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Determining Great Bittern *Botaurus stellaris* laying date from egg and chick biometrics

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Capsule Great Bittern breeding phenology can be estimated from egg and chick biometrics.
Aims To estimate egg or chick ages in order to back-calculate egg-laying dates.
Methods Bittern nests were searched for in six French and three Belarussian sites between 1999 and 2004. Eggs and chicks were measured at each visit. By using a subsample of nests with known egg-laying (or hatching) dates, regression equations are determined using egg density and tarsus length in order to estimate, respectively, egg and chick ages. Additionally in Belarus, the 'water test' was used to estimate the incubation stage of the clutch.
Results A total of 141 Bittern nests were found. Egg density decreased linearly from 1.063 at laying to 0.915 the day before hatching. A regression equation therefore allows estimation of egg age from its density. A scale was also constructed to estimate egg age from its position in water, and the accuracy of the two methods is compared. Chick growth rates were similar between the two countries. Before the age of 25 days, chicks are best aged by tarsus length compared to other measurements (weight, bill length). No data were available after that age because chicks were no longer found on nests.
Conclusions Egg-laying date can be estimated to within ±3 days using egg density, and to within ±5 days, using the 'water test'. Tarsus length can be used until the age of 25 days to age chicks to within ±2 days. These simple measurements provide efficient and accurate methods to record the breeding calendar of this endangered species.

The Great Bittern *Botaurus stellaris* (hereafter 'Bittern') is a species of high conservation concern in Europe, declining in western Europe (Birdlife International/European Bird Census Council 2000), possibly decreasing also in eastern Europe (Kushlan & Hafner 2000), and currently listed as a conservation priority species in the Birds Directive. The main factors affecting Bittern populations are the progressive reduction of habitat availability and quality due to the destruction of marshes with extensive wet vegetation cover (Hagemeijer & Blair 1997, Tyler *et al.* 1998, Kushlan & Hafner 2000), and possibly the reduction of food supply (mainly fish: Cramp & Simmons 1977, Voisin 1991, Gilbert *et al.* 2003). Bittern conservation programmes have been implemented in several European countries (UK, France, Germany, Belgium), but these programmes have suffered from the lack of data on breeding biology. Indeed, the Bittern is difficult to study in the wild because of its habitat (tall marshy grass stands) and behaviour (cryptic plumage, crepuscular activity). Therefore, until recently, available information on its breeding biology (egg-laying, hatching periods, clutch size) was very poor (Gentz 1965, Gauckler & Kraus 1965, Hermansen 1972). In this paper we report data on egg biometrics and chick growth rates from six French and three Belarus sites (a total of 141 nests surveyed). Our aim was to provide regression equations based on both egg and chick biometrics to allow back-calculation of laying dates, which can then be used to predict hatching dates (or chick ringing periods) without the need for repeated nest visits that may adversely affect brood survival.

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METHODS

Study species

Bitterns are highly secretive. Males are apparently polygynous, while females alone build the nest and care for eggs and young (Cramp & Simmons 1977, Voisin 1991). Several female nests (up to four) can be found within a male display territory (authors, unpubl. data). Bitterns are generally reported to nest on wet vegetation stands (usually, but not exclusively, reed-beds *Phragmites australis*) where the water depth is between 0 and 80 cm. Clutch size is three to six eggs, rarely seven, and mean incubation time is 25 days (range 22–27) with a laying interval of one to three days. Laying dates range from early March to early June, with a peak between mid-April and mid-May (Puglisi & Bretagnolle 2005).

Study sites

The French part of the study was carried out at six sites holding nearly one-third of the French breeding population.[a] The principal habitat of the sites was reed-bed and they ranged in size from a few hectares to 1760 ha.

