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Authors: Stermin, Alexandru N., Moraes, Isabela S., Retez, Gabriele, Pinto, Jean S., Saint-Clair, Rafael S., et al.

Source: Journal of Vertebrate Biology, 74(24123)

Published By: Institute of Vertebrate Biology, Czech Academy of Sciences

URL: <https://doi.org/10.25225/jvb.24123>

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Survival rate of embryos after experimental water immersion in one of the most adaptable bird species, the common gallinule (*Gallinula galeata*)

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► Received 15 November 2024; Accepted 24 February 2025; Published online 21 March 2025

Abstract. Birds nesting in wetlands are potentially among the most affected by the impacts of climate change. Its effects on precipitation dynamics and sea-level rise may significantly and directly impact nest flooding. As an adaptive response to water level fluctuations, wetland species express plasticity in nesting behaviour and the egg and embryo, the common gallinule (*Gallinula galeata*) appears to be highly adaptable in these respects. Its plasticity is characterised by its breeding biology, social interactions and habitat use. In the present study, we conducted an experiment to explore embryo survival rate in relation to water immersion period and compared it with related and unrelated species. We collected the eggs from the Lagoa Rodrigo de Freitas, Rio de Janeiro, RJ, Brazil. The eggs were randomly divided into three groups; a control (n = 23) was incubated in standard conditions; meanwhile, the other two groups were exposed to experimental water immersion during incubation, one group for two hours (n = 23) and the other one for three hours (n = 21). The experiment was designed to compare the data with a previous study on water rail (*Rallus aquaticus*) and Japanese quail (*Coturnix japonica*). A binomial proportional test was applied to evaluate the relative hatching success of each species. The results showed no statistically significant differences in the hatching success of common gallinule eggs between the control group and eggs immersed in water ($P = 0.502$). The impact of water immersion on hatching success was most pronounced in the Japanese quail ($P < 0.001$); water rail showed significant differences between control eggs and those flooded for three hours ($P = 0.005$). The results contribute to understanding the biology of the common gallinule and also reveal the differing capacities among species to cope with environmental change.

Key words: developmental tolerance, hatching success, nest flooding, Rallidae

Introduction

Climate change can affect bird behaviour, distribution and population dynamics and may put some species

at risk of extinction. It was estimated that extinction rates could vary from 2% to 72%, depending on region, climate scenario, and potential for birds to adapt. For Mexican birds, for example, a 2-3% extinction rate

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was estimated under a minimum climate scenario, and a 49–72% extinction rate for birds in Australia's Wet Tropics bioregion under a maximum climate change scenario (Thomas et al. 2004).

Recently, it has been shown that in European birds, global warming was likely the single most important contributor to temporal trends in laying date, body condition, and offspring number (McLean et al. 2022). Although numerous species are adapted to nesting in wetland habitats, including shorelines, floating mats, or above-water (i.e. on branches), these species are among the most affected by climate change (Hughes 2004). The effects of climate change on precipitation patterns and sea-level rise have been identified as significant factors potentially influencing nest survival (Hughes 2004, Erwin et al. 2006), with nest flooding emerging as a major risk for clutch survival (Bayard & Elphick 2011). While some species are highly affected, others exhibit plasticity and adapt to changing climate conditions (McLean et al. 2022).

With a distribution from SE Canada to Argentina, Hawaii, the Greater and Lesser Antilles and the Galapagos (del Hoyo & Collar 2014), the common gallinule was, until recently, considered to be *Gallinula chloropus*. The highly distinctive cackle, less yellow on the lower mandible, slightly higher, more squared-off frontal shield, juveniles with yellower bill and legs, and darker smoky-grey face and foreneck currently characterise a new species, *Gallinula galeata*. New molecular phylogenetic analyses support splitting the species formerly known as *G. chloropus* into two species, retaining the name for all Old World populations and designating the New World populations *galeata* (Groenenberg et al. 2008). Seven subspecies are accepted, including *G. g. galeata* present in Brazil (del Hoyo & Collar 2014).

