A Comparison of Reproductive and Energetic States in a Marine Apex Predator (the Tiger Shark, *Galeocerdo cuvier*)

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ABSTRACT

To fuel the high energetic demands of reproduction, vertebrates employ different tactics of resource use. Large sharks exhibit long gestation periods and have relatively few well-developed young, which likely incurs high energetic costs. However, information on the relationship between the reproductive and energetic states for most shark species is lacking. In the present study, we used a noninvasive approach to assess relationships among reproductive stage, plasma triglyceride levels, body condition, and circulating reproductive hormones in free-ranging female tiger sharks (*Galeocerdo cuvier*). A total of 57 sharks were sampled (19 immature, 15 mature/nongravid, and 23 gravid). Circulating plasma triglycerides did not significantly differ among female tiger sharks of different reproductive stages, but body condition values were significantly higher for mature/nongravid sharks (1.29 ± 0.23) compared with gravid (1.15 ± 0.08) and immature (1.13 ± 0.07) sharks. For gravid and mature/nongravid sharks, no significant correlations existed among values of reproductive hormones, plasma triglycerides, and body condition. However, for immature sharks, estradiol values were negatively correlated with body condition values. Additionally, progesterone levels were positively correlated with testosterone levels in immature females. Our results suggest that this large generalist predator may not necessarily be easily characterized as a pure capital breeder, as has been previously hypothesized for ectotherms, but may rely on a mix of energy stores and opportunistic feeding to support reproduction. We present several hypotheses to explain these patterns and discuss our results in terms of energetic reproductive strategies.

Introduction

Reproductive decisions by free-ranging animals are typically influenced by energetic costs associated with physiological maintenance, growth, and survival (Zera and Harshman 2001). Balancing these costs can determine the optimal values for life-history traits, such as age at maturity and reproductive output (Werner and Anholt 1993; Ricklefs and Wikelsky 2002; Gagliano et al. 2007). To fuel the high energetic demands of reproduction, animals employ different life-history strategies and tactics of resource use.

Capital breeders secure food resources in advance of breeding and store them endogenously until they are needed to fuel offspring production (Jönsson 1997; Bonnet et al. 1998; Houston et al. 2007). In contrast, income breeders feed during breeding to concurrently provision their developing young and adjust their exogenous intake accordingly, without reliance on energy stores to nourish developing offspring (Jönsson 1997; Bonnet et al. 1998; Lewis and Kappeler 2005; Houston et al. 2007). Capital breeding is believed to be the predominant strategy among ectothermic species due to the relatively low energetic costs associated with storage, maintenance, and utilization of body reserves (Bonnet et al. 1998). In contrast, income breeding is believed to be more common among endotherms due to the high costs of thermoregulation (Bonnet et al. 1998). However, food availability can also influence whether animals exhibit capital breeding, income breeding, or a mix among the two (Houston et al. 2007; Stephens et al. 2014). While these energetic and breeding strategies have been investigated in a variety of taxa, studies of this kind involving sharks have been relatively few. For the most part, it remains relatively unknown whether and how individual variation in energetic states may influence reproductive states in sharks despite well-documented relationships in a variety of other species, such as birds, reptiles, insects, and land and marine mammals as well as

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freshwater and marine teleosts (e.g., Lambert and Dutil 2000; Jessop et al. 2002; Krockenberger 2003; Elkin and Reid 2005; French et al. 2007; Roscoe et al. 2010). Determining such resource-linked breeding tactics is important for understanding the ecology and fitness potential of sharks as well as how they may respond to natural or human-induced stressors (e.g., climate change).

Large sharks are known to undergo extensive ocean-wide migrations over thousands of kilometers that are energetically costly. For example, satellite-tagged white sharks (*Carcharadon carcharias*) that undertake extensive migrations (4,000 km) in the eastern Pacific Ocean exhibit consistent increases in vertical drift rate, speculated to be due to a decrease in buoyancy caused by depleted lipid reserves during migration (Del Raye et al. 2013). Large sharks also exhibit long gestation periods, have relatively few well-developed young, and experience long periods between reproductive events. This life history is likely tied to the high energetic requirements to fuel their developing embryos, which are born requiring no postnatal parental care (Koob and Callard 1999; Lucifora et al. 2002). The liver is the largest organ in most shark species and plays a large role in lipid metabolism during migrations and throughout gestation (Ballantyne 1997). In many animals, including sharks, large quantities of lipids are stored in the liver before reproduction for subsequent use during vitellogenesis and nutrition of developing young during gestation (Glazier 2000). For example, Lucifora et al. (2002) found that gravid sand tiger sharks (*Carcharias taurus*) in the southwest Atlantic Ocean are capital breeders, depleting their liver lipid reserves to fuel both metabolism and embryonic development/nourishment during gestation, during which they do not feed (Lucifora et al. 2002). However, comparable information on the relationship between the reproductive and energetic states for many shark species is lacking.

