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RESEARCH PAPER

Survival of water rail *Rallus aquaticus* (Aves, Rallidae) embryos exposed to experimental flooding

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Abstract. Rails (family Rallidae) are adapted to wetland habitats, nesting in emergent vegetation where flooding is one of the main factors that contributes to nesting failure rates. We conducted an experiment to test the capacity of water rail and Japanese quail embryos to survive flooding. During the experiment, 52 water rail and 60 quail eggs were divided into three groups. One control group was artificially incubated in standard conditions and the other two groups were submerged during artificial incubation for 2 hours and 3 hours, respectively, in the third week of the incubation period in water at 21 °C. A total of 88.2% of water rail eggs hatched in the control group, 83.3% in the 2 h flooded group and 29.4% in the 3 h flooded group. For the quail eggs, 75% hatched in the control group, 10% in the 2 h flooded group and 5% in the 3 h flooded group. Water rail embryos were five times more resistant to 2 h flooding conditions, and approximately twice as resistant to a 3 h immersion compared to quail embryos. The elevated survival rate of water rail embryos may be related to their capacity to enter torpor.

Key words: avian embryo, egg flooding, hypoxia, incubation

Introduction

In wetland birds, water levels around the nest can be a barrier that reduces the accessibility of mammalian predators (Robertson 1972, Picman 1988, Koons & Rotella 2003). However, nests located in vegetation close to the water surface also risk flooding (Macikunas 1993, Conway 1995, Mazzocchi et al. 1997, Habib & Davidar 2017). In this context, the benefits of choosing nest sites surrounded by water that is sufficiently deep to limit nest predation represents a trade-off against costs associated with the risk of flooding (Clauser & McRae 2016a).

Optimization theory predicts that adaptive traits will evolve to maximize evolutionary success (Brooks & McLennan 1991, Price et al. 1997, Adamík et al. 2003). Occupying a dynamic environment will tend to favour behavioural plasticity as a response to environmental change (Robertson & Olsen 2015). Most rail species (family Rallidae) are adapted to dense wetland habitats, nesting among emergent vegetation, above or adjacent to standing water (Taylor & van Perlo 1998). Recent studies have shown that flooding is one of the factors that contributes to nesting failure in rails (Conway 1995, Schwarzbach et al. 2006, Stermin 2012).

Some rallid species are known to continually build up their nests in response to rising water levels (Taylor & van Perlo 1998, Robertson & Olsen 2015, Clauser & McRae 2016b), with this behavioural plasticity in nest building positively associated

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with nest survival (Robertson & Olsen 2015). King rails (*Rallus elegans*) exhibit plasticity in nest building by constructing taller nests in areas with greater water level fluctuations (Clauser & McRae 2016b). Similarly, Virginia rails (*Rallus limicola*) and soras (*Porzana carolina*) add material to their nests throughout the nesting season in response to increases in water-level (Robertson & Olsen 2015). In rainy periods, water rails (*Rallus aquaticus*) can raise their nests 30 to 60 mm in three days, inserting nest material under the clutch or over the clutch (Stermin 2012). In little crakes (*Zapornia parva*), we previously identified a case where the addition of material raised the nest by 100 mm over two previously laid eggs during a period of heavy rain (Stermin 2012).

While many bird species are adapted to nesting in wetlands, over recent decades the effects of climate change and sea-level rise have had significant direct impacts on a number of species (Hughes 2004, Erwin et al. 2006), and nest flooding has become a major risk for clutch survival (Bayard & Elphick 2011).

An adaptive response to water level fluctuations can occur both through plasticity in nesting behaviour and also in the egg and embryo. The embryos of bird species that occupy habitats that flood regularly may be more resistant to harmful effects of flooding (Bongiorno 1970, Mangold 1974). Many species of duck have water-repellent surfaces on their eggshells (Welty 1975), which help resist the entry of water. The hatching success of clapper rail (*Rallus longirostris*) nests that were flooded at least once daily at high tide were not adversely affected (Mangold 1974, Meanley 1985). Nests of laughing gulls (*Larus atricilla*), a species that nests in salt marshes, are often inundated during the breeding season, though embryos are still able to survive (Bent 1921, Bongiorno 1970). Burger (1979) showed that experimental immersion of laughing gull eggs in salt water for two hours during the third week of incubation, still resulted in 63% of eggs hatching successfully.

