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REGULAR PAPER

Variation of body condition and plasma energy substrates with life stage, sex, and season in wild-sampled nurse sharks *Ginglymostoma cirratum*

Shannon G. Moorhead[1] | Austin J. Gallagher[2,3] | Liza Merly[4] | Neil Hammerschlag[1,5]

[1]Department of Marine Ecosystems and Society, Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, Florida

[2]Beneath the Waves, Herndon, Virginia

[3]Fish Ecology and Conservation Physiology Laboratory, Carleton University, Ottawa, Ontario, Canada

[4]Department of Marine Biology and Ecology, Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, Florida

[5]Leonard and Jayne Abess Center for Ecosystem Science and Policy, University of Miami, Coral Gables, Florida

Correspondence
Shannon G. Moorhead, Rosenstiel School of Marine and Atmospheric Science, University of Miami, 4600 Rickenbacker Causeway, Miami, FL 33149, USA.
Email: s.moorhead@umiami.edu

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Abstract

Reported here are the relationships among morphological (*i.e.*, body condition) and biochemical (*i.e.*, plasma concentrations of triglycerides, cholesterol, free fatty acids, and ketone bodies and ketone body ratios) parameters related to energy storage and use, as well as the variation of such parameters, for 107 free-ranging nurse sharks *Ginglymostoma cirratum* sampled off South Florida. Immature *G. cirratum* exhibited a higher variance in body condition, plasma free fatty acid concentrations and ketone body ratios compared to adults. Mature female *G. cirratum* had significantly higher body condition than mature males, driven by a seasonal increase in mature female body condition during the wet season. Mature male *G. cirratum* showed a decrease in the ketone body β-hydroxybutyric acid during the dry season. Taken together, this study provides a baseline assessment of body condition and internal physiological state for a data-poor marine species and demonstrates significant ontogenetic, sexual and seasonal variation in *G. cirratum* energetic state. As concluded by other studies of energy metabolism in free-ranging sharks, this research highlights the importance of considering intraspecific patterns and sampling context for inferring the drivers of variation.

KEYWORDS
body condition, cholesterol, free fatty acids, *Ginglymostoma cirratum*, ketone bodies, triglycerides

1 | INTRODUCTION

The amount of energy available to an organism determines its ability to perform essential activities and life functions (Lambert & Dutil, 1997). Therefore, understanding the intrinsic and extrinsic factors that create variation in energetic state, or condition, can illuminate how changes in environmental conditions may affect populations of species (*e.g.*, Costa & Gales, 2003). These relationships are especially important for large marine species that carry high rates of extinction risk and are increasingly challenged by a dynamic ocean environment (Roberts & Hawkins, 1999). However, there has been relatively less attention given to these relationships in elasmobranch species, despite growing interest in their conservation physiology (Lyons *et al*., 2019).

A limited number of published studies have explored morphometric and biochemical parameters related to elasmobranch condition and energetic state. These studies have highlighted the unusual metabolic organization of elasmobranch fishes (*e.g.*, Metcalf & Gemmell, 2005; Watson & Dickson, 2001; Zammit & Newsholme, 1979), characterized by a lack of adipose tissue, a limited ability to oxidize lipids in extrahepatic tissue and

an increased reliance on ketone bodies and amino acids for energy transport to such tissues, especially the brain, cardiac muscle and skeletal muscle (Ballantyne, 1997; de Roos, 1994; Speers-Roesch & Treberg, 2010; Watson & Dickson, 2001). Instead of adipose tissue, elasmobranchs store energy in the form of triglycerides and other lipids in their large, fatty livers. Consequently, certain previous studies (*e.g.*, Hussey *et al.*, 2009) have evaluated elasmobranch body condition by calculating the ratio of liver weight to total body weight, also known as the hepatosomatic index (HSI). However, because HSI can only be determined *via* lethal sampling, span condition analysis, an alternative and nonlethal method, has been used to assess body condition in large sharks. Developed by Irschick and Hammerschlag (2014), span condition analysis is based on various measures of shark girth along the dorsal surface relative to their length. This method can be readily integrated with nonlethal blood sampling of various energetic biochemical parameters for a more holistic assessment of energetic state (Gallagher *et al.*, 2014; Hammerschlag *et al.*, 2018).

Many studies (*e.g.*, Gallagher *et al.*, 2017a; Speers-Roesch *et al.*, 2006; Valls *et al.*, 2016) of elasmobranch condition and energetic state have investigated blood plasma concentrations of lipids (*e.g.*, triglycerides, cholesterol, free fatty acids) and associated metabolites (*e.g.*, ketone bodies). The concentrations of circulating triglycerides and other lipids (*i.e.*, cholesterol and free fatty acids) measured in elasmobranch plasma are lower than those measured in most other vertebrates, which may reflect the limited capacity of this taxa to oxidize lipids in extrahepatic tissues (Ballantyne, 1997; Gallagher *et al.*, 2014; Speers-Roesch & Treberg, 2010). Conversely, concentrations of circulating ketone bodies (*i.e.*, acetoacetate, β-hydroxybutyric acid) measured in elasmobranch plasma are much higher than those measured in other taxa, reflecting high rates of ketone body oxidation in elasmobranch heart and red muscle and, ultimately, the heightened reliance on ketone bodies as a metabolic fuel in elasmobranchs (Ballantyne, 1997; de Roos, 1994; Watson & Dickson, 2001; Zammit & Newsholme, 1979). The ratio of ketone body concentrations (*i.e.*, acetoacetate/β-hydroxybutyric acid) in blood plasma can reflect cellular energy status and, in mammals, is thought to be indicative of liver function; considering the enhanced metabolic role of ketone bodies in elasmobranchs, the potential of ketone body ratio as a metric of elasmobranch energetic state warrants exploration (Ballantyne, 1997; Tanaka *et al.*, 1979; Yamamoto *et al.*, 1980).

This study investigated variation in body condition and biochemical parameters related to nutritional condition in a wild population of South Florida nurse sharks *Ginglymostoma cirratum* (Bonnaterre 1788) through morphological measures (span condition analysis) and nonlethal sampling of plasma metabolite concentrations (triglycerides, cholesterol, free fatty acids, acetoacetate and β-hydroxybutyric acid). *G. cirratum* is widely distributed throughout coastal waters of the subtropical and tropical western Atlantic Ocean (Heithaus *et al.*, 2007; Ward-Paige *et al.*, 2010). Off South Florida, this species is the most abundant large predator in marine ecosystems, where it feeds at a low trophic level on invertebrates and small teleosts (Castro, 2000; Phenix *et al.*, 2019). Despite its abundance, *G. cirratum* is listed as "Data Deficient" by the International Union for the Conservation of Nature (IUCN) due to a lack of information on its population dynamics and connectivity (Rosa *et al.*, 2006). The body condition and some plasma metabolites of *G. cirratum* have been measured in prior research (*i.e.*, AtallahBenson *et al.*, 2020; Gallagher *et al.*, 2017a; Irschick & Hammerschlag, 2014), however, intra-annual and intraspecific variation within this population has yet to be explored. Moreover, measures of ketone bodies in this species have not been assessed, despite their importance to elasmobranch energetics. To address these knowledge gaps, the present study sought to answer two primary research questions: (a) Do any correlations exist among body condition and various plasma metabolite concentrations (*i.e.*, triglycerides, cholesterol, free fatty acids and ketone bodies, as well as ketone body ratios) in *G. cirratum* and (b) do body condition and plasma metabolite concentrations vary with life stage, sex or season? The results of this study contribute to the current knowledge base on condition, energy storage and energy utilization in wild elasmobranch populations, providing insight into how these parameters may be related and how they vary with intrinsic and extrinsic factors.

