

## Reproductive Stage and Clutch Size Incur Energetic and Oxidative Costs in an Endangered Iguana, *Ctenosaura oedirhina*

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**ABSTRACT.**—The Roatán Spiny-tailed Iguana (*Ctenosaura oedirhina*) is an endangered species endemic to only two islands in the Bay of Honduras and a few of its surrounding cays. *Ctenosaura oedirhina* is currently threatened by different impacts, including habitat modification, invasive predators, and illegal collection and harvesting. Although previous work has monitored population numbers, animal distributions, and genetic variation, very little is known about the reproductive ecology and physiology of the species. Physiological indicators provide valuable information regarding the health of animals and populations. Commonly used metrics include circulating hormone concentrations, energetic indicators, and immunological indices. However, the aforementioned physiological measures can also be difficult to interpret because they are not static but rather vary according to sex, season, reproductive state, and body condition. Therefore, to understand the health status of an individual better, it is important to concomitantly measure multiple related and relevant physiological systems, and to examine these responses under different individual and environmental contexts (e.g., reproductive state, site). In the current study, we measured circulating plasma oxidative stress markers and energy metabolites in five populations of Roatán Spiny-tailed Iguanas during the prenesting season. We found significant physiological variation in females according to breeding state, and males according to population. Understanding the natural variation in the physiology of free-living animals will allow us to determine the significance of physiological measures such as health indicators in the future and thus better manage these threatened populations.

The Roatán Spiny-tailed Iguana, *Ctenosaura oedirhina*, is endemic to only two islands in the Bay of Honduras and a few of their surrounding cays, and is listed as Endangered by the International Union for Conservation of Nature because of habitat fragmentation and illegal hunting (Goode and Pasachnik, 2019). In order to maintain a stable population, successful reproduction and recruitment is critical; however, little is currently known about reproductive ecology of *C. oedirhina* (Pasachnik, 2013), or its reproductive physiology. Producing offspring is widely accepted as a costly process for most species (Linden and Møller, 1989; Shine and Schwarzkopf, 1992), and reproduction would thus be expected to accrue significant costs in *C. oedirhina* as well.

*Ctenosaura oedirhina* is an oviparous species and therefore has distinct stages of follicle development (vitellogenesis, gravidity, and oviposition; Pasachnik, 2013). The oviparous condition provides a unique opportunity to quantify specific stage and female investment, as the associated costs vary with different stages of reproduction in most oviparous species. For example, during gravidity, reproductive females are susceptible to higher levels of predation because of increased basking and lower mobility (i.e., sprint speeds; Sinervo et al., 1991; Shine and Schwarzkopf, 1992). Additionally, females that have an experimentally increased number of follicles have lower survivorship than females with fewer follicles, presumably because of the high cost of reproduction (Olsson et al., 2001). In addition to the variety of ways that reproductive costs can be measured, inherent differences between sex, stages of reproduction, and reproductive strategies make quantifying the costs of reproduction difficult (Shine, 2015; Webb et al., 2019).

Recent work has demonstrated that oxidative stress is a relevant and widespread cost of reproduction found in many species. Oxidative stress is an accumulation of oxidative damage induced in an organism's cells by reactive oxygen species that arise from metabolic activity (Monaghan et al., 2009). Reactive oxygen species can be neutralized by antioxidants, which are substances that chemically stabilize reactive oxygen species so that they are no longer reactive (Shahidi and Ho, 2007). It is ideal to keep oxidative stress at a minimum, as high levels are damaging to cells that, when left unbalanced, can result in increased aging and mortality (Monaghan et al., 2009; Pizzino et al., 2017). Reproduction is one way in which oxidative damage can increase (Webb et al., 2019). During vitellogenesis, there is an increase in oxidative damage in reproductive females as compared to nonreproductive females; there is also more oxidative damage during vitellogenesis than other stages of reproduction (Webb et al., 2019). Oxidative damage may be caused by the deposition of antioxidants into yolk during vitellogenesis, rendering them unavailable to neutralize reactive oxygen species in the mother. In addition, high levels of triglycerides and glycerol are being circulated during vitellogenesis to both fuel the process and to reallocate energy into the yolk for nutrition. However, in birds and other reptiles reproduction, including the production of vitellogenin, may actually protect females from increased oxidative costs during reproduction and offspring rearing (Costantini et al., 2014; Lindsay et al., 2020). Reactive oxygen species are known to readily react with lipids which may be another reason for the increased levels of oxidative damage that occurs during vitellogenesis because there is more substrate with which oxidative species can react (Pérez-Rodríguez et al., 2015). For example, in *Coleonyx brevis*, caudal reserves, including lipids, are transported during vitellogenesis and depleted at oviposition

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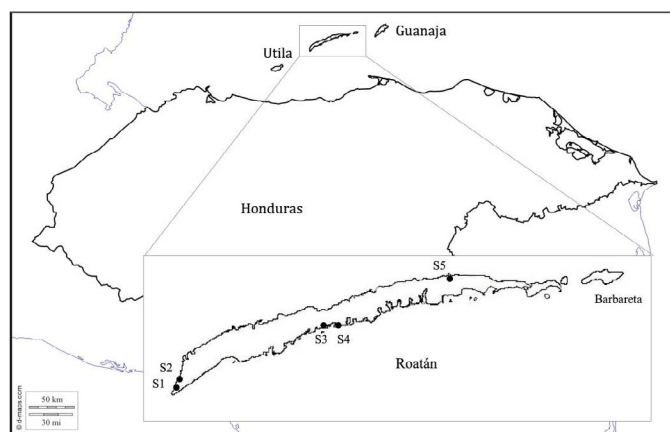


FIG. 1. Map of study populations on the island of Roatán Honduras labeled S1–S5 from west to east across the island.

