FEN), Brasilien, markiert worden waren. Die Ergebnisse sprachen für einen wahrscheinlichen Zusammenhang zwischen den Parametern und dem beobachteten Bewegungsmuster. Die vorläufige Analyse spricht dafür, dass das Hormonprofil zumindest teilweise mit dem beobachteten Habitatwechsel verbunden ist; dies wiederum ist vereinbar mit der regulierenden Rolle des Endokrinsystems als eine evolutionäre Anpassung, die geschaffen wurde, um bei weit umherstreifenden Arten wie dem Tigerhai interspezifischen Wettbewerb zu vermeiden oder die Wirksamkeit des Nahrungssuch-Verhaltens zu steigern.

Sommario
I comportamenti associati alla riproduzione, alla difesa territoriale e alla posizione gerarchica all'interno di una popolazione sono fortemente influenzati dal sistema endocrino e possono condizionare la riproduzione, l'alimentazione e gli schemi migratori. Questo studio ha esaminato il legame tra parametri riproduttivi endocrini [livelli plasmatici di testosterone (T), progesterone (P_4) e 17β-estradiolo (E_2)], colesterolo totale e comportamento degli spostamenti di tre giovani maschi di dimensioni simili dell'Arcipelago Fernando de Noronha (FEN), Brasile, etichettati con un trasmettitore satellitare. I risultati hanno suggerito un possibile collegamento tra i parametri e il modello di movimento osservato. Questa analisi preliminare indica che il profilo ormonale potrebbe quindi essere,

almeno in parte, correlato allo spostamento dell'habitat osservato, essendo coerente con il ruolo regolatore del sistema endocrino come adattamento evolutivo modellato per ad es. evitare la concorrenza interspecifica o ottimizzare l'efficienza del foraggiamento nelle specie che si muovono in ampi areali domestici come lo squalo tigre.

INTRODUCTION

Migration is defined as the movement of organisms between two or more distinct geographical locations, a behavior that may be driven by seasonal/predictable changes (e.g., resource flutuation), unpredictable changes (e.g., weather conditions) and the impact of social interactions (e.g., food and sexual competition). All variations cited above are able to increase or reduce the individual motivation to move (Jachowski & Singh 2015). For several years, researchers described movement patterns as prompted by a combination of extrinsic factors (Martin et al. 2013) but the internal state of the animal has seldom been taken into account (Biuw et al. 2007). Currently, the imperative role of physiological paths in the migratory behavior is well recognized, being now considered a central component on ecological and conservation studies (Lennox et al. 2016).

In vertebrates, reproductive steroid hormones play a mechanistic role in social behaviors and spatial ecology (Lynn 2008). These hormones regulate all aspects of reproduction and modulate general behaviors such as aggression, territorial defense and hierarchical positioning (Maruska & Gelsleichter 2011). The study of movement and behavior of large marine predators has always been challenging because of the high mobility of these species and the logistic constraints imposed by the marine environment (Hammerschlag et al. 2011). Recently, the development of satellite-tracking technology has been helping scientists to increase the knowledge of habitat use and movements of marine predators (Hammerschlag et al. 2011) however, the physiological paths that modulate movement behavioral responses are still poorly understood.

The tiger shark, *Galeocerdo cuvier* (Péron and Lesueur, 1822), has a cosmopolitan distribution in tropical and warm temperate waters, being found in both coastal and oceanic waters. The species is known by its migratory behavior, exhibiting seasonal variability in habitat use manly related to prey availability (Heithaus et al. 2007) and reproductive purposes (e.g., give birth) (Papastamatiou et al. 2013) performing that way, an important role in trophic links between diverse habitats. According to Randall (1992), the size at maturity in males is 226-290 cm (TL) and in females 250-350 cm (TL). Here we studied the possible influence of physiological parameters in decision-making based on habitat use and distribution shifts, through plasma T, P_4, E_2 and total cholesterol essays in male juvenile tiger sharks.