All three study sites in Belarus were located in the southern part of the country in the Polesye region. Beloe fish-farm is a complex of small and large fish-ponds (total area of ponds about 1800 ha), used for commercial aquaculture. About 14% of the area (255 ha) is covered by tall vegetation dominated by *Typha angustifolia* (72% of vegetated area) and *Phragmites australis* (18%). Each year 25–35 male Bitterns were present. The second study site, Zvanets, is the largest intact mesotrophic fen in Europe (about 15 000 ha) dominated by sedges (*Carex* sp.) and common reed, with numerous sandy islands. The number of male Bitterns fluctuated between years from several to 100 birds. The third study site was the River Pripyat floodplain (two study sites 20 km apart). *Phragmites* grows in small and isolated patches (0.5–2 ha) within lakes and some mires. The usual Bittern density is about one booming male/km^2 of open swamped floodplain (authors, unpubl. data).

Nest searching

Two methods were used to search for the nests: watching females flying between nest and foraging grounds, and active cold prospecting by foot in the reed-beds. Presumably, the first method causes less disturbance to Bitterns, but it cannot be used everywhere, as females may not always fly to forage (Adamo *et al.* 2004). Moreover, although foraging flights are more common during chick-rearing, chicks leave the nest when more than ten days old, and in many cases empty nests were found.

Cold searching involved first selecting a potentially favourable site (e.g. presence of booming males, and/or an area covered by wet reed-bed), then walking with three to five persons (extremes: one to eight) 2–3 m apart (depending on the density of vegetation). In smaller plots, some landmarks and a compass were enough to ensure that no area was ignored; in larger reed-beds, a GPS was necessary. A rope was held tight between fieldworkers to maintain a regular distance. The major disadvantage of the latter method is the disturbance it generates within the reed-bed, which however is minimized by the short time spent in a given place. No direct desertion resulting from the discovery of a nest has been proven (no deserted nests were found during the incubation period after an earlier visit; n = 75 nests).

Egg and chick measurements

When possible, a nest found during the incubation period was visited again around the expected date of hatching, and later again to ring the chicks (from around nine days: G. Gilbert pers. comm.). The following measurements were made: egg length and width, egg weight, length of tarsus and length of bill (from the feathers to tip) and weight of chicks. Length measurements were made using vernier calipers to ±0.1 mm, while weight was measured with a Pesola to ±0.5 g or an electronic balance to ±0.1 g.

Egg volume was obtained following the Hoyt (1979) equation:

$$\text{length} \times (\text{width})^2 \times 0.507$$

Egg density was calculated as egg weight (g) divided by egg volume (cm^3). The age–density relationship was determined from eggs for which a date of laying was known (n = 54 eggs). Laying date was either determined directly from nest visits (using incomplete clutches, as revealed by an increased number of eggs in a subsequent visit), or estimated by backdating from known hatching date (assuming 25 days' incubation for an egg: Puglisi & Bretagnolle 2005; see also results below). In the latter case, we assumed an interval of two days between laying of successive eggs, and then aged all eggs from a given clutch on the basis of knowledge of hatching date of a single egg and ranking eggs on their relative densities. The original data set with eggs of known laying date comprised 138 measurements (egg weights) for 88 different eggs. However, we excluded from this data set sterile eggs (i.e. those that did not hatch, n = 16), eggs in the process of hatching (pipping eggs, n = 17), and 27 egg weights that came from deserted nests and/or measurements taken by inexperienced observers that provided density values clearly outside the range of values found in birds in general and Bitterns in particular. Therefore, the final data set used for establishing a function for back-calculating egg age comprised 78 measurements for 54 eggs.

We also used the 'water test' (Michelson *et al.* 1963) to determine the incubation stage in Belarus. The water test is an easy field method, as it does not require any specific equipment nor any particular calculation, and allows age estimate directly in the field.[b] In order to evaluate the accuracy of the water test method, we used only eggs for which laying date was known at ±1.5 days (n = 105), assuming an incubation period of 25 days. The test consists of dipping each egg of a clutch into water, then evaluating the egg position in water. After the test, an egg was not wiped in order to leave superficial wax on its surface. When the weather was cold, eggs were warmed in the hands before replacement in the nest.