Similar experiments were performed by 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). Herring gull embryos survived immersion during the first and third week of development equally well, whereas more chicken embryos survived immersion during the first week, but with no significant differences between the species in relation to the duration of immersion (Ward & Burger 1980).

In this context, we designed an experiment to evaluate whether water rail embryos possess the capacity to survive flooding. For comparison we used the Japanese quail (*Coturnix japonica*) as a model for ground nesting species with similar eggs.

The aims of our study were: (1) to test the capacity of water rail and Japanese quail embryos to survive flooding, and (2) to quantify differences in the survival rates of embryos between the two species. We predicted that water rail embryos would have higher survival rates than those of Japanese quail as an adaptation to the greater likelihood of nest inundation.

Material and Methods

Between 15th and 18th of May 2012, the eggs from six monitored water rail nests located in the Sic Reedbeds (north-west Romania) were collected under permit (Romanian Commission for the conservation of Nature Monuments, Romanian Academy, permit no. 2766/05.03.2012), in the third trimester of the incubation period (between the 12th and 16th day of incubation).

The Sic Reedbeds (46.914566°, 23.910051°) are the largest reedbed in the Transylvanian Plain (Stermin et al. 2011) and second largest in Romania after the Danube Delta. With an area of 252.68 ha it is 98% covered by compact reeds with small patches of cattail and reed mixture, with an edge strip of *Carex* and *Juncus* (Stermin et al. 2019).

Because egg collection took place at the start of the breeding season, the pairs from which eggs were taken were likely to have laid a replacement clutch (Taylor & van Perlo 1998), and the study was considered to have minimal impact. Collected eggs were transported in thermos-insulated boxes and incubated at 37.5 °C using an electric eggs incubator (Cleo 5DTH). The time from egg collection to the start of experimental incubation was less than 2 hours.

The collected eggs (n = 52) were divided into approximatively equal three groups. One control group (n = 17) was incubated in standard conditions

(37.5-38 °C, without flooding) while the other two groups were exposed to experimental inundation during incubation, for 2 hours (group "I", n = 18) and 3 hours (group "II", n = 17).

The flooding treatment was imposed after four days of experimental incubation. Each egg from groups I and II were placed in a 200 ml plastic cup filled with water at 21 °C for 2 and 3 hours, respectively. The water was collected from the Sic Reedbeds. During flooding, due to the elevated temperature of eggs under incubation, the water temperature ranged from 21-23.5 °C. After exposure to flooding, each egg was incubated again in standard conditions.

The same experimental protocol was performed with 60 Japanese quail eggs, divided into three groups of 20 eggs each. Groups I and II were flooded on the 14th day of incubation, in the same conditions as the water rail eggs. The Japanese quail eggs were collected from domesticated breeding birds of mixed sex groups. Eggs were matched for size to water rail eggs.

The experiment was designed to flood the eggs in the third week of the incubation period, with the rationale that water rail nests tend to flood at this time. All hatched water rail fledglings water rails were hand-reared and released in the Sic Reedbeds when they reached an age of 50 days.

Data were analysed with a Generalized Linear Model with a binomial distribution, performed in JMP® 7.0.1, and using a log link function, with the regression coefficients interpreted on the exponential scale.

Results

At the end of the incubation period, 15 (88.2%) water rail eggs hatched successfully in the control group, 15 (83.3%) in group I, and 5 (29.4%) in group II. For Japanese quail eggs, 15 (75%) hatched in the control group, while only 2 (10%) and 1 (5%) hatched in groups I and II respectively (Fig. 1).

There was no significant group × species interaction in the model (Table 1), indicating the independence of species and treatment. We consequently analysed treatment differences separately for each species. There was a statistically significant difference between all three flood treatments groups for water rails: $\chi^2 = 16.59$, df = 2, $p < 0.001$, and Japanese quail: $\chi^2 = 29.86$, df = 2, $p < 0.001$, as well as in the intercept of control and experimental groups (I and II combined) (Table 2).

