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Interactive Effects of Food Supplementation and Snake Fungal Disease on Pregnant Pygmy Rattlesnakes and Their Offspring

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Abstract.—**In viviparous organisms, the ability to feed while pregnant may mitigate energetic trade-offs experienced during the reproductive process and enhance fecundity. However, anorexia during pregnancy has been reported in many crotaline snakes. The potential costs and benefits of feeding while pregnant are not completely described in the literature, and experimental studies have been conducted in a limited number of taxa, rendering our understanding of the forces that may underlie the evolution of anorexia in pregnant snakes incomplete. Here, we examine the impact of food supplementation during mid to late pregnancy on mothers and offspring in a viviparous crotaline snake species, the Pygmy Rattlesnake (*Sistrurus miliarius*). Specifically, we offered multiple large meals to mothers held in outdoor enclosures and measured a suite of maternal and offspring traits including maternal body condition, offspring length and mass, maternal disease state, and offspring foraging behaviors. We focused on interactions between feeding, clinical signs of snake fungal disease (SFD), and the presence of its causative agent, *Ophidiomyces ophiodiicola*, in mothers. Pregnant females fed readily when offered food, but feeding did not impact offspring traits. Food supplementation significantly increased maternal postparturient body condition, but also increased clinical signs of disease in mothers and led to a significantly higher reproductive failure rate in the treatment group, particularly in mothers afflicted with SFD. Our results suggest that food supplementation during pregnancy may disrupt the reproductive process in pregnant rattlesnakes, and that such disruption is particularly pronounced in mothers suffering from SFD.**

Life history theory posits that reproductive effort constrains maternal energy budgets and, when energy is limited, results in trade-offs between current reproductive success and competing energetic functions (e.g., immunity or energy storage) that promote maternal survival and future fecundity (van Noordwijk and de Jong, 1986; Doughty and Shine, 1997; Lourdais et al., 2004). The ability to forage successfully during pregnancy would help mitigate such trade-offs, enhance maternal immunocompetence, and increase residual reproductive value (Gregory and Skebo, 1998; Gregory, 2006). However, observational studies suggest that many crotaline snakes are anorexic during pregnancy (Keenlyne, 1972; Macartney and Gregory, 1988; Beaupre, 2002; Crane and Green, 2008; Webber et al., 2012). Although the maternal benefits of feeding during pregnancy are intuitive, few studies have examined potential benefits to offspring beyond size or condition, and little work has demonstrated potential costs or trade-offs that may underlie the evolution of anorexia during pregnancy.

Offspring provisioning strategies are diverse in snakes and may fall along a spectrum from strict lecithotrophy to partial placentatrophy/matritrophy (Bonnet et al., 1998; Winne et al., 2006; Van Dyke and Beaupre, 2012; Van Dyke and Griffith, 2018). Viviparous crotaline and natricine snakes, for example, are predominantly lecithotrophic (Blackburn and Stewart, 2011), but can transfer nutrients, including amino acids, to developing offspring across a placenta (Van Dyke and Beaupre, 2012). In the limited number of taxa studied, stored energy and food intake during vitellogenesis influence litter characteristics (Ford and Seigel, 1989; Weatherhead et al., 1999; Bonnet et al., 2001; Lourdais et al., 2002, 2003). However, food supplementation during pregnancy increases maternal postpartum condition, but not offspring size characteristics (Gregory and Skebo, 1998; Lourdais et al., 2002; Gregory, 2006). Observed effects of feeding on reproductive traits can vary at fine phylogenetic scales (Seigel and Ford, 2001), and few studies have examined the effect of food supplementation during pregnancy on maternal or litter characters in crotaline snakes (but see Taylor et al., 2005).

