DOI https://doi.org/10.30456/AVO.2021203 Avocetta 45: 169-176 (2021)

The effects of egg laying onset, nest size and egg size on the hatching success of the Little Grebe *Tachybaptus ruficollis*

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Abstract - In 2008, we investigated the relationships between the hatching success of the Little Grebe *Tachybaptus ruficollis* with egg laying onset, nest diameter, as a proxy of nest size, and egg weight, as a proxy of egg size, in an artificial wetland in Mazandaran Province, northern Iran. The first egg was laid on April 23 and the last chick hatched on July 27. From 118 eggs on 25 nests, we recorded a total of 72 hatchlings (hatching rate: 61%). A generalized linear model (GLM) revealed that while egg size and nest diameter were not significant factors predicting hatching success, egg laying onset predicted significantly the hatching success as early breeders were likely to produce less hatchlings than late breeders. Further studies will be necessary to elucidate the underlying mechanisms of such results.

Keywords: Breeding time, clutch size, egg size, Iran, Little Grebe, Mazandaran, nest size, Podicipediformes

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INTRODUCTION

Studies on habitat characteristics and their effects on productivity and survival of avian species (Martin 1992) are important for conservation planning (Chalfoun & Schmidt 2012, Rocha et al. 2013). Nest-site selection is the most important stage of habitat selection and territory acquisition (Gochfeld 1997). Preference for high-quality nests or nest-sites increases survival and reproductive performance (Clark & Shutler 1999, Lambrechts et al. 2012, Cancellieri & Murphy 2014). Avian breeding success might also be influenced by egg laying onset, clutch size and egg size (Moreno et al. 2008, Broggi & Senar 2009, Mainwaring & Hartley 2009), which vary within and between species (Barati et al. 2011).

The right timing of laying onset often allows parents rearing their chicks with maximum food availability. It also likely increases clutch sizes and breeding success (Arnold 1992, Harrison et al. 2010, Newlon & Saab 2011). Nest size (e.g. nest weight, nest height, and nest depth) is an index of individual phenotype and breeding site quality (Moreno et al. 2008, Vergara et al. 2010). Larger nests, due to earlier nest occupation, can improve the breeding performance (Álvarez &

Barba 2008, Vergara et al. 2010, Britt & Deeming 2011).

Water level near nests is an important parameter of nest-site selection for water birds. For example, predation risk (Caro 2005, Hoover 2006, Mikula et al. 2014, Ibáñez-Álamo et al. 2015) and probability of nest flooding may influence breeding success and are both directly related to local water levels. A decline in water level could lead to decrease in local food supplies (e.g. insects, invertebrates, Brandis et al. 2011), which can also negatively affect breeding success (Leslie 2001, Brandis et al. 2011).

Clutch and egg size characteristics are often predictors of breeding success. Larger clutches and larger eggs often have higher probability of breeding success (Bolton 1991, Krist & Remeš 2004). Egg shape index, a measure of sphericalness, is an important criterion of egg quality (Duman et al. 2016) that varies with nest type, nest size, and clutch size (Duursma et al. 2018). Environmental and climatic pressures are known to determine egg shape (Duursma et al. 2018). For example, in warmer regions, eggs are more spherical, which also affects incubation rates (Jaeckle et al. 2012) and the risk of eggs cracking (Duman et al. 2016). Egg weight, another parameter of egg size, is directly related to the quantity and quality of albumen, yolk, and shell, which can improve breeding success as well as offspring growth and survival (Alkan et al. 2015). However, these relationships are often debated as there are studies showing contrasting results, which calls for more investigations, especially on waterbirds because they often breed in unstable, fluctuating environments (Athamnia et al. 2015, Amininasab et al. 2021). In particular, more studies are especially needed on the factors that influence breeding habitats and nest or nest-site selection (Wang et al. 2018).