2 | MATERIALS AND METHODS

2.1 | Ethical statement

All capture, handling and sampling protocols described here were approved by the Florida Fish and Wildlife Conservation Commission (license #SAL-18-0957-SR), the National Park Service (permit #BISC-2017-SCI-0017) and the University of Miami Institutional Animal Care and Use Committee (IACUC, protocol #18-154-LF).

2.2 | Study site and sampling procedure

Free-ranging *G. cirratum* were sampled between April 2015 and July 2018 within subtropical U.S. federal waters (*i.e.*, >5.6 km from shore) and Florida state waters (*i.e.*, <5.6 km from shore), primarily within the confines of Biscayne Bay (25.56°N, 80.22°W). Situated along the southeast coast of Florida, Biscayne Bay is a shallow coastal lagoon that encompasses a variety of estuarine and marine ecosystems (*i.e.*, mangrove forests, seagrass beds, coral reefs); the northern portion of the bay is bordered by a series of barrier islands, and the central and southern portions are bounded by the northernmost islands of the Florida Keys archipelago (Lirman *et al.*, 2008; Serafy *et al.*, 2003). Paralleling Florida's mainland, about 2–5 km oceanside of Biscayne Bay, is the Florida Reef Tract, the third largest barrier coral reef system in the world (Banks *et al.*, 2008). *G. cirratum* are the most abundant large predator in the ecosystem (Phenix *et al.*, 2019).

G. cirratum were primarily captured using a standardized drumline system, similar to that described by Hueter and Tyminski (2007). Briefly, the drumline system is composed of a submerged weighted drum (∼20 kg) that is attached to a pair of surface floats with ∼10 m of braided nylon rope. From the weighted drum, ∼22 m of monofilament (∼400 kg test) extends along the seafloor, terminating at a baited 13/0 or 16/0 offset circle hook. Ten drumlines were set in a transect line and allowed to soak for 1 h, after which the drumlines were retrieved and checked for

captured sharks. A small subset of sampled *G. cirratum* (n = 8) were captured *via* rod and reel fishing, which utilized a 7/0 offset circle hook. Captured sharks were reeled in and restrained, either alongside the vessel or atop a semi-submerged platform (drumline system) or on a concrete seawall (rod and reel). While restrained, a standardized sampling process was carried out (*i.e.*, sex determination, mark-recapture tagging, morphological measurements for span condition analysis, blood collection for metabolite concentrations). Each sampling process took no more than 7 min, after which sharks were released at their capture site. *G. cirratum* have a high tolerance for capture and handling stress; this species performs low-intensity exercise and often rests on the bottom when hooked on fishing line, which is reflected by blood glucose and lactate concentrations, common metrics of elasmobranch stress, that are much lower than those of other species (Bouyoucos *et al*., 2018; Gallagher *et al*., 2017b; Jerome *et al*., 2017). Because of this reduced response and the short duration of sampling time, the effect of capture and handling stress on *G. cirratum* plasma metabolites was considered negligible.

2.3 | Morphological measurements and determination of body condition and life stage

During the routine sampling process, a measuring tape was used to collect five measurements across the dorsal surface of each shark for span condition analysis (A_{SC}), measured to the nearest centimetre (Irschick & Hammerschlag, 2014): (a) precaudal length (L_{PC}), the distance from the tip of the shark's snout to the caudal peduncle; (b) lateral span (S_L), the distance spanning across the surface of the shark between the anterior insertion points of the pectoral fins; (c) frontal span (S_F), measured at the anterior insertion point of the first dorsal fin, the distance spanning the surface of the shark between lines parallel to the frontal plane that extend from the pectoral fins to the caudal fin; (d) proximal span (S_P), measured at the posterior insertion point of the first dorsal fin, the distance spanning the surface of the shark between lines parallel to the frontal plane that extend from the pectoral fins to the caudal fin; and (e) caudal keel circumference (C_{CK}), measured at the caudal peduncle, the circumference at the base of the caudal fin (*Figure* 1). From these measurements, the body condition of each shark was calculated using the span condition analysis equation: $A_{SC} = \Sigma\ (S_L + S_F + S_P + C_{CK})/(L_{PC})$. Total length ($L_T$) was also measured from each shark to estimate its life stage based on published length at maturity relationships in the study region: female sharks with $L_T \geq 223$ cm, and male sharks with $L_T \geq 214$ cm or calcified claspers were considered "mature", while sharks that did not meet this criteria were considered "immature" (Castro, 2000).

2.4 | Blood collection and metabolite analysis

A 6–10 ml blood sample was collected from the caudal vein using a 2–3 cm, 18-gauge hypodermic needle and 10 ml syringe during the routine sampling process. To separate plasma from other blood constituents, about 6 ml of whole blood was immediately aliquoted into

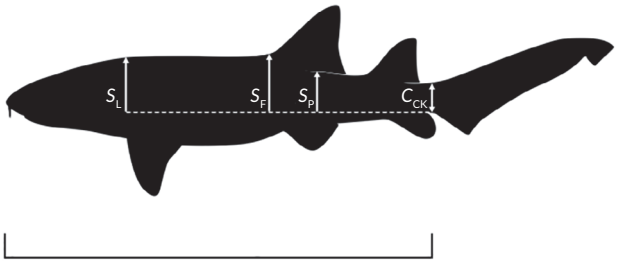

FIGURE 1 Diagram of *Ginglymostoma cirratum* depicting the morphological measurements used to calculate body condition *via* span condition analysis (A_{SC}). Lateral span (S_L), frontal span (S_F), proximal span (S_P) were measured across the body between lines parallel to the frontal plane that extend from the pectoral fins to the base of the caudal fin (dotted line). Caudal keel circumference (C_{CK}) *was measured around the base of the caudal fin.* Precaudal length (L_{PC}) was measured from the snout to the base of the caudal fin

1.5 ml microcentrifuge tubes and centrifuged at 5000–6000 rpm for 2 min *in situ*, mitigating any clotting. The resulting plasma was immediately transferred to 2 ml cryovials and placed in an ice-slurry until they could be transported to the Rosenstiel School of Marine and Atmospheric Science at the University of Miami, where they were permanently stored in a freezer at −20°C until analysis. This standardized procedure was performed by thoroughly trained individuals for the collection of each plasma sample. It should be noted that what was collected and referred to here as "plasma" was not true plasma because an anticoagulant was not used during collection. However, the sample was more similar to plasma than serum as it was extracted from unclotted blood, thereby retaining clotting proteins that are not present in serum (Issaq *et al*., 2007).

