Capstone Title: “An Overview of Reproduction and Development in *Rhacodactylus auriculatus*”

Written November 2021 by Ingrid Schoonover in completion of American University’s BS in Biology curriculum.

Background – What is a Gargoyle Gecko?

Species: *Rhacodactylus auriculatus* (Gargoyle gecko)

Gargoyle geckos are one of a few species that are considered “Giant Geckos”, adults weigh 40 to 60 grams and are 105 to 122 millimeters from snout to vent. These geckos are endemic to New Caledonia, an island in the Pacific Ocean (Figure 1). New Caledonia can be characterized by a warm subtropical to tropical climate, ultramafic (metallic) substrate, mountains, and high levels of biodiversity (58 endemic gecko species). The ancestor to all New Caledonian geckos arrived on the island ~22.4 million years ago via long-distance overwater dispersal. The gargoyle gecko has ancient roots and diverged approximately 14 million years ago (Skipwith, Bauer, Jackman, and Sadlier, 2016). Today all of the New Caledonian biodiversity is threatened by mining, invasive species, and logging.

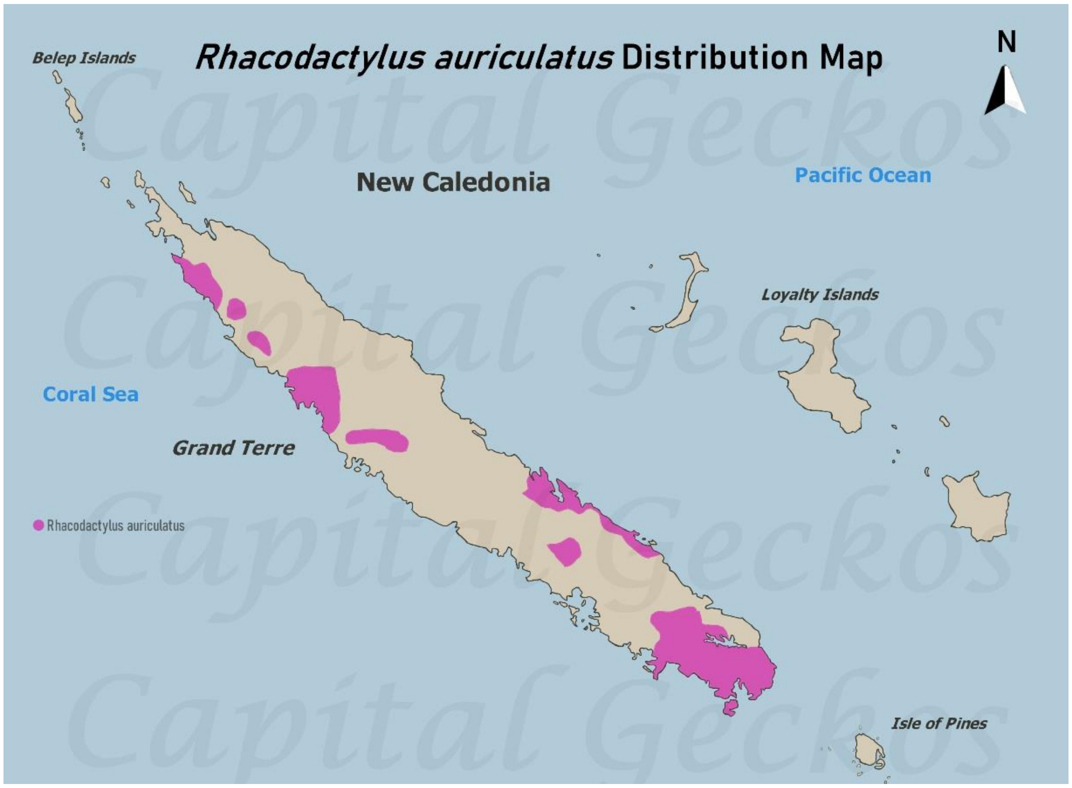


Figure 1: Distribution map of Rhacodactylus auriculatus based on historical sightings and suitable habitat.

Experimental Design

Experimental Goals: (1) Characterize reproduction and development in *R. auriculatus*, (2) investigate the factors resulting in reproductive success, and (3) explore the interaction between developmental variation and the environment (developmental plasticity).

Methods:

- Sample population: 11 adults and 38 offspring.
- Controls: all individuals reared under same environmental conditions: same diet, seasonal variation in temperature/humidity/light cycles corresponds to NC climate.
- Data collection: Frequently measured and weighed all individuals, clutch information (date, relative egg mass, clutch order, incubation temp. and length, fertility), growth rates.
- Statistical analysis to interpret data and determine significant interactions.

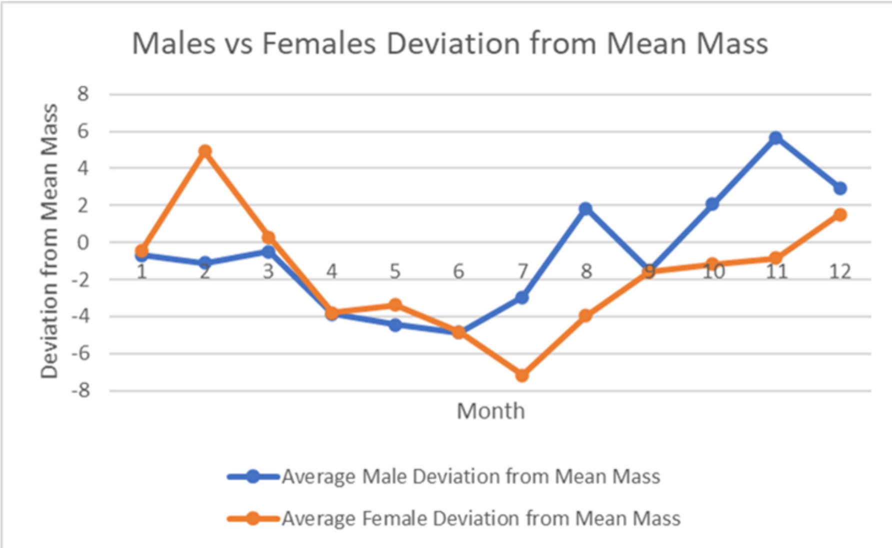
This presentation will be divided into 4 sections: (1) Breeding behavior and reproduction, (2) Oviposition and eggshell morphology, (3) Embryonic development and incubation, and (4) Growth and development.

