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Clutch size and egg repeatability in three elusive bird species: Little Bittern (*Ixobrychus minutus*), Little Crake (*Zapornia parva*) and Water Rail (*Rallus aquaticus*) from north-west Romanian populations

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SUMMARY. Data were collected from Little Bittern, Water Rail and Little Crake nests located in North-Western Romania, between April and July of 2002 - 2006 and 2010 - 2012. The repeatability was calculated as intra-class correlation of length, breadth and egg volume coefficients. The total number of active nests considered for the present study was as follows: 43 for Little Bittern, 25 for Water Rail and 9 for Little Crake. The clutch size in Little Bittern ranged from 4 to 7 eggs, in Water Rail from 4 to 10 and in Little Crake from 4 to 8 eggs. The repeatability values of egg parameters varied between 0.844 to 0.860 in Little Bittern, 0.262 - 0.374 in Water Rail and 0.310 - 0.574 in Little Crake. Short-term environmental impact was strong in case of Water Rail and Little Cracke, while the genetic component had little influence. For these two species, the low repeatability could be explained by larger numbers of eggs in a clutch, reflecting in turn a longer laying period.

Keywords: breadth, egg volume, laying period, length.

Introduction

Clutch size and egg characteristics vary among and within bird species (Lack, 1947; Klomp 1970; Figuerola and Green, 2005). These variations are important in revealing the individual's fitness (Rockwell *et al*., 1987, Brisk and Sealy, 1989; Horak *et al*., 1997) and aspects about species evolution (Martin, 1995; Figuerola and Green, 2005).

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In bird species, clutch size may depend on many factors, such as the effective coverage capacity of the eggs during incubation (Rice and Kenyon, 1962) or the pressure from predators during nesting (in the sense that, the more eggs are being laid, the more exposed to predators they are) (Slagsvold, 1982; Arnold *et al.*, 1987). The chances in offspring survival of large clutches usually decrease, in the sense that as the number of the offspring is larger, the possibility of being detected by predators increases (Cooch, 1961; Hilden, 1964; Lessells, 1986). Other factors influencing the clutch size are: food resources available during egg laying (Lark, 1974) and temperature during egg production (Great Tits, *Parus major* roosting in cooled nestboxes laid eggs 14% smaller than those roosting in heated nestboxes (Nager and Noordwijk, 1992)). In addition, significant effects on clutch size and egg characteristics are reported from parameters like: female age and experience (a statistically significant increase in egg size with age or experience was found in almost half (17/37) of the studies (Christians, 2002)); female mass and size; and other aspects of the phenotype (Christians, 2002).

The egg size and their intraclutch and interclutches variations are important in determining the offspring body size and weight, their survival probability and therefore the reproductive success (Schifferli, 1973; Williams, 1994; Surmacki, 2003). Variations in egg size are the consequences of genetic and environmental action (Surmacki, 2003), repeatability dimensions characterizing environmental conditions and food resources during laying period (Stermin *et al.*, 2008).

By calculating the repeatability of egg size we can split the phenotypic variation in egg size into within-individual and among-individual components. Variation in egg size among individuals is caused by a combination of genetic and environmental differences, while the variation in egg size within an individual during several years is caused by temporary environmental differences between reproductive attempts (Falconer, 1989; Flint *et al.*, 2001).

In this context, analyzing the repeatability in egg size can reveal information about the ecology and biology of bird species. These data bring valuable information about several bird species which are poorly studied, due to their elusiveness and rather inaccessible habitats. Some of the less studied bird species in Europe are Little Bittern *Ixobrychus minutus*, Little Crake *Zapornia parva* and Water Rail *Rallus aquaticus* (Marion *et al.*, 2000; Stermin *et al.,* 2011; Stermin *et al.*, 2013).

Those three species coexist in dense wetland habitats and during their evolution each has adapted to occupy specific microhabitats inside the wetlands (Taylor, 1998; David, 2008; Stermin *et al.*, 2012). Little Bittern and Little Crake are protected species and, along with the Water Rail, are now being affected by habitat loss and fragmentation (Marion *et al.*, 2000; Brambilla *et al.*, 2012). New information about their biology and ecology is important not only in solving the mystery of their elusive life, but also in designing and implementing conservation programs.

Thus, the main goals of our study are: (i) to describe the variation of clutch size and egg parameters in Little Bittern, Water Rail and Little Crake populations from the Fizeş Basin (N-W Romania) and (ii) to calculate the repeatability and compare the results to other studies on other bird species by analyzing them in relation to the effect of the environment (sensus Bańbura and Zieliński, 1998).

