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PROXIMATE FACTORS AFFECTING INTRACLUTCH EGG-SIZE VARIATION IN THE SQUACCO HERON *ARDEOLA RALLOIDES*

By

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ABSTRACT

PROXIMATE FACTORS AFFECTING INTRACLUTCH EGG-SIZE VARIATION IN THE SQUACCO HERON *ARDEOLA RALLOIDES*

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I studied certain aspects of the breeding biology of the Squacco Heron (*Ardeola ralloides*), a poorly known species, focusing on egg-size variation within clutches and the proximate factors that may affect it. I found that, in most cases, this heron's clutch profile (pattern of intraclutch egg-size variation) is arched, that is both first and last eggs are significantly smaller than middle ones. I tested Parsons's (1976) hypotheses that the size of final eggs is a) physiologically constrained due to hormonal changes associated with the onset of female incubation, or b) nutritionally limited by shortages in female resources for egg formation. I also examined the possibility that the onset of incubation may restrict female feeding opportunities, and thus limit egg-resource availability and last-egg size. I was unable to directly assess either female nutritional resources or the timing of the onset of incubation, but I inferred the levels of these factors, respectively, from a) the average egg volume of clutches (AvVol), and b) their hatch span (H-Span), that is the time interval between the hatching of their first and final eggs. I also tested the hypotheses that the relative size of first eggs may be nutritionally constrained or limited anatomically by an initial inelasticity of the oviduct.

Neither of the hypotheses concerning the size of first eggs was supported: a) first eggs became relatively smaller in 4-egg clutches with higher AvVol (the opposite of what

the nutritional hypothesis predicted); b) they were not significantly thinner than later eggs, neither were their volume and width/length ratio positively correlated (as the anatomical hypothesis predicted). There was no correlation between clutches' H-Span and AvVol values, which refutes the hypothesis of nutritional constraints on egg size related to the onset of incubation.

H-Span affected clutch profile in the way predicted by Parsons's hormonal hypothesis: between samples of long and short H-Span (early and late incubation onset), the profile's linear trend changed from negative to positive, and the relative size of final eggs increased. The same trend was observed between 5-egg and 4-egg clutches (which have median H-Span values of 4.5 and 3.0 d, respectively), but only among high-AvVol nests (where resources for egg formation were presumably abundant). AvVol had a positive effect on the linear trend of clutch profile and on relative last-egg size (as the nutritional hypothesis predicted), but only in 4-egg clutches, where H-Span was relatively short (and hormonal constraints were presumably weak). Therefore, it seems that both hormonal and nutritional factors can influence the relative size of last eggs in this heron, but each one's effects become apparent only in the absence of interference from the other. The hormonal and nutritional hypotheses also predicted, respectively, that H-Span should decline, while AvVol should increase along a gradient of clutch profile types where relative last-egg size progressively increases. The former prediction was confirmed, but the trend in AvVol was quadratic, and clutches with irregular profiles also had a low mean AvVol. These results further support the hypothesis of hormonal constraints on final-egg size that interact with nutritional limitations.

To my Parents

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LIST OF TERMS AND ABBREVIATIONS

AvVol	Average egg size of a clutch.
Clsz	Clutch size ; 4- and 5-egg clutches are symbolized as C4 and C5, respectively.
Egsz	Egg Size ; among-nests factor representing level of AvVol (low vs high).
FSH	Follicle-stimulating hormone.
Gn-RH	Gonadotropin-stimulating hormone.
H-Span	Hatch span of a clutch (interval between the hatching of first and last eggs).
LH	Luteinizing hormone.
Profile of a clutch	Pattern of intraclutch egg-size variation. Clutch profile types: a) Decline (decrease in egg size with laying order); b) I-max (clutch profile is arched and intermediate egg is largest); c) P-max (clutch profile is arched and penultimate egg is largest); d) Rise (increase in egg size with laying order); e) Irregular (clutch profile does not fit any of the above patterns).
Rank of an egg	Position in the laying sequence. Symbolized by A-D in C4 and by A-E in C5. Modified egg ranks in C5 are first, intermediate (average of B and C), penultimate, and last. First and last can be referred to as *marginal*, others as *middle*.

I. INTRODUCTION

The avian egg provides a protective environment, the genetic information and all necessary nutrients for the development of avian embryos, which only requires the input of thermal energy and gas exchange with the outside world (Burley and Vadehra 1989). Given the egg's importance for embryonic growth, biologists have been interested both in the factors that influence its size and quality (Perrins 1996; Meijer and Drent 1999), and in the effects of the latter on nestling fitness (Williams 1994).

Differences in egg size among females of the same species may reflect individual variation in body size (Ojanen et al.1979; Nol et al.1984), age (Reid 1988; Willebrand 1992), and nutritional condition (Houston et al.1983; Leblanc 1989; Wiebe and Bortolotti 1995). Egg size also shows consistent patterns of intraclutch variability (*clutch profiles*) in diverse avian taxa, including cormorants (Coulson et al.1969), herons (Custer and Frederick 1990), anatids (Owen and West 1988; Williams et al.1993), coots (Arnold 1991), shorebirds (Nol et al.1984; Amat et al.2001), larids (Runde and Barrett 1981; Meathrel and Ryder 1987; Kilpi et al.1996), and passerines (Ryden 1978; Ojanen et al. 1981; Rofstad and Sandvik 1985; Heeb 1994).