(Dial and Fitzpatrick, 1981). Thus, oxidative damage and energy metabolites are highly correlated with reproduction and should be measured accordingly as accumulating damage and depletion of energy reserves are costly to survival.

Lastly, we are interested in comparing oxidative damage, reproductive investment, and energy metabolites across years and across the different geographic locations where *C. oedirhina* occurs. Our recent use of ultrasonography makes it possible to determine if females are breeding and to assess their clutch size and stage (size of follicles). *Ctenosaura oedirhina* are thought to be biannual breeders, which should allow us to compare both reproductive and nonreproductive individuals during the same season. In the current study we tested 1) whether reproductive investment (size and number of follicles) impacted energy metabolites and reactive oxygen species in the blood, 2) whether populations of iguanas from different sites differed in reproductive energetics and oxidative status, and 3) annual variation in these same parameters. We predicted that reproductive animals would have greater energy metabolites and reactive oxygen species than nonreproductive ones, and that these metrics would correlate with the degree of reproductive investment. We also expected variation across populations because of the environmental variability across sites where the iguanas are found, including both human impacts and habitat differences (e.g., substrate, habitat type—mangrove versus forest; Goode et al., 2016). Finally, because of the relatively constant climatic conditions, we did not expect significant annual differences.

#### MATERIALS AND METHODS

**Iguanas and Sampling.**—The Endangered *Ctenosaura oedirhina* is endemic to the Honduran islands of Roatán, Barbareta, and several small cays (Pasachnik et al., 2015) and is distributed narrowly in anthropogenically modified habitats that allow some protection from harm (Goode et al., 2016). Animals (male,  $n = 109$ ; female,  $n = 35$ ) were caught by lasso or by hand in April of 2018 and 2019 at five different localities across the island of Roatán (numbered S1–S5 from west to east; Fig. 1). Animals were individually marked using PIT tags, as well as visually by beads (Rodda et al., 1988) and by nontoxic paint so that we avoided the capture of the same individuals during each of the sampling periods. Adults were determined to be 150 mm and larger (Pasachnik, 2013). Animals were in breeding condition during

April and most females were vitellogenic at the time of capture. A blood sample was collected from the caudal vein of adult iguanas within 3 min to control for stress-induced changes in physiology, and the average time to sample collection was  $153.32 \pm 8$  ( $\pm$ SE) sec. Blood samples did not exceed 0.5 mL and were collected with a 25-gauge heparinized 3-mL syringe (305270, BD Integra, New Jersey, USA). Blood samples were collected between 0837 and 1405 h. There was no correlation between any physiological measure and time of day ( $r^2 < 0.017$ ) or time to acquire blood sample ( $r^2 < 0.001$ ). Iguanas were temporarily held in cloth bags until processing to obtain morphometric data and determine sex by probing for hemipenes. Female reproductive state and follicle number were determined by ultrasonography (for procedure details see French et al., 2017). Because females were still investing yolk in follicles, size of follicle (diameter in centimeters) was used to stage females in a continuous fashion and not as a measure of investment. Number of developing follicles or clutch number was used to assess investment. Blood samples were stored on ice for up to 6 h, after which a whole-blood glucose test was performed (Aviva AccuChek Aviva Plus, Roche Diagnostics, Indiana, USA) with subsequent separation of plasma by centrifugation at 6,000 rpm ( $2,000 \times g$ ) for 10 min. Plasma samples were stored at 4°C for up to 6 d until processing and were never frozen prior to our assays. All animal handling and procedures were approved by Utah State University IACUC (2530 USU).

**Reactive Oxygen Metabolites.**—Oxidative status was measured using reactive oxygen metabolite species (dROMS). Circulating dROMS were quantified using an assay kit (MC435, Diacron International, Italy) which detects levels of hydroperoxides that oxidize an alkyl-substituted aromatic amine (A-NH<sub>2</sub>). Plasma was diluted in the provided acidic buffered solution (10  $\mu$ L: 100  $\mu$ L) following manufacturer instructions for ‘endpoint’ mode and French et al. (2017) methods and incubated for 90 min at ambient temperature. An additional 800  $\mu$ L of dH<sub>2</sub>O was added just prior to reading absorbance to attain the necessary volume to read samples from a semimicro 1.5-mL cuvette (cell) with a portable spectrophotometer at 505 nm (Hatch DR1900, Loveland, Colorado, USA). Values were calculated as absorbance change relative to the standard. The intra-assay coefficient of variation (CV) was 2.8% (2018), 3.4% (2019), and the interassay CV was 4.7%.

**Energy Metabolites.**—Two lipid-related plasma components (triglycerides and free glycerol) were measured using sequential enzymatic color endpoint assays (F6428, T2449, and G7793, Sigma-Aldrich, Missouri, USA). The manufacturer’s protocol and a dilution protocol (Guglielmo et al., 2002) were followed. The free glycerol reagent was added to 10  $\mu$ L of plasma, incubated for 15 min at ambient temperature, and the absorbance was measured at 505 nm with a portable spectrophotometer (Hatch DR1900). The procedure allowed for the measurement of free glycerol, which is indicative of triglyceride breakdown at the time of blood collection. Next, a lipase reagent was added to dissociate the fatty acids from the glycerol backbone of triglyceride molecules. The cuvette was again incubated for 15 min at ambient temperature, and absorbance was measured at 505 nm to estimate triglyceride concentration. The intra-assay coefficient of variation (CV) was 6.2% (2018), 5.3% (2019), and the interassay CV was 8.6%.