The Little Grebe *Tachybaptus ruficollis* (Order Podicipediformes; Family Podicipedidae) is a monogamous waterbird that builds their nests among submerged plants (Hockey et al. 2005). Females lay 4-7 eggs that both parents incubate for 20-25 days (Del Hoyo et al. 1992, Fjeldsa 2004, Fazili et al. 2008). Although the Little Grebe is not threatened under the IUCN Red List of Threatened Species (Samraoui et al. 2013), its breeding ecology and the effects of anthropogenic pressures on its reproductive habits are poorly documented because of its elusiveness (Moss & Moss 1993). In this observational study, we investigated several ecological hypotheses regarding the relationships between breeding, nest, and nest-site characteristics with breeding success indices of the Little Grebe.

We hypothesized that hatching success depends on i) time of egg laying onset, ii) nest size and iii) egg size, expecting higher hatching success among earlier breeders, in larger nests and from larger eggs, respectively.

MATERIALS AND METHODS

This study was carried out in the Zarrin-kola man-made wetland (327.5 ha), also called Ab-bandan, in Mazandaran Province, Iran (36° 42' 42'' E and 52° 58' 50'' N). Ab-bandan is managed by local communities and has an average depth of approximately 1.5 m. Having been developed as water reservoirs for agricultural activities, Ab-bandan has attracted many species of waterbirds and has become a unique human-made ecosystem with high biodiversity value (Safaeian & Shokri 2003).

Reproductive phenology of the Little Grebe was surveyed in the study area during the 2008 breeding season. Little Grebes laid eggs from April 23 until July 28. Nest sites were discovered using a boat and nest positions were recorded via a Garmin GPS 72H (Bacon & Rotella 1998). Nest sites were checked three times a week from nest-building initiation to determine when the first egg was laid, when the first egg was hatched, and clutch size of each nest. Egg laying onset was the number of days between the first laying day in each nest and April 23. We took into account first clutches only for which we did not observe any nest failure, abandonment or predation.

Four parameters of nest size and nest characteristics, namely nest diameter, nest height, nest depth, and water level near the nest, were recorded with a metal tape measurer attached to a stick. Three parameters of egg size characteristics including clutch size, egg shape index, and egg weight were also recorded. Egg shape index was measured as E.I. = $[\frac{\text{egg breadth}}{\text{egg length}}] \times 100$ (Hoyt 1979). Egg breadth and length were measured with a digital vernier caliper (precision = 0.01 mm) and weighed to the nearest 0.01 g using an electronic digital balance. All measurements were done by the same individual to avoid bias. Hatching success was determined as the proportion of eggs that hatched in each nest (Si Bachir et al. 2008, Ashoori & Barati 2013).

To determine the effect of egg laying onset, nest size and site characteristics, and egg characteristics of Little Grebes on their hatching success, pair-wise Pearson correlation coefficients were calculated to check for multicollinearity between the eight explanatory variables. Only the most relevant and independent predictors were kept for further analyses. We used a generalized linear model (GLM) with binomial distributions to consider whether egg laying onset, nest size, and egg size might be significant predictors of hatching success. We started the analysis with the maximal model including all independent variables and subsequently removed the least significant variable at each step to find the minimal adequate model (where all variables were significant; $p < 0.05$). All statistics were performed using R version 3.1.0 (R Core Team 2015).

RESULTS

The results of the study revealed that the first egg was laid on 23 April. The hatching period lasted from June 17 to July 27. We did not find more than one brood per nest. From 118 eggs laid in 25 nests, 72 chicks successfully hatched (61%). Means and standard deviations of egg laying onset, four characteristics of nest size and site, and three characteristics of egg were calculated (Tab. 1).

Table 1. General breeding parameters of Little Grebe *Tachybaptus ruficollis* (number of nests = 25, number of eggs = 118).

