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Communal egg-laying behaviour and the consequences of egg aggregation in the brown anole (*Anolis sagrei*)

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Abstract

Communal nesting is a behaviour that involves multiple females laying eggs in the same nest or nesting site. This behaviour may be a consequence of a shortage of preferable nest sites (constraint hypothesis) or an adaptation generated by fitness benefits associated with egg aggregation (adaptive hypothesis). Experimental tests of these hypotheses require information about maternal nest site choice and its fitness consequences. To address these, we studied a lizard (brown anole; *Anolis sagrei*) that produces single-egg clutches, but often aggregates eggs in nest sites. In a lab study, females were given the option of nesting in (a) soil previously used as nest substrate vs. fresh soil and (b) soil with eggshells vs. without eggshells. We also experimentally examined the effects of egg aggregation by incubating eggs singly, in groups of four, and in groups of nine. We recorded egg surface temperature, water uptake, and hatchling morphology. Females were more likely to nest in pots with used soil and with eggshells than in pots with fresh soil or without eggshells. We observed no effects of egg aggregation on egg survival, egg temperature, or most measures of hatchling morphology. However, singly-incubated eggs absorbed more water than eggs incubated in the four and nine egg aggregations and this resulted in offspring with greater body condition (i.e. heavier for their length) at hatching. The behavioural experiment demonstrates that females actively choose nest sites that have been used previously (as expected under the adaptive hypothesis), but the egg-aggregation experiment shows no benefits to offspring based on the variables measured. Thus, results of the behaviour study support the adaptive hypothesis; however, results from our egg-incubation study do not. Likely, the adaptive and constraint hypotheses are not mutually exclusive, and a diversity of factors influence the evolution of communal nesting behaviour.

KEYWORDS
developmental plasticity, hatchling phenotypes, maternal effects, nesting behaviour, nest-site choice, oviparous reptiles

1 | INTRODUCTION

Nest-site selection can be an important determinant of offspring survival for oviparous species (Refsnider & Janzen, 2010; Mitchell, Maciel, & Janzen, 2015). Consequently, nesting behaviours can be adaptive, providing benefits to offspring development and/or parental survival (Radder & Shine, 2007). For example, variation in nest microhabitats selected by females can be linked to variation in egg survival or fitness-relevant phenotypes of hatchlings (Mitchell et al., 2015; Pruett, Addis, & Warner, 2019). This is particularly important for oviparous species that do not provide parental care after nesting (e.g. most non-avian reptiles; henceforth "reptiles") because eggs are left to develop under prevailing environmental conditions. For these reasons, nesting behaviour has been of great interest to evolutionary ecologists for its utility in answering basic questions about the evolution of behaviour (e.g. Nilsson, 1984; Refsnider & Janzen, 2010; Warner, 2014) as well as forecasting the effects of human-induced global change on wildlife (e.g. Carlo, Riddell, Levy, & Sears, 2018; Mainwaring et al., 2017; Refsnider & Janzen, 2012). Oviparous reptiles have played a significant role in understanding the adaptive significance of nesting behaviour and the consequences of nest environments for offspring (Mitchell, Maciel, & Janzen, 2013; Refsnider & Janzen, 2010; Shine & Harlow, 1996; Warner & Shine, 2008a). Yet, despite decades of study, many aspects of nesting behaviour are still poorly understood in reptiles (Doody, Freedberg, & Keogh, 2009; Magnusson & Lima, 1984; Tiatragul, Hall, Pavlik, & Warner, 2019).