**Statistical Analysis.**—For all analyses except glucose, the sexes were analyzed separately because of known significant sex differences in both morphological and physiological variables for most iguanid species (French et al., 2017; Webb et al., 2019). We statistically tested for glucose differences among the sexes in the

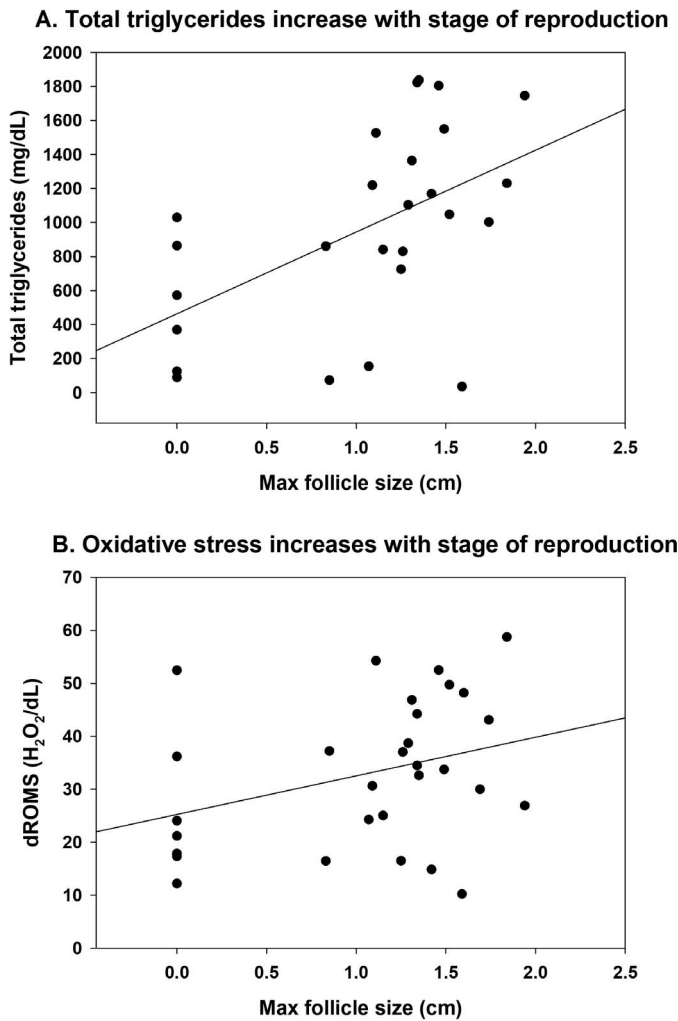


FIG. 2. The relationship between (A) maximum follicle size (cm) and circulating blood triglycerides and (B) maximum follicle size (cm) and reactive oxygen metabolites in female iguanas. There was a significant positive relationship between maximum follicle size (indicative of reproductive stage) and both parameters ( $P < 0.05$ ).

present study and found no significant differences ( $F_{1,135} = 0.409$ ,  $P = 0.524$ ). All data were log transformed to meet assumptions of normality. We used ANOVAs and GLMs to test for sex, site, and annual differences in body condition, energy metabolites, clutch size (females only), and reactive oxygen species. We followed a similar procedure for glucose, but omitted sex from the models. Tukey's honestly significant difference (HSD) post hoc comparisons were made to probe specific differences. Finally, correlations among clutch size, reactive oxygen metabolites, and energy metabolites were conducted in females using bivariate analyses. All analyses were conducted using JMP 12.1.0 (SAS Institute, Inc.) with an  $\alpha = 0.05$ .

## RESULTS

**Reproductive Effects in Females.**—Within females, body condition (residual of the regression of body mass on snout-to-vent length) does not significantly affect reproductive investment, nor do any of the physiology measures ( $F \leq 1.573$ ,  $P \geq 0.221$  in all tests), and so we did not include body condition in other models. Maximum follicle size is indicative of stage of investment, because females are not fully vested (i.e., vitellogenic and eggs