Factors	Parameters	Mean	SD	N	Range
Nest size and site characteristics	Nest diameter (cm)	31.3	11.80	25	20.0-53.5
	Nest height (cm)	5.52	0.83	25	4.5-7.0
	Nest depth (cm)	9.96	1.02	25	8.5-12.0
	Water level under the nest (cm)	159.64	11.97	25	145-180
Egg size and characteristics	Clutch size	4.72	0.94	25	4-6
	Egg shape index	73.13	11.27	118	65-100
	Egg weight (g)	2.74	0.33	118	2.31-3.57
Egg laying onset	Date of egg laying onset (April 23 = day 1)	18.08	11.03	25	1-42
Hatching Success	Hatching rate	0.61	0.15	25	0.5-1.0

Multiple collinear variables were identified via Pearson correlations (Tab. 2). Thus, the number of predictors was reduced to those most relevant and independent including egg laying onset, nest diameter as a proxy of nest size, and egg weight as a proxy of egg size. The GLM revealed that nest diameter and egg weight were not significant factors predicting hatching success. The first day laying instead predicted significantly hatching success ($P = 0.017$), indicating that early breeders had a lower hatching success (Tab. 3).

Table 2. Pearson correlation matrix of breeding variables in Little Grebe.

	Nest depth	Nest height	Water level under the nest	Egg weight	Egg shape index	Clutch size	Egg laying onset	Hatching success
Nest diameter	0.681***	0.630***	0.045	0.108	0.234	0.594*	-0.066	-0.361
Nest depth		0.894***	0.166	0.199	0.341	0.642***	-0.008	-0.343
Nest height			0.236	0.342	0.397*	0.460*	0.092	-0.232
Water level under the nest				0.288	0.530**	0.173	0.662***	-0.417*
Egg weight					0.819***	0.061	0.007	-0.246
Egg shape index						0.327	-0.351	-0.388*
Clutch size							-0.248	-0.460*
Egg laying onset								0.525**

Table 3. Summary of the GLM with binomial distribution of the effect of egg laying onset, nest diameter, and egg weight on hatching success of the Little Grebe.

	Estimate	Standard Error	t-Value	P-value
Maximal model				
Intercept	0.548	1.065	0.515	0.612
Nest diameter	-0.014	0.010	-1.353	0.190
Egg weight	-0.053	0.372	-0.143	0.888
Egg laying onset	0.029	0.012	2.480	**0.022***
Minimal model				
Intercept	-0.061	0.235	-0.260	0.797
Egg laying onset	0.030	0.012	2.575	**0.017***

DISCUSSION

The results of the GLM model revealed that among the egg laying onset, nest size and site characteristics, and egg size characteristics, only egg laying onset significantly affected hatching success indicating that early breeders had a lower hatching success than late breeders. This contradicts our expectation that earlier egg laying should result in a higher rate of hatching success. However, environmental conditions, such as local weather and habitat quality, are also known to affect eggs hatchability (Ritz et al. 2005, Gill et al. 2008). The timing of local vegetation growth may also affect onset of egg laying by providing both nesting cover and habitat for insects as food sources (Samraoui et al. 2007, 2013). Breeding success usually declines over the course of the season, which may be a direct effect of breeding time, an effect of environmental quality, or a combination of the two. Perrins (1970) reported that the onset of egg laying is an important parameter for migratory nesting birds. Early breeding may be a special attribute of high-quality adults that can provide good parental care, and chicks that hatched early may grow faster than those that hatched (Nisbet et al. 1998, Ritz et al. 2005). However, in the current study, contrasting other studies on waterbirds (Huxley & Wood 1976, Gibbons 1986, Cempulik 1993, Samraoui et al. 2013), early breeders were less likely to produce eggs that hatched, which may be related to other unmeasured environmental (e.g. habitat) and regional characteristics. Further studies will be necessary to elucidate the underlying mechanisms.