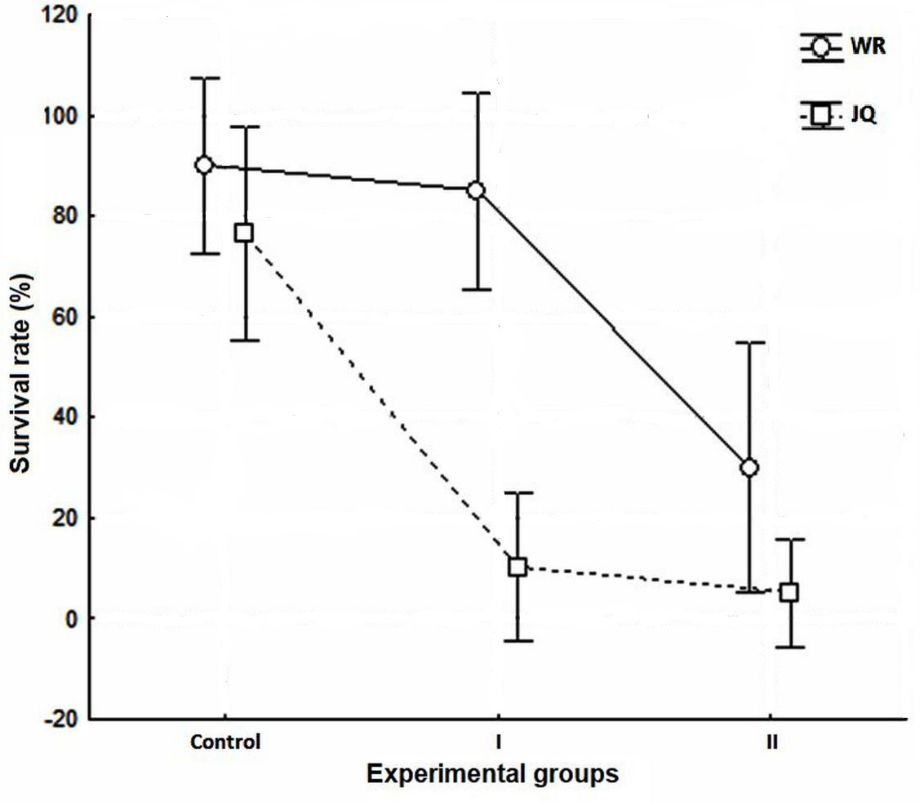

Fig. 1. Survival rate of water rail (WR) and Japanese quail (JQ) embryos in control and experimental groups of eggs (I and II).

Table 1. Analysis of the effects of species and treatment on the hatching success of water rail and Japanese quail embryos.

	df	χ^2	*p*
Species	1	11.78	< 0.001
Group	2	35.91	< 0.001
Group*species	2	4.68	0.098

Table 2. Analysis of the intercept of the interaction term (control x groups I/II) related to the hatching success of each species.

	Regression coefficient	SD	χ^2	*p*
Water rail	–0.92	0.37	6.87	0.009
Japanese quail	1.34	0.45	12.15	< 0.001

The regression coefficient of the hatching rate of the two species was 3.10. The difference to 1 of this value (i.e. 2.10) suggested that, for the entire experiment, the hatching probability of water rail eggs exposed to flooding was approximately double that of Japanese quail eggs.

The results indicated that in the third week of incubation (between days 14 and 21-22), water rail embryos expressed a high rate of survival after egg immersion in fresh water at 21 °C for 2 h than after 3 h. In terms of hatching rates, the difference was lower between group I and control (5%), and higher (59%) between the control group and group II.

For the Japanese quail eggs, the hatching rate was low in both experimental groups. The differences between the control group and group I was around 65%. A similar difference was recorded with the 3 h immersion, when the difference was around 70%.

The hatching probability, calculated in relation to the regression coefficient, was 568% higher for a water rail egg flooded for 2 h, compared with a Japanese quail egg, and 181% higher in the case of water rail eggs after 3 h.

Discussion

Water rail embryos were shown to be significantly more resistant to immersion than Japanese quail embryos; water rail embryos were five times more resistant after 2 h of flooding, and around twice as resistant after a 3 h, in comparison with Japanese quail embryos. A possible explanation for this difference is that the water rail, which inhabits wetlands, has evolved specialised adaptations to enable it to occupy marshes with variable water levels (Olson 1973, Taylor & van Perlo 1998). Because Japanese quail are a ground-nesting species, their nests are also susceptible to the risk of flooding (Orcutt & Orcutt 1976, Skagen & Adams 2012), but at a lower frequency and for shorter periods. Thus, while Japanese quail embryos expressed some resistance to flooding, it is to a significantly lower degree than in water rails.