To date, this species' plasticity has been characterised in terms of breeding biology, social interactions, and habitat use. There are populations that have two or more clutches per breeding season, as in Miami, North America (Bannor 1997), Panama, Central America (McRae 2011), and Brazil (Wallau et al. 2010). Also, in the case of nest predation or clutch loss due to environmental factors, a breeding pair can produce a replacement clutch (Relton 1972, Post & Seals 2000).

In a population from Rio Grande do Sul, Brazil, it was observed that breeding pairs were typically accompanied by four or five small young offspring and two or three larger offspring raised in the same territory (Wallau et al. 2010). These larger offspring

were likely from different clutches. It is known that the first brood of juveniles remains close to the nest for 18 days on average after the second brood is hatched and assists the breeding pairs in protecting their territory and in obtaining food (Skutch 1935, 1961, Eden 1987).

A population of common gallinule was observed in 1996 at the Lagoa Rodrigo de Freitas, Rio de Janeiro, RJ, Brazil (M.A.S. Alves, pers. observ.). These birds nest in the vegetation surrounding the lagoon, where the water level fluctuates significantly due to precipitation and connections via a channel called Jardim de Alá (Alves & Pereira 1998, Alves et al. 2012). The probability of egg nest and egg flooding is high under such conditions.

The likelihood of nest flooding is a problem facing many species, and it has become more likely in the last decade, possibly due to climate change. The increased risk is due to more extreme and unpredictable weather patterns, including increased frequency and intensity of storms and heavy rainfall, which disrupt precipitation regimes (Prowse et al. 2009, Marengo et al. 2012). As an adaptive response to water level fluctuations, some bird species exhibit plasticity in nesting behaviour in the eggs and embryos. The embryos of species that occupy habitats that flood regularly may be more resistant to the harmful effects of flooding (Bongiorno 1970, Mangold 1974, Stermin & David 2020).

Several studies have been conducted to investigate embryo survival due to flooding. One such experiment involved immersing laughing gull (*Larus atricilla*) eggs in salt water for two hours during the third week of incubation, resulting in a 63% hatching success rate (Burger 1979). After immersing herring gull (*Larus argentatus*) and domestic chicken (*Gallus gallus*) eggs in saline and fresh water at two different temperatures (7 and 26 °C) for different intervals (30 and 120 min) and at different stages of incubation (first and third week) it was observed that herring gull embryos survived immersion during the first and third week of development equally well. In contrast, more chicken embryos survived immersion during the first week. There were no significant differences between the species in relation to the duration of immersion (Ward & Burger 1980).

During an experiment involving one species of rallid; the water rail (*Rallus aquaticus*), and a phasianid; the Japanese quail (*Coturnix japonica*), eggs were exposed to water immersion for two hours and three hours in the