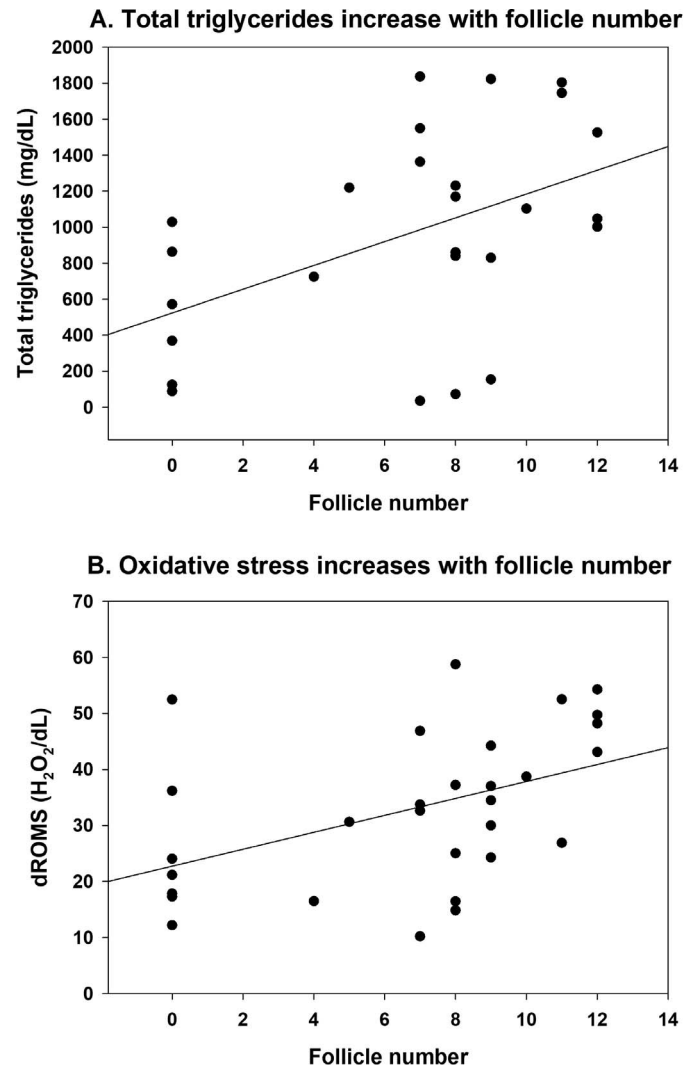


FIG. 3. The relationship between (A) clutch size (number of developing follicles) and circulating blood triglycerides and (B) clutch size and reactive oxygen metabolites in female iguanas. There was a significant positive relationship between clutch size (indicative of reproductive investment) and both parameters ( $P < 0.05$ ).

not yet shelled). Maximum follicle size is positively related to both total and true triglycerides ( $F \geq 5.627$ , all  $P \leq 0.0260$ ;  $R^2 = 0.19$  in all models; Fig. 2A), but was not related to free glycerol or glucose ( $F \leq 0.168$ ,  $P \geq 0.685$  in all models), and only a weak relationship with dROMS (Fig. 2B;  $F_{1,28} = 3.301$ ,  $P = 0.080$ ;  $R^2 = 0.11$ ). When we restricted our analyses to reproductive females the relationships between maximum follicle size with triglyceride measures and with dROMS were not significant ( $F \leq 1.877$ ,  $P \geq 0.188$  in all models). Therefore, breeding status (e.g., reproductive vs. nonreproductive individuals) rather than current follicle size appeared to be driving the observed relationships.

Total number of follicles (i.e., clutch number) was positively related to total and true triglycerides and with dROMS ( $F \geq 4.630$ ,  $P \leq 0.042$ ,  $R^2 \geq 0.16$  in all models; Fig. 3), but not free glycerol or glucose ( $F \leq 0.435$ ,  $P \geq 0.515$  in all models). When we considered only reproductive females, the relationships among follicle number and dROMS and free glycerol were significant ( $F \geq 6.224$ ,  $P \leq 0.021$  in all models) but triglyceride measures were not related to the other variables ( $F \leq 1.111$ ,  $P \geq 0.306$ ). Therefore breeding (e.g., reproductive vs. nonreproduc-

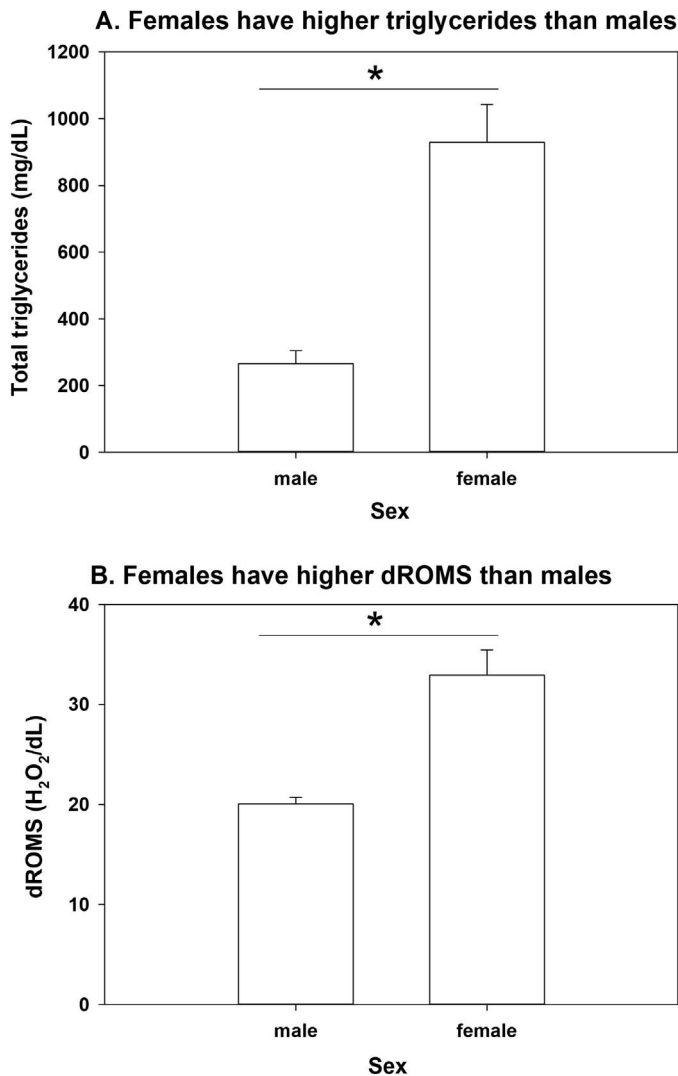


FIG. 4. Differences in circulating blood (A) triglycerides and (B) reactive oxygen metabolites between female and male iguanas. Asterisks denote statistically significant difference ( $P < 0.05$ ). In both cases females have significantly higher circulating levels ( $P < 0.05$ ).

tive individuals) is driving the relationship with triglycerides rather than follicle number *per se*.