Although communal nesting is a common behaviour among reptiles, its adaptive significance is uncertain for most taxa (Doody et al., 2009). Communal egg-laying is a clumped distribution of egg clutches (Doody et al., 2009), and typically refers to several females depositing eggs in a single nest (e.g. some lizards; Radder & Shine, 2007; Robinson, Kircher, & Johnson, 2014) or multiple females depositing clutches in separate nests that are clustered within a relatively small area (e.g. sea turtles; Miller, 2017). Communal egg-laying is likely an ancestral behaviour that has persisted throughout reptile evolution (Horner, 1982), and has maintained some adaptive value. Current explanations of communal egg-laying assume it occurs for one of two reasons: a shortage of preferable nesting sites (i.e. constraint hypothesis) or due to fitness benefits to parents, offspring, or both (i.e. adaptive hypothesis) (Doody et al., 2009; Radder & Shine, 2007). In some environments, a paucity of suitable nest sites may require females to lay eggs in common areas (Robinson et al., 2014; Tiatragul et al., 2019); thus, there may be no adaptive significance to this behaviour, per se. On the contrary, communal egg-laying can potentially benefit females and/or offspring via reduced likelihood of egg depredation, reduced energetic cost to females due to searching for nest sites and creating nests, as well as metabolic benefits to eggs (van Doody et al., 2009; de Merwe, Ibrahim, & Whittier, 2006). There are, however, potential costs to communal egg-laying for both mothers and offspring; thus, the adaptive and constraint hypotheses are not mutually exclusive. For example, communal nesting may result in greater detection probability of eggs and/or nesting females by predators, increased competition among eggs for oxygen and water, or greater likelihood of disease (Brown & Shine, 2009; Kiesecker & Blaustein, 1997). Indeed, water uptake during egg incubation is necessary for the successful development of most reptiles, yet absorption of water from the nest substrate may be hindered in the presence of conspecific eggs (Marco, Díaz-Paniagua, & Hidalgo-Vila, 2004; Warner & Chapman, 2011). Thus far, most of what is known about communal egg-laying in reptiles comes from field observations rather than experimental manipulations (e.g. Rand, 1967; Doody et al., 2009; but see Marco et al., 2004; Radder & Shine, 2007; Warner & Chapman, 2011). These types of observations can determine the prevalence of communal egg-laying across taxa, but alone cannot identify the underlying causes of communal egg-laying (hypotheses of constraint vs. adaptation) or determine its effects on offspring.

In reptiles, communal egg-laying is most common in lizards (Doody et al., 2009). Most lizards lay multi-egg clutches, so even in the absence of communal egg-laying, eggs usually incubate in aggregates. The number of eggs within a clutch can influence water uptake during development (Brown & Shine, 2009; Marco & Díaz-Paniagua, 2008), and such effects can be compounded when females lay eggs communally. *Anolis* lizards, however, lay single-egg clutches approximately once every 1 to 2 weeks and can potentially reduce or avoid costs associated with incubation in multi-egg nests (Warner & Chapman, 2011). Despite the evolution of a fixed, single-egg clutch, anole nests often contain several or dozens of eggs, presumably because females return to the same nesting site, nest communally, or both (reviewed by Rand, 1967; Robinson et al., 2014; see also Greenberg & Noble, 1944; Godfrey, Duberstein, Mota, & Moore, 2018). Several authors have sought to discern the adaptive value of communal nesting by incubating eggs singly and in aggregates and measuring the effects on egg development and hatchling phenotypes (e.g. Radder & Shine, 2007). These studies have typically used lizards that lay multi-egg clutches. However, because eggs of these species do not incubate in isolation in the wild it is challenging to determine the ecological or evolutionary significance of results. Using anoles as models is preferable because anole eggs naturally incubate both singly and in aggregates.

We had three objectives in this study. First, to determine if brown anoles (*Anolis sagrei*) prefer to lay eggs in locations that have been used by other females for nesting, we performed a choice experiment that gave females the option of nesting in soil that was previously used for nesting vs. fresh soil. Second, to determine if nesting is cued by the presence of hatched eggshells (as in other species; Brown & Shine, 2005), we allowed females to choose between soil with and without eggshells. Finally, to determine the potential consequences of communal nesting on egg incubation and hatchling phenotypes, we incubated eggs singly, in groups of four, or in groups of nine and routinely measured water uptake and egg temperature, recorded egg survival, and measured hatchling phenotypes. If females prefer to nest in sites used by other females, this would indicate egg aggregations in the field are not due solely to a lack of suitable nesting sites, supporting the adaptive hypothesis.

If females exhibit no preference, this would be consistent with the hypothesis that field aggregations are due to constraints imposed by limited preferred nesting habitat. Moreover, if egg aggregation treatments enhance egg survival and/or fitness-relevant phenotypes of hatchlings (e.g. result in larger body size), this would further support the adaptive hypothesis. Conversely, if egg aggregation treatments have negative effects on egg survival or hatchling phenotypes, this would not support the adaptive hypothesis and indicate that females may aggregate eggs in the field due to constraints. Finally, additional outcomes could occur, demonstrating the non-mutually exclusive nature of these hypotheses. For example, females may show no preference, but egg aggregations may benefit offspring development, or vice versa.