Statistical analysis

Chick body measurements were fitted to several models of growth curves (Ricklefs 1983, Zach *et al.* 1984) using a program written using PROC NLIN (SAS Institute 1988). The use of non-linear least-square regression procedure assumes that residuals are randomly distributed, independently of chick age (Zach *et al.* 1984). Two models were compared (Logistic and Gompertz). The best model was chosen with regard to residual distribution, estimated visually by plotting measurement residuals versus chick age. In practice, however, models were sometimes difficult to separate and thus we present both parameter estimates. For body weight, as well as bill length, asymptotic values clearly were not reached at the age of 25 days (last measurements in the wild); therefore we used adult values (separated by sex and country) and assumed that these were attained at the age of 50–60 days, in order to estimate correctly the parameter values of the growth curve.

Values are given only when information on a brood or a nest was complete. Hence, sample size will differ between procedures. Means ± sd are presented. All statistical analyses were made using SAS 8.0 software.

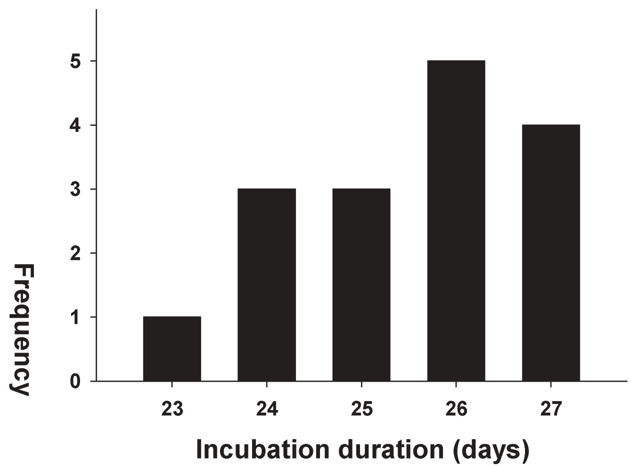

Figure 1. Frequency distribution of incubation duration measured in the field for 16 different eggs.

RESULTS

Volume, density and weight of Bittern eggs

Exact incubation length was obtained in the field for a total of 16 eggs from three nests in France and three nests in Belarus (Fig. 1). However, because laying date was determined from the presence of a new egg between subsequent nest visits, the precision of the length of incubation was only two days (assuming that eggs are laid every second day). The median value of incubation duration was 25.3 ± 1.24 days (n = 16).

Between 2002 and 2004, a total of 181 eggs were measured at the French study sites (three extreme and isolated widths, 34.1, 34.4 and 43.5 mm, were removed from calculations; see Table 1). At the first measurement, mean egg weight = 38.2 ± 3.52 g (min. = 26.0, max. = 47.0, n = 163). However, as is known for other bird species (Furness & Furness 1981), egg weight decreases with age, and egg density therefore also decreases from laying to hatching. The decrease in density in Bittern eggs appeared to be linear (Fig. 2a). Taking into account only eggs for which a direct measure of age could be obtained (54 different eggs for a total of 78 measurements: 37 eggs measured once, 10

Table 1. Egg biometrics of Bitterns (n = 178; three outliers have been excluded).

Parameter	Mean ± sd	Min.	Max.
Length (mm)	52.3 ± 2.1	47.5	59.0
Width (mm)	38.3 ± 1.26	34.8	42.0
Length/width ratio	1.37 ± 0.06	1.21	1.59
Volume (cm^3)	39.01 ± 3.31	31.72	47.67