**Population Membership Effects.**—Sex significantly affected all physiological variables ( $F \geq 35.309$ ,  $P \leq 0.001$ ; Fig. 4), except glucose ( $F_{1,135} = 0.409$ ,  $P = 0.524$ ) and free glycerol ( $F_{1,134} = 0.024$ ,  $P = 0.878$ ). Therefore, we analyzed the sexes separately for all variables except the aforementioned metabolites.

Comparing across populations of both sexes, glucose level was significantly different in one population ( $F_{5,132} = 6.254$ ,  $P < 0.001$ ; Fig. 5), and post hoc comparisons revealed that individuals from the easternmost locality (S5) had significantly higher glucose levels than did individuals from the other localities. Free glycerol did not vary among individuals from populations ( $F_{5,131} = 1.899$ ,  $P = 0.099$ ). When examining sexes separately, we observed several additional population-level differences among males, but no other significant population-level differences for female physiology ( $F_{4,29} < 2.370$ ,  $P > 0.056$ ). For males, there was a locality effect on body condition ( $F_{4,104} = 3.262$ ,  $P = 0.015$ ; Fig. 6A), where body condition was highest at S2 ( $t = 2.65$ ,  $P < 0.001$ ), intermediate at S1, S3, and S4 (i.e., not different from any sites), and significantly lower at easternmost

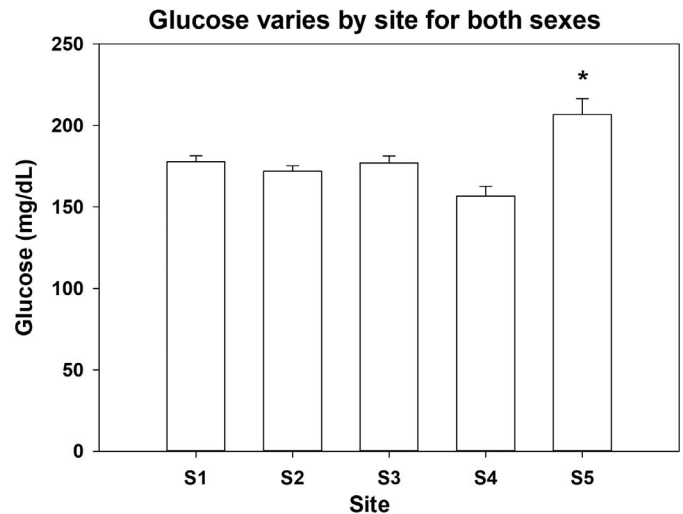


FIG. 5. Differences in blood glucose levels among study sites. Blood glucose varies significantly across different study sites, but not by sex. Asterisks denote statistically significant difference ( $P < 0.05$ ). Sites are numbered S1–S5 from west to east across the island.

site on the island, S5. Therefore, we included body condition in subsequent statistical models. Reactive oxygen metabolites (dROMS) also varied by locality ( $F_{6,101} = 5.840$ ,  $P < 0.001$ ; Fig. 6B), with the highest levels occurring in individuals from S1 ( $t = 4.50$ ,  $P < 0.001$ ), individuals from the three westernmost localities were significantly lower than those from other localities (S3, S4, S5) and those individuals from S2 were intermediate in value. Male energy metabolites did not significantly differ among sites ( $F_{4,81} \leq 1.421$ ,  $P \geq 0.236$  in all tests).

**Annual Differences.**—Free glycerol level in males was higher in 2019 than 2018 (Table 2;  $F_{1,105} = 24.921$ ,  $P < 0.001$ ), but no other annual effects were observed ( $F \leq 2.857$ ,  $P \geq 0.094$  in tests). For females, there were marginal year effects, with greater reproductive investment in terms of size (stage) ( $F_{1,33} = 3.069$ ,  $P = 0.089$ ; Table 2) and number of follicles ( $F_{1,33} = 4.037$ ,  $P = 0.053$ ; Table 2) in 2018. However, the annual effect seems to be driven by a relatively high number of nonbreeding animals in 2019 (Fig. 7) because if we only include reproductive animals in the analysis, the year effect is no longer significant ( $F \leq 1.676$ ,  $P \geq 0.207$  in all models). When we considered only reproductive females, we also found that both total and true triglycerides were higher in 2018 ( $F \geq 4.471$ , all  $P \leq 0.049$  in all models; Table 2). There were no other annual differences in body condition or other physiological variables present in females ( $F \leq 2.862$ ,  $P \geq 0.106$  in all models; Table 2).