ADULTS IN THE STUDY (N=11)

Section 1: Breeding Behavior and Reproduction

Breeding Period

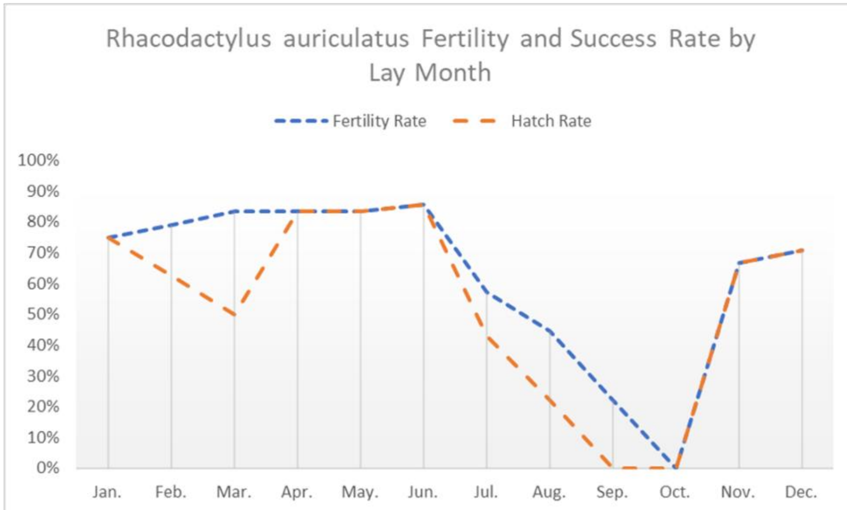
Gargoyle geckos breed during the late winter and spring, during which time both sexes tend to lose a lot of their mass as they invest a lot of energy into courtship and mating. Reproduction and breeding behaviors are energetically costly for both males and females, so they need a recovery period in the summer through the fall. The seasonal variations in their weight correlate with energy invested in reproduction during breeding periods (Graph 1). Tail loss is a common occurrence in the wild populations with 75% of the males and 51% of the females of the population near Noumea in a 2010 survey by Synder et al. with regrown or no tails. The dagger-like nature of their teeth presents an additional risk during copulation, and sometimes the male gargoyle gecko can inflict deep wounds on the shoulders, neck, and head of the female gecko when he bites her in the courtship process. In captivity they form pair bonds and communicate with vocalizations and tail wiggling.



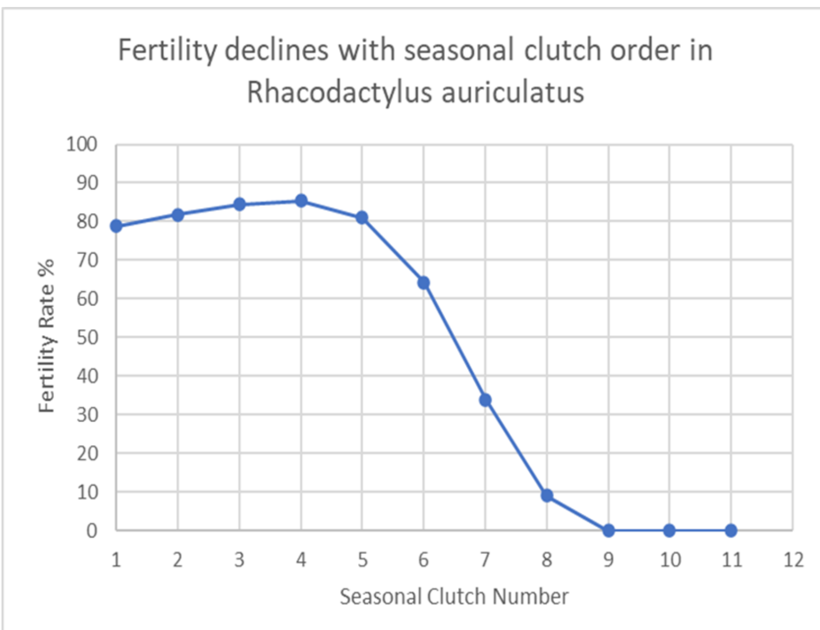
Graph 1: Displaying the seasonal variations in mass observed in the captive population (n=11) of gargoyle geckos. Females increased in weight from December to March before decreasing from April to November. Males increased in weight from August/October until December before decreasing in mass from January to July/September.

Fertility and Hatching Success

Fertility and hatching success correspond with parental energetic investment, with reproductive success being the highest during the breeding period and then dropping down to 0 as they enter their recovery period in the late summer and early fall (Graph 2). This is reflective of maternal investment in egg production and paternal investment in mate guarding plus sperm production. Fertility rates also declines with seasonal clutch order, with the success of fertility in the first five clutches being the highest (Graph 3). Notably, there was no trend between fertility rates and lifetime clutch number, which refers to the total number of clutches the gecko has laid in their life rather than just how many clutches they laid that season. This indicates that the recovery period in the late summer and fall is important for them to focus their energy on foraging and replenishing their fat reserves for next years breeding season.



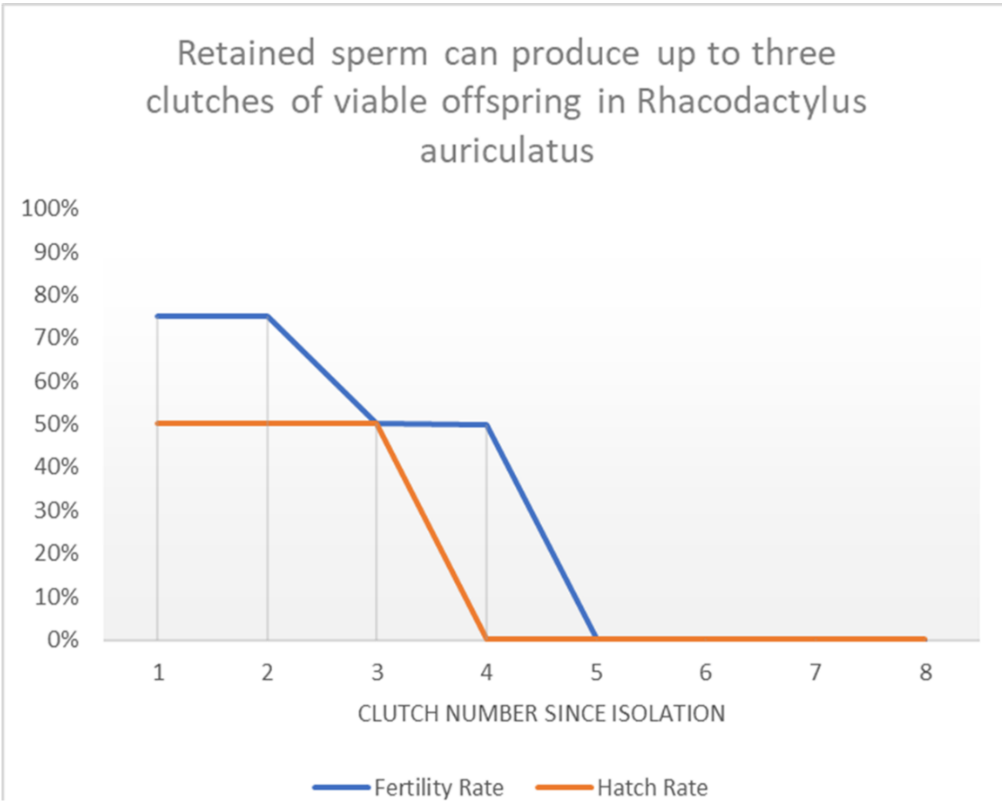
Graph 2: Demonstrating the likelihood of successful fertilization in Rhacodactylus auriculatus by month. The fertility rates are highest during the winter and spring and lowest during the summer. Hatch rate in the graph refers to the likelihood of the fertile egg also being viable, because high rates of non-viable fertile eggs are laid in the fall and winter.



