Variation in incubation onset in birds and its effect on the development of embryos

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Abstract/summary

Incubation predominantly occurs in the class of birds. Many different strategies of incubation are known among this group. The timing of onset of incubation also varies much throughout. Some birds start partially incubating their eggs before the final egg has been laid, while others initiate incubation when the final egg has been laid, at clutch completion. Some birds even decide to delay incubation until a few days have been passed since clutch completion. Various different factors can be attributed to these highly varying strategies. Partial incubation seems to occur in species where ambient temperatures are higher and when clutch sizes are larger. Food abundance peaks early in the breeding season have also been shown to be driving factors. Fitness benefits to partial incubation include protection of the clutch from predation and brood parasitism. Partial migration has also been shown to decrease microbial load on eggshells. A second strategy is incubation at clutch completion, a strategy that is often observed along with the other two. A possible driving factor for this strategy is prevention asynchrony. Food abundance peaks have also been shown to cause parent birds to delay incubation until after clutch completion. This showcases high variability as the same factor is able to be the driving force for two differing strategies. Delayed brood patch development and inclement weather conditions have also been raised as possible driving forces for this strategy. Fitness benefits of partial migration include the ability of parents to reduce brood size when food is scarce. Delaying of incubation can allow parent birds to prepare food reserves for the offspring and to sustain themselves. However, not much research has been done on the effects of the different incubation strategies on the embryonic development. More extensive research needs to be conducted in order to investigate whether such effects exist. It is difficult to perform research on bird embryos due to the fact that they are very vulnerable to changing ambient conditions, making it risky to study them. I conclude that the best way to investigate this is to perform research on a large study population which is known to exert all three main incubation strategies. A comparative analysis using a few known parameters of egg embryo viability (such as infection rates or levels of hormones in yolk) can be performed between the differing strategies, in order to find out whether different incubation onsets do induce different embryonic responses.

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Introduction

The class of birds is a very diverse and large class of animals. Birds show large differences in the process of incubation of the eggs. These differences are often shaped by abiotic factors such as temperature, the abundance of water and the climate. Biotic factors, such as predation, parasitism and vegetation also influence different strategies in birds. Incubation is incredibly widespread among birds and all species incubate in order for their eggs to develop. Without incubation, eggs will not reach temperatures high enough for embryonic development to start. (Drent, 1975). While the incubation temperature differs between species, on average a temperature of 37 degrees Celsius is the optimal temperature for embryonic development (Ehrlich, Dobkin and Wheye, 1988). Temperatures straying too far from this optimum will result in lower embryo survival. Incubation temperature also has an impact on the development of embryos and the hatched offspring alike. Research on Peking ducks (*Anas platyrhynchos*) shows that a small increase of temperature during incubation above the standard 37.5 degrees, has a positive effect on the immune system and survival of the offspring (Shanmugasundaram, Wick, & Lilburn, 2018). Incubation temperatures below the normal level could result in embryo retardation in development of proper homeostasis (Tzschentke & Rumpf, 2011). According to a study performed on domestic chickens (*gallus gallus domesticus*), suboptimal incubation temperatures resulted in decreased growth and hatching rate. These individuals were also found to have an increase in the release of stress hormones (Bertin et al., 2018). Temperatures that are too high during the incubation phase can cause heat stress in embryos. Birds can prevent overheating by shading their eggs (Clauser & McRae, 2017) and to keep incubation temperatures at an even level (Brown & Downs, 2003). Small amounts of heat stress can induce positive effects however, as shown in domestic chicken embryos exposed to temperatures up to 39.5 degrees Celsius for short periods. These individuals were shown to have increased diameters of myofibers and enhanced muscle growth (Piestun, Barak, Halevy, Yahav, & Harel, 2008), suggesting higher survivability after hatching.

Incubation of eggs also comes with costs for the parents. It is an energy-intensive process and reduces the time for other fitness-related behaviours, like foraging and copulating (Thomson, Monaghan, & Furness, 1998). Some parent birds are forced to speed up their foraging times in order to be able to allocate enough time to incubation (Dobbs & Martin, 2007), which can lead to decreased parental fitness. Wild populations have been observed to incubate for longer compared to captive individuals (Jia, Sun, & Swenson, 2010). This is likely to due the fact that birds in captivity are able to feed more efficiently and are therefore able to allocate more energy and time on the nest and eggs.