A further consideration in the present study was that we tested domesticated Japanese quail. With domestication, selection on adaptive traits may be relaxed in comparison with wild individuals.

During egg immersion in cold water, the embryo must cope with a reduced temperature and elevated humidity and hypoxia. Humidity is an important factor on avian nesting success. Studies on the mourning dove (*Zenaida macroura*) showed that eggs incubated at a constant temperature, but with different degrees of humidity (0-5%, 35-45% and 95-100%) suffered different mortality rates, with significantly more embryos (50%) dying before hatching at the highest humidity compared with low and intermediate humidity treatments (Walsberg & Schmidt 1992). Immersing eggs in water has a greater impact on the embryo than humidity, because freshwater can enter eggs, even when an egg is partially submerged (Lippincott & De Pui 1923). Notably, embryos exposed to a high humidity treatment were shown to contain significantly less carbohydrate and protein than those from low humidity treatments (Walsberg & Schmidt 1992). The depletion of proteins or carbohydrates in eggs exposed to a high humidity may indicate greater reliance on non-lipid metabolic substrates. Oxidation of a given mass of lipid generates more water than oxidation of a similar mass of protein or carbohydrate. This outcome suggests that decreasing oxidation of lipids would reduce the amount of metabolic water generated and, therefore, compensate for the decreased water loss occurring in humid environments (Walsberg & Schmidt 1992).

In the context of the present study, egg immersion in water would tend to generate hypoxia. To obtain energy, an hypoxic egg preferentially metabolises carbohydrate, rather than protein and lipid (León-Velarde & Monge 2004). The impact of hypoxia on the embryo is correlated with the time of exposure and the stage of incubation (Lourens et al. 2007). Rate of oxygen consumption increases in direct proportion with embryo age (Grant 1982), and survival rate decreases proportionally with duration of exposure to hypoxia (Sharma et al. 2006, Zhang & Burggren 2012). These metabolic relationships may explain why so few embryos survived 3 h immersion in comparison with 2 h.

In addition to humidity and hypoxia, low temperature can also cause embryo death. Studies on avian incubation temperature have shown that elevated temperatures increase embryonic development only until the second week of incubation. From the third week of incubation, oxygen level influences embryo development more than incubation temperature (Lourens et al. 2007). Our results support the idea that hypoxia, caused by egg immersion, has a more important effect on embryo death than reduced temperature. This conclusion is supported by previous studies that show immersion of herring gull eggs in 7 °C water does not kill significantly more gull embryos than immersion of eggs in water at 26 °C (Ward & Burger 1980). Embryo survival at low temperature is correlated with incubation stage; embryos in the early stages of development can withstand cooling while those at later stages are more susceptible to the effects of suboptimal incubation temperatures (Dougherty 1926, Moreng & Shaffner 1951, Moreng & Bryant 1954, 1956, Ward & Burger 1980).

In the face of this combination of stress factors; water immersion, low temperature and hypoxia, water rail embryos may survive by entering a state of torpor. This hypothesis is supported by the fact that in eggs incubated in an hypoxic environment, the total oxygen consumption can be decreased by lowering the temperature, which would have the effect of slowing down development and decreasing the metabolic rate, but also of increasing hatch time (Lourens et al. 2007). In this way, the low temperature of the water can be advantageous for embryo survival, by decreasing embryonic metabolic rate.

A state of torpor, characterized by a reduced heart rate and decreased metabolic functions (Schleucher 2004) is encountered in adult nightjars (Caprimulgidae), hummingbirds (Trochilidae) and swifts (Apodidae; Wentworth et al. 2009). Decreased environmental temperature, low level of energy reserves and a lack of food are among the causes of torpor (Geiser 1988, Hiebert 1992, Bicudo et al. 2002, Schleucher 2004), which can last from several hours to days (Hiebert 1992). To date, there are no clearly described cases of torpor of bird embryos, though we suggest this as a possibility. Published studies provide evidence that torpor in birds is much more common than previously appreciated and the traditional concepts concerning patterns and determinants of natural torpor are not universally applicable for all the species capable of entering this state (Schleucher 2004).

The present study demonstrated that water rail embryos have a high survival rate when flooded with water at 21 °C for 2 hours, with a lower survival rate after a period of 3 h. Japanese quail embryos, in contrast, had a lower rate of survival after 2 h or 3 h of immersion. Water rail embryos were five times more likely to survive 2 h of immersion than Japanese quail embryos and approximately twice as likely to survive 3 h.