Graph 3: On average the first clutch of the season has a lower fertility than subsequent clutches up until the fifth clutch of the season. After the fifth clutch of the season the fertility rate begins to rapidly decrease, and then only infertile eggs are laid. The greatest number of clutches recorded for one female in a year was 11 clutches of eggs.

Sperm Retention

Gargoyle geckos can use retained sperm to produce up to three viable clutches (R-Squared = 0.80 and P-value= 0.002) (Graph 4), more research in the future will reveal if it's possible for them to store sperm for even longer. The analysis revealed that clutch order rather than time duration is a more important predictor of the success of retained sperm, this indicates that the decreasing fertility rate is the result of mechanical sperm damage from the passage of the eggs through the uterus. Sperm retention allows for delayed reproduction, highly successful dispersal, and sperm competition.



Graph 4: Gargoyle geckos can retain sperm after copulation to produce viable offspring for up to three clutches in isolation.

Section 2: Oviposition and Eggshell Morphology

Gargoyle geckos are oviparous, and females start laying eggs when they are 1.5 to 2 years old (Figure 2). Semi-calcified eggs are typically laid in clutches of two during the spring and summer. Females lay 6.5 (4 to 11) clutches per year every 36 days (10 to 70). Egg mass relative to maternal body mass declines with clutch order (R-Squared = 0.51 and P-value= 0.002) (Graph 5) and Females with a greater seasonal variation in body mass lay larger eggs on average. (R-Squared = 0.13 and P-value= 0.002).

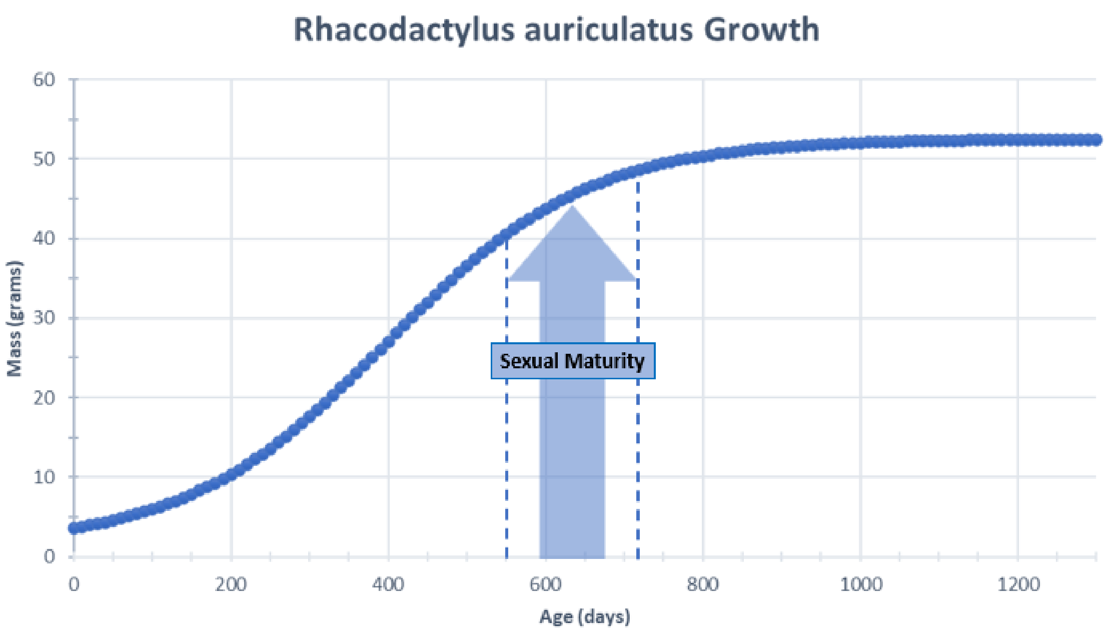
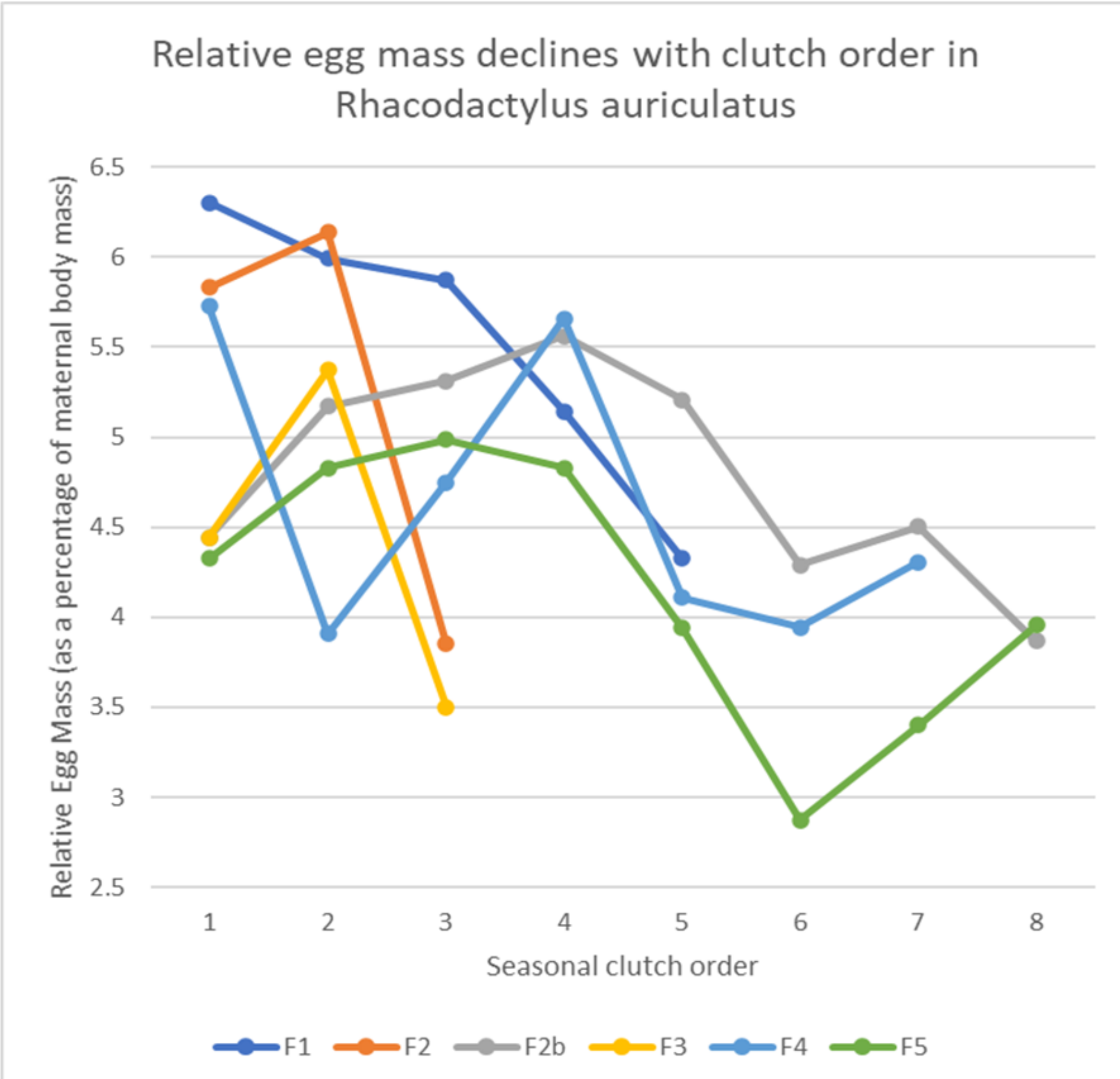