2 | METHODS

2.1 | Study species

The brown anole is a small (~2–5 grams), subtropical, semi-arboreal lizard that is native to the West Indies and its islands (Losos, 2011). Females lay a single egg clutch every 4–14 days across an extended reproductive season (Hall, Buckelew, Lovern, Secor, & Warner, 2018); however, at our study site in Palm Coast, FL (coordinates 29.602199, −81.196211), eggs are often found in aggregations (from 2 to 16 eggs). Eggs increase in size during development via water uptake; thus, egg size can be a proxy for age. Some field aggregations consist of eggs that are roughly the same size, indicating eggs were laid by different females. Others contain eggs that vary in size, meaning the same female may have repeatedly deposited eggs in the same nest (Pruett et al., in press). Regardless, these aggregations indicate either appropriate nest sites are limited or that females prefer to nest communally (or both). Brown anoles make excellent models for lab studies of reproduction and behaviour because methods are established for their captive care and breeding (Sanger, Hime, Johnson, & Diani, 2008), and they are a well-suited model system for studying behaviour (e.g. Irschick, 2000; Simon, 2007) including nesting behaviour (e.g. Reedy, Zaragoza, & Warner, 2012). See Mitchell, Hall, and Warner (2018) for a more complete description of our study site and system.

2.2 | Communal nesting behaviour

On 28 February 2017, 45 female and 18 male *A. sagrei* were collected from Palm Coast, Florida, transported to Auburn University, and housed indoors in ReptiBreeze screen cages (46 × 46 × 91 cm). Room temperature was maintained at 26°C and ambient lighting (described below) provided basking heat. Anoles were housed in groups of 5 females to 2 males per cage (*n* = 9 cages). Twice each week, we provided 21 crickets, dusted with vitamins and calcium, to each cage (i.e., 3 per lizard), and we misted cages with water daily. We provided a 12:12 hr light/dark cycle with Reptisun 5.0 UVB bulbs (Zoo Med Inc.) and plant grow bulbs (model F40; General Electric Co.). For perching, each cage had a single live cut tree branch and one vertical shaft of bamboo nearly equal to the height of the cage with 3–4 perches (tongue depressors) projecting horizontally at various heights. Reptile cage carpet (Zoo Med Inc.) served as a floor substrate and two plastic plant pots (8.5 x 10 cm, height x diameter), filled with moistened potting soil (details below), were provided for egg-laying. From 28 February until the beginning of our study (19 June), eggs were allocated to another experiment, not reported here. For the current study, we collected eggs (*n* = 289) three times per week from 19 June to 6 September 2017.

During the first nesting experiment (19 June to 9 August), we provided each cage with two nesting pots that contained either unused or used soil. Unused soil was a mixture of potting soil and peat moss that had never been used for nesting. Each time an egg was collected from a pot with unused soil, the pot was refilled with fresh, unused soil. Used soil was the same mixture of potting soil and peat moss; however, this soil had been used by nesting females in a previous study. We did not replace this soil throughout the experiment. During the second nesting experiment (16 August to 6 September), we provided each cage with two nesting pots that contained either used soil (as previously described) or used soil with eggshells. Thus, in this experiment, the two pots had identical soils but one pot contained 15 eggshells from recently hatched lizards. These eggshells were distributed evenly throughout the soil in the nest pots; however, due to the amount of substrate in each pot, at least one eggshell in each pot was typically exposed at the soil's surface.

In both nesting experiments, nest pots were checked every Monday, Wednesday, and Friday. Each egg check consisted of dumping the soil from each pot into a different plastic tub and searching through it for eggs. Soil types were not mixed during egg checks. We visually assessed the moisture content of each pot and watered the soil, as needed. We did not quantitatively monitor soil moisture; however, the same two individuals (KW and CR) performed all egg checks for both nesting experiments. Moreover, on each day that we checked for eggs, we checked all cages in a single session and were careful to provide the same amount of water to each pot. Thus, we are confident that the amount of moisture in each pot was consistent among treatments and between the two experiments. We switched the position of the two nest pots after every egg-check to minimize any position effects. For each egg, we recorded the type of pot in which it was found (experiment 1 - used soil vs. unused soil; experiment 2 - used soil without shells vs. used soil with shells). Eggs were then allocated to another study not described here.

2.3 | Potential consequences of communal nesting

From July 17 – September 6, 2017, eggs (139 total) were collected from a breeding colony of adults that were housed among 36 outdoor cages (0.61 x 0.61 x 1.2 m). These lizards were from the same location (Palm Coast, Florida) as those from the nesting behaviour study. Each cage contained six adult lizards, but the sex ratio varied