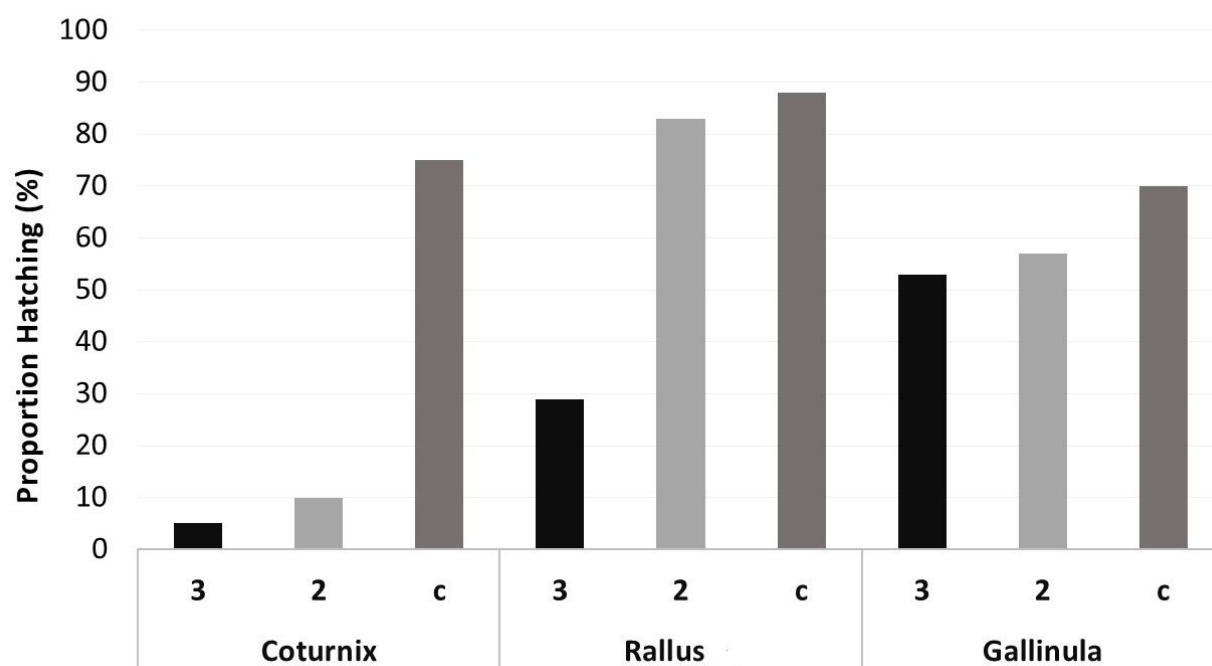


Fig. 1. The proportion (%) of hatching success by the target bird species and experimental groups (Coturnix – *Coturnix japonica*, Rallus – *Rallus aquaticus*, Gallinula – *Gallinula galeata*, 3 – three hours and 2 – two hours of eggs immersed in water during incubation, c – incubated in standard conditions). Original data related to Coturnix – *C. japonica* and Rallus – *R. aquaticus* are from Stermin & David (2020).

third week of the incubation period. It was concluded that water rail embryos were five times more resistant to two hours flooding conditions and approximately twice as resistant to a three hours immersion than quail embryos (Stermin & David 2020).

In this context, we designed an experiment to explore the survival rates of common gallinule embryos in relation to the water immersion period in comparison with confamilial and unrelated species. Our study aimed 1) to evaluate the survival rate of common gallinule embryos to immersion after two and three hours and 2) to compare the results with those from similar experiments conducted on two different species that share similar egg dimensions, the water rail and Japanese quail (Stermin & David 2020).

Water rail, common gallinule, and Japanese quail each occupy distinct ecological niches and biogeographic ranges. The water rail is primarily found in wetlands across Europe, Asia, and parts of North Africa, favouring dense reed beds and marshy areas, relying on thick vegetation for cover and foraging (Taylor & van Perlo 1998). The common gallinule is widely distributed in the Americas, particularly in wetlands, marshes, and coastal habitats from North America to South America, where it prefers shallow waters and dense aquatic vegetation (del Hoyo & Collar 2014). The Japanese quail is native to East Asia but

has spread globally due to domestication, inhabiting grasslands, agricultural fields, and open woodlands, and is often found in temperate climates (Taka-Tsukasa 1967).

Material and Methods

The eggs of common gallinule were collected from the Lagoa Rodrigo de Freitas (−22.973633°, −43.211008°), Rio de Janeiro, RJ, Brazil. At the beginning of the experiment, the breeding pairs' territories were located, and the nest was monitored to evaluate the age of the clutches. Common gallinule incubation lasts 21 days (Helm et al. 1987), similar to the incubation period in water rail (19–22 days) (Taylor & van Perlo 1998). At the beginning of February 2022, we collected 67 eggs from eight complete clutches (min = 6, max = 14, mean = 8.5, range = 8, SE = 1.16).