Different incubation strategies have been observed in bird species, both in wild as well as captive populations. Interestingly, these different strategies have been observed to not only occur interspecifically. The are also known to differ within species and even within populations of the same species (Wiebe, Wiehn, & Korpimäki, 1998) (Álvarez & Barba, 2014). While a lot of research has been done on the driving factors of different incubation differences, studies dedicated to finding out what their effects on embryonic development are remain scarce. My goal is to investigate what the effects of different incubation strategies are on the embryonic development and the survival of the hatched offspring. What potential benefits do different incubation strategies offer for birds? Do these strategies depend on environmental factors? And why is it that these strategies also differ within populations. Three common types of incubation patterns will be discussed here, specifically concerning the different types of onset of incubation.

Types of incubation and their driving factors

Partial incubation

The first type of incubation is known as partial incubation and means essentially that the female will start small bouts of incubation of the eggs before the final egg is laid (Wang & Beissinger, 2011). Usually these bouts last for minutes or hours, as opposed to full incubation, in which case the parent birds only stop incubation to look for food. Some studies refer to this as early incubation. This naturally means that the earliest laid eggs receive the highest amount of heat, while the eggs that were laid last get the least heat transfer. This can lead to differences in the development of the embryos and the hatching chicks, most prominently hatching asynchrony. Early research done to hatching asynchrony in birds found that this hatching asynchrony allows the parents to easily manage their offspring. When size differences exist between offspring, the smallest chicks will often receive less food when food is in short supply. This causes the weaker and smaller chicks to die quickly, while allowing the parents to successfully raise the larger, stronger offspring, since they are more likely to still get enough food to grow and remain healthy (Lack, 1948). Lack suggested that if all offspring were the same size, all offspring would weaken due to low food abundance, a strategy that is generally unfavourable compared to allowing a few individuals to grow strong. This hypothesis was dubbed the Brood Reduction hypothesis and describes how parent birds can easily reduce brood size if needed. While this theory is generally well accepted, not all researchers are satisfied with the theory as an explanation for hatching asynchrony. In a study done on great tits (*Parus major*), the onset of incubation was experimentally manipulated by removing and adding eggs to nests, in an attempt to find evidence for the Brood Reduction Theory. No strong support for the theory was found however, (Podlas & Richner, 2013) and other research performed on great tits also found no support for the theory (Hörak, 1995). Research done to the house sparrow (*Passer* domesticus) shows that they also initiate incubation before the final egg is laid (Aldredge, 2017). The conclusion drawn here is that house sparrows base their decision on a trade-off between high embryo survivability and larger hatched offspring.

Clutch size

Clutch size may have an effect on the decision of parents to initiate incubation earlier. In a study performed on five different species of passerine birds, female birds with larger clutch sizes were found to initiate full incubation earlier than birds with smaller clutch sizes. It was found that partial incubation occurred in many of the birds, with larger clutches causing females to start incubating earlier relative to smaller clutch sizes. (Wang & Beissinger, 2009). The study suggests, however, that a larger clutch size represents higher parental fitness, enabling an earlier onset of incubation. Another study on the same species, the tree swallow (*tachycineta bicolor*), found no such correlation however (Ardia & Clotfelter, 2007). This again showcases the variability within species and suggests that other factors influence the evolution of varies incubation strategies.

Temperature

One of these factors appears to be ambient temperature during egg-laying. In a study performed on Mediterranean Great Tits (*Parus major*) in Eastern Spain, it was found that the higher the temperatures were during the laying period, the earlier the onset of incubation relative to clutch completion (Álvarez & Barba, 2014). The average day of onset of incubation was found to be one day before clutch completion, although the onset varied from three days before clutch completion to six days after clutch completion. Álvarez also concluded that the larger the clutch size, the earlier the onset of incubation.

A similar result in regard to onset of incubation in relation to ambient temperatures has been found in other studies. In king rails (*Rallus Elegans*), the ambient temperature is prone to significantly drop below or rise above the optimal temperatures that lie between 26 and 40.5 degrees. Researchers found that king rails that laid eggs later in the season, when temperatures were higher, would start incubating earlier than their earlier counterparts (Clauser & McRae, 2017). While this was most likely done to shield the first eggs from high ambient temperatures, it also led to considerable hatching asynchrony. In tree swallows, higher ambient temperatures led to early onset of incubation, usually prior to clutch completion (Ardia, Cooper, & Dhondt, 2006).