New methods to assess reproduction through plasma hormones and/or ultrasonography (Jirik and Lowe 2012; Sulikowski et al. 2016), combined with investigations of energy metabolism in sharks inferred by plasma lipid samples (e.g., Speers-Roesch and Treberg 2010; Valls et al. 2016; Gallagher et al. 2017), can be integrated to provide a nonlethal means to assess the relationship between the reproductive and energetic states in free-ranging sharks. However, to our knowledge there are no published studies using nonlethal techniques investigating lipid reserves in large sharks and how they differ with reproductive status, despite its importance for understanding reproductive timing and success. Understanding the physiological processes driving reproductive fitness in large sharks is of particular conservation significance given the widespread overfishing of sharks (Worm et al. 2013).

The tiger shark (*Galeocerdo cuvier*) is an apex marine predator in tropical and temperate seas (Compagno 2001). The species is a dietary and behavioral generalist, consuming a wide variety of prey and also exhibiting both inshore and pelagic movements (Meyer et al. 2009; Hammerschlag et al. 2012; Papastamatiou et al. 2013). The tiger shark exhibits aplacental viviparous (ovoviviparous) reproduction with long gestation periods (up to 16 mo), and it produces large broods of 18–70 pups that are all born at relatively large sizes (~75 cm; Whitney and Crow 2007; Castro 2009, 2011). Tiger sharks are matrotrophic, and embryos can

reach gains of 2,119% in wet weight and 1,092% in dry weight during gestation due to nourishment from both a yolk and energy-rich uterine fluid in the egg case (Castro et al. 2016). Since tiger sharks are large ectotherms, females may exhibit a capital breeding strategy, as hypothesized for ectothermic species (Bonnet et al. 1998).

In the present study, we integrated noninvasive physiological, morphological, and reproductive sampling of female tiger sharks to address three primary questions. First, do levels of triglycerides, a primary lipid used in energy metabolism, differ among female tiger sharks of differing reproductive stages? Second, does shark girth, often a proxy for condition, differ among female tiger sharks of differing reproductive stages? Third, within each reproductive stage, are levels of sex steroid hormones (progesterone, testosterone, and estradiol) related to triglyceride concentrations and/or body condition? Research was conducted at a high-use site for female tiger sharks within the subtropical Atlantic Ocean, nicknamed "Tiger Beach" (Hammerschlag et al. 2012, 2017). Occurring on the northwestern edge of Little Bahama Bank, Bahamas, Tiger Beach consists predominantly of females of mixed age classes, including immature, mature, and gravid individuals (Sulikowski et al. 2016). Sulikowski et al. (2016) suggested that for female tiger sharks, Tiger Beach may serve as both a refuge from male mating harassment and a gestation ground for gravid individuals.

We note up front that although our study is intended to investigate potential relationships among the reproductive state, hormone levels, body condition and triglyceride levels in wild sharks, due to the opportunistic sampling associated with data collection we cannot definitively ascribe any patterns found to specific mechanisms. However, our study approach and results provide a foundation for future investigation of such mechanisms. Accordingly, we present several hypotheses relating to the physiology/life-history nexus (Ricklefs and Wikelsky 2002) and possible foraging-linked reproductive strategies in tiger sharks.

Methods

Capture of Study Animals

This study focused on tiger sharks at Tiger Beach, Bahamas (26.86°N, 79.04°W). Sampling of different sharks occurred on weeklong expeditions to Tiger Beach during December 2011, July 2012, October 2013, May 2014, and November 2014. Sharks were captured using standardized circle-hook drumlines, a passive and minimally invasive fishing technique, following Gallagher et al. (2014). In brief, drumlines were deployed to soak for 1 h before being checked for shark presence. On capture, tiger sharks were brought to the stern of the boat and secured by hand to a partially submerged platform. A saltwater hose was inserted into the shark's mouth to actively pump water over the shark's gills while temporarily immobilized. While sharks were secured, blood samples were obtained and morphological measurements taken (cm); sharks were then tagged for identification and released. This procedure has been approved by the Floridian and Bahamian research permit agencies as well as the University of Miami's Animal Welfare and Care Committee (IACUC protocol 15-238).