Early research on clutch profiles focused on the relative size of last eggs and its potential involvement in reproductive strategies, in association with hatching asynchrony (Howe 1976; Parsons 1976; Clark and Wilson 1981; Slagsvold et al. 1984). Lack (1947) proposed that, in species that suffer low nest predation but depend on unpredictable food resources for raising young, hatching asynchrony is an adaptation that creates competitive asymmetries among broodmates, which ensure the successful growth of senior chicks at the cost of their junior siblings' starvation when food is scarce. Lack's arguments have generated a wealth of studies and alternative hypotheses, adaptive and not (Stoleson and Beissinger 1995), and the evolutionary reasons for the occurrence of hatching asynchrony may vary among species (Magrath 1990). Parental reproductive strategies may then be indicated by the relative size of last eggs (Slagsvold et al.1984): a) small final eggs may further facilitate the demise of junior chicks in brood-reductionist species, while b) large final eggs may reduce the risk of maladaptive brood reduction in species where hatching asynchrony serves functions unrelated to sibling competition (Clark and Wilson 1981). For clutch profile to play such a role, egg size must have significant effects on nestling performance. Egg size has been found to affect the early growth (Howe 1976) and the survival (Grant 1991; Amat et al. 2001) of same-age siblings, and also the early survival of junior chicks in gulls (Parsons 1975a). Other authors, however, have concluded that the effects of clutch profile on siblings' fates are insignificant when compared with those of hatching asynchrony (Stokland and Amundsen 1988; Bollinger 1994; Vinuela 1996).

Since many studies of clutch profile focused on its possible effects on nestling fitness, when such effects were not apparent, authors often attributed the existence of egg-size variation within clutches to unspecified physiological constraints. All traits, however, whether adaptive or incidental, have a physiological basis, the study of which may lead to their better understanding, without prejudice to their possible ultimate functions.

Parsons (1976) investigated the proximate causation of clutch profile in the Herring Gull (*Larus argentatus*) by removing eggs as females laid them. In this species, most females lay 3 eggs, of which the last is significantly smaller than the other 2 (by about 10%), mainly due to a deficiency in albumen. Egg removals delayed the onset of incubation and allowed most females to lay replacement eggs, a phenomenon that has been observed in a large variety of birds (reviews in Kennedy 1991, and in Haywood 1993a). In the enlarged clutches, only final eggs (4th, 5th, etc.) were significantly smaller than early ones, and of similar size to normal last (3rd) eggs. Third or even later eggs were not significantly smaller, as long as they were earlier than last.

Parsons (1976) predicted that, if female resource depletion was responsible for the smaller size of normal final (3rd) eggs, all 3rd and later eggs would be progressively smaller. Since, however, no eggs before the last were affected, whatever their absolute laying order, this resource-limitations hypothesis was refuted. Instead, Parsons (1976) suggested that the Herring Gull's clutch profile is mainly the result of physiological constraints imposed on the female's reproductive system by hormonal changes associated with the onset of incubation. (In my presentation of his proposed mechanism below, I also use supportive evidence from more recent studies.)

The development of incubation behavior is associated with an increase in plasma prolactin in many birds (Goldsmith 1983; Buntin 1996; Vleck 2002). High prolactin levels inhibit the secretion of gonadotropins (Lea et al.1981; Dawson and Goldsmith 1982), which promote the growth of the ovary and its gametogenic and endocrine functions (Murton and Westwood 1977; Scanes 1986). Thus, increased prolactin levels are linked with reduced estradiol secretion (Zadworny et al.1989; Sockman and Schwabl 1999), follicular atresia (Opel and Proudman 1980), and the cessation of egg-laying (Youngren et al.1991). Estardiol stimulates oviduct growth (Murton and Westwood 1977) and albumen protein synthesis (Johnson 1986), whereas high levels of progesterone (as those released by atretic follicles) elicit oviduct regression (Murton and Westwood 1977).

Thus, the suit of hormonal changes triggered by the onset of incubation eventually results in the suppression of ovarian and oviductal function (Buntin and Tesch 1985).

In the Herring Gull, eggs are laid every 2 d, and incubation starts >12 h after the 1st egg is laid (Parsons 1972). By that time, albumen deposition in the 2nd egg is well advanced, and the 3rd egg's yolk is almost complete, but its albumen will not begin to be synthesized for another 1.5 d (Parsons 1976). Therefore, hormonal constraints can limit significantly only the last (3rd) egg's albumen, thus affecting both this egg's size and its composition (Parsons 1976). In species who lay large clutches and begin low-intensity incubation early in the laying sequence, gradual hormonal changes may reduce (without completely suppressing) the function of both the ovary and the oviduct, thus causing a progressive decrease in the size of eggs laid after the onset of incubation without affecting their composition (Arnold 1991; Williams et al.1996).

Houston, Jones and Sibly (1983) proposed an alternative explanation for the smaller size and albumen deficiency of last (3rd) eggs in the Lesser Black-backed Gull (*Larus fuscus*). They found that female protein reserves positively affected clutch size and the dry weight of egg yolks, but not the amount of egg albumen. Assuming that female gulls have to acquire nutrients for egg albumen from their daily diet, like some passerines (Jones and Ward 1979), they proposed that the apparent association between the onset of incubation and the smaller size of final eggs results from the constraints imposed by the former on female foraging time and food ingestion.

More recent experiments on Lesser Black-backed Gulls (Hiom et al.1991; Bolton et al.1992) have shown that food supplements can increase both the average egg size of clutches and the relative size of final eggs. The latter, however, is due to an increase in yolk, rather than albumen, protein (Bolton et al.1992). These results indicate that the yolk of last eggs may be nutritionally constrained, but their albumen is limited by some other factor, which could be the hormonal mechanism proposed by Parsons (1976). Studies on geese (Leblanc 1987; Williams et al.1996), coots (Arnold 1991) and crows (Heeb 1994) were unable to attribute small last eggs to female resource limitations.