among cages for reasons relevant to another experiment that is outside the scope of this study. Cages contained a vertical bamboo perch with artificial vegetation, and a nesting container on the floor. Adults were watered daily and fed 2 crickets dusted with calcium and vitamins twice weekly. To determine the effect of egg aggregation on development, eggs were placed into treatments of one, four, or nine egg aggregations. Upon collection from nesting containers, eggs were weighed and randomly assigned to a treatment. Eggs were placed in their respective formations (Figure 1) in a petri dish that was filled halfway with moist vermiculite, and the dish was sealed with parafilm. The diameter of the petri dish increased with increasing number of eggs (60, 80, 150 mm, for 1, 4, and 9 eggs, respectively), and the quantity of moist vermiculite in each petri dish was proportional to the number of eggs in each treatment. The single egg treatment served as a control, the nine egg treatment allowed us to examine within-aggregation variation based on egg position (details below), and the four egg treatment was an intermediate. These treatments reflect common sizes of egg aggregations observed in the field (Pruett et al., in press). Eggs were incubated at a constant temperature of 28°C which is within the range of field temperatures for *A. sagrei* nests (Pearson & Warner, 2018). During incubation, individual egg temperatures were taken twice weekly by placing a thermocouple (Omega HH800A) directly on the surface of each egg. Rather than opening the petri dish to measure egg temperature, small holes (1/16 of an inch) were drilled in the top of the petri dish over each egg and covered with tape when not in use. Near the end of development (~25 days incubation), eggs were removed from their treatment petri dishes, weighed to determine final egg mass, and placed into individual petri dishes to ensure eggs could be reliably matched to hatchlings. We then checked daily for hatchlings, and when found, we recorded their mass (to the nearest 0.0001g) and measured snout-vent length (SVL) and tail length (to the nearest 0.01 mm) with a digital calliper.

2.4 | Statistical analysis

Because females were housed communally for the nesting experiment, we could not determine which eggs were laid by each female. Thus, to avoid pseudoreplication, we considered each cage (*n* = 9) a replicate. We calculated the percentage of eggs that were laid in a pot with used soil (vs. unused) for choice experiment 1 and for pots with eggshells (vs. without) for choice experiment 2. To generate estimates of choice, we calculated a mean and 95% confidence interval for these percentages. To determine statistical differences, we scored each cage with a 1 if greater than 50% of eggs were laid in a pot with used soil (experiment 1) or soil with egg shells (experiment 2). We scored each cage with a 0 if 50% or less were laid in those pots. We then performed a two-tailed exact binomial test to generate p-values. Our null hypothesis was that 50% of cages should be scored a 1 if females chose nesting sites randomly.

To understand the effects of egg aggregation on embryo development, we examined egg surface temperature, water uptake (i.e. mass gain), egg survival, and hatchling morphology (SVL, mass, body condition, tail length). Body condition was calculated by regressing log mass on log length (SVL) of hatchlings. Residual values from this regression are the body condition for each hatchling; thus, larger values represent lizards that are relatively heavy for their length (Warner, Johnson, & Nagy, 2016). A linear mixed model with petri dish as a random effect and egg mass and treatment as fixed effects was used to test if treatment influenced average egg temperature during development. To test for effects on egg mass gain, we used final egg mass as the response variable and initial egg mass as a covariate. For this analysis, we used only eggs that survived to hatching, as dead eggs only have passive water uptake (Warner, Moody, & Telemeco, 2011a). A generalized linear mixed model with a binomial distribution was used to analyse egg survival with dish as a random effect and treatment as a fixed effect. Finally, a linear mixed model was used to determine the effect of treatment on hatchling phenotypes (SVL, mass, body condition, tail length) with treatment and egg mass as fixed effects and dish as a random effect. Interactions between egg mass and treatment were never significant, and thus, removed from final models.

In addition to analysing the effect of aggregation treatment on embryo development, we also tested the effect of position within the nine-egg aggregation treatment. There are three different positions in this aggregation - a central egg that is surrounded by other eggs at the centre of the aggregation, four corner eggs that are touching other eggs on two sides on the corner of the aggregation, and four middle eggs that are between the corner eggs and touching other eggs on three sides (Figure 1). The position of eggs within an aggregation can influence water uptake and egg survival (Brown &
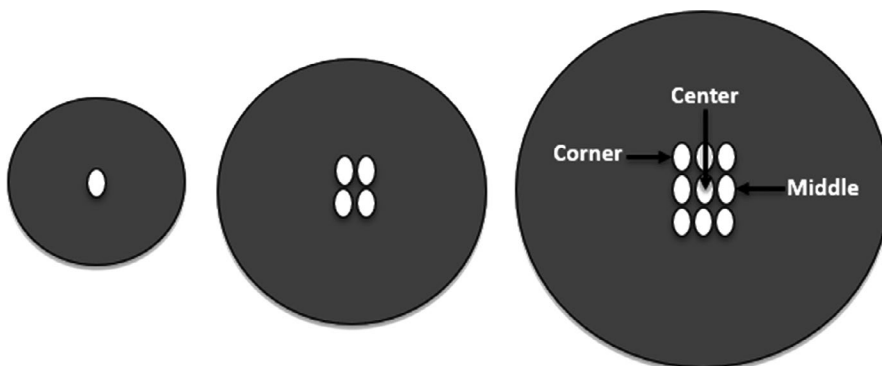