Food availability

Some other factors have been attributed to early incubation. In a study on blue tits (*cyanistes caeruleus*) in Spain it was shown that birds that started incubation early hatched their offspring during peak food abundance (García-Navas & Sanz, 2011).

Full incubation onset at clutch completion

Another strategy for birds is to start incubating the eggs when the final egg has been laid, at the so-called clutch completion. Full incubation at clutch completion could be done to prevent hatching asynchrony in the chicks. This problem can be prevented because all eggs will start their embryonal development at the same time, when the parent start incubating. Not many research has been done specifically on this strategy. Many study populations exhibit this strategy, along with the others strategies, in which small factors influence the individual decision to incubate.

Incubation after clutch completion

Some species choose to start incubation after the last egg has been laid. This is known as onset of incubation after clutch completion. Different driving factors for this strategy have been observed. Some bird species might delay incubation as a strategy to maximize food abundance during the hatching period. In blue tits living in the south of England, about one fifth of an studied group of birds was found to start incubation after clutch completion and that their hatching period was in accordance with the budding of oaks (Stenning, 2008). Because Stenning found a huge variation in incubation delay in clutches that hatched synchronously, he concluded parents delayed incubation of their eggs for reasons other than inducing hatching asynchrony. Since the burst of buds in the oaks in this region indicates caterpillar emergence, he concluded that parent birds delay incubation after clutch completion to allow offspring to hatch during the burst of oak buds and thus, with the peak of caterpillar emergence. Research performed on American robins (*Turdus migratorius*) points towards the same conclusion, as female robins were found to delay incubation in order to maximize food abundance during hatching (Rowe & Weatherhead, 2009).

Observations done on the Magellanic Penguin (*Spheniscus magellanicus*) in Argentina found a correlation between the development of the brood patch and the delay of incubation onset (Barrionuevo & Frere, 2016). In some species of penguins, the brood patch tends to reach full development up to two weeks after clutch completion (St. Clair, 1992). An underdeveloped brood patch is not defeathered and doesn't have the right concentration of blood vessels yet, making it unable to efficiently transfer heat onto the eggs (Bailey, 2007). They concluded that delayed development of the brood patch resulted in delayed onset of full incubation, to maximize the ability of the parents to keep the eggs warm through incubation.

In great tits, incubation delay to after clutch completion was also observed. A study performed on this passerine bird in central/eastern France found that by experimentally increasing the nest-box temperature by 1 degree throughout the laying period, the birds were less likely to delay incubation (Bleu, Agostini, & Biard, 2017). Bleu concluded that birds use ambient temperatures as a cue to keep track of seasonal advancement. He suggests that the parent birds can change their incubation strategy to be in compliance with the season, to improve hatching success.

Effect on embryonic development

Sex ratio

In the house finch (*Carpodacus mexicanus*), when females started incubation when the first egg was laid, a bias in the offspring sex ratio was found (Badyaev, Beck, Hill, & Whittingham, 2006), while such a bias was not found in females that delayed incubation until clutch completion. The study was performed in the states of Alabama and Montana. The first-laid eggs in the Montana clutch were predominantly females, while in Alabama they were mostly males.

Egg embryo viability

Since incubation is vital for the onset of embryonic development, the onset of incubation determines whether an embryo starts to develop early or late. When birds initiate partial incubation, they allow the earliest laid-eggs to begin development before the eggs that are laid last. The American coot (*Fulica americana*) has been shown to partially incubate, with the birds initiating incubation as early as right after the first egg. This is presumably done to maintain embryo viability of the first eggs, as they are susceptible to infections and fluctuating ambient temperatures (Arnold, 2011). Increases in egg temperatures can activate lysozymes, enzymes that can help protect the embryo from gram-positive bacteria (Wellman-Labadie, Picman, & Hincke, 2008). A study on the effects of trans-shell infections by micro-organisms on the domestic chicken suggests that partial incubation might have evolved as a method to combat infections (Cook, Beissinger, Toranzos, Rodriguez, & Arendt, 2003). In great tits, blood sedimentation rate was higher in broods that hatched late and received incubation after clutch completion, as opposed to broods receiving partial incubation (Bleu et al., 2017). High blood sedimentation rates means red blood cells settle quickly, as a response to illness or inflammation. The late-hatched broods corresponded with clutches that were incubated later and received no experimental heating. These results suggest that clutches that receive incubation later are more susceptible to diseases.