The above hypotheses (hormonal and nutritional) concern only the proximate limitation of last-egg size. However, both first and last eggs are significantly smaller than intermediate ones in various birds, including cormorants (Stokland and Amundsen 1988), herons (Jover et al.1993), anatids (Leblanc 1987; Kennamer et al.1997), shorebirds (Nol et al.1984), coots (Arnold 1991), and some passerines (Ojanen et al.1981; Verbeek 1990; Magrath 1992a), while in some other passerines egg size increases with laying order and first eggs are the smallest in the clutch (Howe 1976; Mead and Morton 1985; Slagsvold and Lifjeld 1989). Three proximate hypotheses have been proposed to account for small first eggs in such species, but they have neither been formulated nor tested as rigorously as the ones that concern last eggs.

The size of first eggs seems to be nutritionally constrained in some insectivorous songbirds, who begin to lay eggs while ambient temperature and food availability are still low, but whose food ingestion relative to metabolic needs often improves daily during the laying period (Slagsvold and Lifjeld 1989; Nilsson and Svensson 1993a). In accordance with this hypothesis, food supplements given to Blue Tits (*Parus caeruleus*) before the onset of laying eliminated the size deficit of first eggs (Nilsson and Svensson 1993a).

The more elongate shape of some anatids' first eggs may result from a restriction of their width by an initial inelasticity of the oviduct, which may later expand and thus allow subsequent eggs to be wider (Robertson and Cooke 1993). The deposition of more albumen along the first egg's longitudinal axis may only partially offset the deficit along the transverse axis, as too great an elongation may interfere with embryonic development and lower hatching success (Robertson et al.1994). Thus, both the width and the size of first eggs could be anatomically constrained, but this hypothesis has never been tested.

The physiological efficiency of the ovary (Parsons 1976) and oviduct (Leblanc 1987; Williams et al.1993) may increase as laying progresses, thus allowing subsequent eggs to attain greater sizes than initial ones. However, no specific mechanism has been proposed to underlie this presumed phenomenon. Some supporting evidence for this kind of physiological "inertia" comes from coots (Arnold 1991): the profiles of first clutches are arched, but in continuation clutches (those laid within a few days of the initial clutch's loss), early eggs are the largest, and egg size initially declines with laying order.

Only 2 studies have described clutch profiles in ardeids, one in 3 North American species (Custer and Frederick 1990), and the other in the Purple Heron (*Ardea purpurea*) in Spain (Jover et al.1993). Neither of them, however, examined in detail the proximate causes of the observed trends. I studied the clutch profile of the Squacco Heron (*Ardeola ralloides*), a poorly known species, and found that, usually, both first and final eggs are significantly smaller than intermediate ones. I also collected data on the variation in egg size and hatching intervals among clutches, which allowed me to test the predictions of the above hypotheses concerning the proximate factors that may affect clutch profile.

In the gulls studied by Parsons (1976), clutch size and the timing of the onset of incubation varied little among females. In species with strong variation in incubation patterns (like the Squacco Heron, see *Preliminary Analyses*), the length of time over which hormonal constraints will operate on the reproductive system of females, and the consequent deficit in the size of final eggs, should also vary accordingly. The time of the start of incubation can be inferred from a clutch's hatch span (the interval between the hatching of the first and last eggs), as parents can control their eggs' hatching patterns through incubation in various birds, including raptors (Wiebe et al.1998), larids (Parsons 1972; Nisbet and Cohen 1975), and passerines (Magrath 1992a; Vega and Vinuela 1993). Therefore, Parsons's (1976) hormonal hypothesis would be supported by a negative relationship between hatch span and the relative size of final eggs.

The nutritional hypothesis, as it was formulated by Parsons (1976), predicts a positive relationship between the relative size of final eggs and female resource availability for egg formation, independently of the onset of incubation and hatching patterns. In testing this hypothesis, I considered nutritional resources for eggs as a whole, without distinction between those that are stored in the female's body and those acquired through daily diet (Meijer and Drent 1999). I also inferred the level of female resources from the mean egg size of clutches, an assumption that I will support in the respective section of the *Preliminary Analyses*. This hypothesis thus predicts a positive relationship between the mean egg size of clutches and their last eggs' relative size, but no association between either of these 2 variables and hatch span.

Houston, Jones and Sibly (1983) proposed that the onset of incubation, through a restriction of female foraging time, limits the resources that can be invested in the clutch (which should lower mean egg size), and the size of final eggs. Their hypothesis therefore predicts both a) a negative association between hatch span and the relative size of last eggs (like the hormonal hypothesis), and b) a positive relationship between mean egg size and last-egg size (like the hypothesis of independent nutritional constraints), as well as c) a negative relationship between hatch span and the mean egg-size of clutches. This last prediction is unique to this hypothesis, and thus allows it to be critically tested.

I also examined the variation in mean egg-size and hatch span by year, season, and clutch size, in order to derive secondary predictions about trends in clutch profile among the levels of the latter 3 factors. For instance, I found that hatch span was longer in 5-egg than in 4-egg clutches, while mean egg size did not vary between them. Thus the hormonal hypothesis would predict relatively smaller final eggs in the larger clutches, whereas Parsons's nutritional hypothesis would predict no difference in clutch profile between them. (I will present these secondary predictions in detail in later chapters.)