## DISCUSSION

We find support for our predictions that reproductive females have elevated oxidative stress and circulating energy metabolites relative to nonreproductive females, and these changes also correspond with reproductive stage of investment and overall clutch size. As expected, we also observed population differences in some physiological metrics in male, but not in female, iguanas suggesting some site-specific differences existed. Specifically, body condition and reactive oxygen metabolites varied among sites in male iguanas. However, reproductive physiological costs in females are likely masking any site-level differences that may be present in females. Finally, our

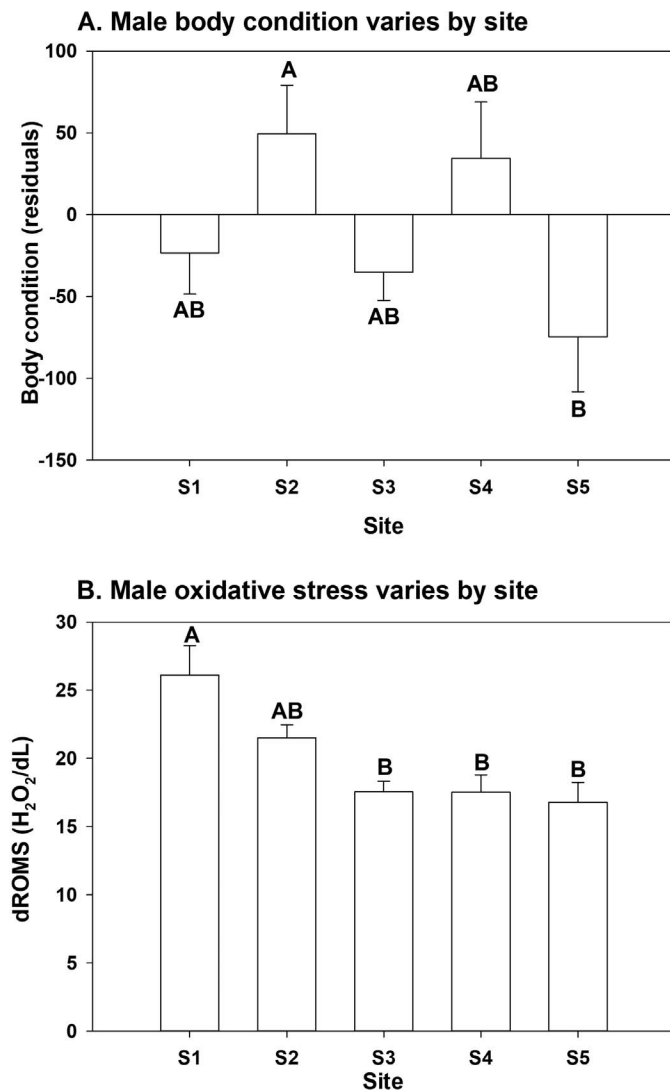


FIG. 6. Differences in mean male (A) body condition and (B) blood levels of reactive oxygen metabolites among study sites. Different letters denote statistically significant difference ( $P < 0.05$ ). Sites are numbered S1–S5 from west to east across the island. No such differences were present for females across sites.

prediction that annual differences would not exist is not supported, instead we see marginal differences between years in energetics and reproductive investment.

Female *C. oedirhina* with larger eggs and clutches exhibited higher reactive oxygen metabolites (dROMS) in their blood plasma that could indicate that reproductive females are in a state of oxidative stress. In Bahamian Rock Iguanas (*Cyclura cyclura*) reproductive investment (follicle number) was associated with higher oxidative stress markers, lower antioxidant capacity, and a higher overall oxidative index, which suggests that reproduction is incurring similar oxidative costs in a related species (Webb et al., 2019). Oxidative stress may therefore be a contributing factor along with other reproductive costs (i.e. energy expenditure) that limit investment into future reproductive events (Plot et al., 2012; Stier et al., 2012; Costantini and Dell'Omo, 2015; Costantini et al., 2016), or lead to reduced longevity or survival probability (Plot et al., 2012; Costantini and Dell'Omo, 2015). However, if animals have sufficient antioxidants to deal with these elevations, then there may be no long-term consequences. Moreover, some changes in dROMS

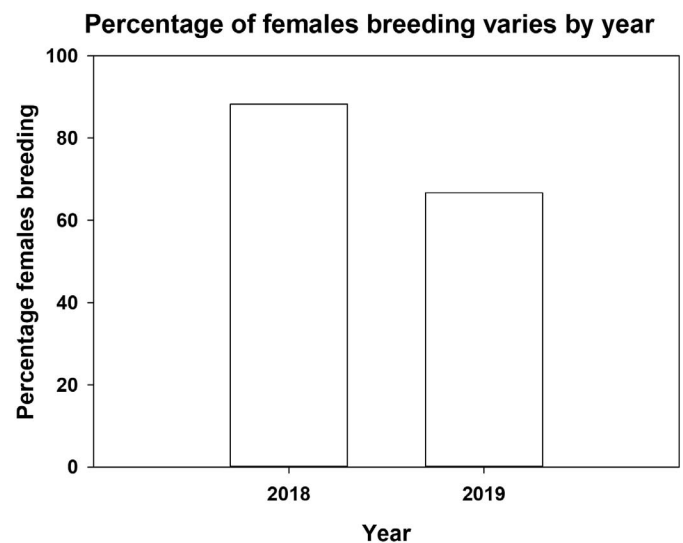


FIG. 7. The percentage of breeding female iguanas in years 2018 and 2019. The percentage of breeding females is lower in 2019 relative to 2018.

may be transient and not exert long term effects (Sudyka et al., 2016). It is also important to note that the relationship between oxidative stress and reproduction in ecology is highly debated (Isaksson et al., 2011; Metcalfe and Monaghan, 2013; Speakman and Garratt, 2014), and so, investigating temporal changes in oxidative physiology throughout the breeding season and across environmental contexts is an important next step.