Blood Collection

Approximately 20 mL of mixed venous blood was extracted through the caudal vein using chilled 18-gauge needles and 10-mL syringes. A subsample was centrifuged at 1,300 g for 5 min to separate plasma from whole blood. Plasma was then removed and stored frozen at −20°C for future hormone and metabolite analyses.

Triglyceride Assays

Plasma triglycerides were assayed using the standard enzyme-linked immunosorbent assay kit (EnzyChrom Triglyceride Assay Kit, ETGA-200; BioAssay Systems, Haywood, CA; Liss et al. 2013), which has been used previously in tiger sharks (Gallagher et al. 2017). Samples were run in duplicate with intra- and interassay coefficients of <20%. Triglyceride data for a subset of these tiger sharks were published in Gallagher et al. (2014).

Hormone Analysis

Steroid hormone concentrations were determined following a modified radioimmunoassay procedure from Sulikowski et al. (2004). Nonradiolabeled P4, T, and E2 were obtained from Steraloids (Newport, RI). The specifics of the nonradiolabeled stock concentrations, radiolabeled steroids, antibody characteristics, and titers can be found in Sulikowski et al. (2004). A Tri-Carb 2900TR liquid scintillation analyzer (PerkinElmer, Waltham, MA) was used to determine radioactivity. The mean intra-assay coefficients of variation for P4, T, and E2 were 11%, 10%, and 6%, respectively, and the interassay coefficients of variation were 13%, 10%, and 10%, respectively. When hormone values for individual samples fell below detectable limits, those samples were concentrated and reanalyzed. When calculating mean ($\pm$ SE) concentrations of each steroid hormone, any value that was not detectable after concentration was assigned the lowest possible concentration that the assay would have been able to detect in the aliquot used (sensu Prohaska et al. 2013).

Body Condition

To quantify the body condition of sharks, span condition analysis (SCA) was calculated for each individual on the basis of five morphological measurements recorded from sharks in the field before release (Irschick and Hammerschlag 2014). These measurements included (1) precaudal length (PCL), the linear distance from the tip of the snout to the insertion of the caudal fin into the body; (2) lateral span (SL), the distance spanning from the insertion point of the anterior edge of one pectoral fin to the same point on the other pectoral fin (i.e., around the curved dorsal surface of the shark); (3) frontal span (SF), the distance spanning from the insertion point of the anterior edge of the dorsal fin to a line oriented parallel to the horizontal plane of the pectoral fin; (4) proximal span (SP), the distance spanning from the insertion point of the posterior edge of the dorsal fin to a line oriented parallel to the horizontal plane of the pectoral fin; and

(5) caudal keel circumference (CCK), the total circumference at the base of the tail as measured at the caudal keel. These measures were then used to calculate SCA as follows: SCA = (SL $\pm$ SF $\pm$ SP $\pm$ CCK)/PCL (Irschick and Hammerschlag 2014). This metric has been demonstrated previously to positively scale with plasma triglyceride concentration in tiger sharks (Gallagher et al. 2014).

Reproductive Status

The reproductive statuses of the tiger sharks investigated here have been published previously in Sulikowski et al. (2016). Pregnancy status was assessed through ultrasonography. In brief, an Ibex Pro portable ultrasound (EI Medical Imaging, Loveland, CO) was used to image the reproductive tract of each female shark; scans were performed on the ventral surface from pectoral to pelvic fins in transverse and longitudinal orientations, and images of the ovary and follicles were taken between the pectoral fins. The presence of follicles or pups in the uterus was used to determine gravid/nongravid status, and published length at maturity data for tiger sharks in the study region were used to distinguish immature from mature/nongravid individuals. For a subset of sonograms of good quality, sizes of follicles and any developing embryos present were measured. For sharks sampled in 2011 and 2012, hormone values were used to infer pregnancy via a predictive formula based on hormone levels of animals assessed with ultrasonagraphy (see Sulikowski et al. 2016). All sharks were then assigned one of three reproductive statuses: immature, mature/nongravid, or gravid.