Feeding habits during pregnancy are variable among the Crotalinae. Some species actively forage and feed throughout pregnancy (Schuett et al., 2013; Perez-Mendoza et al., 2018) whereas others are anorexic (Webber et al., 2012). Several hypotheses may explain the evolution of anorexia in animal life histories (Mrosovsky and Sherry, 1980). In pregnant snakes, the behavioral demands of pregnancy (e.g., thermoregulation and appropriate microhabitat selection) may conflict with behaviors that promote successful foraging (Bonnet and Naulleau, 1996; Gregory et al., 1999). Additionally, the physiological burden of carrying embryos and the associated increase in locomotor costs may impair the ability to forage successfully (Seigel et al., 1987). Others have suggested that physiological or anatomical constraints may underlie anorexia. For example, the position of embryos within the body cavity may constrain space available for large prey items (Gregory et al., 1999; Webber et al., 2012). Indeed, the volume of embryos alone has been shown to increase the costs of respiration and decrease respiratory efficiency in viviparous skinks (Munns and Daniels, 2007; Munns, 2013; Munns et al., 2015). Addition of a large food item to the body cavity may exacerbate this effect. Pregnancy is also associated with decreased immune performance in some pitvipers (Graham et al., 2011). It is possible that the large metabolic increments associated with feeding (specific dynamic action; SDA) and pregnancy (Jackson et al., 2015) push individuals near or over their maximum sustained metabolic scope (Peterson et al., 1990) and may force allocation away from other metabolically costly processes such as immunity (i.e., the metabolic ceiling hypothesis).

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Competing hypotheses explaining the evolution of anorexia during pregnancy in snakes have received little attention in the literature, and the effects of experimental food supplementation on mother and offspring have been described in a limited number of species (Seigel and Ford, 1992; Gregory and Skebo, 1998; Lourdais et al., 2002; Gregory, 2006). Even fewer studies have examined the effect of food supplementation in mothers faced with additional energetic challenges such as disease. Mounting an immune response is energetically costly (Sheldon and Verhulst, 1996; Lochmiller and Deerenberg, 2000), and trade-offs between reproduction and immunity may be particularly important in the face of competing energetic challenges such as snake fungal disease (SFD; Lorch et al., 2016). Snake fungal disease is prevalent in many crotaline populations in the eastern United States (Clark et al., 2011; McBride et al., 2015; Allender et al., 2016; Tetzlaff et al., 2017). The disease is caused by the fungus *Ophidiomyces ophiodiicola* (*Oo*), and clinical signs include dermal lesions, nodules, and crusts that may occlude sensory organs and impair successful foraging (Allender et al., 2011; Lorch et al., 2015, 2016). Infection is associated with reduced body condition (McCoy et al., 2017) and reproductive suppression (Lind et al., 2018a, 2019). Studies have not previously reported the impact of SFD on reproductive outcomes in pregnant female snakes or investigated the outcomes of supplemental feeding in infected mothers. Informed management and husbandry require a clear understanding of the reproductive consequences of SFD and the impacts of supplemental feeding on disease and reproductive outcomes.

Although previous studies suggest that feeding during late pregnancy does not affect offspring size, little work has investigated the possibility for maternal energetic status to impact offspring via maternal effects on offspring phenotypes other than size (Bernardo, 1996). In addition to the potential for provisioning across a placenta, the feeding response in snakes is associated with a suite of endocrine factors (Secor and Diamond, 1998) that may impact the developmental environment of offspring. Exposure to hormones that circulate in relation to energetic status (e.g., adrenal glucocorticoids; Lind et al., 2018b) during development can alter traits in viviparous squamates relevant to offspring fitness (Meylan and Clobert, 2004). In most reptiles, the ability of neonates to acquire food successfully shortly after birth is critical to survival until reproductive age and likely directly linked to fitness. In many viperid species, neonates use caudal luring to increase rates of prey capture (Rabatsky, 2008). Effective use of this behavior involves detecting prey and producing an appropriate response (Reiserer and Schuett, 2008) and can therefore serve as a proxy for offspring foraging ability. Maternal influences on luring behavior have not been investigated in viviparous squamates and may be valuable in identifying cryptic maternal effects of feeding on offspring quality.

Pygmy Rattlesnakes, *Sistrurus miliarius*, are small, viviparous, crotaline snakes native to the southeastern United States (Conant and Collins, 1998). Populations in central Florida breed in late summer and early fall, are vitellogenic in spring, and give birth from late July to early September (Farrell et al., 1995; May et al., 1996; Rowe et al., 2002; Lind et al., 2018b). Snake fungal disease has been endemic in the population for over 20 yr (Cheatwood et al., 2003). Afflicted individuals can survive and clear clinical signs of infection in the field (Lind et al., 2018c), but no work has examined outcomes in reproductive females. To add to our understanding of the impacts of feeding during pregnancy on mothers and offspring, we offered food to pregnant female Pygmy Rattlesnakes held in outdoor enclosures. We examined the amount of food consumed relative to body mass and described the postparturient state of mothers with respect to body condition, presence of clinical signs of disease, and abundance of *Oo*. Additionally, we quantified the rates of reproductive failure within each treatment group and recorded parturition date, and offspring size characteristics (mass and snout-to-vent length [SVL]). To examine potential cryptic maternal effects on offspring quality, we measured caudal luring intensity and latency to lure in response to a prey item 7–10 d after birth. We hypothesized that pregnant Pygmy Rattlesnakes accept food when offered and that supplemental feeding enhances reproductive success and residual reproductive value in mothers. We predicted higher body condition, lower prevalence of *Oo*, and less severe clinical signs of SFD in supplemented mothers postpartum. We did not predict that feeding would affect offspring size traits, but that it might affect offspring foraging behavior.