The temperature in the area during the collection period ranged from 21 °C to 32 °C, with an average of 26 °C. The humidity varied between 50% and 100%, averaging 84%. All eggs in the clutch were collected after the clutches were completed and incubation began. We considered a clutch completed when no new eggs appeared for two or three days.

The eggs were divided randomly into three groups. One control group (group 'c', n = 23) was incubated

Table 1. Percentage of embryo survival rate on three target birds after the experiment of eggs immersed in water for two and three hours (Reference – control group, 'c' n = 23, incubated in standard conditions (37.5-38 °C, without flooding), 2 h and 3 h – groups exposed to experimental inundation during incubation for two hours (n = 23) and three hours (n = 21). Original data related to *Coturnix japonica* and *Rallus aquaticus* are from Stermin & David (2020) experiment.

Species	Nest/eggs	Reference (%)	2 h (%)	3 h (%)
<i>Coturnix japonica</i> (Stermin & David 2020)	60 eggs	75	10	5
<i>Rallus aquaticus</i> (Stermin & David 2020)	6 nests/52 eggs	88	83	29
<i>Gallinula galeata</i>	8 nests/67 eggs	70	57	53

in standard conditions (37.5-38 °C, without flooding) while the other two groups were exposed to experimental inundation during incubation, for two hours (group '2', n = 23) and three hours (group '3', n = 21). Each egg from groups '2' and '3' was placed in a 200 ml plastic cup filled with filtered drinking water at 21 °C. The inundation treatment was conducted just once for each egg.

The experiment was designed to expose the eggs to inundation in the third week of incubation to compare results with data obtained for the water rail and Japanese quail by Stermin & David (2020). Collected eggs were transported in thermos-insulated boxes and incubated at 37.5 °C using an electric egg incubator. The time from collecting the eggs to placing them in the incubator did not exceed two hours. The experiment was approved by the Ethics Committee (protocol number 008/2021).

The eggs were incubated in an incubator (Eclopinto) under standard incubation conditions for domestic hen eggs. The incubation temperature varied between 37.5 and 38.3 °C, with the humidity around 55%; the eggs were automatically rotated five times a day and sprayed once a day with filtered water to follow the protocol from Stermin & David (2020). Fledglings that survived the experiment were released in the wetland at Lagoa Rodrigo de Freitas, close to females with similar age fledglings. It is known that these rallid species frequently adopt cooperative breeding strategies and readily adopt young birds (Taylor & van Perlo 1998, Forman 2001, Wallau et al. 2010).

To analyse the data in a consistent manner, we used the original data from Stermin & David (2020). These original data were included with our own data in statistical tests.

We employed a binomial proportional test to evaluate the relative hatching success of each species across the reference group and two experimental conditions. Specifically, following the method reported by Limpus et al. (2020), we applied Pearson's chi-

squared test statistic, with a significance threshold corrected using the Bonferroni method at an alpha level of 0.01.

Results

On completion of the experiment, 70% of the embryos in the control group survived from the common gallinule eggs. Group '2' (flooded for two hours) survived 57%, and group '3' (flooded for three hours) survived 53% (Table 1).

In comparison to the other two species (water rail and Japanese quail), among the eggs that were not subjected to water immersion, serving as the control group for each species, the water rail exhibited the highest hatching success (88%), followed by the Japanese quail (75%) and the common gallinule (70%).

The impact of water immersion on hatching success was most pronounced in the Japanese quail (Pearson's P -value < 0.001), with a hatching success of 10% after two hours of water exposure and 5% after three hours. Similarly, the water rail showed significant differences between control eggs and those flooded for three hours (Pearson's P -value = 0.005), resulting in a decreased hatching success of 29%. However, eggs flooded for two hours interval shown no statistical difference, with a hatching success of 83% (Fig. 1).

In contrast, common gallinule exhibited no statistically significant differences in hatching success between the control group and eggs immersed in water (P = 0.502). For this species, the hatching success rates remained relatively stable at 57% after two hours of flooding and 53% after three hours (Fig. 1).