Materials and methods

The study area is located in the central part of the Transylvanian Plain, in Romania (24°10' E; 46°50'N). The wetlands surveyed here represent 1/3 from all Transylvanian wetlands. Most wetlands from the Fizeş Basin are man-made and used as fishing ponds; only two of them are natural, and were recently designated "Natura 2000" site (Special Protected Area) (David, 2008). The wetland areas range from 19 to 253 ha, being partially or totally covered by marsh vegetation.

Data were collected between April and July of 2002 - 2006 and 2010 - 2012. During this time, the areas suitable for Little Bittern, Little Crake and Water Rail nesting were checked at intervals of 2 to 5 days. Transect method was used in nest searching (Bibby *et al.*, 2000). The Little Bittern nests were located in Lakes Știucii, Borzaş, Năsal, Ţaga Mare, Sucutard I, Roşieni, Tău Popii, Cătina and Legii Pond; the Little Crake nests were found in Lakes Sucutard I and Cătina, while the Water Rail nests were situated on Sic Reedbeds and Lake Știucii.

The length (L) and breadth (B) of eggs were measured to the nearest 0.1 mm with digital caliper. The volume was calculated using the following formula: $V = k \times L \times B2 / 1000$ (where $k = 0.51$) (Hoyt, 1979). The egg weight was measured in the field using a digital scale.

One-way analysis of variance (ANOVA) was carried out in order to obtain variance components. The repeatability was calculated as intra-class correlation coefficients (Lessells and Boag, 1987; Falconer and Mackay, 1995; Sokal and Rohlf, 1995), standard errors for repeatability values were calculated as described in Becker (1992).

Results and discussion

A total of 223 eggs from 43 active Little Bittern nests, 209 eggs from 25 Water Rail nests and 56 eggs from 9 Little Crake nests were measured. The clutch size in Little Bittern ranged from 4 to 7 eggs, in Water Rail from 4 to 10 and in Little Crake from 4 to 8 (Table 1).

Table 1.

Clutch size characteristics in Little Bittern, Water Rail and Little Crake nests located in the Fizeş Basin (SD - standard deviation)

	Little Bittern	Water Rail	Little Crake
Total number of clutches	43	25	9
Minimum number of eggs/clutch	4	4	4
Maximum number of eggs/clutch	7	10	8
Mean of eggs/clutch ± SD	5.32 ± 0.74	8.36 ± 1.55	6.22 ± 1.39

Egg measurements for Little Bittern, Water Rail and Little Crake are presented in table 2, while repeatability values are depicted in table 3.

Table 2.

Egg measurements from Little Bittern, Water Rail and Little Crake clutches located in the Fizeş Basin (SD - stardard deviation)

		Little Bittern (n = 223)	Water Rail (n= 209)	Little Crake (n=39)
Length (mm)	Minimum	30.80	26.59	28.87
	Maximum	37.80	39.59	34.09
	Mean ± SD	34.92 ± 1.45	35.92 ± 1.64	31.01 ± 1.38
Breadth (mm)	Minimum	22.10	23.88	20.58
	Maximum	29.10	36.93	22.81
	Mean ± SD	25.55 ± 0.92	25.96 ±1.24	21.76 ± 0.57
Volume (cm^3)	Minimum	8.81	8.63	6.33
	Maximum	16.15	24.14	8.42
	Mean ± SD	11.66 ± 1.14	12.38 ± 1.42	7.49 ± 0.58
Weight (g)	Minimum	-	9.9	6.60
	Maximum	-	15.10	8.90
	Mean ± SD	-	12.43 ± 0.95	7.71 ± 0.61

Table 3.

Repeatability (R) of egg length, breadth and volume index in Little Bittern, Water Rail and Little Crake clutches located in the Fizeş Bazin (SE - standard error; Df - degrees of freedom; F - variance ratio; P - significance level)

		R	SE	df	F	P
Little Bittern	Length	0.844	3.055	42	23.374	< 0.001
	Breadth	0.860	1.639	42	6.363	< 0.001
	Volume	0.851	2.225	42	10.932	< 0.001
Water Rail	Length	0.374	9.326	23	8.537	< 0.001
	Breadth	0.355	7.270	23	9.863	< 0.001
	Volume	0.262	15.824	23	28.212	< 0.001
Little Crake	Length	0.574	10.771	8	16.092	<0.001
	Breadth	0.517	3.288	8	7.294	<0.001
	Volume	0.310	3.917	8	24.83	<0.001

Significant statistical differences between species were found regarding egg measurements: length ($F(2.468) = 196.60$, $df = 2$, $P < 0.001$), breadth ($F(2.468) = 262.95$, $df = 2$, $P < 0.001$) and volume ($F(2.468) = 252.80$, $df = 2$, $P < 0.001$). Larger eggs were recorded in Water Rail, while Little Bittern and Little Crake had smaller eggs.