Nest and nest-site characteristics play a key role in the reproductive performance of many bird species (Krause 1994, Minias et al. 2012, Bilal et al. 2013). Breeding success of waterbirds is highly related to nest-site selection since waterbirds build their nests during optimal environmental conditions according to their nest size, aquatic vegetation height and cover, and water and noise levels (Gherib & Lazli, 2016). In current study, Little Grebes built their nests among *Phragmites australis* and *Nymphaea* sp. while Moss & Moss (1993) reported that Little Grebes nest on reed vegetation of *Phragmites sp., Scirpus sp.,* and *Typha sp.* in Britain and Ireland. In Algeria, Athamnia et al. (2015) reported nest locations on *Scirpus lacustris* and *Typha angustifolia*. These reports indicate that a variety of different water habitats are suitable for Little Grebes (Ceccobelli & Battisti 2010). The submerged vegetation provides nesting material and food, especially insects and larvae that Little Grebes are dependent on during the breeding season (Vinicombe 1982, Cramp 1998). Higher hatching success was expected in larger and higher nests based on previous studies of Passeriformes (Lambrechts et al. 2012) and specifically Little Grebes (Athamnia et al. 2015). Bigger nests are likely to be made by older individuals with more experience that have survived longer than younger individuals. Bigger nests are also more tolerant to weather fluctuations (Fjeldsa 2004). However, in our study, nest size did not significantly affect hatching success, which is in contrast with the findings of Athamnia et al. (2015).

Regarding egg characteristics, we expected larger eggs are more likely to hatch. Quality and quantity of available food sources affect egg size (Fenoglio et al. 2003, Forman & Brain 2004). However, some studies indicate that supplemental food doesn't increase egg size (Arnold 1992). Regardless, patterns of egg size variation are relatively good indices of parental quality and/or age (Williams et al. 1993, Blomqvist et al. 1997) and may increase breeding success or performance (Williams et al. 1993, Saino et al. 2004). In Little Grebes, egg size varies between populations in different geographical areas (Athamnia et al. 2015). Across different bird species, there is conflicting evidence on the relationships between egg size, egg quality, and chick development (Williams 1994, also see Perrins 1996). However, it is generally accepted that egg size is positively associated with the nestling body quality, growth, and survival of broods (Arcese & Smith 1988), likely because they contain greater amounts of egg yolk and albumen resulting in greater nutrients (Rohwer 1986, Alkan et al. 2015, Duman et al. 2016,) and energy (Bolton 1991) than small eggs. Egg size may also be correlated with parental care and is expected to be under the influence of embryo genes (Krist & Remeš 2004). In contrast, Williams (1994) found little support for an egg size effect on hatching success while Vinuela (1997) found no relationship between egg mass and growth of nestlings. Thus, the effect of egg mass might be masked by other factors (see Barrett & Runde 1980). Additionally, while egg mass may help increase early growth, perhaps its effect on final size or total growth rate is minimal (Williams 1994). It also might be concluded that chicks from light eggs can survive and fledge at a normal weight rather than heavier eggs (Schifferli 1973). In this study, egg weight was used as an index of egg size, and, based on the GLM, egg weight had no influence on the hatching success. It should be noted that, in addition to the factors discussed above, maternal age and body quality, which were not measured, can also influence egg size (Fjeldsa 2004). Further investigations on the reproduction of Little Grebes should attempt to account for these.

Conclusions

The results of the GLM revealed that only egg laying onset had a significant effect on hatching success. It is indicating, in contrast to expectations, early breeders are the least successful breeders. We did not find a significant effect of nest size, nest-site characteristics, egg size, and egg characteristics on hatching success. Hatching success varies yearly and between different types of water bodies and habitats due to changes in climatic conditions, frequency of nest flooding, predation pressures, parental quality, and other unknown factors. Further investigations across multiple breeding seasons and different geographical areas are needed and may clarify the mechanisms affecting the relationships of nest location, size, and other breeding habitat characteristics with breeding performance (see Lawton & Lawton 1980, Komdeur & Kats 1999). These insights may help inform watercourse management such as dredging and bank vegetation clearance in water bird habitats.

Acknowledgements

We thank Ali Ahmadi Zarrinkolaei and Seyed Abdollah Moosavi for their indispensable help in the field. This work was funded by Sari Agricultural Sciences and Natural Resources University, Iran. Authors' contributions: SMA conceived this study. SMA, SMHM collected and analyzed the data. SMA, SMHM, MM, and CCYX were major contributors in writing the manuscript. SMA, MM and CCYX refined the manuscript. All authors read and approved the final manuscript. All authors declare that they have no conflict of interest.

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Received: 11 September 2021
First response: 10 October 2021
Final acceptance: 16 October 2021
Published online: 16 October 2021
Associate editor: Daniela Campobello