Statistical Analysis

We used one-way ANOVA to separately test whether mean plasma triglycerides and body condition significantly differed between female reproductive stages (immature, mature/nongravid, and gravid). Within each reproductive stage, potential correlations among pregnancy hormones, plasma triglycerides, and body condition were investigated using Pearson correlations. For the analyses using triglyceride data, we also separately tested for patterns where individual shark triglyceride values were normalized by shark length to account for any potential size-based differences within reproductive groups. To meet the assumptions of the statistical tests used, hormone, triglyceride, and body condition values were log transformed (value + 1) before analyses. Statistical significance was declared at $P < 0.05$, and all analyses were performed in SAS software.

Results

A total of 57 female tiger sharks were sampled across our study period (19 immature, 15 mature/nongravid, and 23 gravid). Total lengths (mean $\pm$ SD) of immature sharks were 252.05 $\pm$ 27.67 cm (range, 260–373 cm), whereas mature/nongravid sharks were 349.87 $\pm$ 23.53 cm (range, 301–383) and gravid sharks were 338.78 $\pm$ 27.67 cm (range, 260–373 cm).

The mean values of circulating plasma triglycerides did not differ significantly among female tiger sharks of different repro-
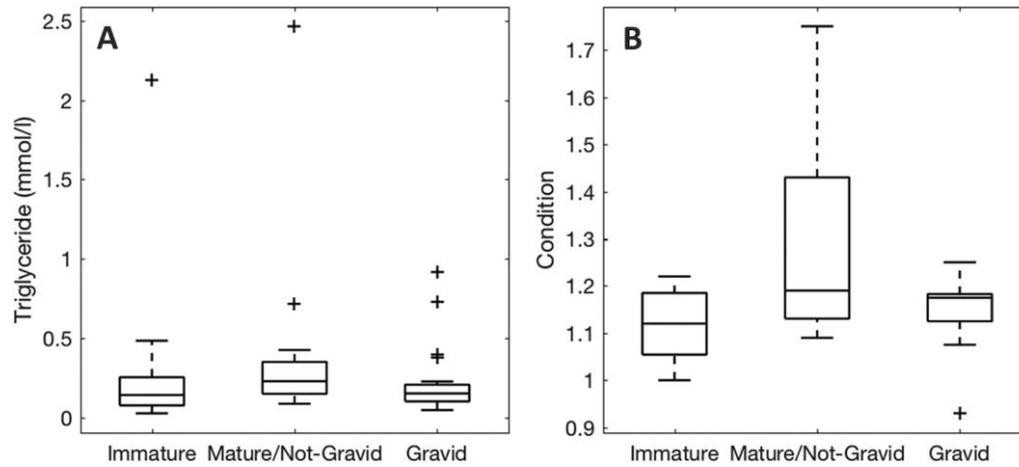
Figure 1. Boxplots of plasma triglycerides (*A*) and body condition (*B*) measured in female tiger sharks of different reproductive stages (immature, mature/nongravid, and gravid).

ductive stages (ANOVA, df $= 2$, $F = 0.83$, $P = 0.44$; fig. 1*A*). Similarly, size-adjusted triglyceride values also did not differ statistically among groups (ANOVA, df $= 2$, $F = 0.06$, $P = 0.94$). In contrast, mean body condition differed significantly among females of different reproductive stages (ANOVA, df $= 2$, $F = 5.09$, $P = 0.01$), with body condition values of mature/nongravid sharks (1.29 ± 0.23) being significantly higher than those of gravid (1.15 ± 0.08) and immature (1.13 ± 0.07) sharks (fig. 1*B*).

For gravid sharks, no significant correlations existed among values of reproductive hormones, plasma triglycerides, and body condition (Pearson correlations; table 1). Body condition was also not correlated with size-adjusted triglyceride values, either overall (Pearson's $r = 0.14$, $P = 0.41$) or within reproductive groupings (immature: $r = -0.26$, $P = 0.37$; mature/nongravid: $r = 0.03$, $P = 0.93$; gravid: $r = 0.51$, $P = 0.11$). Similarly, for mature/nongravid sharks, no significant correlations existed among values of reproductive hormones, plasma triglycerides,

Table 1: Pearson correlations among circulating reproductive hormones (E2, P4, and T), body condition, and plasma triglycerides in gravid tiger sharks

	Condition	Triglycerides	E2	P4	T
Condition:					
r	1	.52	−.31	−.22	−.02
P	...	.1	.32	.5	.95
n	12	11	12	12	12
Triglycerides:					
r	.52	1	−.12	−.07	.06
P	.1	...	.61	.75	.81
n	11	20	20	20	20
E2:					
r	−.31	−.12	1	−.11	−.18
P	.32	.61	...	.63	.41
n	12	20	23	23	23
P4:					
r	−.22	−.07	−.11	1	.22
P	.5	.75	.63	...	.31
n	12	20	23	23	23
T:					
r	−.02	.06	−.18	.22	1
P	.95	.81	.41	.31	...
n	12	20	23	23	23

Note. Data are Pearson correlation coefficients (*r*), *P* values, and sample size (*n*).