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Literature

Adamík P., Korňan M. & Vojtek J. 2003: The effect of habitat structure on guild patterns and the foraging strategies of insectivore´s birds in forests. *Biologia (Bratislava) 58: 275–285.*

Bayard T.S. & Elphick C.S. 2011: Planning for sea-level rise: quantifying patterns of saltmarsh sparrow (*Ammodramus caudacutus*) nest flooding under current sea-level conditions. *Auk 128: 393–403.*

Bent A.C. 1921: Life histories of North American gulls and terns. *U. S. Natl. Mus. Bull. 113: 154–163.*

Bicudo J.E., Bianco A.C. & Vianna C.R. 2002: Adaptive thermogenesis in hummingbirds. *J. Exp. Biol. 205: 2267–2273.*

Bongiorno S.F. 1970: Nest-site selection by adult laughing gulls (*Lurus atricilla*). *Anim. Behav. 18: 434–444.*

Brooks D. & McLennan D. 1991: Phylogeny, ecology and behavior: a research program in comparative biology. *University of Chicago Press, Chicago.*

Burger J. 1979: Nest repair behavior in birds nesting in salt marshes. *J. Comp. Physiol. Psychol. 11: 189–199.*

Clauser A.J. & McRae S.B. 2016a: Plasticity in incubation behavior and shading by king rails (*Rallus elegans*) in response to temperature. *J. Avian Biol. 48: https://doi.org/10.1111/jav.01056.*

Clauser A.J. & McRae S.B. 2016b: King rails (*Rallus elegans*) vary building effort and nest height in relation to water level. *Waterbirds 39: 268–276.*

Conway C.J. 1995: Virginia rail (*Rallus limicola*). In: Poole A. (ed.), Birds of North America. *The Academy of Natural Sciences and The American Ornithologists' Union, Philadelphia.*

Dougherty J.E. 1926: Studies in incubation. I. The effect of low temperatures previous to incubation on hatchability of eggs set. *Am. J. Physiol. 79: 39–43.*

Erwin R.M., Sanders G.M., Prosser D.J. & Cahoon D.R. 2006: High tides and rising seas: potential effects on estuarine waterbirds. In: Greenberg R., Maldonado J.E., Droege S. & McDonald M.V. (eds.), Terrestrial vertebrates of tidal marshes: evolution, ecology, and conservation. *Studies in Avian Biology, Cooper Ornithological Society, Camarillo: 214–228.*

Geiser F. 1988: Reduction of metabolism during hibernation and daily torpor in mammals and birds: temperature effect or physiological inhibition? *J. Comp. Physiol. 158: 25–37.*

Grant G.S. 1982. Avian incubation: egg temperature, nest humidity and behavioral thermoregulation in a hot environment. *Ornithol. Monogr. 30: 1–100.*

Habib M. & Davidar P. 2017: Nesting success of mallard *Anas platyrhynchos* at Kashmir Lakes, India, is associated with nest location. *Ornithol. Sci. 16: 65–69.*

Hiebert S.M. 1992: Time-dependent thresholds for torpor initiation in the rufous hummingbird (*Selasphorus rufus*). *J. Comp. Physiol. 162: 249–255.*

Hughes R.G. 2004: Climate change and loss of saltmarshes: consequences for birds. *Ibis 146: 21–28.*

Koons D.N. & Rotella J.J. 2003: Comparative nesting success of sympatric lesser scaup and ring-necked ducks. *J. Field Ornithol. 74: 222–229.*

León-Velarde F. & Monge C.C. 2004: Avian embryos in hypoxic environments. *Respir. Physiol. Neurobiol. 141: 331–343.*

Lippincott W.A. & De Pui P.L. 1923: Note on incubating eggs lying in water. *Poult. Sci. 3: 25–28.*

Lourens A., van den Brand H., Heetkamp M.J.W. et al. 2007: Effects of eggshell temperature and oxygen concentration on embryo growth and metabolism during incubation. *Poult. Sci. 86: 2194–2199.*

Macikunas A. 1993: Hatching success and replacement success of the black tern (*Chlidonias niger*) of the Kaunas Sea. *Acta Ornitol. Litu. 7–8: 107–114.*

Mangold R.E. 1974: Research on shore and upland migratory birds in New Jersey: clapper rail studies. *New Jersey Department of Environmental Protection, Trenton.*