FIGURE 1 Position of eggs within petri dishes. For the 9-egg aggregation, we categorized eggs based on their position in the aggregation since this resulted in variation in the number of eggs that each egg touched (corner = 2 eggs, middle = 3, center = 4)

Shine, 2009; Socci, Schlaepfer, & Gavin, 2005). We used the same models as previously described for these analyses, but position was a fixed effect instead of treatment. Interactions between egg mass and treatment were never significant, and thus, removed from final models.

3 | RESULTS

3.1 | Communal nesting behaviour

Females selected nest pots with used soil (vs. unused) 60.41% of the time (56.59 – 64.24, 95% CI; Figure 2a). In all 9 cages, greater than 50% of eggs were laid in pots with used soil (0.66–1.00, 95% CI; p = .004). Although females selected nest pots with eggshells (vs. without) 59.69% of the time (48.8 – 70.57, 95% CI, Figure 2b), this was not statistically significant: for 5 cages, greater than 50% of eggs were laid in nest pots with eggshells (0.21–0.86, 95% CI; p = .99).
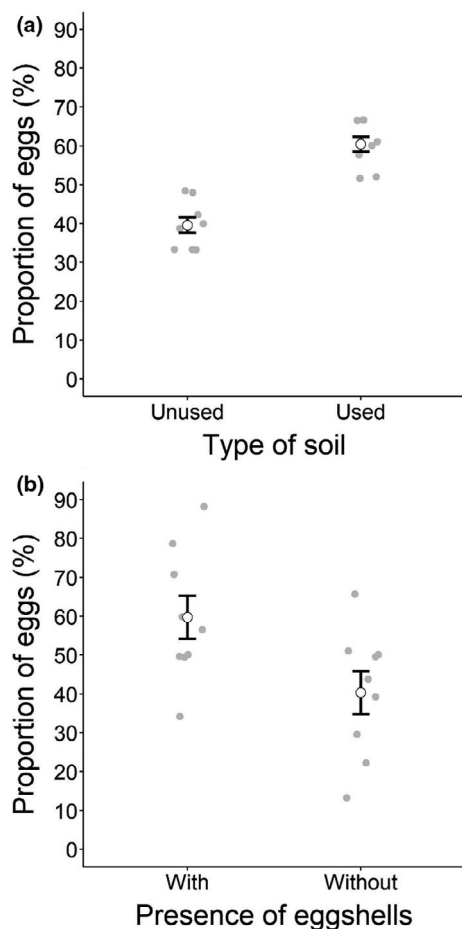

(a)

(b)

FIGURE 2 Nest-site choice for *Anolis sagrei* provided with (a) soil that was (Used) or was not previously used (Unused) by nesting females, and (b) used soil that did (With) or did not (Without) include hatched eggshells. Open circles show the raw means, gray circles show raw data, and bars show the standard error

3.2 | Consequences of communal nesting

Egg aggregation treatment had minimal effects on development and hatchling phenotypes: for eggs, only water uptake was influenced by treatment and for hatchlings only body condition was affected. We observed no effects on average temperature, incubation duration, hatching success, or other measures of hatchling phenotypes (SVL, mass, tail length) (Table 1). Post-hoc analysis for water uptake revealed that single eggs gained 47 (±20 SE) mg more mass during development than those from the four-egg treatment (p = .026) and 53 (±19 SE) mg more than those from the nine-egg treatment (p = .009). Eggs from the nine-egg aggregation were 6 (±10 SE) mg less massive than those from the four-egg treatment, but this was not statistically significant (p = .63) (Figure 3a). Hatchlings from the single egg treatment had 0.091 (±0.005 SE) and 0.011 (±0.005 SE) greater body condition than those from the four-egg treatment (p = .009) and nine-egg treatment (p = .031), respectively. The nine-egg treatment resulted in 0.004 (±0.003 SE) greater hatchling body condition than the four-egg treatment, but this was not significant (p = .21) (Figure 3b).

Within the nine-egg treatment, egg position had little effect on development or hatchling phenotypes; however, position influenced water uptake (Table 2): central eggs gained 49 (±18 SE) mg less (p = .008) mass during development than middle eggs and 75 (±18 SE) mg less (p < .001) than corner eggs. Corner eggs gained 26 (±11 SE) mg more mass than middle eggs (p = .021) (Figure 4).