MATERIAL AND METHODS

Three male juvenile tiger sharks (S1, S2, and S3) were caught in Fernando de Noronha, Pernambuco, Brazil (3°51'13.71" S, 32°25'25.63" W) in July 2014. Maturity was determined by the presence, size and degree of calcification of claspers (Meyer et al. 2009). The total length (TL) of sharks were 182 cm, 251 cm, 250 cm, respectively. Capture was performed individually from a small vessel using baited handlines. The sharks were fitted with a satellite tag (SPOT; Wildlife computers, USA) in the first dorsal fin and a blood sample was taken. The project was approved by Brazilian Ministry of Environment (permit number 15083-8) and by the Ethics Committee on Animal Use (CEUA) 23082.003679/2009 – UFRPE.

After blood withdrawal, the sample was centrifuged for 7 minutes at room temperature (20°C). Plasma was separated and kept frozen at -20°C until analysis. The gonadal steroids testosterone (T), 17β-estradiol and progesterone (P_4) were measured in duplicate in blood plasma using ELISA commercial kits (Cayman Chemical Company, MI, USA for T and E_2 and IBL International for P_4). An ether extraction protocol was used to separate protein and lipid fractions. Plasma proteins were precipitated with ethyl ether at a 1:3 ratio. After centrifugation, the supernatant was transferred and dried with nitrogen. The absorbance measurements were then read in plates with a Spectra Max 250 spectrophotometer (Molecular Devices), and steroid concentrations were calculated by comparing the optic density (405 nm for T and E_2 and 450 nm for P_4) of the samples with the specific standard curve. The quantification of total cholesterol was performed using the automatic biochemical analyzer for clinical analysis (LABMAX 240, version 2.0) with commercial kits (Labtest ®).

RESULTS

The telemetry results showed two different movement patterns in animals of the same sex and sim-

