MOORHEN *GALLINULA CHLOROPUS* FEMALES LAY EGGS OF DIFFERENT SIZE AND β-CAROTENE CONTENT

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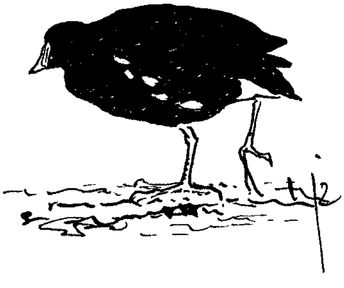

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In birds, carotenoids deposited in the eggs can influence the progeny's prospects of survival. As a potentially limiting resource, carotenoids should be allocated optimally by the mother during egg production. The Moorhen *Gallinula chloropus* produces large clutches. We tested if females differ in their allocation of macronutrients (proteins and lipids) and the key micronutrient, β-carotene. To this end, we measured intra- and inter-clutch variability in egg size and contents. All parameters showed large inter-clutch differences and low intra-clutch variability. The total amount of β-carotene decreased in smaller eggs, indicating that females did not compensate for smaller egg size with a higher content of this micronutrient.

Key words: *Gallinula chloropus* – β-carotene – egg composition – inter-clutch variability.

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There is increasing interest in the influence of maternal effects on the quality of offspring (Qvarnstrom & Price 2001). Since egg production is an energetically expensive process (Nager *et al.* 2001), maternal investment in egg size and composition can have a strong influence on offspring fitness (Williams 1994; Blomqvist *et al.* 1997). Unequal resource allocation among eggs may be the optimal strategy for females with access to limited resources (Reed & Vleck 2001). It has long been known that each female can lay eggs of different size (Lack 1968) or relative amount of yolk and albumen (Carey 1996; Swennen & Van der Meer 1995) and with a different composition (proteins, lipids and other contents such as hormones and carotenoids; Royle *et al.* 2001).

Yolk is the principal energy reserve used by the developing embryo and is particularly important in precocious species (Carey *et al.* 1980). Apart from water, lipids are the primary constituents of egg yolk: developing avian embryos use yolk lipids to provide energy through β-oxidation of fatty acids (Noble & Cocchi 1990). Proteins are another fundamental component of the yolk, reaching almost 1/5 of its total weight in some species, even if they are also present in the albumen (Williams 1994). Carotenoids together with riboflavin are responsible for the red-orange colour of the yolk (Blount *et al.* 2000). These substances have important physiological functions, as lipid antioxidants, in different phases of the ontogeny (Surai & Speake 1998; Royle *et al.* 2001; Surai *et al.* 2001) and in the development of neurosensory networks. They also have a modulatory effect on the immune system (Bendich 1989). The carotenoids are a group of more than 600 different biochemicals; β-carotene is one of the most important carotenoids found in nature (Møller *et al.* 2000).

In several bird species, egg mass is highly repeatable within each clutch but variable between clutches (Christians & Williams 2001). Variations in the size of eggs laid by different individuals of the same population can reflect a combination of genetic and/or environmental effects, while variation between eggs laid by the same individual in a clutch may depend on different investment in reproductive effort (Williams 1996). Apart from total mass, the specific components of eggs can vary considerably in relation to numerous factors, such as parental quality, environmental factors and food quality and availability (Arnold *et al.* 1991; Ward 1995). For this reason, egg components may to some extent be unrelated to size (Alisauskas 1986; Arnold *et al.* 1991). While the protein and lipid composition of eggs has been studied in several species (Carey 1996), less is known about the allocation of substances with metabolic and regulatory functions (i.e. hormones, vitamins and carotenoids, Royle *et al.* 2001). In this study, we report the occurrence of high inter-clutch variability and small intra-clutch variability in egg mass and contents (β-carotene, proteins, lipids) in the Moorhen *Gallinula chloropus*. Since carotenoids are a limiting resource of great physiological importance, we observed in particular if a) females allocate a fixed amount of β-carotene to each egg, irrespective of egg size, or b) the amount of β-carotene increases in larger eggs, which could be an indication of a mechanism of parental investment (Royle *et al.* 2001), or c) β-carotene decreases in larger eggs, which could indicate a mechanism of compensation to decrease the difference among freshly hatched chicks.