Previous studies reported that the Water Rail clutch size ranged from 5 to 16 eggs, with an average of 6 - 11 eggs (Flegg and Glue, 1973; Taylor, 1998). In the present study, the smallest clutch contained 4 eggs and the largest one 10 eggs, most of the clutches having 8 - 9 eggs. Moreover, the average egg size corresponded to those described by other authors, who observed egg sizes of 32 - 40 × 24.1 – 27.2 mm (Cramp and Simmons, 1980; Taylor, 1998). From this point of view, no differences were depicted between European populations and ones from the Fizeș Basin. A similar situation was recorded for Little Crake: its clutch size was reported to range from 4 to 11 eggs (Cramp and Simmons, 1980; Taylor, 1998), with an average size of 7 - 9 eggs. In this study, the 4 egg clutch was a replacement of the first clutch, destroyed by flooding. The average egg size was quite similar to those described in other studies, ranging from 28.5 - 32 × 12.2 - 22.2 mm (Dombrowski, 1912) or from 27.5 - 33.5 × 19 - 23 mm, with a weight of 6.3 - 8.7 g (Taylor, 1998).

In previous studies on Little Bittern, the clutch size ranged from 4 to 7 eggs (Samraoui *et al.*, 2012); 1 to 6 eggs (Pardo-Cervera *et al.,* 2010), 4 to 9 eggs (Cramp and Simmons, 1977), or 3 to 7 eggs (Martínez-Abraín, 1994). This clutch size variation could be a consequence of various habitat quality, since Little Bittern was described as an area-sensitive species (Pezzo and Benocci, 2001; Benassi *et al*., 2009). The variation of egg sizes in the present study was comparable to previous literature, where the means for egg length were 35.1 ± 1.3 mm; for egg breadth 25.7 ± 0.7 mm, and for egg volume 11.8 ± 0.9 cm^3 (Samraoui *et al*., 2012), and the mean egg size 34.2 mm × 25.6 mm (range 30.9 - 37.3 × 23.6 - 26.9 mm) (Pardo-Cervera *et al*., 2010).

Regarding the repeatability, the lowest values were recorded for volume and weight. These low values are normal in volume analysis, since it is a parameter that increases approximately with the cube of the linear size. On the other hand, weight is a parameter proportional to volume and density, and is therefore less related to linear dimensions, thus having a different effect on the repeatability calculation.

Repeatability values in Little Crake (0.574 - 0.260) and Water Rail (0.374 - 0.262) were low compared to those in Little Bittern (0.860 - 0.844), and those calculated in other birds (Boag and van Noordwijk, 1987; Hendricks, 1991; Potti, 1993; Bańbura and Zieliński, 1998; Jerzak *et al*., 2000; Tryjanowski *et al*., 2001; Christians, 2002; Zduniak and Antczak, 2003), which are generally higher than 0.6 (Christians, 2002). However, lower repeatability values were reported in the literature, in Greater Scaup, *Aythia marila* and American Oystercatcher, *Haematopus palliates* (0.36 in both cases) (Nol *et al*., 1984; Flint and Gran, 1999). Higher repeatability values were found in Redshank *Tringa tetanus* (Thompson and Hale, 1991), Northern Pintail *Anas acuta* (Flint and Grand, 1996) and Canada Goose *Branta canadensis* (Leblanc, 1989): 0.87, 0.89 and 0.92, respectively.

These findings highlighted the fact that in Water Rail and Little Crake the environmental impact was strong on short term and the genetic component had very little influence.

The low repeatability in Water Rail and Little Crake could be an effect of the large clutch size, which means a long laying period, compared with Little Bittern (with smaller clutch size and a shorter laying period). During a longer laying period, birds may face environmental variations that affect accessibility to food and thus the amount of resources and nutrients invested into an egg. Previous studies infered that in some bird species habitat quality and food supply may affect the eggs (Christians, 2002). On the other hand, other results show that food availability may have no effect on egg size (Arnold *et al*., 1991; Bolton *et al.* 1993; Jager *et al.,* 2000).

A higher repeatability in Little Bittern could be caused by smaller clutch size, that implied a short laying period of time, and a lower probability of environmental changes.

The wetland habitats have rapidly changing parameters in terms of the water level, vegetation density and availability of food resources (Dyrcz and Zdunek, 1996). In wetlands, adaptability is a condition of survival, in contrast to birds living in urban environments, which have a higher rate of repeatability, living under more constant environmental conditions and where food resources are constant over longer periods (Surmacky *et al*., 2003).

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