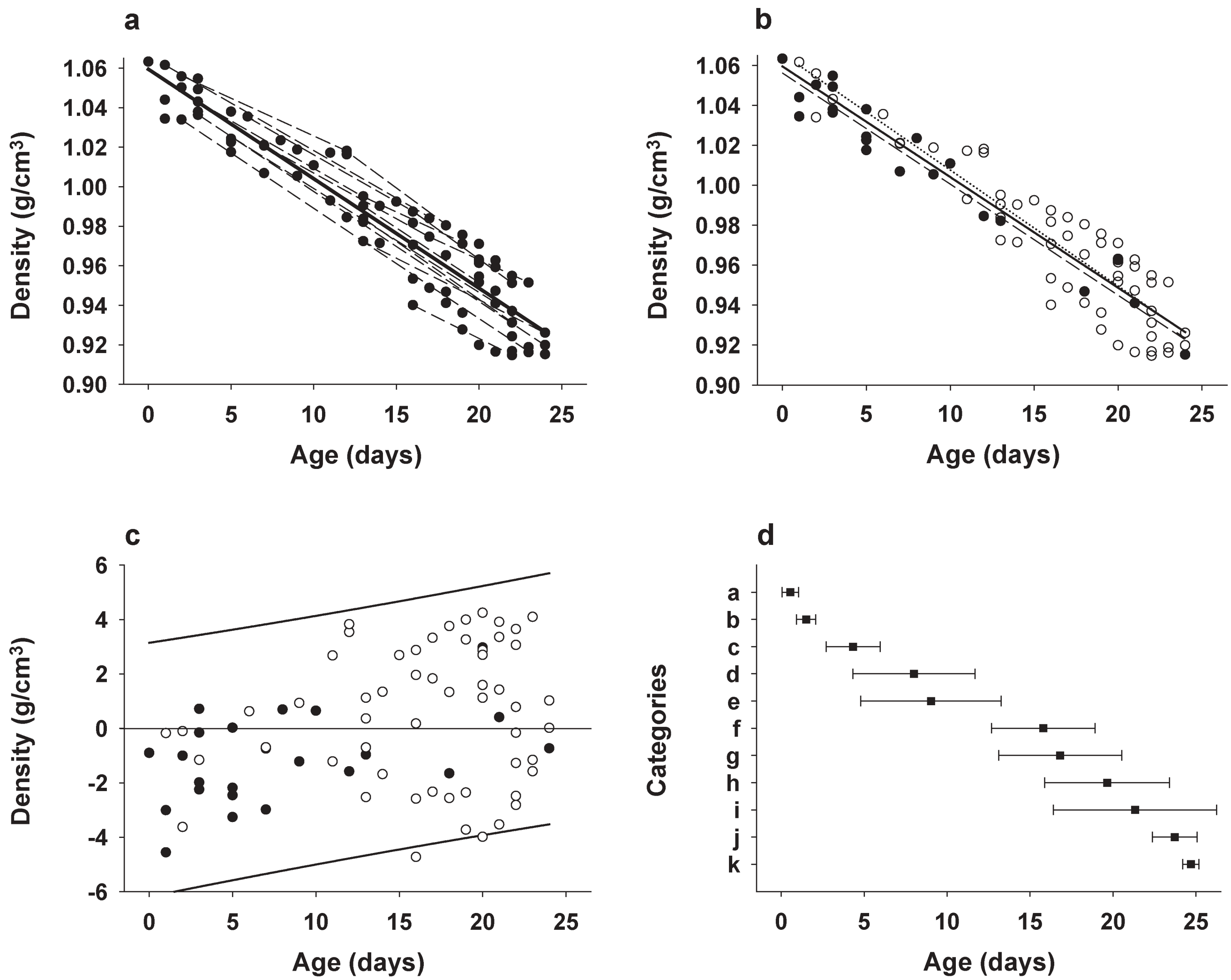

Figure 2. (a) Egg density (g/cm^3) as a function of age. Continuous line for the regression equation, and dashed line for eggs measured at least twice. (b) Egg density as a function of age in clutches of known laying date (●) (n = 22) compared to clutches of known hatching date (○) (n = 56). (c) Residuals of the regression between density and age (n = 78), with clutches of known laying date (●) (n = 22) compared to clutches of known hatching date (○) (n = 56). (d) Egg position in the water as a function of age. Category: a, at the bottom, horizontal (n = 6); b, at the bottom, at an angle of 10–40° (n = 2); c, at the bottom, 45–60° (n = 3); d, at the bottom, 70–85° (n = 8); e, at the bottom, 90° (n = 19); f, touching water surface, but not protruding (n = 5); g, protruding from the water (n = 28); h, hardly protruding (n = 13); i, hardly protruding and at a small angle (n = 3); j, beginning of hatching, small part of egg protruding (n = 13); k, hatching, large part of egg protruding (n = 5).