The size of first eggs has received a lot less attention than that of last eggs, and the existing literature did not help me examine possible factors that may limit first-egg size as closely as those affecting final eggs. Nevertheless, I tested the hypothesis of nutritional constraints on first-egg size by comparing the profiles of small- and large-egg clutches, predicting relatively bigger first eggs in the latter (Nilsson and Svensson 1993a). I also analyzed egg shape (width/length ratio), to test the anatomical-constraint hypothesis' prediction that first eggs would be more elongate than subsequent ones (Robertson and Cooke 1993). I was unable to derive predictions from and test the "physiological inertia" hypothesis (Parsons 1976; Leblanc 1987).

In this study, which offers valuable information about the breeding biology of a poorly known species, I examined the Squacco Heron's clutch profile, as well as certain factors that may affect it proximately, at a level of detail never before applied to heron research, and rarely seen in studies of wild birds.

II . GENERAL METHODS

1. *ARDEOLA RALLOIDES*

The Squacco Heron (*Ardeola ralloides* Scopoli) is a small-sized member of the family Ardeidae, with males weighing 285 ± 37 g (mean ± s.d.) and females 290 ± 38 g (Cramp and Simmons 1977). It has relatively short and strong legs and bill, and its habit of keeping its neck retracted gives it a compact appearance. When not in flight, it displays a cryptic brownish-buff coloration, as its elongated mantle and scapular feathers drape over its white wings (Voisin 1991). The biology of this heron has rarely been studied and some aspects of its natural history are poorly known. The existing information has been summarized by Cramp and Simmons (1977) and Voisin (1991).

The pond herons of the genus *Ardeola* are Old World species, mostly found in tropical areas (Hancock 1999). *A. ralloides* is widespread in sub-Saharan Africa throughout the year, but also has migratory populations that breed in scattered colonies across southern and eastern Europe and western Asia, from Portugal to the Aral Sea (Cramp and Simmons 1977). These Palearctic populations suffered significantly in the late 19th and early 20th centuries from excessive hunting for feather collection, and again later in the last century due to habitat loss (Josefik 1969, 1970).

Squacco Herons arrive in Europe in the spring and start nesting in late April or early May (Cramp and Simmons 1977). They breed colonially, together with other tree-nesting herons, both in coastal and inland areas, but avoid sites that depend exclusively on saline feeding grounds (Voisin 1991). In Camargue, in southern France, they arrive later than other sympatric herons, when most nesting sites in central colony areas and at higher tree elevations are already occupied by heterospecifics. Thus they settle mostly in peripheral areas and at lower tree heights than other species (Hafner 1980). Egg-laying occurs in May and June, and hatching usually begins in early June; nestling care may last into August, but the dispersal of early breeders and their fledglings starts in July (Cramp and Simmons 1977). Fall migration lasts from mid-August into October, and though some birds stop in north Africa, most of the European population winters south of the Sahara (Voisin 1991).

The Squacco Heron's mating, nesting and parental behavior has been studied by Voisin (1980), who found that both sexes share incubation and nestling care. Incubation lasts 22-24 days (Sterbetz 1962). According to Cramp and Simmons (1977), incubation starts after clutch completion and hatching is synchronous, an unusual phenomenon in ardeids (Voisin 1991). My results indicate that, in the Axios river delta, incubation started early in the laying sequence, and hatching was partially asynchronous (see next chapter). Females lay 3-6 eggs in Camargue, where the average clutch size is 4.7 eggs (Hafner 1980). Egg dimensions have been measured (Cramp and Simmons 1977), but egg volume has never been estimated, and egg-size variability has never been examined before in this heron. In Camargue, compared with 3 sympatric ardeids, Squacco Herons suffer similar levels of egg loss, hatching failure and early chick mortality, but lower mortality of nestlings over the age of 15 days (Hafner 1978). Young Squaccos begin flying at about the age of 30 days and leave the colony site approximately 2 weeks later (Cramp and Simmons 1977). The growth and development of nestlings has never been studied in this heron in any detail, though there are some data from a few chicks raised in captivity in Madagascar (Werding 1970).

During the breeding season in Europe, Squacco Herons forage exclusively in freshwater habitats, both in natural marshes and ponds, and in man-made ricefields and vegetated ditches and canals (Sterbetz 1962; Voisin 1978). They are mostly solitary and crepuscular hunters. They often remain concealed in aquatic vegetation, waiting for prey to come within striking distance, or they wade slowly, often at the water's edge, which gives them the opportunity to pursue both aquatic and terrestrial prey (Voisin 1978). In a comparative study with the Little Egret (*Egretta garzetta*), Hafner et al.(1982) found that the Squacco Heron's less active and solitary foraging methods were better suited for the capture of larger, more elusive prey. Stomach analyses from Italy (Moltoni 1936) and Hungary (Sterbetz 1962), as well as nestling regurgitations from Yugoslavia (Szlivka 1986), indicate that this heron's prey consists mainly of small (< 10 cm) fish and amphibians, and of aquatic insects and their larvae, in varying proportions.

2. STUDY AREA AND POPULATION

The Axios river delta (40°30' N, 22°53' E) is part of a greater wetland complex, which extends over 70 km^2 along the west coast of Thermaikos Gulf in northern Greece. (Athanasiou 1990). This area is protected under the Ramsar Convention, having been recognized as internationally important for waterbirds, though large parts of the original wetland system have been reclaimed for agriculture over the last century (Athanasiou 1990). Remaining natural habitats include salt- and freshwater marshes, tidal mudflats, open sea, coastal lagoons and vegetated islets, riparian forest and tamarisk (*Tamarix* sp.) bushland (Kazantzidis and Goutner 1996). Man-made ricefields and irrigation canals also attract various foraging waterbirds, including herons (Kazantzidis and Goutner 1996).