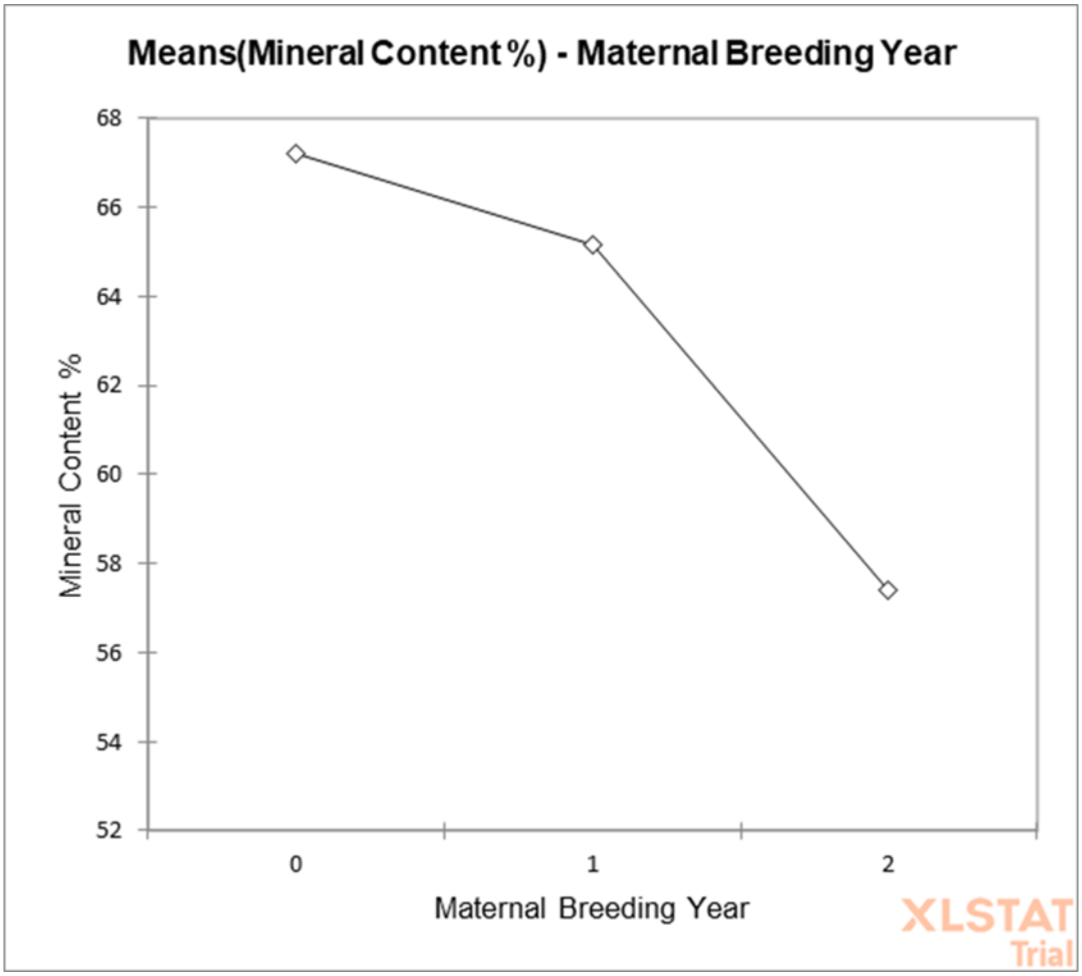
Figure 2: Sexual maturity is reached around the age of 1.5 to 2 years, males are usually quicker to become sexually active.



Graph 5: Each marker corresponds to a different individual female. At the start of the season the female gargoyle geckos lay eggs that are between 4.3% and 6.3% of their body mass, but after the first three or four clutches the eggs become much smaller (2.8% to 4.5% of their body mass) in relation to their body size.

Eggshell Adaptation: High Mineral Content

The average mineral content by dry mass is 64% (55% to 71%) (n=16). This is very unusual because most geckos either have very hard or very soft-shelled eggs, but instead this represents an intermediate condition. In comparison (Robin, 2017): rigid-shelled gecko eggs have a mineral content of 90% to 95% by dry mass, and flexible-shelled gecko eggs have a mineral content of 15% to 16% by dry mass. This intermediate level of mineralization is most likely an adaptive benefit for an arboreal lifestyle because water vapor permeability decreases with increasing mineralization which provides resistance to desiccation, and this would allow them to lay eggs in tree hollows instead of being limited to burying them in wet soil. Furthermore, mineral content declines with breeding season, which is reflective of lifelong reproductive constraints (Graph 6).

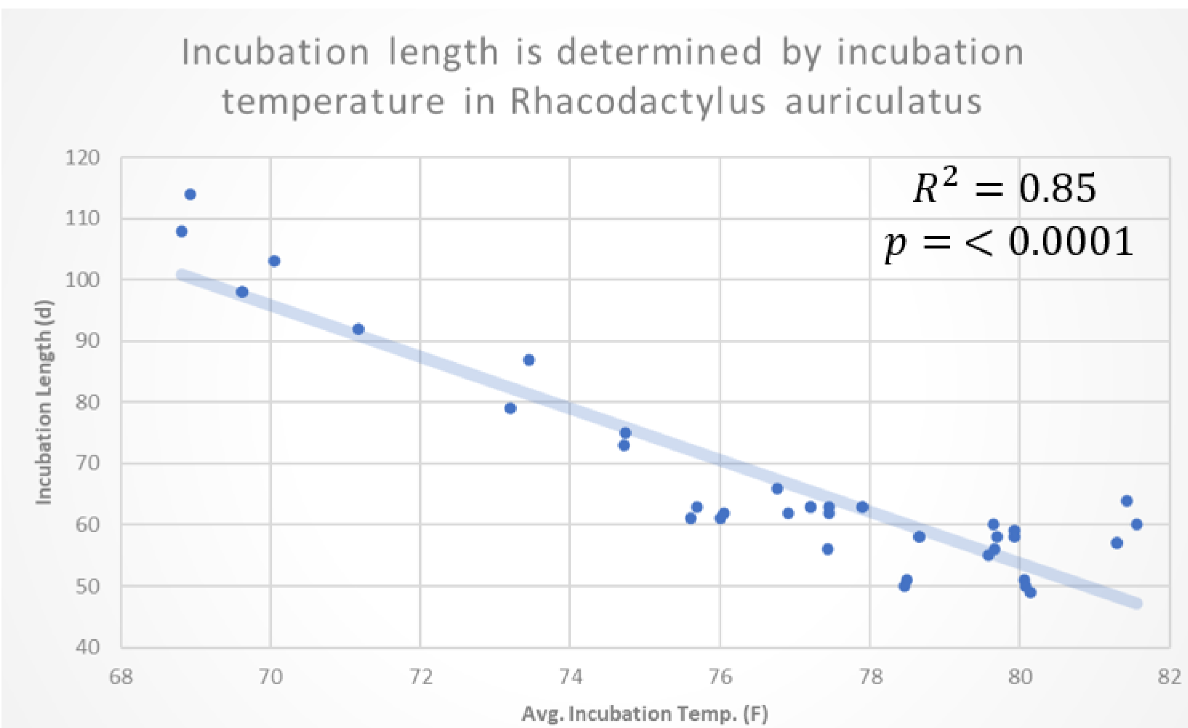


Graph 6: Average mineral content (as a percentage of dry mass) in Rhacodactylus auriculatus eggshells is the highest for virgin geckos and then declines for breeding geckos.