Faster development?

In an aforementioned study on five species of passerine birds, early incubation was not shown to contribute to embryonic development (Wang & Beissinger, 2009). This was concluded because differences in hatching were not found to be related to onset of full or partial incubation. A study conducted on great tits found that partial incubation led to faster development of the embryo and subsequently faster hatching. However, this was linked to the fact that earliest laid eggs received more heat transfer compared to the last eggs, and not that early incubation induces a different physiological response in egg embryos (Lord et al., 2019). Instead, the conclusion was drawn that increased heat transfer due to earlier onset of incubation simply allowed the embryos to develop faster than embryos that were laid at a later date. This would lead to early-laid egg embryos to have shorter development times.

Other effects

In a study performed on black-headed gulls (*Chroicocephalus ridibundus*), yolk testosterone levels were shown to be higher when the mother gull incubated the eggs for a longer period before clutch completion (Müller, Eising, Dijkstra, & Groothuis, 2004). They concluded that elevated testosterone levels are a means of compensation for the hatching asynchrony as a result of the early onset of incubation. Higher testosterone levels would allow the later hatched chicks to be able to survive when food is abundant, or in the case when the early-hatched chicks don't survive.

Other fitness benefits of the strategies

Since many different birds use these different incubation strategies, it would be expected that they provide some sort of fitness benefit, whether it be for the parents or for their offspring.

Partial incubation

Several studies show that partial incubation can lead to increased egg viability. This is commonly referred to as the Egg Viability Hypothesis (Stoleson, 1999). It stems from the idea that exposure to ambient temperatures before incubation results in lowered egg viability ((Arnold, Rohwer, & Armstrong, 1987)). It also includes that eggs that have been subjected to incubation are more likely to have developed enough to be able to handle fluctuating environmental conditions (Ardia et al., 2006). Hatching asynchrony, the phenomenon that often results from partial incubation, is often considered merely a side-effect of the fitness benefits. In the formerly mentioned study on house sparrows, partial incubation did lead to high offspring survivability, but not for the first eggs (Aldredge, 2017). The growth rate of the chicks was also the same among both groups, showing no benefit to early onset of incubation. Many other studies mention hatching asynchrony as a consequence of early onset of incubation and it is often regarded as a strategy in of itself. In precocial birds, whose chicks still rely on their parents for food after hatching, hatching asynchrony immediately poses a problem however. The first hatched chicks get food first and therefore have higher survivability after hatching (Stenning, 1996). Some studies found that the incubation might also provide some kind of protection of the eggs from the environment. In a study performed in eastern bluebirds (*Sialia sialis*), eggshells subjected to early incubation were found to have a decreased microbial load, compared to eggshells that were not incubated during egg laying (Bollinger, Bollinger, Daniel, Gonser, & Tuttle, 2018). Other studies also find similar effects of incubation on bacterial growth on eggshells in pearly-eyed thrashers (*Magarops fuscatus*) (Shawkey, Firestone, Brodie, & Beissinger, 2009).

In the aforementioned study on great tits by Podlas and Richner, the onset of incubation was experimentally manipulated by removing and adding eggs to nests. The goal was to find out whether a difference between offspring mortality, nestling mass and size would be observed when different eggs were subjected to different onset times of incubation and, subsequently, differing conditions after hatching. While during harsh conditions the nestling mass and size was reduced in the asynchronous broods, no strong support for the theory of Brood Reduction was found (Podlas & Richner, 2013). Offspring mortality was found to be the same between the groups. Offspring mortality was found to be the same between the groups

In precocial birds, plenty of evidence exists that early incubation can protect clutches from predation. Black brants (*Branta bernicla nigricans*) have an incubation method in which they allocate incubation time amongst their eggs, while also initiating incubation after the second was laid (Flint, Lindberg, MacCluskie, & Sedinger, 1994). This would prevent predation of the eggs and also reduce the hatching asynchrony in the clutch. Research performed on nocturnal incubation in red-winged blackbirds (*Agelaius phoeniceus*) showed that early incubation did not affect predation rates or increase egg viability, however. They did discover that the clutches that were incubated earlier were not parasitized by the brown-headed cowbird (*Molothrus ater*) (Clotfelter & Yasukawa, 2012) and concluded earlier onset of incubation could protect the clutch from parasitism. A study on wood ducks (*Aix sponsa*) however did not show decreased brood parasitism when incubation was initiated earlier relative to clutch completion (Hepp, 2004).