MATERIALS AND METHODS

Study Animals.—We collected 27 pregnant female Pygmy Rattlesnakes in western Volusia County, Florida, from 2 June to 1 August 2017. We visually examined snakes for clinical signs of skin lesions and nodules consistent with SFD and assigned each snake a skin lesion score between zero and three (see McCoy et al., 2017). We weighed and measured snakes in the field using a squeezebox and a Pesola® spring scale (PESOLA Präzisionswaagen AG, Switzerland) and used sterile swabs to sample the skin of each individual for the presence of *Oo* using methods described in detail by Lind et al. (2018a). We transported collected snakes to an isolated field site and housed them in one half of a subdivided 0.9 × 1.2–m outdoor enclosure identical to those used in Lind et al. (2017a). When possible, treatment and control groups were housed in paired enclosures to minimize any difference in microclimate experienced by each treatment group.

We randomly assigned pregnant females to control or treatment groups. Both groups were given water ad libitum. We offered treatment snakes a live Southern Leopard Frog (*Lithobates sphenocephalus*) approximately once per week (see Appendix). We captured frogs at the field enclosure site to ensure that no foreign pathogens were introduced by feeding. We recorded the mass of each frog on a digital balance in the lab and arranged prey mass to be approximately 10–40% the mass of each fed snake. We checked the enclosures daily following the introduction of the meal to determine if feeding had occurred. We removed frogs that remained in the enclosure after 2 d. Each snake in the treatment group was offered and consumed between one and four meals over the course of the study (see Appendix).

We examined the enclosures each morning for the presence of a litter. Upon observing a litter, we removed both the mother and offspring for processing the following day. We weighed each mother, assigned it a postpartum skin lesion score, and sampled again for the presence of *Oo* using epidermal swabs. We stored swabs at −80°C until shipment to the U.S. Geological Survey–National Wildlife Health Center (NWHC; Madison, Wisconsin, USA) for real-time polymerase chain reaction (PCR) analysis. Real-time PCR methods followed those for the "ITS assay" outlined in Bohuski et al. (2015). We used cycle threshold (Ct) values to estimate the abundance of *Oo* on skin swabs. We considered snakes positive for *Oo* when the threshold was

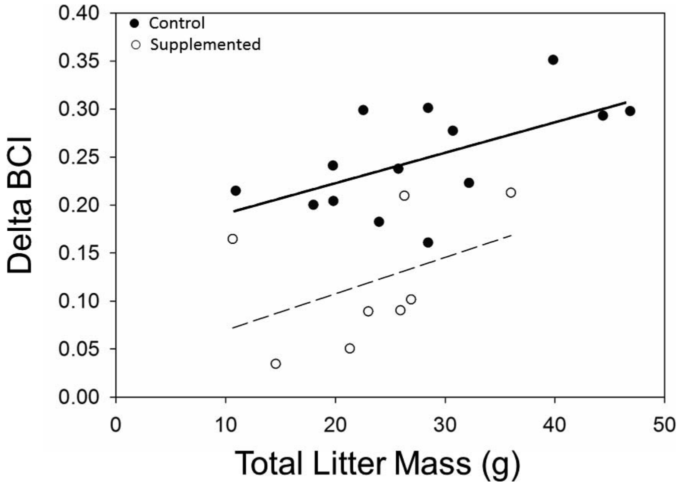

FIG. 1. Scatter plot with linear regression lines describing the relationship between total litter mass and the change in maternal body condition postpartum (Delta BCI) in pregnant *Sistrurus miliarius* collected in central Florida and assigned to either the control or food-supplementation group. Analysis of covariance demonstrated a significant effect of total litter mass and supplemental feeding on Delta BCI.