Mazzocchi I.M., Hickey J.M. & Miller R.L. 1997: Productivity and nesting habitat characteristics of the black terns in northern New York. *Colon. Waterbird. 20: 596–603.*

Meanley B. 1985: The marsh hen: a natural history of the clapper rail of the Atlantic coast salt marsh. *Tidewater Publishers, Centerville.*

Moreng R.E. & Bryant R.L. 1954: Effects of subfreezing temperature-exposure on the chicken embryo. I. Survival and subsequent growth up to time of hatch. *Poult. Sci. 33: 855–862.*

Moreng R.E. & Bryant R.L. 1956: The resistance of the chicken embryo to low temperature exposure. *Poult. Sci. 35: 753–757.*

Moreng R.E. & Shaffner C.S. 1951: Lethal internal temperatures for the chicken, from fertile egg to mature bird. *Poult. Sci. 30: 255–266.*

Olson S.L. 1973: A classification of the Rallidae. *Wilson Bull. 85: 381–446.*

Orcutt F.S., Jr. & Orcutt A.B. 1976: Nesting and parental behavior in domestic common quail. *Auk 99: 135–141.*

Picman J. 1988. Experimental study of predation on eggs of ground-nesting birds: effects of habitat and nest distribution. *Condor 90: 124–131.*

Price T., Helbig A. & Richman A. 1997. Evolution of breeding distributions in the old world leaf warblers (genus *Phylloscopus*). *Evolution 51: 552–561.*

Robertson R.J. 1972: Optimal niche space of the redwinged blackbird (*Agelaius phoeniceus*). I. Nesting success in marsh and upland habitat. *Can. J. Zool. 50: 247–263.*

Robertson E.P. & Olsen B.L. 2015: Behavioral plasticity in nest building increases fecundity in marsh birds. *Auk 132: 37–45.*

Schleucher E. 2004: Torpor in birds: taxonomy, energetics, and ecology. *Physiol. Biochem. Zool. 77: 942–949.*

Schwarzbach S.E., Albertson J.D. & Thomas C.M. 2006: Effects of predation, flooding, and contamination on reproductive success of California clapper rails (*Rallus longirostris obsoletus*) in San Francisco Bay. *Auk 123: 45–60.*

Sharma S.K., Lucitti L.J., Nordman C. et al. 2006: Impact of hypoxia on early chick embryo growth and cardiovascular function. *Pediatr. Res. 59: 116–120.*

Skagen S.K. & Adams A.A. 2012: Weather effects on avian breeding performance and implications of climate change. *Ecol. Appl. 22: 1131–1145.*

Stermin A.N. 2012: Biology and ecology of some problematic species: water rail (*Rallus aquaticus*) and little crake (*Porzana parva*) – studies on the Fizeş Basin's populations. *PhD Thesis, Babes-Bolyai University, Cluj-Napoca.*

Stermin A.N., David A., Holte D. & Schmitz-Ornés A. 2019: Nesting habitat and nest features of water rails *Rallus aquaticus* and little crakes *Zapornia parva* on large wetlands. *Bird Study 66: 398–406.*

Stermin A.N., Pripon L.R., David A. & Coroiu I. 2011: Wetlands management for little crake (*Porzana parva*) conservation in a "Natura 2000" site. *2nd International Conference on Environmental Science and Development, IPCBEE 4: 91–94.*

Taylor B. & van Perlo B. 1998: Rails: a guide to the rails, crakes, gallinules, and coots of the world. *Yale University Press, New Haven*.

Walsberg G.E. & Schmidt C.A. 1992. Effects of variable humidity on embryonic development and hatching success of mourning doves. *Auk 109: 309–314.*

Ward L.D. & Burger J. 1980. Survival of herring gull and domestic chicken embryos after simulated flooding. *Condor 82: 142–148.*

Welty J.C. 1975. The life of birds, 2nd ed. *Saunders, Philadelphia.*

Wentworth B., Cigan J. & Schaaf T. 2009: Tolerance of Japanese quail embryos and young chicks to hypothermia. *Poult. Sci. 88: 1040–1043.*

Zhang H. & Burggren W.W. 2012: Hypoxic level and duration differentially affect embryonic organ system development of the chicken (*Gallus gallus*). *Poult. Sci. 91: 191–201.*