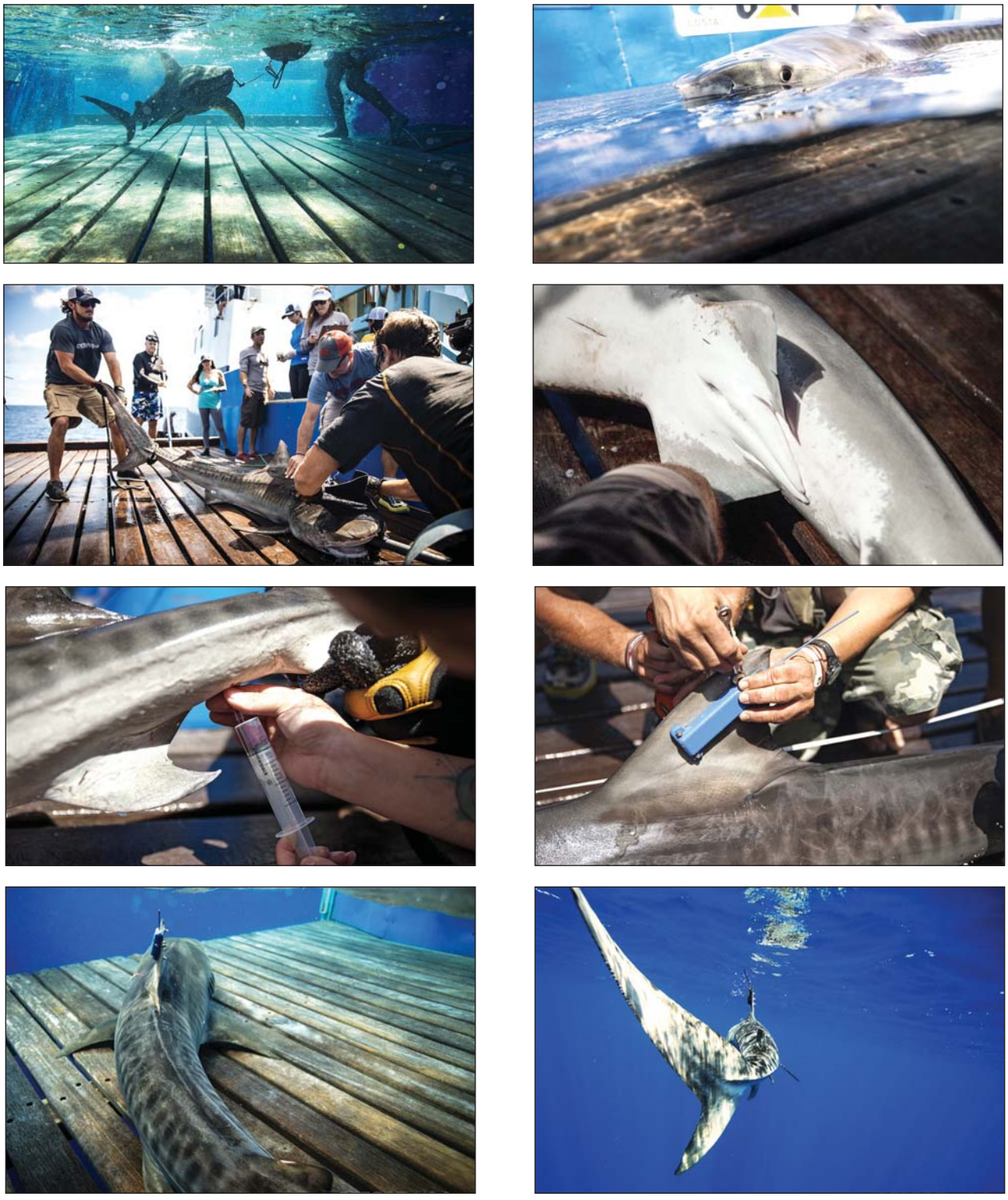

Fig. 1. Handling and sampling steps of the juvenile tiger sharks. From right to left, top to bottom: Tiger shark soon after capture, being lead to the sampling platform; male juvenile tiger shark being removed from water for sampling; tiger shark being restrained for sampling; sex and sexual maturity determination; blood extraction for hormonal and metabolic analyses; satellite tagging (SPOT tag) of the male juvenile tiger shark; male juvenile tiger shark recovering after procedures; secure release of the tiger shark, after fully recovering, into its natural habitat. Photos by R. Snow, OCEARCH.

ilar size/life stage captured in the same area. While one male stayed in the Fernando de Noronha archipelago during the whole span of the track (120 days – S3), two males moved away through great distances towards the northern hemisphere and the African continent during the whole span of the track (90 days – S1) (270 days – S2) (Figure 1). Both males who moved away from the tagging area towards the northern hemisphere of the African continent showed lower values of T (S1: 641.96 pg/ml and S2: 773.48 pg/ml) (Figure 2a) when compared to the resident shark (S3: 1556.28 pg/ml). P_4 (S1: 2.11 pg/ml, S2: 1.9 pg/ml and S3: 3.8 pg/ml) was also lower in the animals that migrated when compared to the resident shark. Plasma concentration of E_2 was similar among sharks (S1: 51,6 pg/ml, S2: 53,0 pg/ml and S3: 53,9 pg/ml) (Figure 2a). Total cholesterol was higher in the shark that did the longest migration (Fernando de Noronha, Northeast Brazil to West Africa) (S2: 76 mg/dL^{-1}) (Figure 2b). The animal that remained in Fernando de Noronha showed intermediate levels (S3: 55 mg/dL^{-1}) of total cholesterol, while the male that performed the shortest migration exhibited the lowest concentration (S1: 32 mg/dL^{-1}) (Figure 2b).