Five separate nutritional metabolic assays were performed on shark plasma samples to determine the concentrations of the following metabolites: triglycerides (TAG, in millimoles per litre, mmol l^{-1}; EnzyChrom Triglyceride Assay Kit, BioAssay Systems, Hayward, CA, USA), cholesterol (CHOL, in millimoles per litre, mmol l^{-1}; EnzyChrom Cholesterol Assay Kit, BioAssay Systems), free fatty acids (FFA, in micromolar, μM; EnzyChrom Free Fatty Acid Assay Kit, BioAssay Systems), and two ketone bodies, acetoacetate (AcAc) and β-hydroxybutyric acid (BOH, in millimolar, mM; EnzyChrom Ketone Body Assay Kit, BioAssay Systems). These kits have been previously validated in fish, including *G. cirratum* (*i.e.*, Gallagher *et al*., 2017a) and other elasmobranchs (*e.g.*, Gallagher *et al*., 2014, 2017a; Hammerschlag *et al*., 2018; Lyons, 2018), as well as teleost fish (*e.g.*, Hanson *et al*., 2012; Jeffery *et al*., 2019; King *et al*., 2016; Liss *et al*., 2013, 2016). TAG, CHOL and FFA assays were assayed in triplicate, while ketone body assays were assayed in duplicate. Because ketone body assays were performed in duplicate, it should be noted that data was retained from this analysis only if the duplicates yielded comparable results; if not, that sample was excluded from analysis. Absorbance values for each of the 96-well plates from all assays were obtained using a Tecan Sunrise absorbance microplate reader (Tecan, Grödig, Austria). CHOL and ketone body assays were read at 340 nm, while TAG and FFA assays were read at 570 nm. Individual metabolite

concentrations were determined using appropriate standard curves, and concentrations that were below the detection limit of the assay kit were treated as zeros. The ketone body ratio (KB ratio) was calculated for each shark by dividing the AcAc concentration by the BOH concentration; any sharks with BOH concentrations below the detection limit of the assay kit (*i.e.*, zero) were excluded from KB ratio analysis to avoid division by zero.

2.5 | Statistical analyses

Prior to any statistical testing, the data were scrutinized for anomalous outliers (value >100 × IQR, interquartile range); one such outlier (155 × IQR) was identified in the KB ratio dataset and removed. The normality of residuals for all data were assessed *via* Shapiro–Wilks tests and visual examination of Q-Q normal plots. For any variable with non-normal residuals, transformations were attempted (*i.e.*, natural log, square root) to meet the parametric assumption of normality; if a transformation successfully normalized the residuals, the inverse of the transformation was performed on the corresponding variable (*i.e.*, raising e to the power of the variable, squaring the variable). If after transformation the residuals remained non-normal, then the transformation that brought the residuals closest to normality was used to minimize the violation of the parametric assumption of normality.

Linear relationships among measured parameters (*i.e.*, body condition, plasma concentrations of TAG, CHOL, FFA, AcAc and BOH, KB ratio) were explored using Pearson's correlation matrix. Correlation matrices were performed for three distinct life stage-sex groupings: immature, mature female and mature male. To assess variation in measured parameters across the three life stage-sex groupings, the Levene's test was used to test for significant differences in variance, followed by a one-way analysis of variance (ANOVA) to test for significant differences in the means. When the Levene's test demonstrated that data did not meet the ANOVA assumption of homogenous variance, a Welch's ANOVA was used. If the results of the ANOVA were significant, the appropriate *post hoc* tests were used to explore the results: Tukey (ANOVA) or Games–Howell (Welch's ANOVA). Similarly, to explore seasonal variation in parameters, Levene's tests and independent sample *t*-tests (or Welch's *t*-test, when appropriate) were used to compare metric variance and means, respectively, in mature sharks sampled in the dry season (November–April) and the wet season (May–October). To further investigate seasonal variation, the same methodology was used to test for differences in metric variances and means by sex, both within and between seasons.

For all analyses, significance was defined as $P < 0.05$. Analyses were performed in SPSS Version 26 (IBM, Armonk, NY, USA).

3 | RESULTS

Body condition and plasma metabolite data were collected from a total of 107 *G. cirratum*; 55 sharks were female and 52 were male. Sharks ranged in length (L_T) from 65 to 259 cm (mean ± S.D. = 196.01 ± 54.35), encompassing both immature and mature size classes. *Table* 1 displays the distribution of sampled *G. cirratum* across life stages, sexes and seasons. *Table* 2 provides the descriptive statistics for body condition, plasma concentrations of TAG, CHOL, FFA, AcAc and BOH, and the plasma KB ratios measured from sampled sharks. *Figure* 2 displays the frequency distributions of each parameter.

Within immature *G. cirratum*, four significant relationships existed among parameters. Body condition was positively correlated with both AcAc (Pearson's correlation, $r_{51} = 0.314$, $P < 0.05$; *Figure* 3a) and the KB ratio (Pearson's correlation, $r_{50} = 0.336$, $P < 0.05$; *Figure* 3b). TAG and FFA were negatively correlated with one another (Pearson's correlation, $r_{51} = -0.379$, $P < 0.01$; *Figure* 3c). Finally, AcAc and BOH were also negatively correlated (Pearson's correlation, $r_{51} = -0.420$, $P < 0.01$; *Figure* 3d).

Within mature female *G. cirratum*, body condition was positively correlated with both TAG (Pearson's correlation, $r_{20} = 0.644$, $P < 0.001$; *Figure* 3e) and CHOL (Pearson's correlation, $r_{20} = 0.495$, $P < 0.05$; *Figure* 3f). Only one significant relationship was detected within mature male *G. cirratum*, a positive correlation between TAG and CHOL (Pearson's correlation, $r_{30} = 0.664$, $P < 0.001$; *Figure* 3g).

Figure 4 displays boxplots of body condition, plasma metabolite concentrations and KB ratios measured in immature, mature female and mature male *G. cirratum*. There were no significant differences in mean plasma metabolite concentrations or KB ratios among immature, mature female and mature male *G. cirratum* (one-way ANOVA, $P > 0.05$). Body condition was significantly different between the three groups (Welch's ANOVA, $F_{2,62.75} = 3.219$, $P < 0.05$). *Post hoc* analysis revealed that, while the mean body condition of immature sharks (mean ± S.D. = 1.07 ± 0.12) was not significantly different than either mature group (Games–Howell test, $P > 0.05$), mature female sharks (mean ± S.D. = 1.09 ± 0.07) had significantly higher mean body condition than mature males (mean ± S.D. = 1.04 ± 0.09; Games–Howell test, $P < 0.05$). With the exception of CHOL, variance in all parameters was significantly different between the three life stage-sex groups. Most notably, variance in body condition (Levene's test, $F_{2,104} = 5.12$, $P < 0.01$), FFA (Levene's test, $F_{2,104} = 7.75$, $P < 0.001$) and KB ratios (Levene's test, $F_{2,100} = 3.62$, $P < 0.05$) were significantly higher in immature sharks than in mature sharks of both sexes.

Boxplots of each parameter measured in mature *G. cirratum* within the dry season (November–April) and the wet season (October–May) are shown in *Figure* 5. Seasonal means of analysed parameters were higher in mature sharks sampled during the wet season than in mature sharks sampled during the dry season, with the exception of FFA.

TABLE 1 Distribution of sampled *Ginglymostoma cirratum* across life stages (*i.e.*, immature, mature), sexes (*i.e.*, male, female) and seasons (*i.e.*, dry season, wet season)

	Immature			Mature		
	Female	Male	Total	Female	Male	Total
Dry season	33	20	53	14	14	28
Wet season	0	0	0	8	18	26
Total			53			54

TABLE 2 Descriptive statistics (*i.e.*, *n*, sample size; range; mean ± S.D., standard deviation; median; C.V., coefficient of variation) for body condition, plasma metabolite concentrations and KB ratios for sampled *Ginglymostoma cirratum*

	n	Range	Mean ± S.D.	Median	C.V.
Body condition	107	0.75–1.38	1.06 ± 0.11	1.06	10.38
TAG (mmol l^{-1})	107	0.01–0.95	0.26 ± 0.17	0.24	65.38
CHOL (mmol l^{-1})	104	0.07–1.78	0.88 ± 0.38	0.85	43.42
FFA (μM)	107	0.00–281.11	52.89 ± 80.97	9.04	153.09
AcAc (mM)	107	0.00–7.05	1.46 ± 1.58	1.12	108.22
BOH (mM)	107	0.00–6.69	1.36 ± 0.75	1.31	55.14
KB ratio	103	0.00–6.74	1.34 ± 1.68	0.64	125.37

Note: C.V., coefficient of variation; *n*, number of individual sampled; S.D., standard deviation.