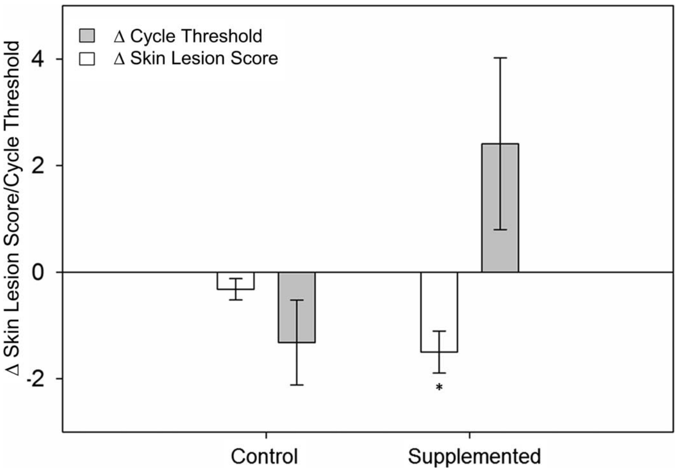

FIG. 2. Mean change in skin lesion score and PCR cycle threshold (field − postpartum) in pregnant *Sistrurus miliarius* collected in central Florida and assigned to either the control or food-supplementation group. Error bars represent the standard error of the mean. Significant treatment effects are indicated with an asterisk.

crossed at or below 40 cycles. Lower Ct values indicate a greater amount of *Oo* DNA in the sample. Three females died during pregnancy, and one litter experienced very high mortality. We stored two deceased mothers and the failed litter ($n = 4$) at −20°C before shipment to the NWHC for PCR and necropsy to identify the cause of mortality.

Neonate Traits.—We weighed and measured the SVL of each neonate with the use of a digital balance and a squeeze box on the day after birth. Between 7 and 10 d after birth, and after the first shed, we moved each neonate into a separate 0.45-m-long by 0.35-m-wide by 0.33-m-high plastic bin that served as an arena for caudal luring trials. After a 24-h acclimation period, we started a luring trial by focusing a video camera on the neonate in an arena and placing a wooden dowel into the arena with a small Green Treefrog (*Hyla cinerea*) tethered with string approximately 20 cm above the substrate (outside of striking distance). We recorded if the snake caudal lured (repeatedly moved its tail back and forth while the rest of the body remained motionless) in the 15 min period after prey introduction. For the snakes that caudal lured, we also recorded luring latency as the time elapsed between prey introduction and the initiation of caudal luring. Neonates were released with their mothers at their point of capture shortly after the study.

Data Analysis.—We calculated maternal body condition upon capture and again postpartum as the residual of the regression of log-transformed mass on log-transformed SVL. Postpartum body condition (BCI) was then subtracted from pregnant BCI to calculate the change in BCI after birth (Delta BCI) for use as a dependent variable. We determined the effect of food supplementation on Delta BCI with the use of analysis of covariance (ANCOVA) in JMP Pro® 11 (SAS Institute Inc., Cary, North Carolina, USA) with total litter mass as a continuous covariate. Regression slopes were homogeneous and no interaction effect was included. We analyzed maternal skin lesion score and PCR cycle thresholds using logistic regression and nonparametric Wilcoxon tests, respectively. We compared the probability of reproductive success between groups using a likelihood-ratio χ^2 test. We analyzed individual offspring mass, SVL, and caudal luring latency in mixed models that included treatment as a fixed effect and maternal identity as a random effect. We quantified parturition date as the number of days before or after 1 August. We examined the effect of food supplementation on parturition date using a Student's *t*-test.

RESULTS

Feeding during Pregnancy.—All 13 females offered prey in the treatment group ate while pregnant. Supplemented pregnant females often consumed large prey items, with 10 of the 13 supplemented females eating a frog that exceeded 25% of their pregnant body mass. The females consumed a mean of 52.62 g of frog (range 19.3–99.8 g) that constituted a mean of 59.18% (range 29.5–107.2%) of their body mass (see Appendix).