Table 2: Pearson correlations among circulating reproductive hormones (E2, P4, and T), body condition, and plasma triglycerides in mature/nongravid female tiger sharks

	Condition	Triglycerides	E2	P4	T
Conditions:					
r	1	−.01	.07	−.19	−.57
P	...	1	.82	.56	.05
n	12	12	12	12	12
Triglycerides:					
r	−.01	1	.04	−.10	.28
P	1	...	.89	.71	.32
n	12	15	15	15	15
E2:					
r	.07	.04	1	−.04	−.32
P	.82	.89	...	.9	.24
n	12	15	15	15	15
P4:					
r	−.19	−.10	−.04	1	−.24
P	.56	.71	.9	...	.39
n	12	15	15	15	15
T:					
r	−.57	.28	−.32	−.24	1
P	.05	.32	.24	.38	...
n	12	15	15	15	15

Note. Data are Pearson correlation coefficients (r), P values, and sample size (n).

and body condition (table 2). For immature sharks, estradiol values were positively correlated with triglyceride levels ($r = 0.72$, $P = 0.001$; fig. 2A) and were negatively correlated with body condition values ($r = -0.56$, $P = 0.03$; fig. 2B). Additionally, progesterone levels were positively correlated with testosterone levels within immature females ($r = 0.48$, $P = 0.04$; fig. 2C). All other tests were not significant (table 3).

Discussion

In the present study, we investigated relationships among reproductive stage, plasma triglycerides levels, body condition, and circulating reproductive hormones in female tiger sharks. While ascribing the patterns found to specific mechanisms is beyond the scope of our study, our approach and results provide an avenue for future investigation of such mechanisms.

The study of plasma lipid dynamics and energetics in sharks is relatively nascent (e.g., Speers-Roesch and Ballantyne 2006; Speers-Roesch and Treberg 2010; Valls et al. 2016; Gallagher et al. 2017). Although the turnover rates of triglycerides in shark blood remain unknown, their levels in plasma are most likely the result of metabolic energy derived from stored endogenous reserves (Larsson and Fange 1977; Beckmann et al 2013). Accordingly, if tiger sharks are purely capital breeders (i.e., feeding in advance of breeding and storing energy that is depleted during gestation), as has been proposed for large ectotherms (Bonnet et al. 1998), we would have expected to have seen the highest triglyceride levels in mature/nongravid females, with values lower

in gravid sharks (fig. 3A); however, our data do not support this view. On the other hand, if tiger sharks are pure income breeders and feed intensely during gestation to nourish developing offspring, we then would have expected triglyceride levels to have gradually increased toward maturity, peaking in gravid females (fig. 3B); however, our data do not support this view either. Given that triglyceride values were consistent across life-history/reproductive stages, it is plausible that tiger sharks may use a strategy that combines both stored energy and recently acquired nutrients to provide a more flexible energetic solution for fueling reproduction and embryo development during gestation.

One possible explanation for a lack of significant differences in triglyceride values among sharks of different reproductive stages could be that sharks are being provisioned by dive tourists, as Tiger Beach is a popular dive site where sharks are fed. However, published tracking data combined with our own personal observations from the site demonstrate minimal effects of provisioning on shark habitat use (Hammerschlag et al. 2012, 2017), with only a few individuals relative to the population being frequent feeders (direct observation). That all sharks were captured using bait further suggests that these sharks may forage irrespective of life-history stage if food is opportunistically available.