Human activities in the area include, besides agriculture, cattle and sheep grazing, fishing and aquaculture along the coast, housing development, sewage and garbage dumping, and hunting in the winter. Many of these activities, and at various degrees, continue to encroach upon and degrade the area's wildlife habitats and diminish the waterbird populations that use them (Athanasiou 1990).

During the years of my study, a Squacco Heron population of 150-200 pairs nested in the Axios delta as part of a 1500-pair multispecific colony, which also included Little Egrets (*Egretta garzetta*), Black-crowned Night Herons (*Nycticorax nycticorax*), Spoonbills (*Platalea leucorodia*), and Great Cormorants (*Phalacrocorax carbo*). The colony site was located in riparian forest along the banks of the Axios river, dominated by 6-8 m high tamarisks, with occasional stands of alder (*Alnus glutinosa*) and willow trees (*Salix* sp.). A thick understory of 1-2 m high blackberry bushes (*Rubus* sp.) was often present within 20 m of the river banks and made parts of the colony site inaccessible.

Squacco Herons started nesting at Axios in early May, when many heterospecific pairs were already established at the site. They built their nests in tamarisks, at a height of 2-4 m, solitarily or in small clusters (personal observations). In 1993 they nested at lower elevations and in shorter tamarisks than Little Egrets and Night Herons, and they were concentrated in the thicker vegetation zone close to the river bank (Charalambides 1994). Egg-laying lasted from early May until early or mid-June (see next chapter), and hatching occurred throughout June and in early July. Young Squaccos were rarely seen around their nests after the age of 30 days, and they dispersed from the colony site along with their parents soon afterwards, so that few birds remained at it after the end of July.

Extensive ricefields in the vicinity of the colony attracted foraging adults soon after they were flooded in May, when mole crickets (*Gryllotalpa gryllotalpa*) became exposed by seeking refuge on levees, and again after they were colonized by amphibians and aquatic insects (personal observations). Adult and juvenile Squaccos also foraged in vegetated irrigation canals and freshwater marshes, but never in saline habitats. The relative frequencies of 295 prey items found in nestling regurgitations were 35% *Rana* frogs and tadpoles, 30% adult and larval aquatic insects, 20% mole crickets, 8.5% small freshwater fish, and 6.5% various or unidentified items (Goutner et al. 2001).

3. DATA COLLECTION

I conducted this study at the Axios river delta heronry during the breeding seasons of 1992, 1993 and 1994. I collected my data with permission from the Ministry of Agriculture (General Secretariat of Forests and Natural Resources) of the Hellenic Republic, and the MSU All-University Committee on Animal Use and Care.

Throughout my study, I visited the colony site in the morning (8:00-12:00) and in the late afternoon (16:00-20:00), in order to avoid exposure of eggs and nestlings to the intense sunlight of midday. Within the study site, I followed standard routs, so as to visit each group of nests at approximately the same time of the day every time. I checked nests in the egg-laying stage every 4 days, except for the early part of the 1992 season, when I checked them every 3 days. I did so in order to minimize disturbance during this potentially sensitive stage of the nesting cycle (Tremblay and Ellison 1979), but at the same time to be able to estimate egg-laying order by differences in eggshell color (see below). I marked nests with small (10×10 cm), numbered wooden tags, which I attached beneath them. I marked and measured the eggs I found in nests that I could access with a 2-m long ladder. I checked the contents of inaccessible nests with a mirror attached to a pole (I use only clutch-size and laying-date data from such nests).

I marked eggs according to their laying order at both ends with small letters made with a soft lead pencil. I did not mark the middle of eggs to avoid interference with the normal egg-turning patterns of incubating parents (Frederick and Collopy 1989), while I marked both ends to avoid possible obliteration of single marks by parental droppings.

Whenever I found ≥2 new eggs, I estimated their laying order from differences in eggshell color: fresh eggs are darker and more greenish, whereas older ones are paler and more bluish (Custer 1991). The validity of these estimates is also supported by hatching-order information. Reversals of egg-laying and hatching order are extremely rare in ardeids (Dickerman and Gavino 1969; Fujioka 1984; Inoue 1985; Custer and Frederick 1990). I observed no reversals in 107 cases where both laying and hatching order were certain (both the eggs and the hatchlings involved were first seen on different days). Therefore, I consider hatching order a reliable index of egg-laying order. In most cases where hatching information was available, it confirmed my laying-order estimates based on eggshell color: 60 out of 62 cases in 1992 (97%); 50/54 cases in 1993 (93%); 64/66 cases in 1994 (97%). Because of this, I considered my laying-order estimates reliable enough to be used even in the absence of hatch-order information (but I corrected the few estimates that were proven wrong by hatching data). However, there are some clutches with uncertain laying order, because I could not estimate it at the time of egg discovery and I also lack hatch-order information from those nests. I excluded such cases from clutch profile analyses, but I used their average egg volume data.

I measured egg length and width with vernier calipers and 0.05-mm accuracy. From each egg I took duplicate measurements at right angles, which I averaged, unless they differed by > 0.2 mm, in which case I took a 3rd measurement and I averaged the 2 closer ones (Custer and Frederick 1990). From these measurements, I calculated egg volume using the formula

$$\text{Volume} = 0.509 \times \text{Length} \times (\text{Width})^2,$$

which I estimated from a sample of 40 eggs where I also directly measured egg volume (details in the next chapter).