Effects of Supplemental Feeding on Pregnant Females.—There was a significant effect of total litter mass ($F_{1,19} = 7.14$, $P = 0.015$) and supplementation ($F_{1,19} = 22.18$, $P < 0.001$) on Delta BCI (Fig. 1). As expected, supplemented females had lower Delta BCI compared to controls and total litter mass was positively related to Delta BCI within each treatment. Delta skin lesion score was significantly different between treatment groups, but there was no significant difference in the change in *Oo* PCR cycle threshold between groups (Fig. 2; Table 1). Supplemented females experienced higher rates of reproductive failure compared to unsupplemented females (Table 1). Three of the 13 snakes in the supplemented group died during pregnancy and 2 gave birth to stillborn litters, resulting in a reproductive success rate (i.e., percent of females that completed pregnancy and gave birth to a live litter) of 61%. Within the treatment group, five snakes were identified as *Oo* positive by PCR either on capture, postpartum, or both; only three individuals in the control group were *Oo* positive. Four of five SFD-positive snakes in the supplemented group failed to produce a live litter (three maternal mortalities and one stillbirth). All three suspected SFD-positive snakes in the control group successfully completed pregnancy and gave birth to a viable litter. Necropsy of two supplemented mothers that died during the study revealed that both were suffering from severe SFD, including >10 dermal lesions. *Ophidiomyces ophiodiicola* was cultured from affected skin of both animals, and microscopic lesions with fungal invasion consistent with SFD

TABLE 1. Descriptive statistics (mean ± standard error) for supplementally fed ($n = 13$) and control ($n = 14$) female *S. miliarius* in central Florida. Statistical results (test statistic and P value) are provided for each response variable. *Ophidiomyces ophiodiicola* (*Oo*) loads were quantified with the use of polymerase chain reaction (PCR) cycle thresholds, and neonate size was assessed by measuring snout-to-vent length (SVL).

Dependent variable	Supplemented	Control	Statistic	P
% successful pregnancy	61.54%	100%	$\chi^2 = 8.55$	0.003
Parturition date (d)	3.77 ± 2.45	2.07 ± 1.96	$t = 0.54$	0.6
Delta skin lesion score	1.50 ± 0.39	0.32 ± 0.20	$Z = 2.47$	0.013
Delta body condition	0.14 ± 0.02	0.25 ± 0.02	$F_{1,19} = 22.18$	<0.001
Neonate SVL (cm)	14.54 ± 0.29	14.96 ± 0.23	$F_{1,19.7} = 1.51$	0.23
Mean neonate mass (g)	4.34 ± 0.22	4.32 ± 0.16	$F_{1,19.7} = 0.28$	0.61
Oo PCR cycle threshold	0.46 ± 0.80	1.32 ± 0.80	$Z = 1.49$	0.13
Mean luring latency (min)	8.73 ± 1.54	7.99 ± 0.90	$t = 0.42$	0.69
Total litter mass (g)	23.07 ± 2.77	27.97 ± 2.74	$t = 1.26$	0.11

(Lorch et al., 2015) were confirmed by histologic examination. However, both snakes also showed gross abnormalities of the stomach or colon and a *Salmonella* sp. was cultured from these tissues; histopathologic analyses supported a diagnosis of salmonellosis. Salmonellosis was thought to be the proximate cause of death in these two snakes.

Effects of Supplemental Feeding on Neonates.—Neonate mass and SVL were not affected by treatment (Table 1). We observed caudal luring within 15 min of prey introduction in 21.4% (24 of 112) of the filmed neonates. The percentage of neonates from supplemented litters that lured (16.1%, 5 of 31) did not significantly differ from the percentage of neonates from control litters that lured (23.5%, 19 of 81; Fisher's exact test, $P = 0.45$). The mean luring latency was slightly higher in neonates from the supplemental feeding group, but this was not a statistically significant difference ($P = 0.69$, Table 1).

DISCUSSION

When given the opportunity to feed in outdoor enclosures, pregnant Pygmy Rattlesnakes are not anorexic during pregnancy. Individuals fed up until the final 3 d of pregnancy (Appendix). The possibility that anorexia may occur in unmanipulated situations as the result of behavioral trade-offs, such as engaging primarily in thermoregulation rather than foraging, was not tested and cannot be ruled out. Offspring size and luring behavior were unaffected by maternal food supplementation. Feeding during pregnancy may, however, come at a cost to mothers. There was a high incidence of reproductive failure in the supplemented group, and supplemented mothers exhibited high skin lesion scores postpartum. Not all skin lesions were PCR positive for *Oo*, suggesting false-negative PCR results or etiologies other than SFD in some cases. Processing large, frequent meals may conflict with the demands of pregnancy and result in reproductive failure and disease in Pygmy Rattlesnakes.