Section 3: Embryonic Development and Incubation

Incubation

Incubation length is a function of incubation temperature, with the length of incubation decreasing with increasing temperatures (Graph 7), in this experiment the length of incubation for the offspring ranged from 49 to 114+ days.



Graph 7: Incubation length is the shortest for the geckos incubated at the high temperatures and the longest for the geckos incubated at low temperatures.

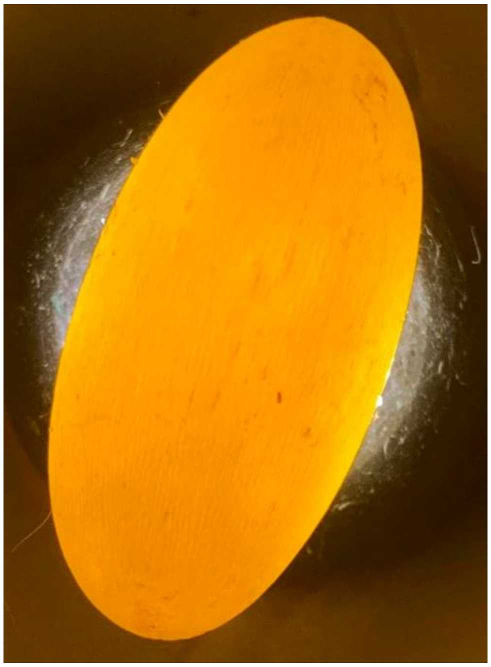


Figure 3: Infertile egg with no vasculature.

Figure 4: Fertile newly laid egg showing the beginning of vasculature development. The location of the embryo was marked with a sharpie so the eggs could be placed with the embryo upright in the incubator to prevent accidentally drowning.

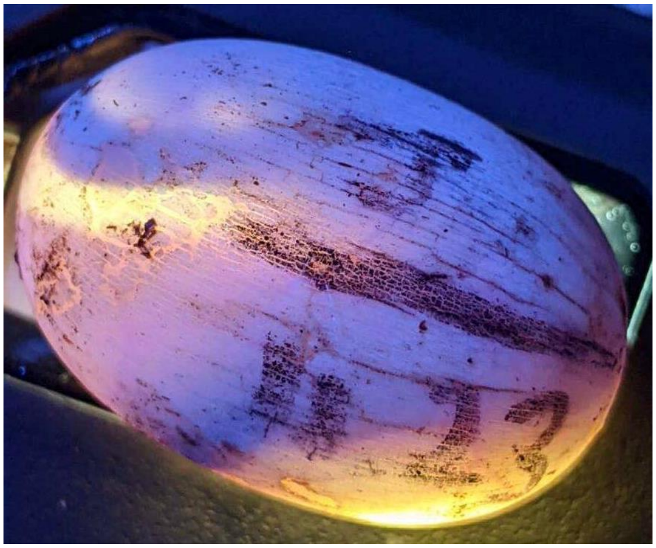


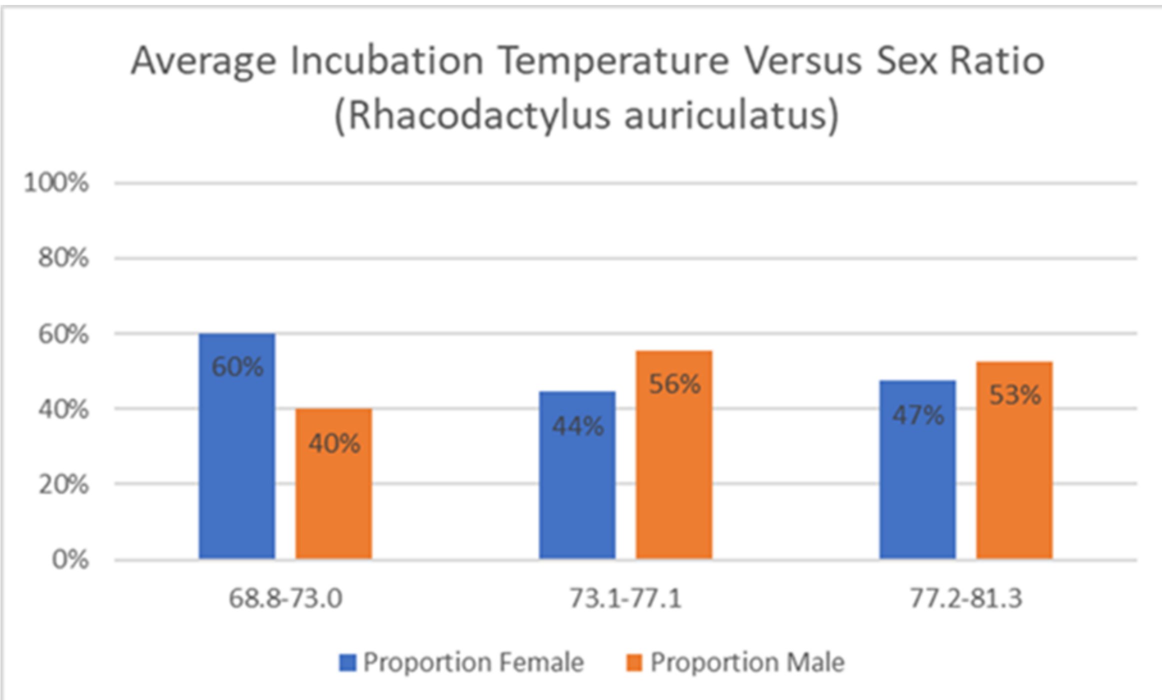
Figure 5: Gargoyle gecko right before hatching.