Since embryos do not develop until incubation is initiated, starting incubation at clutch completion is a way to avoid hatching asynchrony. This is because all eggs are warmed up simultaneously and therefore their embryos will develop synchronously (Stoleson & Beissinger, 2011). In wood ducks, hatching success was found to slowly decrease as the females started incubation later (Walls, Hepp, & Eckhardt, 2011). They found no relation between microbial infection on the eggshells and the delay of incubation, but did see that egg viability only dropped by 8.6% when females initiated incubation 7 days into the egg-laying period. They conclude early onset of incubation is important for reduction of incubation period and prevention of predation. Other studies on wood ducks concluded similarly that early onset of incubation is method used to reduce incubation period (Hepp, 2004).

Onset of full incubation after clutch completion

Fitness benefits attributed to this strategy are somewhat poorly understood. Research suggests incubation after clutch completion allows offspring to hatch while food is very abundant. In American Robins, delay of incubation was not found to be more energetically demanding for the parent birds and might actually allow them to prepare food reserves early in the season, leading to fitness benefits in both the parents as well as their offspring. High food abundance does not always explain delay of incubation after clutch completion, as advancing onset of incubation was also found to lead to these results.

Discussion

Many different theories surrounding the highly differing strategies of incubation within birds have been created. Some studies agreed with each other about the effect of ambient and/or nest temperatures during the laying period. They concluded that increased temperatures during the laying period motivated parent birds to start incubating earlier relative to clutch completion. One of the fitness benefits being attributed to early onset of incubation is higher egg viability. However, not every study supports this claim, with some suggesting early incubation leads to hatching asynchrony and decreased survivability of offspring hatched last. In the case of house sparrows, early incubation actually resulted in decreased egg viability in the very first eggs. A possible reason for this is that the parent house sparrow decide their onset of incubation in order to make a trade-off between high egg viability and larger hatched offspring. It's also possible she wanted to avoid hatching asynchrony being a major issue in her clutch, causing her to start incubation late enough to ensure most chicks hatch synchronously.

Food abundance appears to be influential on onset of incubation in birds. Recent studies have shown that it is very likely some birds delay incubation in order to hatch their offspring in accordance with peak food availability. The peak in food availability differs for nearly every region and species. This explains why some birds choose to incubate early, while others may wait until days after clutch completion. Variability in peak food abundance most likely explains variability in onset of incubation when both strategies have been shown to allow offspring hatching in compliance with said peak food abundance.

The exact effects of differing strategies surrounding incubation onset on embryonic development remain an interesting topic. Research and studies surrounding this topic remain relatively scarce, as most of the research concerning incubation onset focusses on other potential fitness benefits in offspring. Without incubation, no embryonic development will take place. It remains relatively unclear however whether differences in onset of incubation induce different responses in embryos. Furthermore, studying embryos is not as easy as making observations on hatched offspring. Researchers run the risk of (accidentally) manipulating the development of eggs as they are very susceptible to environmental changes, especially in the early stages of development. Many studies conclude partial incubation accelerates embryonic development, but attribute it to the fact that eggs that receive incubation first simply received more heat from incubation compared to last-laid eggs. Not much concrete evidence exists on whether different incubation onsets change embryonic development.

A few studies regarding embryonic development and the onset of incubation do exist, however. The table below highlights the most important studies and their findings.