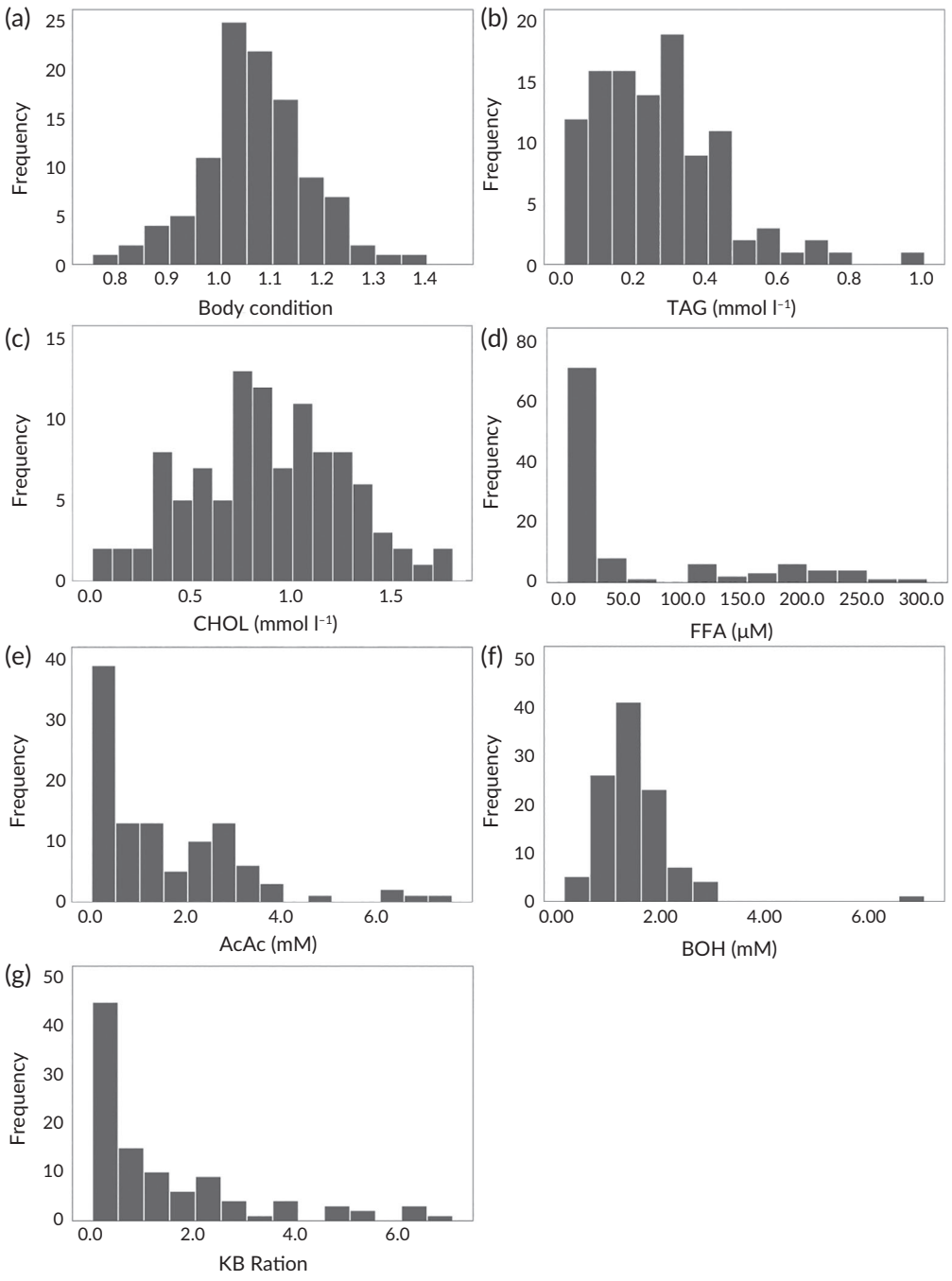

FIGURE 2 Histograms of (a) body condition, (b) plasma TAG concentrations, (c) plasma CHOL concentrations, (d) plasma FFA concentrations, (e) plasma AcAc concentrations, (f) plasma BOH concentrations and (g) plasma KB ratios for sampled *Ginglymostoma cirratum*