Another plausible reason for the lack of significance in triglyceride values found among reproductive groups is related to the opportunistic and irregular timing of our field sampling. In egg-producing animals, there is an active increase in circulating triglycerides in the form of yolk-targeted very low-density lipoprotein (VLDLy) during the rapid yolk development stage of
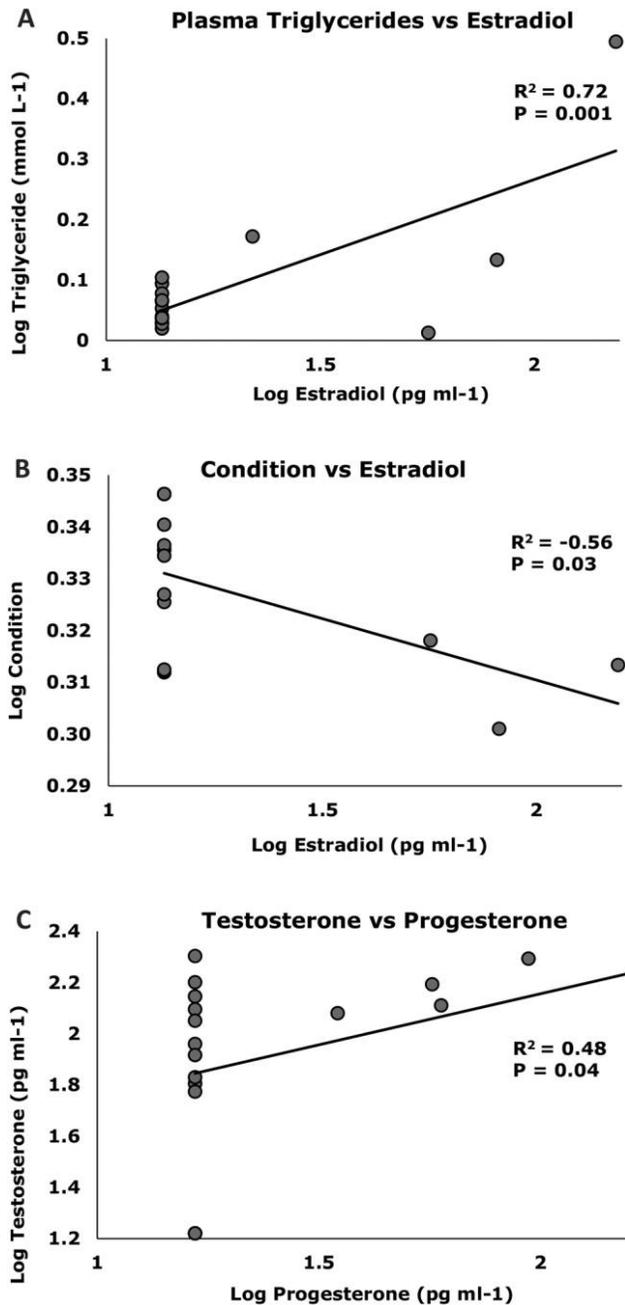
Figure 2. Significant Pearson product-moment correlations found within immature female tiger sharks between plasma triglycerides and estradiol (*A*), body condition and estradiol (*B*), and testosterone and progesterone (*C*). Numbers are log transformed (value + 1).

follicle growth (e.g., Salvante et al. 2007). Once the follicles are fully developed (ovulated), VLDLy production ceases, and triglyceride values return to prebreeding levels (Salvante et al. 2007). Thus, it is possible that our sampling periods missed gravid females during this early stage of development. If we had sampled females in the early stages of pregnancy, we may have found higher levels of triglycerides in the gravid versus nongravid individuals, due to upregulation of VLDLy.

It is also worth considering that triglyceride values could vary across reproductive stages due to reliance on recent feeding (income) or because sharks are relying on lipid stores in the liver (capital). To better address this question, future studies of this kind should consider measurements of plasma ketone bodies, which are synthesized from energy stores in the liver (Speers-Roesch and Treberg 2010). Indeed, several studies have demonstrated that starvation intensifies the mobilization of ketone bodies by elasmobranchs, increasing plasma levels that subsequently drop after feeding (e.g., Wood et al. 2010).

Our finding of increased condition in mature but nongravid tiger sharks may be because they are not investing energetic reserves toward future breeding but instead are investing energy in needed somatic growth or for migration. Moreover, the act of mating itself can be traumatic in sharks (Pratt and Carrier 2001), which might manifest as reduced body condition at the onset of gravidity. While not mutually exclusive, the high condition values in mature/nongravid sharks may be a function of reproductive readiness, such as sharks needing to be of sufficient condition, with adequate energy stores built up, to support energetically costly gestation following mating. For example, if tiger sharks in the study area are indeed biennial, as previously reported (Castro 2011), then mature sharks that have given birth in the prior year could be preparing for the next reproductive season by storing energy, resulting partly in the higher body condition documented in this group.