Data were collected in May 2001 at the Stork and Wildfowl Centre of Racconigi (NW Italy). The centre is a 3-ha reserve characterised by a series of ponds surrounded by grassfields and reedbeds (Fenoglio *et al.* 2000a). Since 1985, the centre has supported a collection of waterfowl for which food was provided twice daily, and as a result the reserve has accommodated a large population of wild Moorhens. Twenty-two randomly distributed Moorhen nests were found, and 131 eggs were weighed with an electronic balance (± 0.01 g accuracy) while their length and breadth were measured with a calliper (± 0.1 mm). The lean mass was estimated non-invasively by total body electrical conductivity (TOBEC) measurements (Williams *et al.* 1997). This gives an indication of lean mass, since the contribution of lipid to conductivity is negligible. Each egg was placed in the detection chamber (32 mm diameter) of an SA-3000 Small Animal Body Composition Analyzer (EM-SCAN Inc., Springfield, Illinois) for 5-6 sec and the electrical conductivity index was recorded as: TOBEC index = (S - E) / R, where S = measurement with the specimen, E = empty measurement, and R = reference number (EM-SCAN operator's manual). We conducted a chemical analysis (β-carotene, lipids, proteins) on a subsample of 29 eggs collected from 13 randomly selected nests (mean 2.23 eggs/nest ± 1.14 SD). The collected eggs were chosen in order to represent a wide range of sizes, while their rank in the

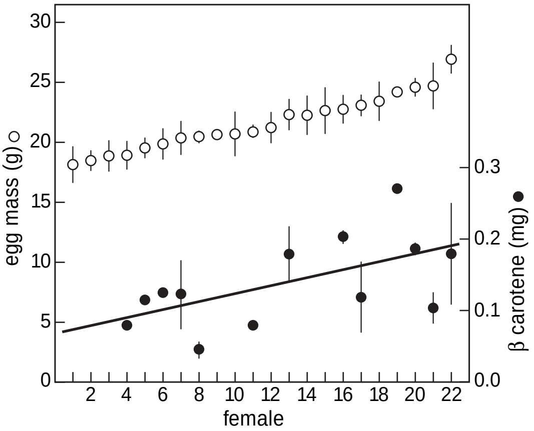

Fig.1 Variation in egg masses and β-carotene content in egg yolks produced by each female. Female identification numbers were assigned to females by ranking them according to mean mass of their eggs.

laying sequence was not assessed in order to minimise disturbance in the laying period. Lipids were extracted with a Soxhlet apparatus, protein content was assessed with the Kjendall method, and β-carotene was measured with the 958.05 AOAC (1990) official method. Inter- and intra-clutch differences were investigated by ANOVA and, because multiple tests were conducted on several measured variables for the same eggs, Bonferroni correction was applied.

Most variation in egg mass and size was due to differences among clutches produced by different females. Each female produced similar eggs, while there was a significant difference among females in the mass (Fig.1, n = 131 eggs, ANOVA $F_{21,109} = 11.8$, $P < 0.001$), as well as in length (mean = 44.3 mm ± 1.81 SD, $F_{21,109} = 5.45$, $P < 0.001$), breadth (mean = 30.2 mm ± 1.36 SD, $F_{21,109} = 2.42$, $P < 0.002$) and lean mass (TOBEC index, mean = 158.4 ± 74.9 SD, $F_{21,109} = 6.54$, $P < 0.001$). A comparison of chemical parameters, determined in 29 eggs, revealed significant differences among females in the contents of lipids (mean = 2.03 g ± 0.34 SD, ANOVA $F_{9,16} = 3.07$, $P < 0.01$) and proteins (mean = 2.21 g ± 0.26 SD, $F_{9,16} = 3.88$, $P < 0.009$), as well as in total β-carotene content ($F_{9,16} = 9.08$, $P < 0.001$). Heavier eggs contained more proteins (Fig.1, $r_{29} = 0.79$, $P < 0.001$), lipids (Fig.1, $r_{29} = 0.38$, $P < 0.05$) and absolute β-carotene content ($r_{29} = 0.37$, $P < 0.05$). Females did not compensate for smaller egg mass with a higher content of micronutrient, thus the concentration of β-carotene (egg yolk mean = 18.5 µg/g ± 7.8 SD) did not decrease in heavier eggs ($r_{29} = 0.22$, P = n.s.), but rather tended to increase slightly with mass.