twice and 7 three times), the age of each egg could be calculated following the formula ($r^2 = 0.89$, $P < 0.0001$):

$$\text{Age (days)} = -160.93 \times \text{density (g/cm}^3\text{)} + 172.02$$

Eggs for which two or three measurements were available showed similar slopes to the general regression line (Fig. 2a). More eggs were aged by knowing their hatching date (56 measurements) than by knowing their laying date (22 measurements); however, there was no significant difference between the two regression lines ($F = 0.75$, $P = 0.38$; Fig. 2b). According to our sample, Bittern egg density decreases from 1.063 at laying to 0.915 the day before hatching, and daily weight loss was 0.195 ± 0.32 g (min. = 0.125, max. = 0.250, n = 20). The precision of our method was tested on eggs of known laying date, by comparing the residuals of density to the regression line (Fig. 2c). The precision was around three days (see Fig. 2c).

The water test was applied to 105 Bittern eggs from Belarus (for which laying or hatching dates were known). We discerned 11 different egg positions in the water (a–k in Fig. 2d). Non-incubated (fresh) egg was always positioned horizontally, while as soon as incubation started, the larger end of the egg rose. In general, a large overlap was found between the estimated incubation stages (Fig. 2d). On average, the estimated age of eggs was correct, but variability was very high (Fig. 2d): for instance, at nine days of age, the range of estimates was from three to 20 days, and accuracy of age estimates varied from ±2 to ±5 days according to chick age, based on range values (Fig. 2d).

Growth rates and ageing of chicks

A general growth curve was established for each biometric character using all values from all chicks, but considering as a first step Belarus and French localities as separate strata. Growth patterns differed among the three parameters considered (Fig. 3, Table 2): tarsus grew more quickly than other parameters, attaining asymptotic length at about 25 days. In contrast, body weight was still increasing at 30 days when the last birds were measured.

Restricting our data set to values relating only to known date of hatching, the age of chicks may be calculated from the tarsus length[c] (logistic growth curve; n = 227 values for chicks):

$$\text{Age (days)} = -\{\ln[(96.8261/\text{tarsus}) - 1] - 0.1548 \times 12.0566\}/0.1548$$

Tarsus growth rate did not differ between the two countries (Fig. 3a), so data were combined. The