Offspring size was unaffected by feeding during pregnancy, which is consistent with previous studies (e.g., Gregory and Skebo, 1998; Lourdais et al., 2002; Taylor et al., 2005; Gregory, 2006). Although the potential for placental transfer of nutrients exists in rattlesnakes (Van Dyke and Beaupre, 2012), nutrients transferred from food acquired during late pregnancy did not alter the mass or length of neonates in Pygmy Rattlesnakes. There was also no significant effect of feeding on our indirect measures of offspring foraging ability. The proportion of offspring that caudal lured when presented with prey and the latency of caudal luring in the snakes that did lure was not significantly affected by supplementally feeding their mothers. Although supplemental feeding had negative impacts on offspring survival to birth, it did not have clear effects on the size or behavior of neonates. Results should be interpreted with some caution, given the increased incidence of reproductive failure and clinical signs of disease in the treatment group. Supplemented mothers were, however, in significantly better postparturient body condition, and may have been more likely to accrue enough stored energy to reproduce in the following year (Naulleau and Bonnet, 1996). Future work should attempt to demonstrate this empirically.

Supplemented females experienced more negative outcomes compared to controls both in terms of reproductive success and disease progression. Five snakes in the treatment group were SFD positive, indicated by clinical signs and PCR (either on capture or postpartum). Three SFD-afflicted individuals in the supplemented group died prior to giving birth and the fourth aborted a litter, producing stillborn offspring. Only one SFD-positive snake in the supplemental group gave birth to a live litter; however, only one of her six offspring survived past 10 d postpartum. The cause of death of the neonates could not be determined upon necropsy. Neither gross nor histopathologic lesions consistent with SFD were observed in the neonates, although one neonate was PCR positive for *Oo* at a very high Ct value (37.28), suggesting that the snake may have been harboring the fungus. Four out of the five incidents of reproductive failure in the supplemented group were associated with SFD. The *Oo*-negative individual in the supplemented group that gave birth to a stillborn litter had a postpartum skin lesion score of 3 (most severe). As this snake survived the experiment, a necropsy was not performed to determine the cause of the skin lesions, and the possibility of a false-negative diagnosis by PCR cannot be ruled out. The presence of gross skin lesions suggestive of SFD did not result in reproductive failure in the control group. Three individuals in the control group had clinical signs consistent with SFD and were *Oo* positive by PCR. All three *Oo*-positive control snakes survived and gave birth to viable litters, suggesting that pregnant snakes with SFD can carry offspring to term successfully, but supplemental feeding of SFD-afflicted mothers resulted in a high incidence of reproductive failure.

The causal mechanisms underlying the observed effect of food supplementation on SFD and reproductive outcomes were beyond the scope of the study. Necropsy revealed that the likely proximate cause of mortality in two SFD-positive mothers was salmonellosis. *Salmonella* is a common component of the gut microbiota in rattlesnakes (McLaughlin et al., 2015) and does not typically lead to pathology in healthy individuals. Two hypotheses may explain why an enteric bacterium would cause mortality in supplemented females. It may be that processing large meals while pregnant caused alterations to the microbial gut flora that favored *Salmonella* or damage to the gastrointestinal tract that allowed gut-associated bacteria to become systemic. Alternatively, the additive metabolic demands of pregnancy and SDA may have forced snakes to divert energy away from immune function. This may have shifted the host and pathogen (*Oo*) dynamic, allowing the disease to progress more rapidly. Already compromised from SFD, the animals may have then been more vulnerable to secondary processes such as salmonellosis. Nine of the 13 snakes in the supplemental group had postpartum skin lesions (compared to 2 of 14 in the control group), suggesting a disruption of normal immune function in supplemented snakes. Alternatively, the increased skin lesion score in the treatment group could have resulted from a more robust immune response (i.e., increased localized inflammation resulting in larger lesions) in fed snakes; however, the high reproductive failure rates observed in the treatment group suggest a reduced ability to cope with infection. In previous studies on this population, skin lesions were associated with presence of *Oo* in 85% of cases, and all snakes that scored as severe on the skin lesion scale were PCR positive for *Oo* (Lind et al., 2018a, 2019). However, here we report severe skin lesions in three postpartum mothers in the supplemented group that were negative for *Oo* by PCR, suggesting false-negative PCR results or other etiologies. All three of these mothers had a skin lesion score of zero upon capture. Only 2 of the 14 control snakes showed any increase in skin lesion score, and both individuals were PCR positive for *Oo*. Future work should examine the metabolic ceiling hypothesis and demonstrate the combined metabolic increment associated with pregnancy and SDA in snakes that consume large meals relative to body size. Previous investigations of the combined metabolic increments associated with feeding and pregnancy have been conducted in natricine snakes that were fed smaller food items relative to body size (10% body mass; Jackson et al., 2015) and likely had a proportionally smaller metabolic increment associated with SDA (Zaidan and Beaupre, 2003).