We also found evidence of locality-specific context effects on iguana physiology indicating that there are environmental pressures that cause differences in physiology across populations. Female and male *C. oedirhina* at eastern sites on Roatán exhibited higher circulating blood glucose levels compared to individuals at other sites, meriting several potential explanations. Altered blood glucose levels through food supplementation by tourists could indicate that *C. oedirhina* populations across the islands may vary in their diet. Endangered Bahamian Rock Iguanas (*Cyclura cyclura*) exhibit higher blood glucose levels on islands where tourists visit and feed grapes to the iguanas in contrast to populations on nonvisited islands (Knapp et al., 2013). Alternatively, differences in blood glucose could also be indicative of an acute stress state. Vertebrates immediately respond to external stressors by the release of glucose from glycogen into glucose, so that glucose can be diverted to other parts of the body (Wingfield et al., 1998; Sapolsky et al., 2000). Hence, higher blood glucose levels could indicate iguanas at the most eastern site (S5) are in a state of physiological stress. Furthermore, males from eastern populations are in lower body condition that could exacerbate the effects of physiological stress (Kitaysky et al., 1999). Blood glucose levels are highly labile following internal and external changes; thus, it is unclear whether population glucose varies due to diet or levels of stress. Future work should focus on what other site-specific pressures may be leading to differences in physiology among populations, such as habitat fragmentation, food availability, and human disturbance, all of which may affect reproductive physiology, output, and the costs reproduction incurs. In particular, characterization of diets among populations, including human provisioning that has been documented at all sites (Pasachnik, pers. obs.), and measuring blood corticosterone, an energy-

TABLE 1. Mean physiological parameters in male and female iguanas across study sites.

Site	Sex	n	Glucose (mg/dL)	Max follicle diameter (cm)	Follicle number	dROMS (H <sub>2</sub> O <sub>2</sub> /dL)	Total triglycerides (mg/dL)	True triglycerides (mg/dL)	Free glycerol (mg/dL)	SVL (mm)	Weight (g)
S1	M	20	182.30 ± 3.84			26.10 ± 2.16	3.77 ± 1.11	3.48 ± 1.06	0.24 ± 0.03	243.85 ± 7.37	613.50 ± 48.72
	F	11	167.22 ± 8.47	1.07 ± 0.18	7.82 ± 1.26	36.21 ± 4.91	9.73 ± 2.15	9.06 ± 2.03	0.38 ± 0.06	206.73 ± 3.47	393.55 ± 19.63
S2	M	30	171.67 ± 3.74			21.51 ± 0.97	2.87 ± 0.71	2.47 ± 0.68	0.34 ± 0.04	263.23 ± 7.42	819.20 ± 69.79
	F	6	173.40 ± 8.74	0.96 ± 0.32	6.00 ± 2.21	33.34 ± 6.21	13.53 ± 2.50	12.78 ± 2.32	0.28 ± 0.06	211.83 ± 11.42	466.67 ± 87.04
S3	M	34	174.00 ± 4.23			17.54 ± 0.79	2.10 ± 0.72	1.64 ± 0.64	0.33 ± 0.04	236.65 ± 5.80	552.21 ± 36.77
	F	11	185.30 ± 12.52	0.94 ± 0.18	6.91 ± 1.19	32.22 ± 3.88	8.73 ± 1.62	8.21 ± 1.54	0.26 ± 0.05	203.00 ± 6.70	363.55 ± 31.38
S4	M	11	155.09 ± 6.77			17.52 ± 1.27	1.36 ± 0.33	1.01 ± 0.30	0.37 ± 0.05	285.82 ± 7.59	959.27 ± 75.55
	F	2	165.50 ± 11.50	0.75 ± 0.75	3.50 ± 3.50	25.52 ± 8.21	8.37 ± 7.12	7.75 ± 7.02	0.36 ± 0.18	208.50 ± 13.5	525.00 ± 240.00
S5	M	14	205.92 ± 10.05			16.77 ± 1.48	9.79 ± 7.43	9.22 ± 7.24	0.93 ± 0.59	221.50 ± 10.50	408.71 ± 46.59
	F	5	209.00 ± 26.05	1.20 ± 0.32	5.60 ± 1.63	30.74 ± 9.04	8.77 ± 3.04	7.84 ± 2.94	0.69 ± 0.33	184.80 ± 15.83	259.20 ± 56.57

TABLE 2. Mean male and female iguana physiology by year.

Sex	Year	n	Glucose (mg/dL)	Max follicle diameter (cm)	Follicle number	dROMS (H <sub>2</sub> O <sub>2</sub> /dL)	Total triglycerides (mg/dL)	True triglycerides (mg/dL)	Free glycerol (mg/dL)
Male	2018	36	176.44 ± 4.33			21.31 ± 1.01	5.48 ± 2.70	0.43 ± 0.23	5.18 ± 2.63
	2019	73	177.07 ± 3.24			19.41 ± 0.83	2.55 ± 0.57	0.38 ± 0.03	2.05 ± 0.54
Female	2018	17	175.85 ± 12.09	1.20 ± 0.12	8.06 ± 0.93	35.64 ± 3.54	12.36 ± 1.62	0.26 ± 0.03	11.67 ± 1.53
	2019	18	184.17 ± 7.65	0.84 ± 0.16	5.33 ± 0.98	30.82 ± 3.57	6.86 ± 1.19	0.46 ± 0.11	6.18 ± 1.12



mobilizing hormone involved in the vertebrate stress response, would both help to further elucidate this relationship.