4 | DISCUSSION

Choice of nesting microhabitat can have important effects on offspring fitness, especially when no parental care is provided (Brattstrom, 1974; Resetarits, 1996; Resetarits & Wilbur, 1989). For example, oviparous reptiles choose nest sites based on many macrohabitat features that provide shelter or suitable hydric/thermal conditions for developing offspring (Shine & Harlow, 1996; Warner & Shine, 2008b). Indeed, eggs require optimal moisture levels since they are prone to desiccation in dry soil and prone to fungal infections in soil that is too moist (Tracy, 1980). Females also choose sites with optimum temperatures due to thermal effects on egg survival and a suite of offspring phenotypes (Warner & Andrews, 2002; Noble, Stenhouse, & Schwanz, 2018). In this study, we observed that female brown anoles prefer to lay eggs in locations previously used as nest sites by other females, which is a behaviour that facilitates communal nesting. The presence of conspecific eggs or hatched eggshells may be a cue that a potential nest site is suitable for successful development (Brown & Shine, 2005); however, few studies have assessed the potential for females to select nest sites based on these features and few have determined the effects of communal nesting on offspring (Pike, Webb, & Andrews, 2011).

Our experiment suggests that females may use olfactory cues, visual cues, or both to choose suitable nesting sites. Chemosensory function is well documented in lizards and enables them to detect

| Dependent variable	Independent variable	denDF	numDF	F-value	p-value
Average temperature	Egg mass	87	1	0.010	.868
	Treatment	24	2	0.142	.919
Final egg mass	**Egg mass**	**87**	**1**	**38.769**	**<.001**
	Treatment	**24**	**2**	**4.033**	**.03**
Incubation duration	**Egg mass**	**87**	**1**	**15.260**	**<.001**
	Treatment	24	2	1.632	.1594
Hatching success	Egg mass	87	1	0.963	.327
	Treatment	24	2	0.845	.466
Hatchling SVL	**Egg mass**	**87**	**1**	**29.557**	**<.001**
	Treatment	24	2	1.282	.296
Hatchling mass	**Egg mass**	**87**	**1**	**80.658**	**<.001**
	Treatment	24	2	2.274	.125
Hatchling tail length	**Egg mass**	**87**	**1**	**13.294**	**<.001**
	Treatment	24	2	3.176	.060
Hatchling body condition	**Egg mass**	**87**	**1**	**30.781**	**<.001**
	Treatment	**24**	**2**	**3.656**	**.041**
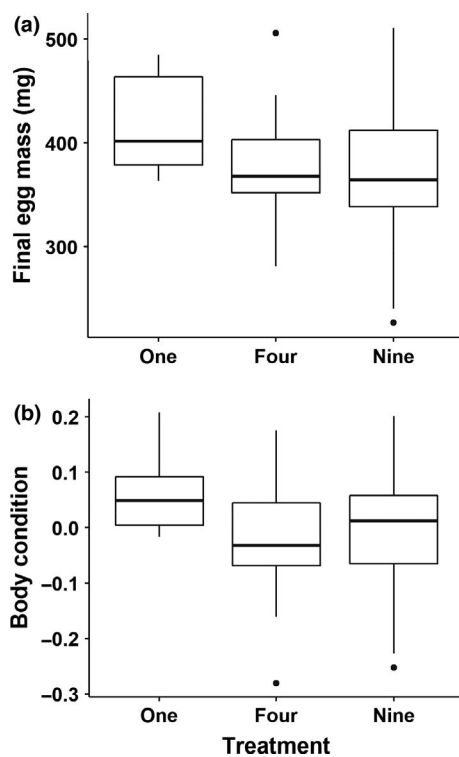
FIGURE 3 Effects of egg aggregation on final egg mass (a) and hatchling body condition (b). The bold, horizontal line represents the 50th percentile, and lower and upper bounds represent the 1st and 3rd quartiles, respectively. The whiskers extending from the boxes represent the 1st and 3rd quartiles ± 1.5 × interquartile range and the closed circles show outliers

food, predators, and conspecifics (Baeckens, Driessens, & Van Damme, 2016; Reilly, McBrayer, & Miles, 2007); however, the use of chemosensation for nest sites is poorly studied in reptiles (Grant,