While not mutually exclusive of this hypothesis, reduced body condition in gravid sharks may also be due to maternal investment of energetic reserves to support embryo nourishment during gestation via production of uterine fluid (Castro et al. 2016). Moreover, barring recent large feeding events, circulating triglyceride levels are usually maintained within some basal level, which for the gravid females may require a greater reliance on these endogenous somatic reserves to liberate triglycerides for both her own use and to nourish the developing offspring; hence, the lower condition factor. It is also possible that tiger sharks of different reproductive states may have similar energy acquisition strategies, as indicated by a lack of differences among triglyceride values, although they have different energy requirements, reflected in the differences in body condition values found. That said, the wide variation in condition values documented among individuals, especially in mature but nongravid females, suggests that more studies are needed to determine the biological significance of the patterns found.

The highest triglyceride values were found in two sharks: a 296-cm (total length) female classified as immature and a 356-cm (total length) female classified as a nongravid/mature. Accordingly, we considered whether the reproductive stage assigned to these two sharks could have been incorrect and they were actually females in the early stages of pregnancy, with the high values related to upregulation of VLDLy during vitellogenesis. However, we can rule out this possibility for two reasons. First, in the case of early pregnancy, we could determine differences between active and inactive ovaries on the basis of size measurements made with the ultrasound, and we found no differences in the sizes of follicles present on the opportunistically sampled

Table 3: Pearson correlations among circulating reproductive hormones (E2, P4, and T), body condition, and plasma triglycerides in immature female tiger sharks

	Condition	Triglycerides	E2	P4	T
Condition:					
r	1	−.32	−.56	.21	−.02
P	...	.24	.03	.45	1
n	15	15	15	15	15
Triglycerides:					
r	−.32	1	.73	.30	.11
P	.24	...	.001	.26	.67
n	15	16	16	16	16
E2:					
r	−.56	.73	1	.05	−.07
P	.03	.001	...	.84	.79
n	15	16	19	19	19
P4:					
r	.21	.30	.05	1	.48
P	.45	.26	.84	...	.04
n	15	16	19	19	19
T:					
r	−.02	.11	−.07	.48	1
P	1	.67	.79	.04	...
n	15	16	19	19	19

Note. Data are Pearson correlation coefficients (r), P values, and sample size (n).

ovaries (Sulikowski et al. 2016), suggesting that vitellogenesis was not occurring, at least in the sharks designated as mature. This is likely due to the time at which sampling occurred. Given the reproductive cycle proposed for tiger sharks in the North Atlantic (Castro 2011), vitellogensis likely occurs during the summer, a time when we were not sampling. Second, tiger sharks in the study region are believed to have a biennial reproductive cycle (e.g., Castro 2011). As such, vitellogensis and pregnancy are not concurrent, as in the case of annual breeders. However, it is worth considering that despite using various methods for assigning sharks to one of three reproductive states (Sulikowski et al. 2016), we cannot rule out the possibility of misclassification.

Our finding of no significant relationship between body condition and triglyceride values differed from the findings of Gallagher et al. (2014), who found a positive relationship at the same study site. There are several possible reasons for the difference. The previous study did not distinguish between reproductive states and was based on fewer individuals. Comparing data between the two studies demonstrates the high degree of individual variation in shark energetics and condition among tiger sharks at the study area. The positive relationship between triglycerides and condition found by Gallagher et al. (2014) was primarily driven by a few sharks that had high values of both, whereas the lack of a significant relation in the present study was driven by three females of high body condition that had low triglyceride values. It is also possible that the additional individuals sampled in the present study were sampled at a time when condition and energy is typically lower within the population—or that there may have been some interannual differences in both parameters.

Accordingly, repeated longitudinal sampling of tiger sharks in the study area may help provide insights and further elucidate this relationship.

Among the immature sharks, several correlations occurred between reprodutive hormones, triglyceride values, and condition. However, these patterns were largely driven by a few points. Thus, we do not feel that it is appropriate to overspeculate as to the drivers of these patterns. However, we provide some considerations for future research. Progesterone is converted into testosterone, which is then converted into estradiol. An increase in estradiol is often a signal for the production of vitellogenin from the liver, a precursor for yolk production (Callard et al. 2005). The pattern found in immature sharks, where progesterone was positively correlated with testosterone and estradiol values were positively correlated with triglyceride levels, could be related to young females on the cusp of maturity physiologically preparing for their first mating. The negative relationship between estradiol and body condition in immature sharks could be related to the females in the initial stages of preparing for becoming reproductively active in the following years, such as increasing somatic growth via storing fats.