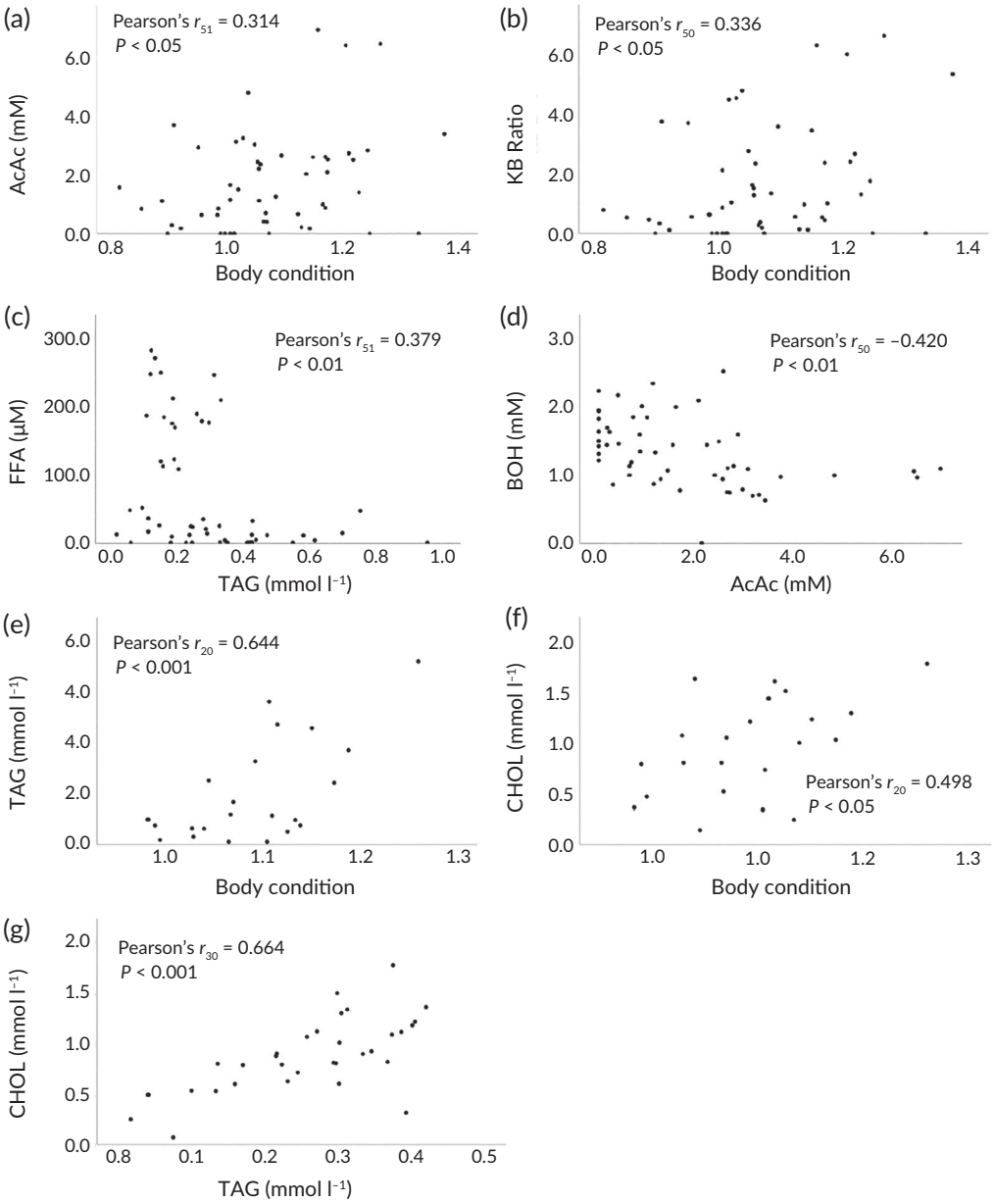

FIGURE 3 Scatter plots depicting significant correlations between parameters detected *via* Pearson's correlation matrix for (a)–(d) immature *Ginglymostoma cirratum*, (e) and (f) mature female *G. cirratum* and (g) mature male *G. cirratum*. Four significant correlations were detected within immature *G. cirratum*: (a) a positive correlation between body condition and plasma AcAc concentrations; (b) a positive correlation between body condition and plasma KB ratio; (c) a negative correlation between plasma TAG concentrations and plasma FFA concentrations; and (d) a negative correlation between plasma AcAc concentrations and plasma BOH concentrations. Two significant correlations were detected within mature female *G. cirratum*: (e) a positive correlation between body condition and plasma TAG concentrations; and (f) a positive correlation between body condition and plasma CHOL concentrations. One significant correlation was detected within mature male *G. cirratum*: (g) a positive correlation between plasma TAG concentrations and plasma CHOL concentrations

However, none of these differences were significant (independent sample *t*-test, $P > 0.05$). Variance in CHOL was significantly higher in the wet season ($\sigma^2 = 0.230$) than in the dry season ($\sigma^2 = 0.107$; Levene's test, $F_{2,52} = 4.88$, $P < 0.05$). However, no other parameters showed a significant difference in variation between seasons.

Figure 6 depicts boxplots of each parameter measured in mature female and mature male *G. cirratum*, separated by season. Mean body condition of mature female sharks was significantly higher in the wet season (mean ± S.D. = 1.13 ± 0.07) than in the dry season (mean ± S.D. = 1.07 ± 0.05; independent sample *t*-test, $t_{20} = -2.419$, $P < 0.05$); accordingly, this seasonal increase in female body condition drove a significant difference between mature female and mature male sharks in the wet season (independent sample *t*-test, $t_{24} = 3.357$, $P < 0.01$).

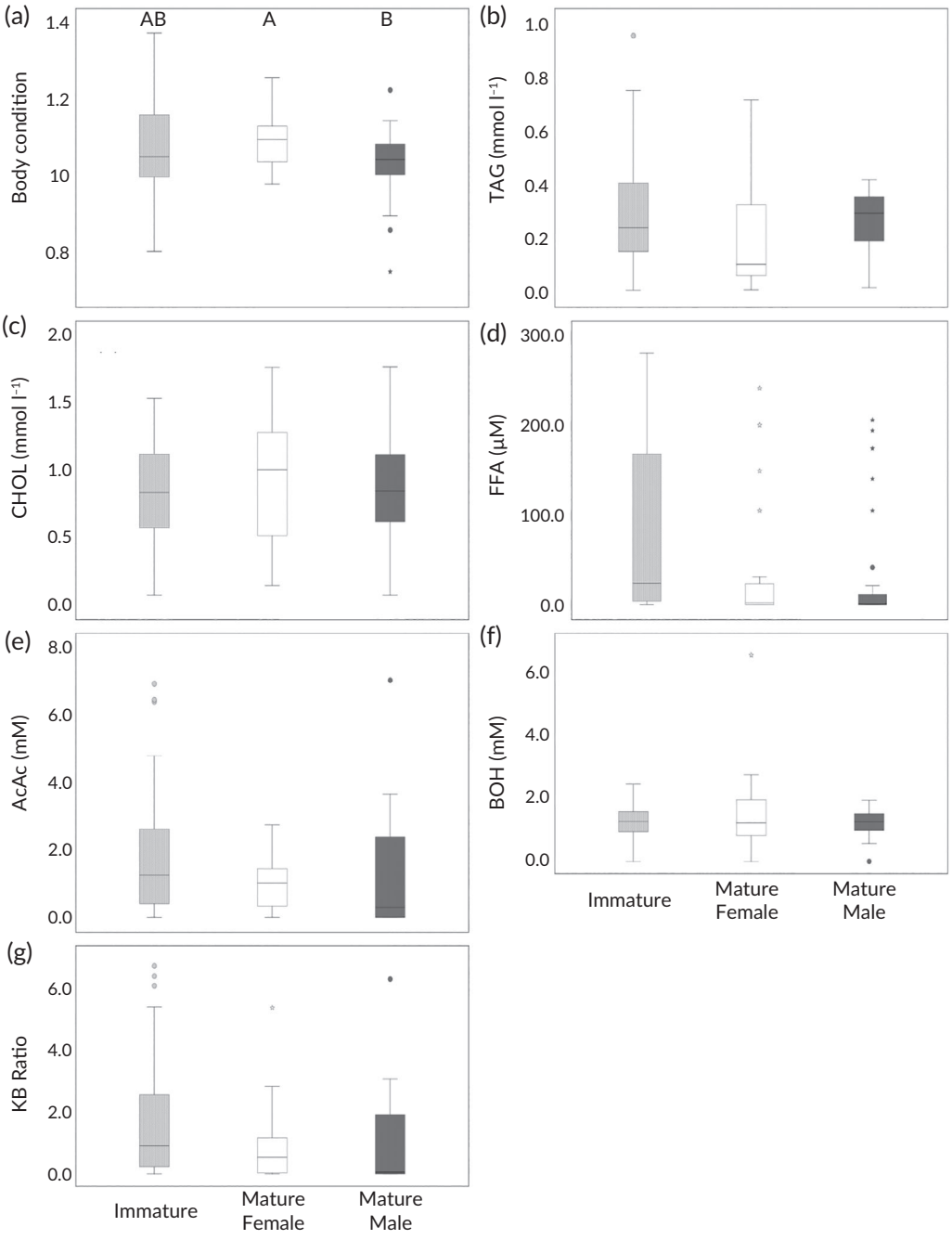

FIGURE 4 Boxplots depicting values of (a) body condition, (b) plasma TAG concentrations, (c) plasma CHOL concentrations, (d) plasma FFA concentrations, (e) plasma AcAc concentrations, (f) plasma BOH concentrations and (g) plasma KB ratios for immature, mature female and mature male *Ginglymostoma cirratum*. Immature *G. cirratum* are represented by light grey boxplots, mature female *G. cirratum* are represented by white boxplots and mature male *G. cirratum* are represented by dark grey boxplots. Circles represent outliers (*i.e.*, values outside 1.5 × IQR), and stars represent extreme outliers (*i.e.*, values outside 3 × IQR). Capital letters (*i.e.*, A, B) denote groups with significantly different means; statistic was assessed by Welch's ANOVA (immature *G. cirratum* body condition (AB) ×mature female *G. cirratum* body condition (A) × mature male *G. cirratum* body condition (B), $P < 0.05$) and Games-Howell test ($P < 0.05$)

Within the dry season, mature female sharks (mean ± S.D. = 1.59 ± 0.73 mM) had a significantly higher mean BOH than mature male sharks (mean ± S.D. = 1.13 ± 0.30 mM; Welch's independent sample *t*-test, $t_{17.275} = 2.168$, $P < 0.05$). No other parameters showed significant differences in means within and between seasons (independent sample *t*-test, $P > 0.05$).