Anderson, & Twitty, 1968; Greenberg, 1993; Owens, Commuzzie, & Grassman, 1986), and is rarely addressed in anoles, likely because this genus depends primarily on visual cues for most behaviours (e.g., detecting prey, predators, and conspecifics; Fleishman, Loew, & Leal, 1993; Singleton, 2011). Regardless, females preferred to nest in used vs. unused soil, suggesting a capacity for chemosensory function when selecting nest sites. The presence of intersexual chemo-sensation in brown anoles (Baeckens et al., 2016) indicates that females may be able to pick up cues from other females when selecting nest sites. Moreover, the few accounts of nesting behaviour in anoles demonstrate that females dig prospect nests and behave in ways that indicate they are using vision and olfactory information to find a suitable site (Propper, Jones, Rand, & Austin, 1991). In some other reptiles, the commencement of egg-laying is cued by the presence of eggs or eggshells. For example, green snakes (*Opheodrys aestivus*) were more likely to deposit clutches where other females had previously oviposited in the lab (Plummer, 1981), and keelback snakes (*Tropidonophis mairii*) preferred to lay eggs in sites that included conspecific eggshells rather than sites with no eggshells (Brown & Shine, 2005).

The presence of eggshells may be evidence of successful hatching in previous nesting attempts; therefore, choosing nests with eggshells may enhance offspring survival (Brown & Shine, 2005). Moreover, females may benefit from selecting a previously used location because it saves time and energy that would have been spent searching for a new location, in addition to decreasing predation risk (Doody et al., 2009; Elphick, Pike, Bezzina, & Shine, 2013). This allows females to repeatedly choose nest sites that result in relatively high offspring survival without expending extra energy searching for nest sites. There are, however, potential costs to communal oviposition. Females may accidentally disturb eggs of other lizards, negatively affecting their development (Elphick et al., 2013). Communal

TABLE 2 Effects of egg position (centre, middle, corner) on egg development, hatching success, and hatchling phenotypes. Bold text denotes statistical significance (alpha = 0.05)

Dependent Variable	Independent Variable	denDF	numDF	F-value	p-value
Average temperature	Egg mass	57	1	0.003	.953
	Egg position	57	2	0.669	.516
Final egg mass	**Egg mass**	**57**	**1**	**43.939**	**<.001**
	Egg position	**57**	**2**	**9.456**	**<.001**
Incubation duration	Egg mass	57	1	10.482	.002
	Egg position	57	2	0.705	.498
Hatching success	Egg mass	57	1	1.272	.259
	Egg position	57	2	0.213	.774
Hatchling SVL	**Egg mass**	**57**	**1**	**32.126**	**<.001**
	Egg position	57	2	2.241	.116
Hatchling mass	**Egg mass**	**57**	**1**	**56.741**	**<.001**
	Egg position	57	2	0.504	.607
Hatchling tail length	**Egg mass**	**57**	**1**	**12.096**	**<.001**
	Egg position	57	2	1.499	.232
Hatchling body condition	**Egg mass**	**57**	**1**	**14.402**	**.004**
	Egg position	57	2	0.326	.723

nests may be easier for predators to detect if many eggs are laid in the same place and time each year (Blouin-Demers, Weatherhead, & Row, 2004). Eggs laid near other eggs are more likely to spread pathogens between eggs and into the soil, which can harm eggs laid in later years (Socci et al., 2005; Patino-Martinez et al., 2012). For communal egg-laying to be adaptive, the benefits must outweigh the costs. Although costs and benefits associated with communal nesting were not fully addressed in our study (e.g. maternal energy expenditure, egg depredation), the tendency for females to nest in previously-used soil supports an adaptive explanation.

Although females exhibit a tendency to aggregate eggs, we found that egg aggregation has no effect on many aspects of development (i.e., incubation length, hatching success, hatchling size) and
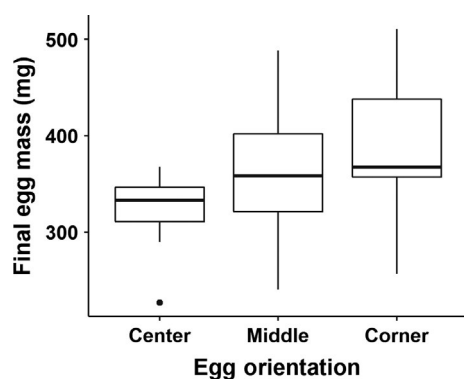