After ascertaining clutch completion (the same eggs present on 2 consecutive visits), I only checked nest contents with the mirror every 4 or 8 days (depending on workload). I restarted monitoring nests closely 3 weeks after the laying of their 1st eggs, when hatching was imminent. In 1992 I checked nests in the hatching stage every day, and I considered dry and wet newfound hatchlings as 0.5 and 0.0 d old, respectively (details in the next chapter). In the other 2 years, I made hatch checks every other day, in order to minimize potential disturbance to the colony. In all years, I took certain body measurements from hatchlings (of ages of 0.0 to 1.5 d), in order to estimate sibling ages and hatching intervals with greater accuracy in 1993 and 1994 (see section 6 in the next chapter). I weighed hatchlings with a spring scale to the nearest 0.05 g, and I measured twice, with vernier calipers and 0.05-mm accuracy, culmen and tarsometatarsal lengths. In 1993 and 1994, I also measured the bill+head length (from tip of culmen to occipital condyle) of wet hatchlings, which I correlated with egg volume, but could not use for age estimation. Again, I averaged duplicate measurements, unless they differed by >0.2 mm, in which case I took a 3rd measurement and I averaged the 2 closer ones.

4. STATISTICAL ANALYSES

In data analyses I generally followed the guidelines of Zar (1996) and those of the SYSTAT 6.0 statistical manual (SYSTAT 1996). For some procedures I also consulted additional sources, which I will cite in later sections where I present the relevant methods.

I analyzed categorical data, like clutch size frequencies, with X^2 or Fisher exact tests, depending on the number of groups compared. In continuous quantitative variables, I tested the normality of sample distributions with Lilliefors tests, and the homogeneity of variances among samples with Bartlett's X^2 tests. When these conditions were met, I used parametric procedures (e.g. ANOVA), and when not, their non-parametric alternatives (e.g. Kruskal-Wallis test). In the following chapters, I will not present in detail the testing of parametric assumptions, unless they were violated.

I analyzed clutch profile, and the effects of various factors on it, with Repeated Measures ANOVA (von Ende 1993), a procedure that requires the fulfillment of certain additional conditions, and which I will discuss in detail in Chapter IV.

In the presentation of results, I will abbreviate the names of certain variables. These can be found in the *List of Terms and Abbreviations* (p. xii).

III . PRELIMINARY ANALYSES

In this chapter I present and discuss results that provide necessary background information and pave the way for the clutch profile analyses, which are the focus of my dissertation. The text is divided into sections according to the data analyzed. The first 4 sections concern basic aspects of breeding biology, including egg-laying intervals, clutch laying dates, and clutch size, as well as the estimation of egg volume from linear egg dimensions.

In sections 5 and 6, I will present the analyses of clutches' average egg volume (AvVol) and hatching intervals, with emphasis on the total hatching span (H-Span). The hypotheses I examine with regard to the proximate causation of clutch profile postulate that the latter is affected by resource limitations or hormonal constraints associated with the onset of incubation. I have been unable to evaluate these 2 factors directly, and I infer their levels, respectively, from measurements of AvVol and H-Span. (I will discuss the suitability of these 2 variables as indices of the above factors later in this chapter.)

I can directly derive from the nutritional and hormonal hypotheses the predictions that last eggs will be relatively smaller in clutches with lower AvVol and longer H-Span, respectively (see *Introduction*). Furthermore, if AvVol or H-Span vary significantly among the levels of other factors (year, season, clutch size), the respective hypotheses would predict a corresponding difference in clutch profile among the levels of the same factors. For example, I will show that clutches of 4 and 5 eggs have a similar AvVol, while H-Span is significantly longer in the larger clutches. Therefore, the nutritional hypothesis will predict no variability in clutch profile between clutch sizes, whereas the hormonal hypothesis will predict relatively smaller final eggs in the larger clutches. The derivation of such secondary predictions about the variation in clutch profile among the levels of factors other than AvVol and H-Span is the reason for the detailed analyses of these 2 variables in this chapter.

In the last (7th) section, I will examine a) the association between AvVol and H-Span, and b) the hypothesis that last-egg size is constrained by resource limitations imposed on laying females by the restriction of their feeding time due to the onset of incubation (Houston et al.1983). In each of the following sections, I will begin with some introductory comments (including data characteristics and statistical analyses employed), and then I will proceed with the presentation and discussion of results.

1. EGG-LAYING INTERVALS

Studies of such disparate avian species as the Adelie Penguin *Pygoscelis adeliae* (Astheimer and Grau 1985) and the Eurasian Kestrel *Falco tinnunculus* (Meijer et al. 1989), indicate that egg-laying intervals correspond to the intervals between the onset of rapid yolk deposition in successive ovarian follicles. As the laying of an egg precedes the ovulation of the next follicle usually by <1 h (Johnson 1986), the laying interval also represents the period when the latter egg passes through the oviduct, where albumen, the egg and shell membranes, and the eggshell are deposited (Burley and Vadehra 1989).

Egg-laying intervals may vary among and within clutches in various bird groups, including anatids (Schubert and Cooke 1993), ptarmigans (Wiebe and Martin 1995), kestrels (Aparicio 1994a), and parrots (Beissinger and Waltman 1991). Longer laying intervals in Eurasian Kestrels (Aparicio 1994a) and gaps in the normal laying sequence of Blue Tits (Nilsson and Svensson 1993 b) were associated with food shortages. In the Common Eider (*Somateria mollissima*), the average egg-laying interval decreases with increasing clutch size, and the interval between the last 2 eggs is shorter than all previous ones (Watson et al.1993).