4 | DISCUSSION

This study explored ontogenetic, sexual and seasonal variation in both morphological and biochemical parameters related to energetic state in *G. cirratum*. Results demonstrated that relationships among parameters were highly dependent on both life stage and sex, emphasizing

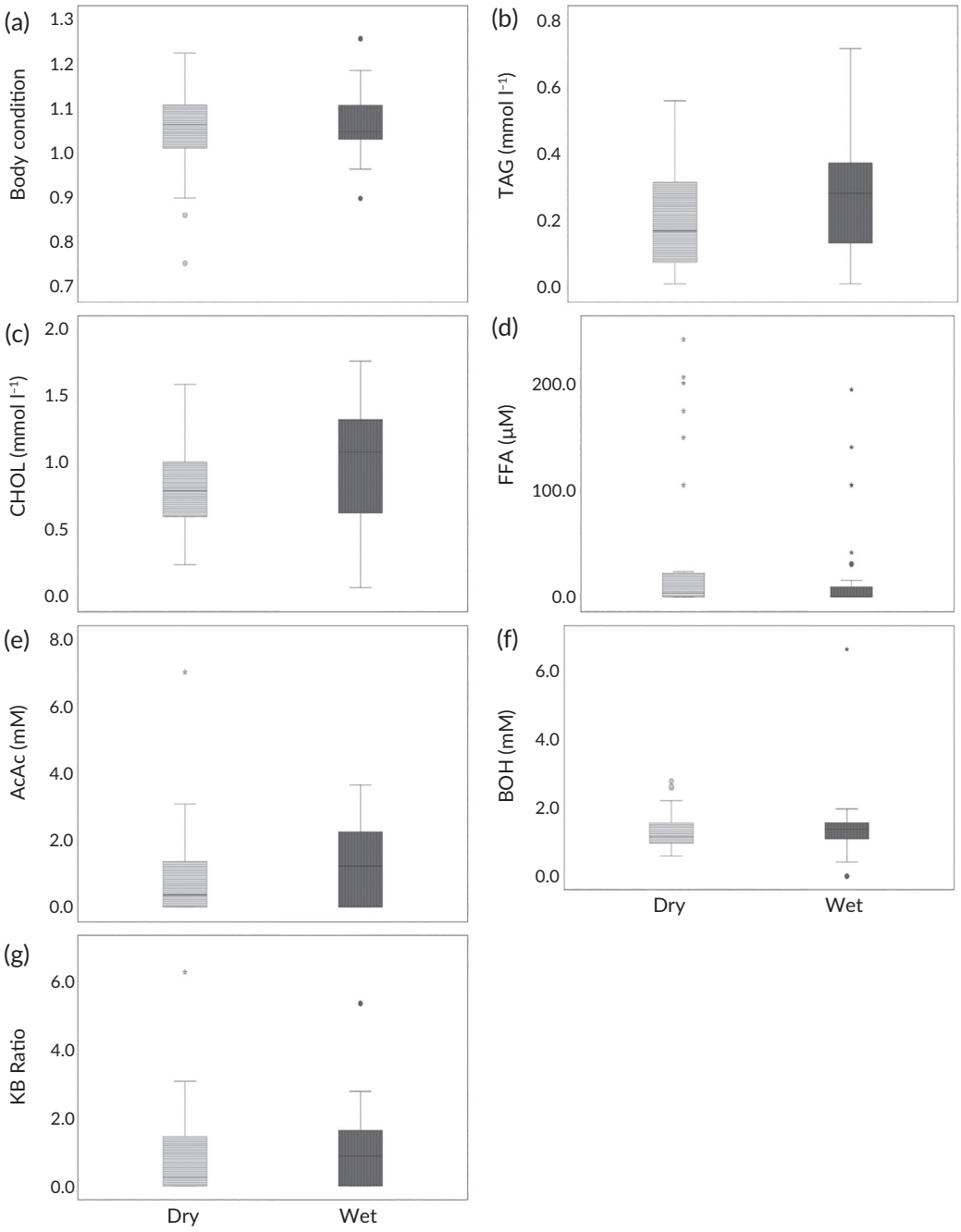

FIGURE 5 Boxplots depicting values of (a) body condition, (b) plasma TAG concentrations, (c) plasma CHOL concentrations, (d) plasma FFA concentrations, (e) plasma AcAc concentrations, (f) plasma BOH concentrations and (g) plasma KB ratios for mature *Ginglymostoma cirratum* in both the dry and wet seasons. *G. cirratum* sampled in the dry season are represented by light grey box plots and *G. cirratum* sampled in the wet season are represented by dark grey boxplots. Circles represent outliers (*i.e.*, values outside 1.5 × IQR) and stars represent extreme outliers (*i.e.*, values outside 3 × IQR)

the importance of assessing parameters both at the population level and for specific ontogenetic and sexual groups within the population. Furthermore, observed differences in parameters between mature female and male sharks were driven by seasonal variation within each sex, possibly related to reproduction, highlighting the need to incorporate temporal analysis when assessing shark body and nutritional condition.

The mean and range of *G. cirratum* body condition values found in the current study were much lower than those measured *via* span condition analysis by Irschick and Hammerschlag (2014); conversely, AtallahBenson *et al.* (2020) also employed span condition analysis to assess *G. cirratum* body condition, and the reference intervals established by the authors correspond with values reported in the present study. Similarly, mean values of TAG and FFA were