Author	Study species	Title	Source	Findings
Badyaev, et al. (2006)	House finch	The Evolution of Sexual Size Dimorphism in the House Finch. V. Maternal Effects.	Evolution	Females that incubated partially laid eggs with sex ratio bias
Arnold (2011)	American coot	Onset of Incubation and Patterns of Hatching in the American Coot.	The Condor	Partial incubation leads to increased egg viability
Wellman Labadie et al. (2008)	Domestic chicken, turkey, duck and goose	Comparative antibacterial activity of avian egg white protein extracts.	British Poultry Science	Increased temperature from incubation activates lysozymes that can battle infections
Cook, et al. (2003)	Domestic chicken	Trans-shell infection by pathogenic micro-organisms reduces the shelf life of non-incubated bird's eggs: A constraint on the onset of incubation?	Proceedings of the Royal Society B: Biological Sciences	Reduced infection by pathogenic micro-organisms in partially incubated eggs
Bleu, et al. (2017)	Great tits	Nest-box temperature affects clutch size, incubation initiation, and nestling health in great tits.	Behavioral Ecology	Embryos that received incubation later had higher blood sedimentation rates
Wang & Beissinger, (2009)	Five different species of passerine	Variation in the onset of incubation and its influence on avian hatching success and asynchrony	Animal Behaviour	Partial incubation does not contribute to embryonic development
Lord, et al (2019)	Great tits	Incubation prior to clutch completion accelerates embryonic development and so hatch date for eggs laid earlier in a clutch in the great tit Parus major	Journal of Avian Biology	Partial incubation led to faster development, due to earlier heat transfer compared to other clutches
Müller et al. (2004)	Black-headed gulls	Within-clutch patterns of yolk testosterone vary with the onset of incubation in black-headed, gulls.	Behavioral Ecology	Clutches that received earlier incubation had higher yolk testosterone levels

Some research suggests early incubation can have a positive effect on the ability of the embryos and eggs to combat infections. Once again, this may simply be attributed to the fact that due to early incubation, the first-laid eggs simply receive more heat. Therefore, they have been allowed to develop more and would be able to combat environmental factors such as diseases and fluctuating temperatures better. Similar conclusions can be drawn to studies that conclude early incubation leads to decreased development time. Evidence also exists that higher yolk testosterone levels in egg embryos that were subjected to partial incubation, which could ensure these embryos will have better survival chances later on.

In order to study whether different types of incubation onset induce different kinds of responses in egg embryos, more extensive research needs to be performed. An idea would be to try and combine much of the research that has been performed on the subject before and apply it to a very large population that is known to have all three main types of incubation onset. For example, researchers can look into infection rates of egg embryos and compare whether one type of incubation onset is better than the others. Perhaps the study can be extended way into the fledgling period, to test whether offspring survival is affected too.

As hatching asynchrony often leads to reduced survivability in the last offspring, it seems as an odd strategy for parent birds to perform. While research suggests it could be done to ensure that parent birds will be more capable of controlling for food allocation during food shortages, some research actually suggests that parent birds aren't able to properly do this. They are capable of ensuring their own survival, but this doesn't explain why they use a strategy that causes hatching asynchrony.

Another plausible reason for parents to start incubation early is to ensure their brood and eggs are safe and healthy. In eastern bluebirds, partial incubation lead to decreased microbial load and in red-winged blackbirds, early incubation seemed to prevent parasitism from a different bird species. In the case of the blackbirds, this makes sense as the presence of the parental birds could scare potential threats away. Not many studies support the theory that early incubation prevents predation rates, however.

Early incubation was often linked to shorter incubation periods for the parents. Incubation is an energy-intensive process, both the act of incubation itself as well as the time constraints it puts on the parents' foraging behaviour. If early onset of incubation often allows for shorter incubation periods, this might a method of the parent birds to alleviate costs for itself.

The effect of incubation onset at clutch completion is relatively poorly studied. I believe the reason for this is that many population in studies show three types of incubation strategies as a whole. Some populations massively incubate partially, while others delay incubation until after clutch completion. Usually whenever incubation at clutch completion occurs however, the study population shows a huge variation in incubation onset, ranging from several days before clutch completion to several days after clutch completion. The individual decision to incubate at a certain point highly depends on the environmental conditions around the individual parents, leading to differences within a population. This means not a definite conclusion can be drawn as to why parents would decide to incubate at clutch completion. The only potential fitness benefit is that it can prevent hatching asynchrony, which is actually a phenomenon that is often described as beneficial. Perhaps in the future it could be interesting to look for populations or species where incubation at clutch completion is a very common phenomenon. Then it could be interesting to attempt to find which environmental factors drive said populations to exhibit this specific strategy, and also investigate what benefits it could have, both for embryonic development as well as offspring survival.

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