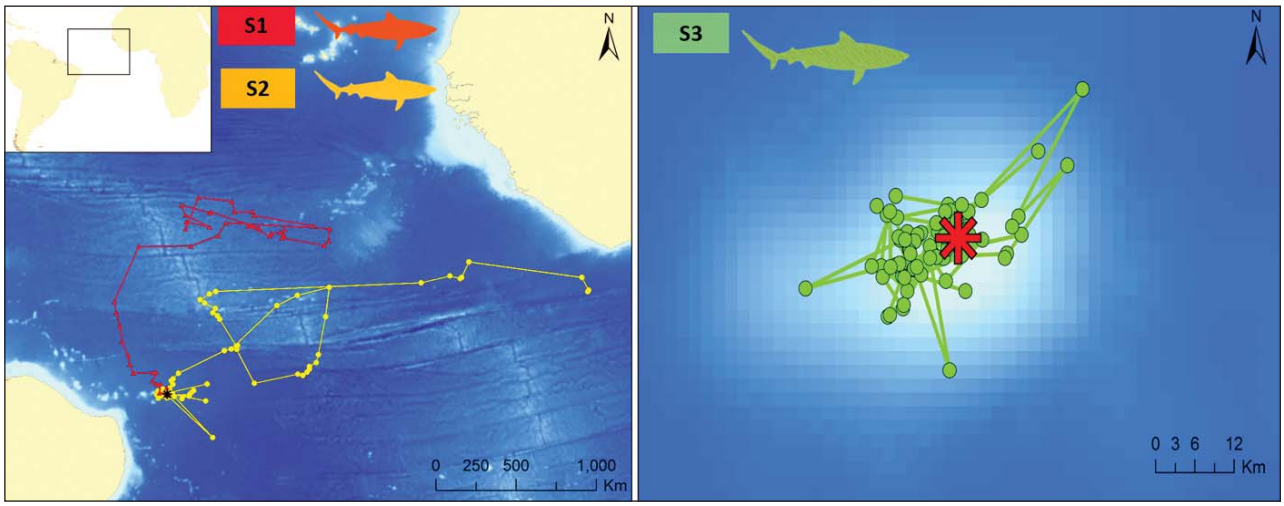

Fig. 2. Movement patterns in juvenille tiger sharks. Tracking data from juvenile male tiger sharks caught and tagged in Fernando de Noronha (FEN).

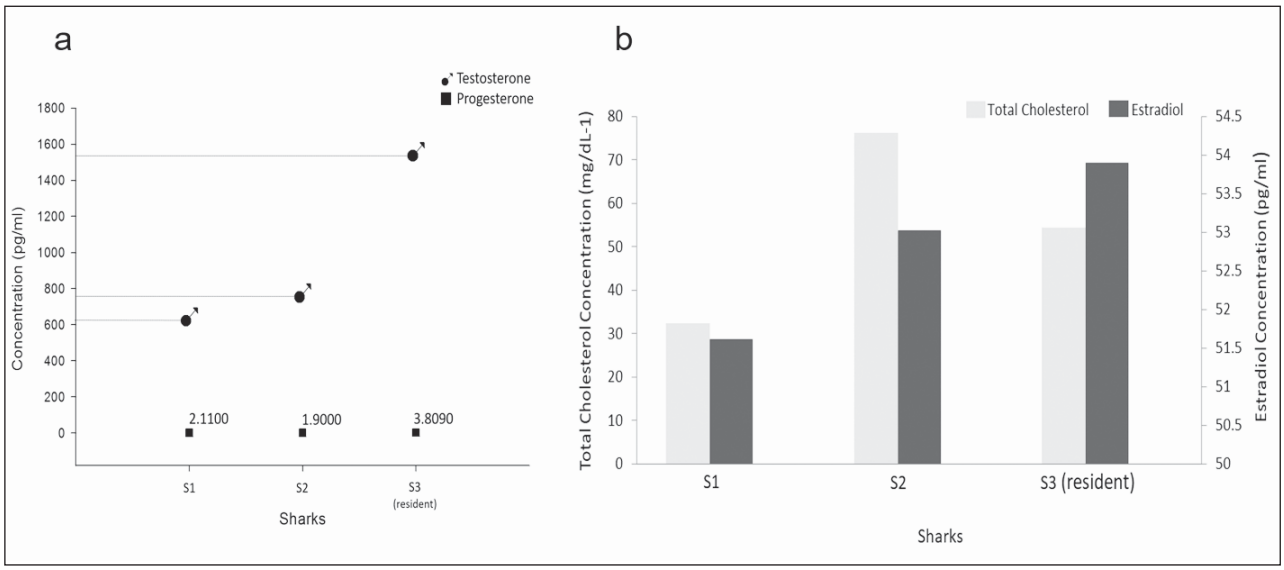

Fig. 3. Endocrine profile in juvenile tiger sharks. (**a**) Plasma concentrations (pg/ml) of Testosterone (T) and Progesterone (P_4); (**b**) Plasma concentrations (mg.dL^{-1} and pg/ml) of Total Cholesterol and 17β-estradiol (E_2) of the sampled animals.