Taken together, our results suggest the possibility that tiger sharks may be able to mitigate possible metabolic costs associated with gestation by opportunistically integrating both stored and acquired food to aid reproduction (a mix of both capital and income breeding). Our data suggest that large tiger sharks may not necessarily follow the pattern of building up both triglycerides and body condition as a precursor to reproduction, as would be expected from pure capital breeders (fig. 3A). Instead, the
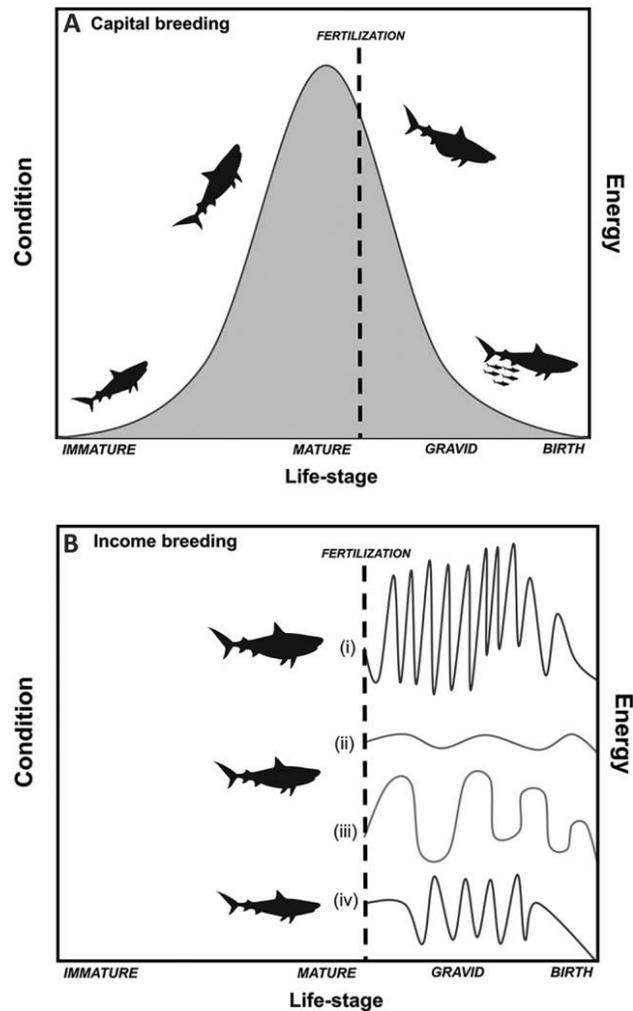
Figure 3. Conceptual model of potential tiger shark resource use strategies (condition and energy, on opposing axes) as they relate to reproduction and life stage. *A*, Capital breeding, where individual sharks build up energy reserves and improve body condition before mating and fertilization. Condition and energy stores then decrease together as internal stores fuel pup development. *B*, Income breeding, where individual sharks enter gravidity/pregnancy with varying levels of stored energy/condition factors and feed throughout this period to fuel pup development in real time. Individual variation in feeding rates and behavior may reflect a subset of different income-based strategies (i–iv), which in turn may affect fecundity and fitness. A color version of this figure is available online.

capability of using stored energy and actively feeding during gestation may be necessary given that tiger sharks are matrotrophic, whereby embryos are nourished throughout pregnancy, first from a yolk and then from an energy-rich uterine fluid in the egg case (Castro 2016). Such a feature may provide biological and ecological benefits to tiger sharks. For example, if prey availability is high or if sharks with higher energy reserves or foraging abilities are fertilized (fig. 3*B*), feeding during gestation may offer increased fecundity, a larger brood size, larger offspring size, and/or a shorter gestation period. Indeed, it has been suggested previously that the availability and seasonality of food resources may

influence where animals are positioned on the capital breeding/income breeding continuum (Stephens et al. 2014), and individual variation in condition may better explain this phenomenon. It is worth considering whether the relatively high intraspecific variability in gestation period (12–16 mo) and/or brood size (18–70 pups) documented in tiger sharks (Whitney and Crowe 2007; Castro 2009) could in part be driven by the variation in nutritional intake during gestation based on the availability and quality of prey resources (fig. 3). Future research at various sites would be needed to evaluate such a hypothesis.

In sharks, there are very few approaches to evaluate the strategies of maternal energy allocation via nonlethal means (Hammerschlag and Sulikowski 2011; Awruch et al. 2014). However, our approach provides a nonlethal alternative for exploring the reproductive life histories of large sharks, and our results underscore the complex nature of energy metabolism, behavior, and reproduction in sharks, providing avenues for future research.

Acknowledgments

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