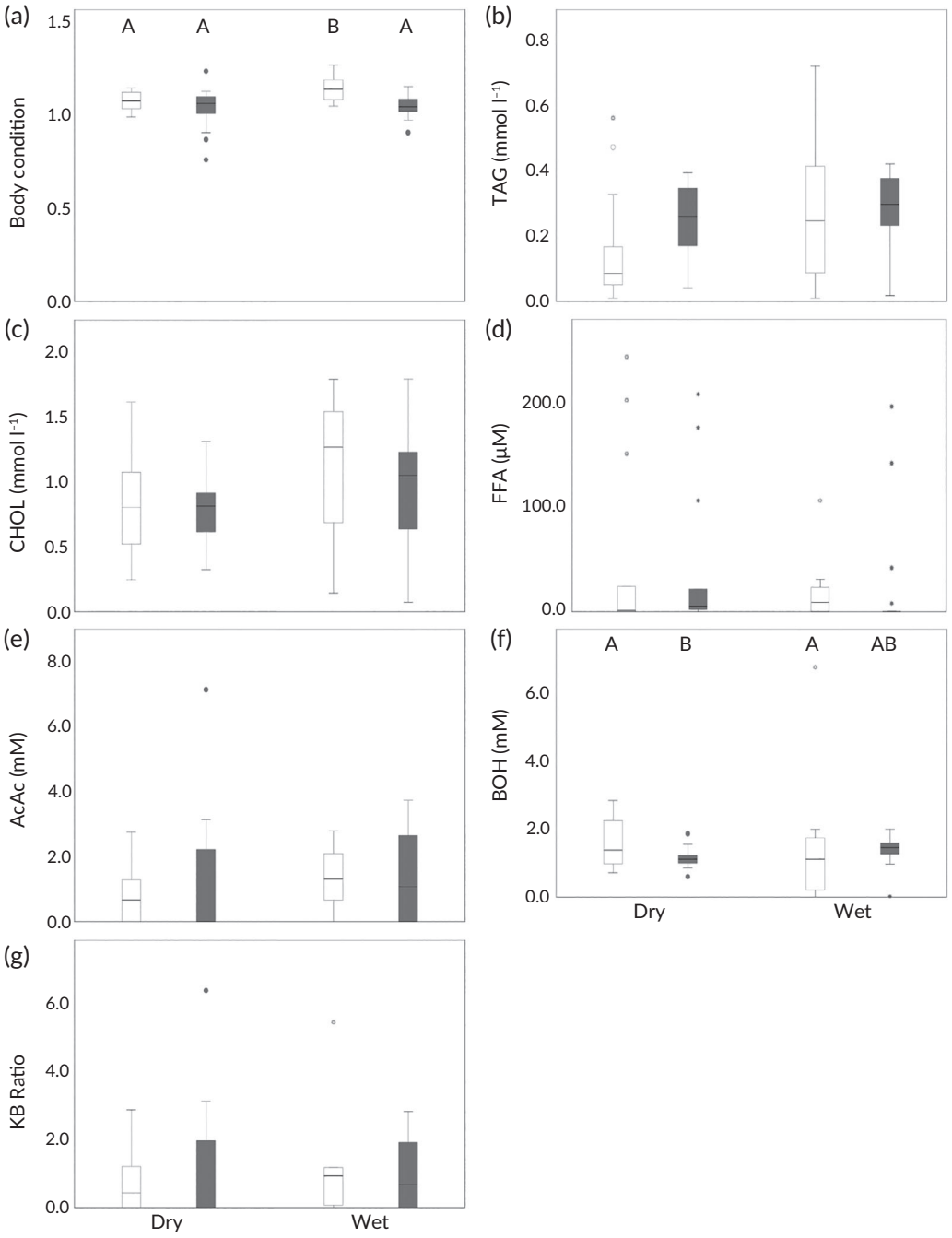

FIGURE 6 Boxplots depicting values of (a) body condition, (b) plasma TAG concentrations, (c) plasma CHOL concentrations, (d) plasma FFA concentrations, (e) plasma AcAc concentrations, (f) plasma BOH concentrations and (g) plasma KB ratios for mature female and mature male *Ginglymostoma cirratum* in both the dry and wet seasons. Mature female *G. cirratum* are represented by white boxplots and mature male *G. cirratum* are represented by dark grey boxplots. Circles represent outliers (*i.e.*, values outside 1.5 × IQR) and stars represent extreme outliers (*i.e.*, values outside 3 × IQR). Capital letters (*i.e.*, A, B) denote groups with significantly different means. Statistics were assessed by independent sample *t*-tests (mature female *G. cirratum* body condition in the wet season (B) × mature female *G. cirratum* body condition in the dry season (A), $P < 0.05$; mature female *G. cirratum* body condition in the wet season (B) × mature male *G. cirratum* body condition in the wet season (A), $P < 0.01$; mature female *G. cirratum* plasma BOH in the dry season (A) × mature male *G. cirratum* plasma BOH in the dry season (B), $P < 0.05$)

considerably lower than those reported for *G. cirratum* by Gallagher *et al.* (2017a), which utilized the same laboratory methodology as the present study. The noted discrepancies between the findings of the present study and prior research may be explained by differences in study site (Valls *et al.*, 2016). Though both Irschick and Hammerschlag (2014) and Gallagher *et al.* (2017a) sampled *G. cirratum* within Biscayne Bay and the Miami area, additional sampling occurred in the Florida Keys and the Bahamas, and Gallagher *et al.* (2017a) conducted further sampling within Everglades National Park. While all sampling locations occur within the subtropical western Atlantic

Ocean, they are discrete systems subject to differing abiotic factors and levels of anthropogenic influence; these differences may manifest as changes in habitat quality or prey availability between systems, and thus *G. cirratum* diet (Castro, 2000; Seal, 1978; Valls *et al*., 2016). Indeed, Biscayne Bay is the least protected of these sampling locations and may experience greater fishing pressure and anthropogenic impacts than other sites due to its proximity to the city of Miami, which may contribute to the relatively low values for body condition and plasma lipids reported here (Ault *et al*., 2001, 2020; Caccia & Boyer, 2005; Carey *et al*., 2011; Kellison *et al*., 2012).

Similar to previous ecophysiology studies (*e.g*., Garcia-Garrido *et al*., 1990), the different relationships among body condition and plasma metabolites, as well as KB ratio, found here in *G. cirratum* were dependent on both life stage and sex. These differing relationships may be related to the physiological roles of the plasma metabolites measured here and how these roles may vary with life stage and sex (Ballantyne, 1997). This was reported by Garcia-Garrido *et al*. (1990), which observed that male and female small-spotted catsharks *Scyliorhinus canicula* (L. 1758) mobilize different serum metabolites (*i.e*., cholesterol and triglycerides, respectively) during gametogenesis; furthermore, it is reasonable to assume that immature sharks that are not yet preparing for reproduction may mobilize these metabolites for other purposes (*i.e*., growth).

KB ratio is considered an indicator of cellular energy status in the liver, especially in mammals, where ketone bodies are exported into circulation and provide metabolic fuel during fasting (Rui, 2014). Given the increased importance of the liver and ketone bodies in elasmobranch energy metabolism relative to mammalian energy metabolism, measures of plasma KB ratio may be more related to elasmobranch energetic state than measures of plasma lipids (Ballantyne, 1997; Iwata *et al*., 1991; Lusseau & Derous, 2019; Tanaka *et al*., 1979; Yamamoto *et al*., 1980). Accordingly, the positive relationship found here between body condition and KB ratio in immature *G. cirratum* may suggest that body condition, measured *via* span condition analysis, is a good indicator of energetic state during this life stage. The lack of such a relationship in mature *G. cirratum* may imply that morphological body condition parameters may only reflect an organism's energy reserves during certain life stages or reproductive states (Hammerschlag *et al*., 2018; Hussey *et al*., 2009; Mesa & Rose, 2015; Næsje *et al*., 2006; Sardenne *et al*., 2016). Future research should continue to explore plasma KB ratio as an indicator of energetic state in elasmobranchs and its relationships with other parameters related to elasmobranch condition (*e.g*., HSI).

Within mature female sharks, positive relationships between body condition and TAG and body condition and CHOL were detected. Both metabolites are considered good indicators of nutritional condition in other taxa (*e.g*., Peres *et al*., 2014; Seal *et al*., 1975; Wagner & Congleton, 2004). Garcia-Garrido *et al*. (1990) noted positive correlations between liver weight and serum concentrations of both TAG and CHOL in female *S. canicula*; such a relationship in mature female *G. cirratum* may be mirrored by positive correlations between body condition, a rough proxy for liver size, and plasma TAG and CHOL concentrations. However, without integrated information on animal behaviour or reproductive state, it is still unclear whether concentrations of TAG in elasmobranch plasma are a result of endogenous or exogenous processes (Ballantyne, 1997; Gallagher *et al*., 2014; Garcia-Garrido *et al*., 1990).