Hatchlings

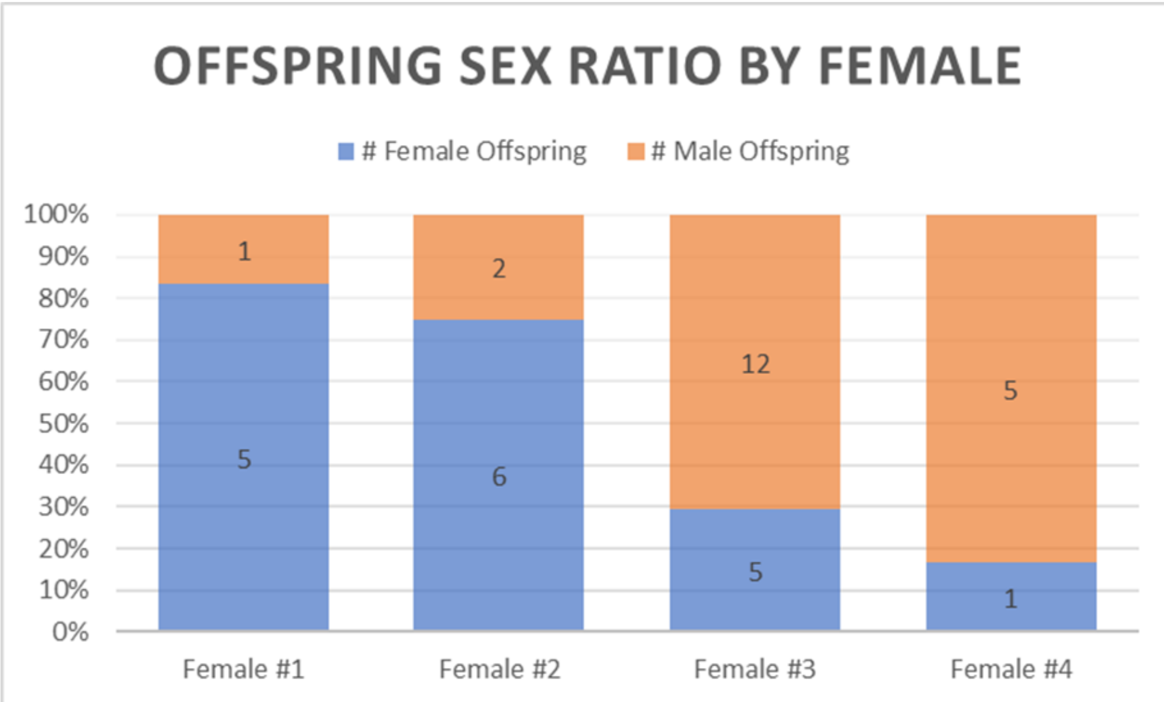
Hatchlings emerge weighing 2.6 grams (1.8 to 3.1 g) and are 39 millimeters in length from snout to vent (35 to 45 mm). Hatchling mass increases with increasing relative egg mass (R-Squared = 0.41 and P-value= 0.000003). In total, 67% of the variation in hatchling mass can be explained by the combination of three variables: (1) Relative egg mass, (2) SD in maternal mass, and (3) maternal mass at oviposition. (R-Squared = 0.67 and P-value= 0.0001).

Sexual Determination

Gargoyle gecko sex phenotype is not exclusively determined by incubation temperatures, with both sexes being produced across the entire range of incubation temperatures (Graph 8). However, there were significant differences in the sex ratio among offspring by maternal identity (Graph 9). Overall, the captive population produced close to a 1:1 sex ratio, but there was considerable variation between individuals. Two females produced a high ratio of female offspring (75% to 83% female), and two females produced a low ratio of females (17% to 30% female). One possible way to explain this observation is that the interaction between genotype and environment is responsible for determining the sexual phenotype, however, the sample size is small so it's also possible that this skew is a result of sampling error. Surveys of wild populations showed a moderate female bias, with 1.6 females for every 1 male (n=73), but it is unclear whether this is the natural sex ratio of the population or if the skew is the result of male-on-male aggression.



Graph 8: There was no clear relationship between the sex ratio of the offspring and what temperature they were incubated at, but on average the highest proportion of females were produced from lower incubation temperatures. Overall, the sex ratio of the entire captive population was close to 1:1.



Graph 9: Demonstrating the variation in the sex ratio of Rhacodactylus auriculatus offspring by maternal identity. Notice how female #1 and female #4 had opposite trends in terms of bias, this could possibly be explained by the females having different genotypes if it turns out that the mechanism of sex determination is the result of a genetic x environmental interaction.

First Confirmed Observation of Parthenogenesis in the Diplodactylidae family

Parthenogenesis is a form of asexual reproduction, in which embryonic development proceeds without the fertilization of the egg by sperm. This is incredibly rare and only seen in 0.1% of all species. In this experiment 1/19 (~5%) of "virgin" gargoyle gecko eggs developed into viable parthenogenetic offspring (Figure 6 and 7). This is the first case of parthenogenesis reported for the entire Diplodactylidae gecko family (136 species). This would be considered an example of facultative parthenogenesis because the gargoyle geckos can still reproduce sexually, in fact the female that produced the parthenogenetic offspring later went on to have six sexually produced offspring. This method of parthenogenesis is different than the obligate parthenogenesis which is usually seen in other gecko species.

Figure 6: Parthenogenetic offspring right after hatching, its identification code is Oct1 named after it's mother "Octave".

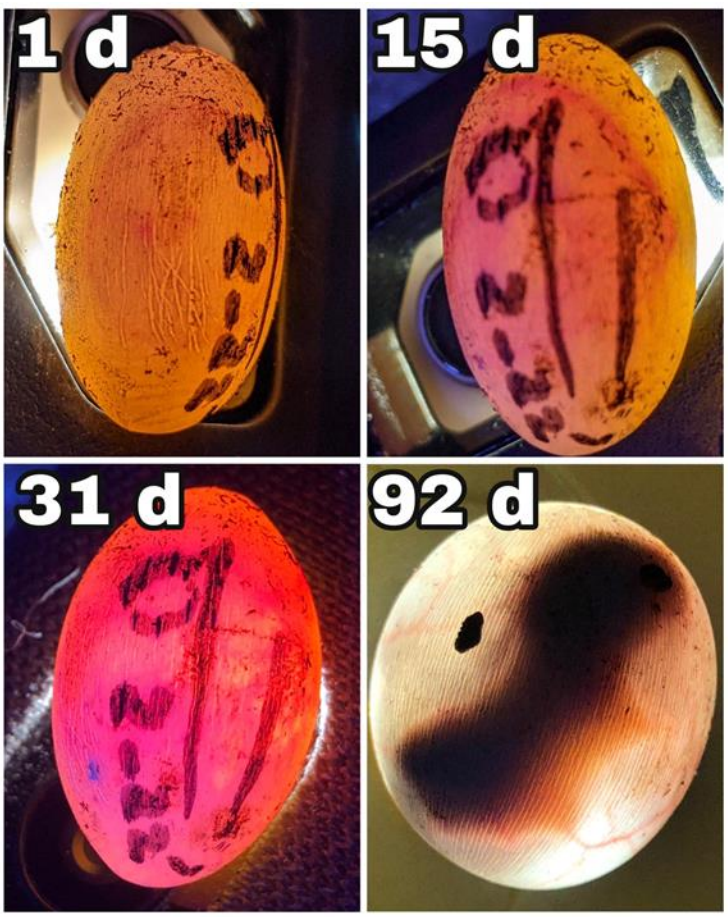


Figure 7: Development of the parthenogenetic gecko from day 1 to day 92 of incubation.