FIGURE 4 Effects of egg position on water uptake. Centre, middle, and corner eggs were in contact with other eggs on 4, 3, and 2 sides, respectively. The bold line represents the 50th percentile, and lower and upper bounds represent the 1st and 3rd quartiles, respectively. The whiskers extending from the boxes represent the 1st and 3rd quartiles ± 1.5 × interquartile range and the closed circles represent outliers

negative consequences for others (i.e., egg water uptake, hatchling body condition). Egg aggregation during incubation did not affect egg temperature or incubation period, suggesting that metabolic heating (as shown in embryos of other reptiles; van de Merwe et al., 2006) plays little to no role in anole development. Indeed, because of the relatively small size and, thus, large surface area to volume ratio of anole eggs, any external heat they gain is quickly lost (Hulbert et al., 2017). Moreover, aggregation treatment had no effect on hatching success, but aggregations did reduce water uptake. This phenomenon appears to be "dose-dependent" in the nine-egg treatment, where water uptake decreased as the number of eggs surrounding a focal egg increased (Figure 4). Thus, we conclude that this reduction in water uptake is due to competition for water among eggs. Water uptake is necessary for successful development of reptile embryos (Packard & Parkard, 1988), and is influenced by the percentage of the egg that is in contact with other eggs and with the surrounding substrate or exposure to moist air (Warner & Chapman, 2011). Past studies show that egg aggregation often reduces the moisture absorbed by eggs (Marco et al., 2004; Radder & Shine, 2007); however, under dry nest conditions, egg aggregation can reduce water loss to the nest substrate, enabling eggs to better retain moisture (Marco & Díaz-Paniagua, 2008). Thus, the effects of communal egg-laying on water uptake are likely context-dependent, and the results of our study may have been different had we used different moisture conditions for incubation. Moreover, the reduction in water uptake due to egg aggregation was not sufficient to reduce egg survival, and this small amount of water may have little influence on hatchling survival later in life (Marco et al., 2004). However, some anoles have communal nests with many more eggs than our largest aggregation (e.g. n = 65, Novo Rodríguez, 1985), which may result in much greater competition for water than observed in our study.

Although many studies have been conducted to understand the effects of nest microhabitats on the development of reptile eggs, most work has studied factors other than moisture (Warner, Du, & Georges, 2018) and more research is required to understand the context-dependent effects of nesting behaviour on water uptake by eggs. Although, treatment-specific patterns of water uptake and body condition did not mirror each other (Figure 3), singly incubated eggs absorbed more water and generated hatchlings with greater body condition compared to eggs incubated in aggregates. This pattern is consistent with previous studies (Reedy et al., 2012; Warner, Moody, Telemeco, & Kolbe, 2011b). Body condition is assumed to be a measure of robustness for lizards (Warner et al., 2016). Although the importance of body condition is contested (e.g. Cox & Calsbeek, 2015), most work has only assessed this in adults, and its effects on hatchlings has not, to our knowledge, been previously evaluated.

Anolis is a unique and potentially useful system for examining the evolution of communal nesting behaviour. Though the production of a single-egg clutch affords the opportunity for eggs to incubate in isolation, anecdotes from the field demonstrate that eggs often incubate communally. Our results indicate that females choose sites that have been previously used for nesting, supporting the adaptive hypothesis for communal nesting in anoles. Conversely, our incubation study demonstrates that there are negative effects of egg aggregation; thus, communal nesting in the field may have costs to offspring fitness, indicating that field aggregations may be due to constraint. Support for one hypothesis does not preclude potential support for the other. For example, even in the presence of negative effects of egg aggregation on offspring, communal nesting may still be adaptive if it enhances maternal survival, which was not measured in our study. When nest sites are limited, communal nesting may reduce energetic costs associated with nesting and allow more time for feeding and thermoregulation (Doody et al., 2009). Moreover, this behaviour may decrease the likelihood of maternal depredation during egg-laying (Burger, 1993). For these reasons, females sometimes prioritize their own fitness over that of their offspring (i.e. fecundity-survival hypothesis), which may result in females laying eggs in maladaptive aggregations (Refsnider & Janzen, 2010). In lab and field studies, anoles select nesting sites that enhance offspring survival (Reedy et al., 2012; Tiatragul, Hall, & Warner, 2020); however, no studies have assessed how females might prioritize their own survival over that of offspring during nesting. Given that brown anole life-history is characterized by a short lifespan and relatively high reproductive effort, females might prioritize offspring survival over their own (Clark & Martin, 2007). However, in the field, we often find communal nests beneath cover objects (e.g. rocks, fallen palm fronds). Such environments conceal nesting females, potentially reducing depredation, and provide microenvironments that are highly suitable for egg development (i.e. stable temperatures and moist substrate). Thus, in this species, communal nesting may minimize trade-offs between maternal and offspring fitness. Field studies addressing these issues are warranted. Regardless, support for both the adaptive and constraint hypotheses in the current study indicates there may be interesting trade-offs that characterize the evolution of communal nesting behaviour, and we suggest that anoles will make excellent models for studying these trade-offs in the future.

CONFLICT OF INTEREST

The authors declare no conflicts of interest for this study.

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