Subsequent analyses require some information about the time interval between the laying of successive eggs. First, an estimate of the modal laying interval is needed for the calculation of clutch initiation dates (see next section). Second, consistent patterns of heterogeneity in this variable could confound my hatching interval analyses (see section 6 in this chapter). Hatching intervals depend on laying intervals and on incubation patterns (timing of onset and intensity) during egg-laying (Wiebe et al.1998). As I mentioned earlier, I intend to use the total hatch span (H-Span) of clutches as an index of female incubation behavior. I can do this safely only if I ascertain that laying patterns remain on the average constant among groups of clutches that may vary in H-Span.

I was unable to measure egg-laying intervals with accuracy, because they were shorter than the intervals between nest visits (3 d in the early 1992 season and 4 d in all other periods). The data I use are frequencies of cases where different numbers of eggs were laid during 3-d and 4-d nest-check intervals. Overall results, excluding cases where only the first or last egg was laid between nest visits, are shown in Table 1.

Results from the larger sample of 4-d visits indicate that eggs must usually be laid every other day (in 85% of the cases, 2 eggs were laid in 4 d). Even the laying of 3 eggs in 4 d does not necessarily indicate shorter laying intervals: the 1st of these eggs could sometimes have been laid soon after the 1st visit, the last egg just before the 2nd visit, and the middle egg in the middle of the 4-d interval.

Table 1. Frequencies of cases where different numbers of eggs were laid during 3-d and 4-d nest-check intervals.

3-d nest-check intervals			**4-d nest-check intervals**		
# eggs laid	# cases	% cases	# eggs laid	# cases	% cases
1	32	27	2	231	85
2	88	73	3	40	15
Total	120	100	Total	271	100

When I visited nests every 3 d, I never found single new eggs after 2 successive intervals. In 80% of 63 cases where I have 2 observations from each nest, a total of 3 eggs (1,2 or 2,1) were laid during 6 d (2 successive 3-d intervals). So, again, in the majority of cases, eggs seem to be laid every other day. In the remaining 20% of those 63 cases, a total of 4 eggs were laid in 6 d (2 eggs in each of 2 successive 3-d intervals). Again, there may have been cases where the 1st of those 4 eggs was laid right after the 1st visit, the 4th egg just before the last visit, and all egg-laying intervals were about 2 d long.

I calculated a rough average for egg-laying intervals for both observation regimes by dividing the time interval between observations by the number of eggs laid in it, and then averaging values over all cases. The resulting averages were 1.90 d for 3-d nest-check intervals, and 1.83 d for 4-d intervals.

In conclusion, my observations indicate that eggs must usually be laid every 2 d, and occasionally at shorter intervals, and that results are independent of the time interval between nest visits. These results agree with data from a variety of middle-sized ardeids studied in temperate areas (Jenni 1969; Maxwell and Kale 1977; Tremblay and Ellison 1980; Fujioka 1984). Grey Herons *Ardea cinerea* (Milstein et al.1970) and Cattle Egrets

Bubulcus ibis (Fujioka 1984) lay their eggs mostly in the early morning, so their egg-laying intervals often take values of integer days. Little Egrets in Japan, however, may lay eggs at various times of the day, at an average interval of 37 h (Inoue 1985).

In calculating clutch initiation dates, I will assume a constant 2-d egg-laying interval. As long as laying intervals do not consistently vary among groups of nests, this practice will be safe, and my hatching interval results will also be free of bias. My data indeed suggest that laying intervals do not vary significantly among the levels of any factor also examined for its effect on hatching intervals.

Since I visited nests every 3 d only in the early 1992 season, data from this period cannot be used for comparisons among years or between early and late clutches (initiated before/after annual median laying dates). Moreover, since I lack 4-d visit data from the early part of the 1992 season, I examined the seasonal variability in egg-laying intervals using data from 1993 and 1994 only. This variability was low and non-significant (Table 2). Therefore, the use of only late-nest data from 1992 should not bias the results of the comparison among years. Again, the relative frequencies of cases where 2 or 3 eggs were laid in 4 d did not vary significantly among samples (Table 3).

Table 2. Frequencies of cases where 2 or 3 eggs were laid in 4 d for early and late clutches, and result of statistical comparison.

	Early clutches		**Late clutches**	
	#	**%**	**#**	**%**
2 eggs in 4 d	87	86	86	81
3 eggs in 4 d	14	14	20	19
Total	101	100	106	100
2-tailed Fisher exact $P = 0.354$ n.s.				

Table 3. Frequencies of cases where 2 or 3 eggs were laid in 4 d by year, and result of statistical comparison.

	1992 #	1992 %	1993 #	1993 %	1994 #	1994 %
2 eggs / 4 d	51	88	74	80	106	88
3 eggs / 4 d	7	12	19	20	14	12
Total	58	100	93	100	120	100
Pearson $X^2 = 3.623$ d.f.= 2 $P = 0.163$ n.s.						

In the comparison of laying intervals between clutches of 4 and 5 eggs, I used data from both 3-d and 4-d observations, but I analyzed them separately. Both data sets failed to show a significant difference in laying intervals between clutch sizes (Table 4). In a later section, I will show that hatching intervals between successive eggs increase with laying order. Here I examine whether a similar increase in the length of laying intervals contributes to this phenomenon. I compared laying intervals between early (1st-3rd) and late (3rd-5th) eggs, again analyzing data from 3-d and 4-d nest visits separately. In both data sets, the effect of egg-laying order was weak and non-significant (Table 5).