A positive relation between egg size and chick survival is generally acknowledged (Amundsen & Stockland 1990). Sources of variation in egg size may include population and individual differences. Populations can differ because of geographical, climatic and seasonal patterns, while individual differences mainly reflect heterogeneity of the local habitat and food abundance, as well as parental genetic quality (Carey 1996).

In our study of the Moorhen, mean egg size was similar to that of another northern Italian population (Realini 1984), and to the values reported for most European countries (Engler 1980; Glutz von Blotzheim *et al*. 1994). Interestingly, eggs of southern populations are smaller than English ones (Cramp & Simmons 1980). This is probably related to the smaller size of adults (Cucco *et al.* 1999). We found low intra-clutch variability and significant inter-clutch diversity in egg size and composition. Each female produced eggs of similar size, while there were significant differences between individual females. These results are in line with those for other species, in which each female lays eggs that are similar to each other but different from those of other females (Arnold 1991; Swennen & Van der Meer 1995; Carey 1996; Christians & Williams 2001). In some species, this pattern is maintained from one year to another (Jager *et al.* 2000) and there is little influence of environmental factors or food abundance (Arnold 1994; Nager & Zandt 1994).

We found similar individual constancy in the composition of Moorhen eggs. Previous studies of intra-specific variation in egg size have frequently assumed that size variation reflects variation in egg quality, but this assumption is not necessarily valid (Christians & Williams 2001). In this study, macronutrient measures of egg quality (lipids and proteins) did show variations in parallel with fresh egg mass. This result is similar to the findings of Swennen & Van der Meer (1995) and Christians & Williams (2001), and contrasts with previous findings of Ricklefs (1984). We focussed particularly on the β-carotene content, a substance of key physiological importance during development (Blount *et al.* 2000). Total β-carotene content was similar to that reported for Moorhen eggs from England (13.8 mg/g; Surai *et al.* 2001). This result, as well as that reported for the American Coot *Fulica americana* and Black-backed Gull *Larus fuscus* (Surai *et al.* 2001), contrasts with data from eggs of the domestic chicken, which contain little or no β-carotene, and underlines the importance of this specific pigment in the physiology of wild species. In our study, each female produced eggs of similar β-carotene content, in the same way as for lipids and proteins, while there were significant differences between hens. A pattern in which inter-clutch variability is significantly higher than intra-clutch variability was also found for other key substances, i.e. maternally derived androgens (testosterone and androstenedione), in the American Coot (Reed & Vleck 2001). Therefore, the egg composition data suggest that females differ in reproductive quality, a fact that will influence the prospect of survival of the offspring from the early growth stages (Bolton 1991). In particular, an important positive metabolic role of carotenoids in chick development has been shown in poultry, in the Barn Swallow *Hirundo rustica* and in the Moorhen. In these species, supplementary carotenoids improve the chick's immune response and make it more reactive (Bailey & Chinnah 1995; Saino *et al.* 2000, Fenoglio *et al.* 2002b). A main result of this study was that the concentration of β-carotene did not decrease with egg mass: for this reason, larger eggs had more β-carotene. This indicates that females did not allocate a fixed amount of β-carotene to each egg, irrespective of its size, and that they did not try to compensate for smaller egg size with a greater abundance of key substances (Schwabl 1993). More data are needed to better understand how the allocation of maternally derived substances is regulated in birds, as there is growing evidence that allocation of key substances (e.g. androgens and carotenoids) may provide an adaptive mechanism for parental favouritism (Royle *et al.* 2001; Hõrak *et al.* 2002).

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De allocatie van nutriënten in eieren speelt een cruciale rol bij de overlevingskansen van kuikens. De grote legsels van Waterhoentjes *Gallinula chloropus* lenen zich uitstekend om variatie in de depositie van macro- (eiwitten en vetten) en micronutriënten tussen en binnen legsels te bekijken. De hoeveelheid β-caroteen, een belangrijke micronutriënt, varieerde nauwelijks tussen eieren binnen een legsel, en was onafhankelijk van eigrootte. Tussen de eieren van verschillende vrouwtjes bestonden wel forse verschillen in hoeveelheid β-caroteen. Dit zou erop kunnen wijzen dat er tussen vrouwtjes grote verschillen bestaan in reproductieve kwaliteit. (RGB)

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