It's too early to tell the mechanism but I suspect terminal fusion automixis.

- Apomixis = Mature egg cells develop into embryos without meiosis. Resulting offspring should be full clones of mother.
- ***Automixis*** = Meiosis occurs as is normally seen in sexual reproduction, but then egg fuses with a polar body instead of sperm. Resulting offspring should be half-clones of mother.
- Endomitosis = Chromosomal duplication occurs before meiosis and then meiosis restores diploidy. The resulting offspring can be full clones or half-clones of the mother depending on the mechanism.

The consequence of parthenogenesis in this case is a craniofacial deformity (Figure 8 and 9), which probably results from reduced heterozygosity. Otherwise, it has been developing normally and is now six months old.

Figure 8: Left eye of the parthenogenetic offspring, the pupil is fixed in place and nonresponsive to light.

Figure 9: Right eye of the parthenogenetic offspring, this pupil responds normally to light and appears to operate as it should.

Section 4: Growth and Development

Growth rates are highly variable and depend on genetic and environmental influences (Figure 10).

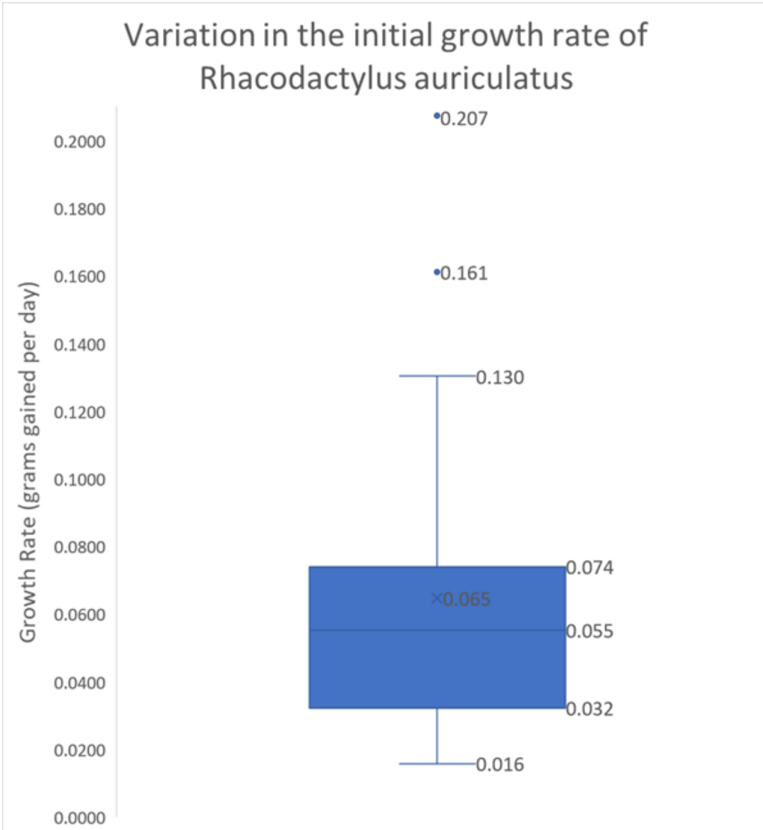


Figure 10: There is significant individual and family variation in the growth rate of Rhacodactylus auriculatus. Growth rate was measured as the rate of change in mass during the first 3 months of a hatchling geckos' life.

Growth Rate is Determined by Initial Body Condition and Maternal Influence

85% of the variation observed in the growth rate of R. auriculatus offspring can be explained by (R^2=0.85 & p=0.00001):

(1) hatchling body condition [g/mm]

(2) relative egg mass: offspring from larger eggs grow faster than offspring from smaller eggs

(3) maternal body mass and body condition [g/mm]: offspring from high quality females grow faster than offspring produced by poorer quality females

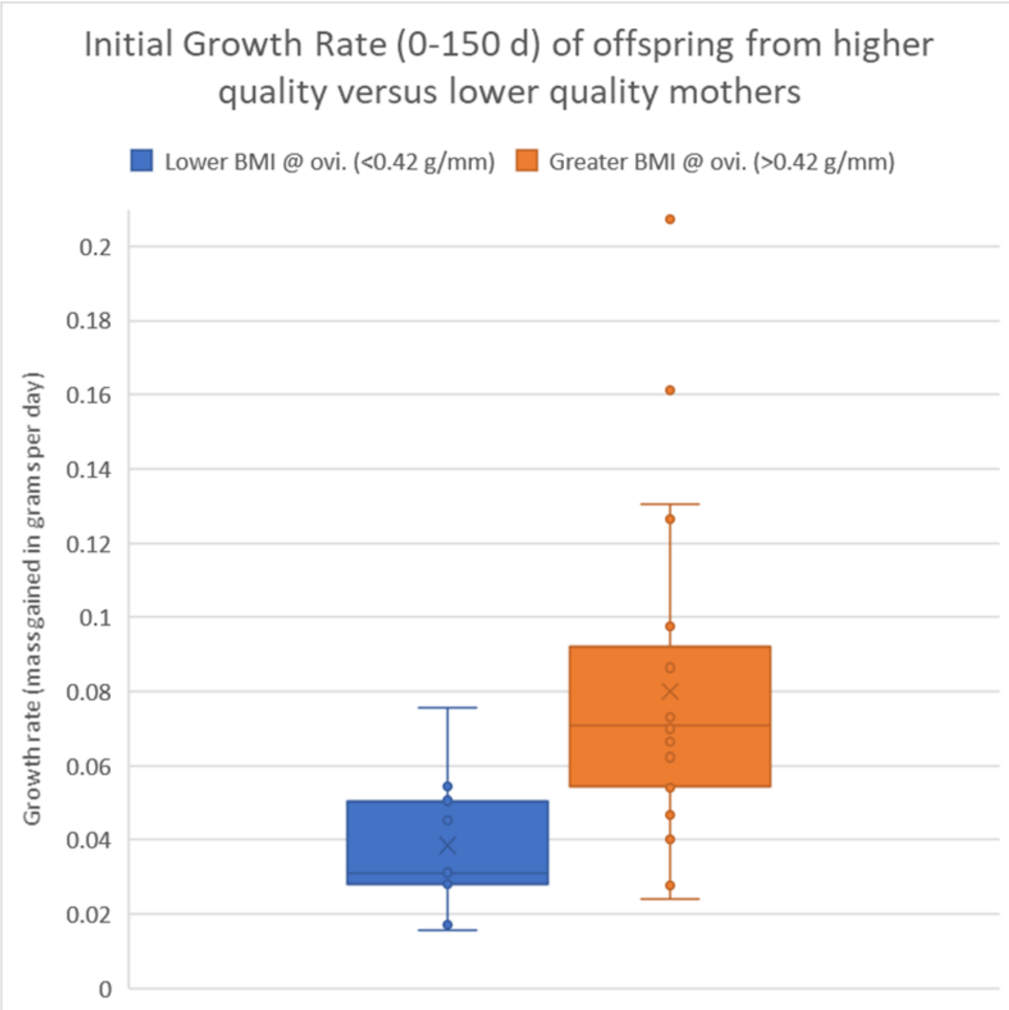


Figure 11: Maternal body condition is an important predictor of offspring growth rate in Rhacodactylus auriculatus. Body condition was measured as the mass of the gecko in grams divided by the snout to vent length in millimeters. The offspring from the higher quality females (heavier) grew twice as fast as the offspring from the lower quality females (lighter).