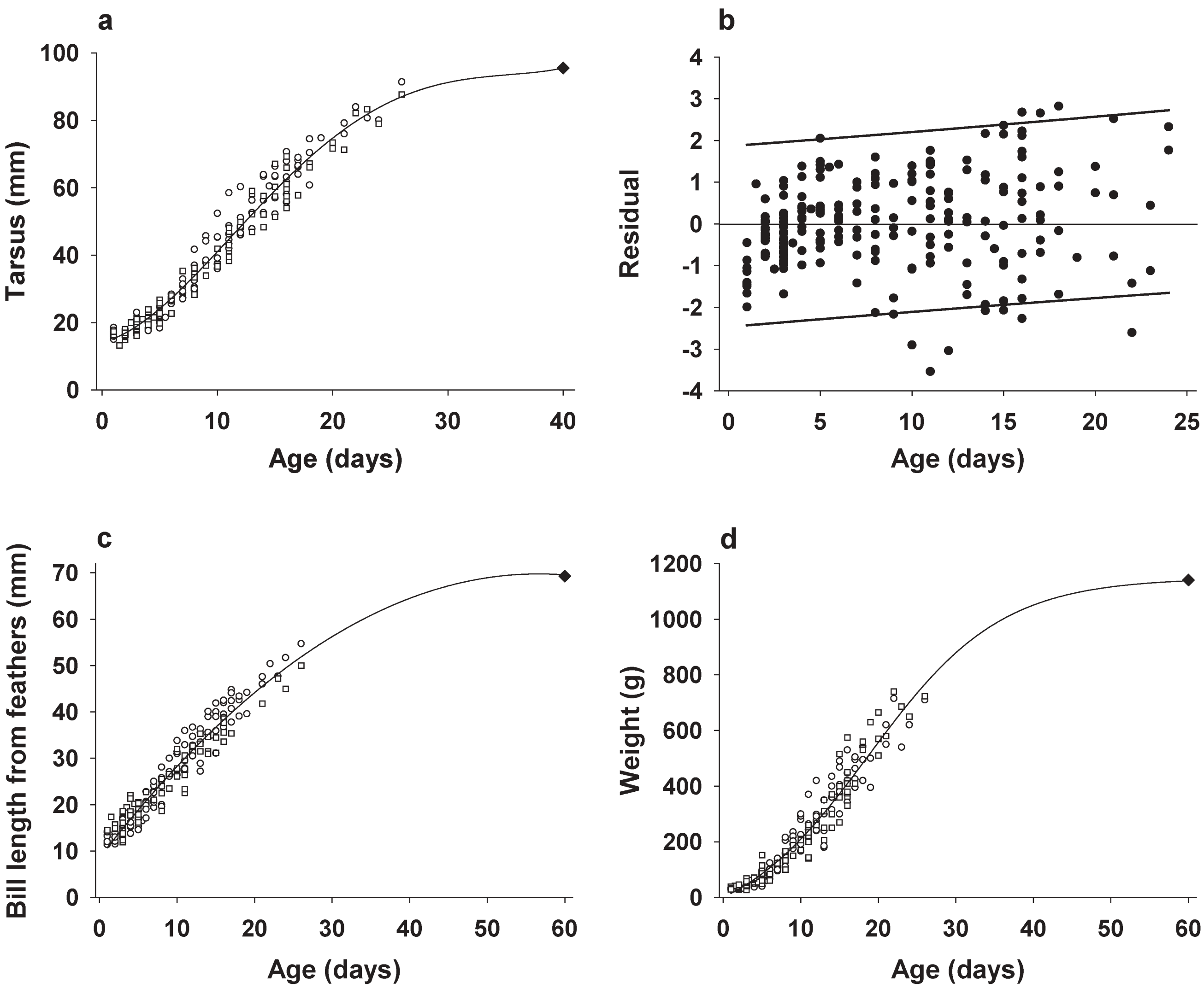

Figure 3. (a) Tarsus length as a function of age (n = 227) in Belarus (○) (n = 127) and France (□) (n = 100). (b) Residuals of the regression between tarsus length and age (n = 227). (c) Length of bill (from feathers to tip) as a function of age (n = 197) in Belarus (○) (n = 133) and France (□) (n = 64). (d) Body weight as a function of age (n = 240) in Belarus (○) (n = 133) and France (□) (n = 107).

Table 2. Average parameters of the growth models applied to the biometric data of Bittern chicks.

Parameter	Model	Parameter estimates		
		K ± se	A ± se	T ± se
Tarsus length	**Logistic**	**0.15 ± 0.004**	**96.8 ± 1.61**	**12.1 ± 0.27**
	Gompertz	0.09 ± 0.003	106.0 ± 2.35	9.15 ± 0.29
Bill length	Logistic	0.11 ± 0.003	68.2 ± 1.31	13.78 ± 0.40
	Gompertz	**0.07 ± 0.02**	**71.3 ± 1.49**	**9.2 ± 0.34**
Body weight	Logistic	0.16 ± 0.005	1100.3 ± 30.33	19.38 ± 0.40
	Gompertz	**0.09 ± 0.003**	**1167.5 ± 32.1**	**16.4 ± 0.38**

A, asymptotic value; K, growth coefficient; T, age at the inflection point of the curve. The tarsus length model shown in bold is recommended for age derivation and the resulting curve is shown in Fig. 3a. Also in bold are the best-fit models for bill length and body weight which are shown in Fig. 3.

accuracy of the tarsus formula was about two days, based on residuals between estimated and observed age (Fig. 3b).

DISCUSSION

Although it is well known that eggs lose weight (due to water loss) during incubation, enabling eggs to be aged from their density, this useful technique has not been widely used (Furness & Furness 1981; see Green (2004) for a review of the method). For the Bittern we were able to provide a formula that allows back-dating laying dates for a clutch, with an accuracy of about ±3 days. Therefore, some caution is advocated when using this method, because error in applying this method can reach up to 25% of the incubation duration.