DISCUSSION

Based on several telemetry studies performed with *G. cuvier* worldwide, it seems that the species exhibit two main movement patterns: (1) some animals moving surprisingly far, and (2) some traveling minimal distances or staying around the same area, for long periods (Wirsing et al. 2006; Heithaus et al. 2007; Meyer et al. 2009; Papastamatiou et al. 2013). Some recent studies have demonstrated that both patterns appear to be linked to environmental factors (Heithaus et al. 2007; Papastamatiou et al. 2013), sex (Papastamatiou et al. 2013) and life stages (Werry et al. 2014; Lea et al. 2015). However, the physiological factors that regulate behavioral shifts in habitat use remain unclear, being essential taking into account intrinsic factors (e.g. hormonal patterns), which are still poorly investigated in sharks (Papastamatiou et al. 2013).

The main androgen in elasmobranchs is testosterone (T). In males, this hormone plays a role in the development and maturation of spermatocysts (Awruch et al. 2008) and in the regulation of the final stage of sperm maturation (Hoffmayer et al. 2009). In the present study, T levels differed between the sharks that moved away from the tagging area and the shark that remained in Fernando de Noronha. These results suggest that, as observed in other vertebrates, while higher testosterone levels appear to be related to a resident behavior, lower levels may be linked to the decision to move/migrate. Recent studies have shown that the competitive behavior regulated by T is not exclusive to adults, since newborns and juveniles demonstrate the same pattern (Anestis 2006). That way, it is possible that the decision to migrate taken by the two juvenile sharks has been at least partially influenced by a competition for resource in the area that may be related to the higher T levels exhibited by the resident juvenile male, as observed in juvenile birds (Boncoraglio and Groothuis 2013).

Plasma P_4 levels in male carps *(Cyprinus carpio)* vary among reproductive stages and it is correlate to the testicular production of T (Chishti et al. 2013). Our results corroborate, at least partially, the pattern described for teleost fish. The resident male showed higher levels of P_4 when compared to the animals that migrated, a physiological trait that could be linked to the higher levels of T also described here for the animal that stayed in the archipelago. Thus, it is possible that the increase in circulating P_4 may be closely related to the increase in T production observed. E_2 appears to be similar among sharks, an expected result since estradiol plays a secondary role in males, with higher levels commonly associated with feminization in fish (Gross-Sorokin et al. 2006).

Regarding the total cholesterol levels exhibited by the sharks in the present study, no clear pattern was observed. While the shark that performed the longest migration showed the highest levels, the other shark that migrate exhibited the lowest levels, even when compared to the resident shark. Studies on salmon migration showed a close relationship between total cholesterol and movement patterns, with a significant increase in total cholesterol levels during migration, being more elevated in the first portion (250 miles), when compared to the last portion (last 450 miles) of the path (Idler & Bitners 1958). So, it is possible that the total cholesterol play a role in shark migration as observed in salmons, however, more studies are needed in order to validate this metabolite as a physiological tool in movement studies.

CONCLUSION

Despite the small sample size and inability to statistically compare the results among sharks, the present report brings preliminary data based in a new perspective taking into account intrinsic factors that may be, at least partially, related to movement behavior in the species. The link between the endocrin system and behaviors is well stablished for several vertebrates, however, due the challenges related to shark research, physiological traits are rarely taken into account. That way, based in the differences observed here, we recommend that researchers that have access and financial resources to tag an greater number of animals take this theory into consideration, thus allowing a better understanding of this link in the group.

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