Maternal body condition has a significant impact on the growth rate of the offspring (Figure 11), with female geckos with a BMI score below 0.42 g/mm producing offspring that grew at the rate of 0.038 g/day +/- 0.017 (n=11), and female geckos with a BMI score above 0.42 g/mm had offspring that grew at the rate of 0.080 g/day +/- 0.043 (n=21).

Summary

- Seasonal reproductive success in *Rhacodactylus auriculatus* is constrained by energy reserves. This claim is supported by four observations: (1) reproductive period characterized by weight loss followed by recovery period of weight gain, (2) fertility rate highest when parents invest more energy into reproduction (decline in mass), (3) fertility rate declines with clutch order, (4) relative egg mass declines with clutch order.

- Sperm retention allows for delayed fertilization of up to three viable clutches.
- Lay 6.5 clutches of two eggs during each reproductive season.
- Novel eggshell morphology (64% mineralization) – possible adaptation for exploiting arboreal nesting sites.
- Developmental plasticity – variation in hatchling body condition and initial growth rate is influenced by environmental conditions.
    - Maternal investment (seasonal variation in mass as a proxy for energy expenditure) corresponds to relative egg mass.
    - Higher quality females lay larger eggs and produce faster growing offspring.
    - Initial hatchling body condition is in part determined by relative egg mass, which in turn influences the growth rate.
- First case of facultative parthenogenesis in the Diplodactylidae gecko family

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Questions and Answers:

Q1: *"Based on your research so far and what you have observed, what do you think your future direction is? Is there any aspects you want to go back and research further or do you have other ideas for how you want to progress your research?"*

A1: I am hoping that having scientific data about the reproduction of these geckos will help in captive breeding programs and can help identify the factors that are most important for reproduction in wild populations. For example, knowing that reproduction in Rhacodactylus auriculatus is constrained by seasonal energy reserves means that conservation efforts should prioritize maintaining adequate food sources for them. Unfortunately, at the moment there are no conservation programs for them in the wild, but I am hoping that increased awareness puts pressure on the New Caledonia government to implement new policies for the protection and research of these geckos. I plan on continuing research with New Caledonia gecko species over the next few years, this presentation was just focused on *Rhacodactylus auriculatus* but I am also working with 5 other species: *Rhacodactylus leachianus, Mniarogekko chahoua, Correlophus ciliatus, Correlophus sarasinorum*, and *Bavayia cyclura*. I have more experiments planned with the gargoyle geckos for 2022 to try to determine the mechanism of sexual determination, also when the parthenogenesis baby is sexually mature, I want to test if it is capable of producing viable offspring. I'd like to do more research with parthenogenesis to see how common it is and under what conditions it arises.

Q2: *"Are the findings from the Gargoyle Gecko study applicable to other types of Geckos, and what are any predicted Gecko genetics-genome implications?"*

A2: I don't have enough data collected for other New Caledonia gecko species to be able to comment on the applicability to other gecko species, but I also conducted eggshell analysis for two other genera of New Caledonia geckos and found that they had similar levels of mineralization. So, I suspect that the novel eggshell is widespread across the giant gecko species (genera: *Rhacodactylus, Correlophus,* and *Mniarogekko*) and represents a condition derived from a common ancestor. The New Caledonia environment is pretty unique, there is really nowhere else like it so all of the animals there experienced the same selective pressures from the environment, so I would imagine that the seasonal energy constraints are also experienced by the other New Caledonia giant geckos because they have similar diets. But there is also a lot of variability within the new Caledonia Gecko clade so really all of those species need to be studied independently without just making assumptions, because for example in the same genus as the gargoyle gecko there are two other species with some notably distinct differences; one of the two species is actually viviparous and gives birth to one young per year, and the other species (*R. leachianus*) has more complex social hierarchies and relationships between individuals. The Gargoyle Geckos can get along in pairs, but they are not strictly monogamous, and you can switch around their mates without much issue, but in the leachianus gecko they appear to be strictly monogamous and if you try to pair them with multiple mates or switch pairings they will kill the new partner. But some of the other findings are definitely more general, there is a whole area of behavioral ecology that looks at how energy and physiological constraints relate to behavioral decisions regarding parental investment, egg size, etc. Similarly, a study (see below) with the gecko species *Heteronotia binoei* found that hatchling size is also influenced by relative egg mass and that relative egg mass is determined by maternal investment. In regard to the genome implications, I haven't done any research with that yet mainly because it is not my area of specialty so I would need a lot of help from others to be able to afford the sequencing and interpret the results. However, I would definitely like to do more with that and in particular, I would like to look at the genetics of the mother that produced the parthenogenesis baby versus the parthenogenesis offspring versus her sexually produced offspring. In class, I was talking about the different types of parthenogenesis and if I knew what the genetic relatedness was between the mother and her asexual baby then it would be a start to identify the mechanism, and knowing the mechanism is important for being able to tell if this is a widespread occurrence and whether or not it could be an evolutionarily stable strategy. Here are some related articles that I came across if you would like more resources about energy constraints and reproduction: 1. Andrewartha, S., Mitchell, N., and Frappell, P. 2010. Does Incubation Temperature Fluctuation Influence Hatchling Phenotypes in Reptiles? Physiology and Biochemical Zoology. 83(4):597-607. And 2. Koch, L., and Meunier, J. 2014. Mother and offspring fitness in an insect with maternal care: phenotypic trade-offs between egg number, egg mass and egg care. BMC Evolutionary Biology. 14:125.

Q3: *"Your research was super impressive! How long have you been working on your research with Gargoyle Geckos? Do you plan to continuing researching their breeding habits and behaviors or are you hoping to explore another aspect of their behaviors in the future?"*

A3: Thank you for your feedback and support! I have been doing research with gargoyle geckos since May 2019 and I plan on continuing research with them and other New Caledonian gecko species for a while because I still have more questions than I have answers. I think that understanding their reproduction is critical for being able to implement conservation policies because with this knowledge we can assess what the biggest threats are to their survival as a species and what areas of their ecology to focus resources into. This being said there is more work that needs to be done to determine the mechanism of sexual determination, lifetime reproductive fitness (as opposed to just seasonal behavior and trends), and occurrence of parthenogenesis. So, I will definitely continue with the reproductive research, but I would also like to explore other aspects of their behavior such as the role of color in their communication. They have the ability to move pigments very quickly from the surface of their skin to a deeper layer, this has the effect of quickly lightening or darkening them (kind of like a chameleon). I think that it's really cool that they have the ability to do this, but I am not sure what the significance this is, and this would be a really cool area to look at! I would also love to be able to travel to New Caledonia to collect data and brainstorm some conservation strategies.


Fired up


Fired down