We detected a rather high variability in Bittern egg weights when measured in the field, although we did not investigate the repeatability of weight measurements. We suggest that humidity of the eggshell (presumably highly variable in this aquatic species) may account for part of this variability. Consequently, we had to dismiss several egg weights that fell clearly outside the range of possible density values (e.g. values above 1.2).

We also detected some observer effect (possibly due to different equipment), but considered that this did not affect parameter estimates in the equations as one of us made 46% of all measurements. The accuracy of the equation also appeared to be higher when eggs came from clutches of known laying dates rather than clutches of known hatching dates (Fig. 2c). This could result from some females, late in the incubation period, deserting their nest temporarily (e.g. for feeding), thus increasing variability in incubation length. It may also result from a non-linear density decrease with age, as found in several seabird species (Furness & Furness 1981, Thomas & Lumsden 1981). Compared to density calculations derived from precise egg weights, the water-derived density method provided consistent results but, as expected, its estimates were less precise (±5 days, as opposed to ±3 days accuracy).

We provide the first chick growth curve for Bitterns (and for any bittern species). As evidenced by Fig. 3, the growth strategy of Bittern chicks is mainly biased towards tarsus growth, and thus may be a reflection of its strong adaptive value in young chicks. Rapid tarsus growth would allow chicks to leave the nest early in their life, and escape potential predators by walking on reeds (pers. obs.). Consistent with this anti-predator strategy, we found that Bittern families broke up as soon as chicks left the nest, with all Bittern chicks dispersing away from each other. Bill length and body weight appeared to increase more slowly than tarsus length, attaining their asymptotic value at 30–40 days old compared with 25 days for the tarsus. We did not collect precise data on wing growth, but it appeared to be the character that grew most slowly. From these different growth curves, tarsus length provided the best estimate of age, irrespective of breeding locality, at least until the age of about 25 days. At that age, the tarsus had almost completed its growth. Compared to tarsus length, we found that bill length was difficult to measure accurately on the smallest chicks (because of its very small size), while body weight varied markedly (by almost 45%; pers. obs.) according to whether the chick had just been fed. Moreover, when handled, chicks that had been fed recently tended to regurgitate food (for instance, a 180-g chick regurgitated 82 g of food). After 25 days of age, body weight probably provides a useful alternative to tarsus length until 35 days, and after that only wing length can be used.

In conclusion, we have provided simple ways to age eggs and chicks in Bitterns. We suggest that this is very useful for researchers working on this endangered species, as it provides a way to minimize the number of visits to the nest when determining breeding biology parameters and estimating when chicks from study nests will attain an age suitable for ringing.

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ENDNOTES

a. The six French study sites are the north bank of the estuary of the River Seine (49°27′N, 0°17′E), Brenne (46°44′N, 1°17′E), the low plain of the River Aude (43°16′N, 3°09′E), the Charnier-Scamandre, western Camargue (43°38′N, 4°22′E), the Vigueirat Marshes, eastern Camargue, (43°29′N, 4°48′E), and Tour du Valat, Camargue (43°31′N, 4°36′E).

b. Although water density varies slightly according to temperature and chemical composition (thus possibly affecting egg flotation), we did not take these parameters into account.

c. A simplified formula could be derived from the fact that tarsus growth was linear between the ages of 5 and 20 days ($r^2 = 0.9192$, $n = 141$):

$$\text{Age (days)} = 0.261 \times \text{tarsus} - 0.6344$$

Similar equations could be obtained for bill length (using Gompertz model; $n = 197$):

$$\text{Age} = -\{\ln[\ln(71.301/\text{bill})] - 0.07 \times 9.2351\}/0.07$$

or body weight (using Gompertz model; $n = 240$):

$$\text{Age} = -\{\ln[\ln(1167.5/\text{weight})] - 0.0865 \times 16.4052\}/0.0865$$

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