Discussion

The results of this study highlight differences in the plasticity of similar aquatic bird species and their adaptability. In the previous study involving water



rail and Japanese quail eggs, the variation in embryo survival rates under different treatments was attributed to habitat adaptation (Stermin & David 2020). Water rail embryos were shown to be significantly more resistant to immersion than Japanese quail embryos. This finding can be explained as an adaptation to the habitat characteristics in the environment where they evolved. It is important to note that there may also be inter-population variations. The water rail inhabits wetlands and has evolved specialised adaptations to marshes with variable water levels and a higher risk of egg flooding. In contrast, the Japanese quail is a ground-nesting species that nests in a variety of habitats, including grasslands, agricultural fields, and forest edges, favouring areas with dense vegetation for cover and proximity to open spaces for foraging (Cheng et al. 2010). Nests of the Japanese quail are susceptible to flooding (Olson 1973, Taylor & van Perlo 1998, Skagen & Adams 2012), albeit at lower frequencies and for shorter durations. It is also recognised that some adaptations of wild bird species to their environment may be lost upon domestication (Price 1999). For further insights into the physiological processes underlying these adaptations, see Stermin & David (2020).

Our findings revealed no statistically significant differences in hatching success between the control group and eggs exposed to flooding in common gallinule. However, a significant difference was observed in the water rail experiment. This difference suggests substantial differences in plasticity and adaptability even between bird species that use similar habitats for nesting and are members of the same family (Rallidae).

These results underscore the likely differences in the capacity of bird populations to respond to climate change. Climate change has been linked to rising sea levels and an escalation in the frequency and intensity of extreme weather events, with projections indicating that these trends might persist in the future (Watson et al. 1996, IPCC 2023). Rio de Janeiro has experienced significant changes in precipitation patterns due to climate change, with an increase in the frequency and intensity of extreme rainfall events, leading to more frequent flooding (Dereczynski et al. 2013)

When predicting the impacts of climate change on birds, it is crucial to recognise that the potential adaptive capacity of species can vary significantly, even among those belonging to similar taxonomic

groups, utilising similar nesting habitats, and sharing common characteristics such as egg size and nesting strategies. This variability in response is evident in the case of the common gallinule and water rail, as demonstrated in the present study. On the other hand, the results reveal the adaptations of common gallinule embryos to their environment, shedding light on key aspects of the biology of this species and explaining its ability to expand.

The results of our study contribute to understanding the biology of the common gallinule and highlight differences in the capacity of related species to cope with the potential consequences of climate change, such as flooding, even when they occupy similar ecosystems. This understanding is crucial for supporting species protection efforts. The common gallinule is classified as of Least Concern by the International Union for Conservation of Nature (IUCN) in Brazil, but its status can vary regionally due to local habitat degradation and environmental change, particularly in wetland areas.

Acknowledgements

We are grateful to Caio Max B. Araujo and João Bezerra for invaluable help in the field, and also other members of Bird Ecology and Behaviour (Laboratório de Ecologia de Aves e Comportamento) of the University of the State of Rio de Janeiro (UERJ). The Brazilian National Research Council (CNPq: Conselho Nacional de Desenvolvimento Científico e Tecnológico) provided Maria Alice S. Alves with a research fellowship (PQ 308.615/2022-0). The Rio de Janeiro State Research Foundation (FAPERJ: Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro) supported the research of Maria Alice S. Alves through the CNE project (CNE E-26/201.126/2022). UERJ also provided a fellowship to Maria Alice S. Alves (Prociência).

Author Contributions

A.N. Stermin – conception of the study, conceptualisation of ideas, methods, data collection, writing the first version and revision of the manuscript; G. Retez – data analysis and revision of the manuscript; I.S. Moraes – field work, revision of the manuscript; J.S. Pinto – field work, revision of the manuscript; R.S. Saint-Clair – field work, revision of the manuscript; Maria Alice S. Alves – conception of the study, conceptualisation of ideas, methods, data collection and curation, project administration and revision of the manuscript.

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