In mature male sharks, plasma concentrations of TAG and CHOL were positively correlated. A positive relationship between these lipids has previously been reported in the serum and plasma of elasmobranchs of both sexes by Garcia-Garrido *et al*. (1990) and Valls *et al*. (2016) for *S. canicula*, and by Gallagher *et al*. (2017a) for pooled data from *G. cirratum*, *Carcharhinus leucas* (Müller and Henle, 1839) and *Carcharhinus limbatus* (Müller and Henle, 1839) sampled in subtropical Florida and the Bahamas. Although Valls *et al*. (2016) theorize that concentrations of TAG and CHOL are both related to food availability, as stated above, the extent to which exogenous processes drive lipid concentrations in elasmobranch plasma remains unknown (Gallagher *et al*., 2014).

The significantly higher variance in body condition, FFA and KB ratio present in immature *G. cirratum* may indicate that the energetic state of these sharks is more variable than that of mature individuals. It is likely that the foraging success of sharks increases with experience, size and age (*e.g*., Martin *et al*., 2009), therefore it is not surprising that immature *G. cirratum* would exhibit more variability in foraging success than mature sharks. Such variability could contribute to variability in energy reserves, manifesting as variation in body condition and KB ratio, as well as variability in plasma FFA values, which are known to fluctuate with recent feeding events (Wood *et al*., 2010).

Variance in plasma CHOL concentrations of mature sharks was significantly higher in the wet season than in the dry season, which may be related to increased variation in environmental salinity during this season. Results of studies that investigate the effect of salinity on CHOL in elasmobranch blood have been inconsistent. Griffith *et al*. (1973) noted that acclimation of freshwater stingrays (*Potamotrygon* sp.) to dilute seawater had no apparent effect on serum CHOL concentrations. Conversely, Armour *et al*. (1993) reported that the plasma CHOL concentrations of *S. canicula* increased with increasing plasma osmolality and environmental salinity. Studies (*e.g*., Evans & Nunez, 2015) have also shown that plasma concentrations of 1α-hydroxycorticosterone (1α-OH-B), an elasmobranch corticosteroid that may play a role in osmoregulation, vary with salinity. As CHOL is a corticosteroid precursor, it would be reasonable to assume that plasma concentrations of CHOL may change with demand for 1α-OH-B (Anderson, 2012). Environmental salinity within the Biscayne Bay study site is more variable during the wet season, especially near water management canals that pulse-release freshwater into the estuary (Armour *et al*., 1993; Ault *et al*., 2001; Lirman *et al*., 2008); more variable salinity may contribute to the increased variation in CHOL during the wet season reported here.

The significant sexual and seasonal variation in body condition reported here may be related to reproduction in mature *G. cirratum*. Sexual variation in body condition was driven by a significant increase in mature female body condition during the wet season; this spike may occur as females accumulate energy reserves to prepare for vitellogenesis, beginning in June or July (Castro, 2000; Hammerschlag

et al., 2018). Once vitellogenesis begins, females mobilize lipids from the liver for yolk formation, depleting their energy reserves until ovulation occurs between May and June of the following year (Castro, 2000). It is reasonable to assume that liver size is reduced as liver energy reserves are depleted, thereby reducing body condition value; this would result in mature female *G. cirratum* with higher body condition in the wet season (May–October), at the beginning of vitellogenesis, and lower body condition in the dry season (November–April), when the sharks approach ovulation and eggs are fully formed. A similar relationship was observed in *S. canicula* by Gutiérrez *et al*. (1988), where HSI and gonadosomatic index (GSI) were inversely correlated, and GSI reached a maximum just before spawning.

Given the high capacity for oxidation of BOH in elasmobranch red muscle and heart ventricle, it is thought that increased plasma concentrations of this ketone body can be indicative of heightened physical activity (Valls *et al*., 2016; Watson & Dickson, 2001). It is plausible that the pattern of increased BOH found in mature males during the wet season is related to the onset of mating, which greatly escalates the amount of physical activity male *G. cirratum* perform. Pratt and Carrier (2001) describe *G. cirratum* mating behaviour as "violent", reporting that male sharks swim more actively while "patrolling" for potential mates, frequently mate multiple times each day, compete with other males for access to mates and forcefully thrash to position willing females for copulation. Laboratory studies (*e.g*., de Roos, 1994; Wood *et al*., 2010; Zammit & Newsholme, 1979) also suggest that plasma BOH levels increase with fasting and starvation. Some studies on free-ranging sharks (*e.g*., Springer, 1967) have hypothesized that thin livers found in mature male sharks resulted from fasting during mating. Pratt and Carrier (2001) noted that they have not observed feeding, nor evidence of feeding, by sharks of either sex at the Dry Tortugas Courtship and Mating Ground (DTCMG). Therefore, it is possible that fasting does contribute to seasonal fluctuation in the plasma BOH levels of mature male *G. cirratum*; however, if feeding has not been observed in sharks of either sex, seasonal fluctuations in the plasma BOH levels of mature female *G. cirratum* would be expected as well.

It is not entirely clear why mature female *G. cirratum* have a higher BOH than male sharks throughout the year. While Pratt *et al*. (2018) reported that male and female *G. cirratum* partake in separate partial migrations in some areas, it is unknown if this results in higher activity levels for females than males. There is limited evidence to suggest that female *G. cirratum* feed less often than males, though some studies (*e.g*., Hussey *et al*., 2009) have hypothesized feeding is reduced for female sharks during gestation. If this occurs in *G. cirratum*, such fasting could result in elevated plasma BOH for mature females across seasons, as gestation begins in the wet season but ends in the dry season (Castro, 2000).

5 | CONCLUSIONS

Taken together, the results of this study highlight the significant influence of life stage, sex and season on the energetic state of *G. cirratum*. Relationships among measured parameters were unique to distinct life stage-sex subgroups, highlighting the need to evaluate condition and energetic state for not only the population as a whole, but also for subgroups of the population that may have differing energetic requirements. Sexual and seasonal variation in body condition and plasma BOH may be related to reproduction and mating, therefore future work should consider incorporating measures of reproductive state (*i.e*., ultrasounds, reproductive hormone analysis) to examine this relationship. Furthermore, integrating metrics of behavioural state into condition studies would help to further elucidate how energetic state is affected by physical activity. This study is limited by the exclusion of immature *G. cirratum* sampled during the wet season; future work should look to explore both seasonal variation in energetic state and how the incorporation of individuals sampled during the wet season alters relationships among parameters within immature *G. cirratum*. It is possible that the variations reported in this study are driven by factors that have not been measured here, such as spatial variability in environmental conditions or prey availability. Assessing spatial variation in these parameters will allow for further investigation into the factors that affect the energetic state of elasmobranchs and, possibly, lend insight to differences in habitat quality between areas. The results reported here provide important baseline data on parameters related to energetic state in *G. cirratum*, how these parameters vary and how these parameters are related to one another; given the continuous significant anthropogenic influence present in the study site, such baseline data is critical for future assessments of the energetic state of this population.

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CONTRIBUTIONS

S.G.M., A.J.G. and N.H. developed the ideas and research methodology. S.G.M. and N.H. collected data in the field. S.G.M performed data analysis, and S.G.M., A.J.G. and L.M. interpreted the data. S.G.M wrote the manuscript, with revisions by A.J.G., L.M. and N.H. S.G.M. prepared the manuscript for submission. Funding to support this research was secured by N.H.

ORCID

Shannon G. Moorhead https://orcid.org/0000-0001-7716-7616
Austin J. Gallagher https://orcid.org/0000-0003-1515-3440
Liza Merly https://orcid.org/0000-0002-0316-8197
Neil Hammerschlag https://orcid.org/0000-0001-9002-9082

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