Even though we did not observe population differences in body condition, energy metabolites, or oxidative stress markers in female *C. oedirhina*, we suspect that our observations may have been due to the confounding effect of reproductive state in females. In fact, Pasachnik (2013) observed differences in body condition across populations when sampling throughout the year. During vitellogenesis, yolk precursors are produced in the liver and begin to circulate in the blood (Wallace, 1985). Increased circulation of fats and proteins could very well influence energy metabolite levels and metabolic rate. In European Starlings (*Sturnus vulgaris*), metabolic rate increased 22.4% during follicular development compared to females possessing no follicles (Vézina and Williams, 2002). In some viviparous snake species, metabolic rate can increase up to 30% during vitellogenesis (Van Dyke and Beaupre, 2011). Therefore, failing to account for the physiological change during reproduction may mask potential relationships or trends. Given that metabolic processes involved in vitellogenesis may lead to a change in oxidative state (Webb et al., 2019), the increase in metabolic demands of egg formation may alter female oxidative physiology as well. Our sample sizes, however, were too small across populations to determine whether reproductive state (presence or absence of follicles) affected physiology and varied across populations. Thus, increasing sampling of female iguanas across populations during the breeding season will allow us to understand landscape variation in reproductive rates and how female physiology changes with breeding condition better.

It is clear that body condition and oxidative stress markers varied in male iguanas across populations. Specifically, we found that males from the westernmost populations (i.e., sites S1, S2) on the island exhibited higher body-condition values and higher oxidative stress markers when compared to eastern populations. We posit that the geographic patterns could be due to a combination of factors, including variation in human presence and associated food provisioning, variation in food abundance and quality, variation in population density, or variation in genetics. Specifically, the western populations of *C. oedirhina* occupy more populated vacation resorts and tourist parks and experience increased food provisioning from locals and tourists compared to eastern populations, which may increase body condition (Pasachnik, 2013). Males are also more aggressive and competitive and therefore more likely to procure supplemental food resources from tourist sites than would females, thereby explaining why geographic patterns in body condition and oxidative stress were not evident in females. Enhanced diet because of anthropogenic activities has been shown to lead to higher body-condition values compared to unfed populations in several large reptilian species (Jessop et al., 2012; Knapp et al., 2013; Smith and Iverson, 2016), mammals (Eley et al., 1989), and avian reptiles (Auman et al., 2008). However, higher body condition via food supplementation does not necessarily equate to being healthier. Rock Iguanas on tourist-visited islands exhibited higher body condition but also had higher parasitic infections and biochemical indicators of poorer health (Knapp et al., 2013) that may suggest a health cost to anthropogenic feeding. Alternatively, variation in body condition across sites may be driven by food availability, diet quality (Naya et al., 2007), diet diversity (Goode et al., 2016, 2020), or population density that may lead to differential competition for resources (Reading and Clarke, 1995; Gaidet and Gaillard, 2008).

Although diet can affect body condition, it may also influence oxidative status. Males from westernmost populations had higher oxidative stress markers compared to those in more eastern populations. Antioxidants are important for maintenance of cellular processes and neutralize ROS that would otherwise cause damage to biomolecules (Shahidi and Ho, 2007). The effects of diet-derived antioxidants, such as carotenoids, Vitamin C, Vitamin E, and polyphenols, on oxidative stress is largely unstudied in reptiles, and its actual effectiveness at mediating oxidative stress is debated (Pamplona and Costantini, 2011). However, obtaining dietary antioxidants via frugivory is a possible dietary antioxidant strategy. Fruit-eating bats species had higher antioxidant capacity and lower reactive oxygen metabolites compared to omnivorous bats (Schneeberger et al., 2014). *Ctenosaura oedirhina* regularly consume fruit (Gandola and Hendry, 2011; Pasachnik, 2013), suggesting a potential link between diet and oxidative status, but this must be empirically tested with the addition of antioxidant level measurements. Conversely, there is genetic differentiation among populations of *C. oedirhina* (Pasachnik and Hudman, 2016), which may lead to underlying differences in oxidative status or susceptibility (Costantini and Dell'Omo, 2006). Without further investigation, differences in body condition and oxidative stress of male *C. oedirhina* across Roatán remain unresolved.

Finally, we observed differences in proportion of animals breeding between years with 88.2% in 2018 and 66.7% in 2019. Within breeding animals, there were also significantly higher circulating triglycerides that reflect relatively high reproductive effort. Looking at annual weather differences, 2018 was a warmer and drier year than 2019 (WorldWeatherOnline.com). Annual differences in reproduction that correspond with weather have also been observed in Jamaican Iguanas (*Cyclura collei*; Pasachnik, pers. obs.) whereby in drier years animals breed sooner and incubation time is relatively short. In the current study we also found that free glycerol was higher in males in 2019 relative to 2018. Similarly, Galápagos Marine Iguanas (*Amblyrhynchus cristatus*) used subtle differences in food quality, associated with seasonal but annually variable changes, to initiate breeding (Rubenstein and Wikelski, 2003). It is unclear if the observed patterns in Marine Iguanas were due to reduced reproductive efforts at the time of sampling as in the females, or environmental changes in resource and energy availability causing a shift in timing of reproductive activities.

Overall, the addition of physiological indicators of energy and oxidative stress to iguana ecology and conservation provide valuable information regarding the health of individuals and populations. Studying the reproductive costs of this species will allow us to understand which physiological measures are health indicators and allow for the better maintenance of the species' survival (Wikelski and Cooke, 2006). Presently, we are not only observing differences emerge in terms of locality level effects, but also in reproduction. Information on geographic variation in oxidative stress and reproduction will inform us as to what resources are necessary to maintain reproductively viable individuals, and which potential perturbations may be problematic for maintaining healthy reproducing populations. Furthermore, quantification of oxidative stress and reproductive variables over space and time may also reveal climatic or environmental changes that influence both the physiology and reproductive success of populations.

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