Although outcomes in the control group show that pregnant Pygmy Rattlesnakes can complete pregnancy and produce viable offspring while likely afflicted with SFD, our findings are cautionary in the context of utilizing supplemental feeding of pregnant females as a potential intervention for SFD-afflicted snakes (McCoy et al., 2017). All SFD-afflicted snakes in the supplemented group failed to produce a viable litter. The feeding regime in the current study included large, frequent meals. In a previous study on Pygmy Rattlesnakes, a single feeding during late pregnancy did not appear to disrupt the reproductive process, although the SFD status of individuals in the study was not established (Lind et al., 2017b). If feeding disrupts the immune system in free-ranging females, coping with SFD while pregnant may force energetic trade-offs that negatively impact fitness. The mechanisms that underlie reproductive failure and disease in the treatment group were not definitively established, but may have resulted from either disruption of the integrity of the gastrointestinal tract or metabolic effects on immunocompetence. These alternative hypotheses should be examined in future investigations, and conservation efforts aimed at mitigating the reproductive costs of SFD should carefully evaluate the potential costs and benefits of food supplementation.

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APPENDIX. Snake fungal disease status indicated by PCR, initial pregnant mass, capture date (Cap date), parturition date (Part date) and amount of food offered and eaten by pregnant *S. miliarius* on each of six feeding dates. Total mass consumed as a percentage of initial pregnant body mass is reported. The reproductive outcome for each snake is categorized as either successful completion (S), mortality (M), or abortion of stillborn offspring (A).

Snake ID	SFD status (PCR)	Field mass (g)	Cap date	Part date	17 Jun	27 Jun	7 Jul	12 Jul	20 Jul	27 Jul	Meals	Total offered (g)	Total eaten (g)	Percent body mass	Outcome
2	Negative	73.0	16 Jun	12 Aug	–	18.73	12.84	–	11.41	13.36	4	56.34	56.34	77.18	S
3b	Negative	80.0	22 Jun	17 Aug	–	22.51	22.31	–	16.06	15.78	4	76.66	60.88	76.10	S
3a	Positive	125.0	15 Jun	NA	36.87	–	–	–	–	–	1	36.87	36.87	29.50	M
9	Negative	111.0	10 Jun	5 Aug	–	–	–	38.64	24.25	16.89[a]	2	79.78	62.89	56.66	S
11	Negative	111.0	7 Jul	27 Jul	–	–	–	45.92[b]	30.29	–	2	76.21	76.21	68.66	S
12	Positive	91.5	6 Jun	31 Jul	26.3[a]	28.74[a]	11.37[a]	33.38	19.83[a]	17.96[a]	1	137.58	33.38	36.48	A
13	Negative	105.0	19 Jun	9 Aug	–	31.55	29.65	–	20.4	18.22	4	99.82	99.82	95.07	S
15	Negative	81.5	2 Jun	9 Aug	20.88	24.53	21.53	–	18.41	16.75[a]	4	102.1	85.35	104.72	S
18	Positive	81.0	14 Jun	NA	15.05[a]	22.94[a]	16.6	–	17.34	16.5[a]	2	88.43	33.94	41.90	M
19	Positive	73.0	14 Jun	NA	–	8.4	14.29	–	12.07	13.48[a]	3	48.24	34.76	47.62	M
21	Positive	89.0	30 Jul	30 Jul	26.03	27.12	25.36	–	18.56[a]	16.89	4	113.96	95.4	107.19	S[c]
23	Negative	65.0	23 Jun	27 Jul	–	7.36[a]	9.71	–	9.63	–	2	26.7	19.34	29.75	S
25	Negative	75.5	6 Jun	9 Aug	–	–	–	24.82	12.86[a]	14.12	2	51.8	38.94	51.58	A

[a] Meal offered but not eaten.
[b] Meal was regurgitated.
[c] High mortality after birth.