Table 4. Comparison between clutch sizes of frequencies of cases where different numbers of eggs were laid during 3-d or 4-d intervals.

3-d nest-check intervals					**4-d nest-check intervals**				
	4-egg nests		**5-egg nests**			**4-egg nests**		**5-egg nests**	
	#	**%**	**#**	**%**		**#**	**%**	**#**	**%**
1 egg / 3 d	9	35	20	31	2 eggs / 4 d	75	82	156	84
2 eggs / 3 d	17	65	45	69	3 eggs / 4 d	11	18	29	16
Total	26	100	65	100	Total	86	100	185	100
2-tailed Fisher exact $P = 0.805$ n.s.					2-tailed Fisher exact $P = 0.586$ n.s.				

Table 5. Comparison between early and late eggs of frequencies of cases where different numbers of eggs were laid during 3-d or 4-d intervals.

3-d nest-check intervals					4-d nest-check intervals				
	Early eggs		Late eggs			Early eggs		Late eggs	
	#	%	#	%		#	%	#	%
1 egg / 3 d	16	27	15	25	2 eggs / 4 d	93	82	137	87
2 eggs / 3 d	43	73	46	75	3 eggs / 4 d	20	18	21	13
Total	59	100	61	100	Total	113	100	158	100
2-tailed Fisher exact $P = 0.836$ n.s.					2-tailed Fisher exact $P = 0.390$ n.s.				

In summary, none of the factors I examined (year, season, clutch size, and laying order) seems to affect egg-laying intervals. Therefore, any variability in hatching intervals among the levels of these factors can be attributed to differences in incubation patterns.

2. LAYING DATE

The timing of avian egg-laying is generally considered to have evolved so as to maximize breeding success by synchronizing the energetically demanding nestling growth stage with the period of highest food availability in the environment (Lack 1954, 1968; van Noordwijk et al.1995). A seasonal decline in reproductive success may result merely from the fact that parents of higher intrinsic or territory quality breed both earlier and more successfully (Newton and Marquiss 1984; De Forest and Gaston 1996). Nevertheless, experimental studies have demonstrated seasonal trends in reproductive output that track environmental variability and are independent of parental attributes (Verhulst and Tinbergen 1991; Brinkhof et al.1993; Barba et al.1995).

Population variability around a common optimal laying date may result from nutritional constraints on egg formation (Perrins 1970). Alternatively, individual optima may vary according to parental or territory quality and the prevailing seasonal trends in food availability and offspring value (Daan et al.1989; Aparicio 1998). In colonially-nesting birds, like ardeids (Krebs 1978), this variation is often reduced, as nest synchronization may decrease predation (Parsons 1975b; Forbes 1989; Hatchwell 1991), facilitate mate attraction (Draulans 1988), or enhance feeding success (Krebs 1974) through the exchange of information about profitable foraging sites (Ward and Zahavi 1973).

Proximately, the hormonally controlled egg production is triggered by changes in photoperiod, which sets a reproductive window (Follet and Robinson 1980; Meijer 1989). Within this window, in a variety of avian species, egg-laying may be hastened by high ambient temperatures (Slagsvold 1976; Perdeck and Cave 1989; Meijer et al.1999), increased food supply (Arcese and Smith 1988; Korpimaki 1989; Nol 1989; Soler and Soler 1996), and improved female nutritional condition (Jones and Ward 1976; Dijkstra et al.1988; MacCluskie and Sedinger 2000).

My interest in the laying dates of Squacco Herons during my study is 2-fold. First, annual variation at the colony level may reflect differences in environmental conditions among years, which could also affect other reproductive variables. Second, I need clutch laying dates and annual population median values in order to examine seasonal trends in other clutch characteristics (egg size, hatching intervals, etc.).

I consider as the laying date of a clutch the date on which its first egg was laid, and I express it as number of days after April 30 (thus May 1 and June 1, for example, take the values of 1 and 32, respectively). In all years, I discovered most nests during the laying stage. To estimate the laying date of such clutches, I assumed that the last of the eggs I found on my first visit to a nest was laid on the previous day (half of the 2-d egg-laying interval), and I counted back 2 d for every other egg present. However, in each year, I discovered 10-20% of observed nests after clutch completion. In these cases I used information about incubation intervals to estimate laying date.

I considered as an egg's incubation interval the period between its laying and hatching dates, even though each egg may not have been effectively incubated throughout this period. Actually, within clutches, the median incubation interval decreases with egg-laying order (Table 6). This is because Squacco Herons do not begin full incubation with the first egg of their clutch, but at some later point in the laying sequence (see discussion of hatching intervals in section 6 of this chapter). In nests under observation during both the laying and hatching stages, I calculated incubation intervals by subtracting eggs' laying dates from their hatching dates. Statistics on this variable, which is not normally distributed, are shown in Table 6. In all samples, the median and mode were equal. I used the percentage of values equal to a sample's median (%M in Table 6) as an inverse index of sample variance. In both clutches of 4 and 5 eggs, the incubation intervals of the last 2 eggs were less variable than those of earlier ones (they have higher %M values). For consistency, in the estimation of laying date, I used the median incubation interval of the 4th egg (20 d) in both clutch sizes, unless that egg failed to hatch. Then I used the median interval of the 3rd egg in clutches of 4, and that of the 5th egg in clutches of 5 (both of which are 20 d). I estimated the focal egg's laying date by counting back 20 d from its hatching date, and then I counted back another 2 d for every earlier egg in order to estimate the clutch's laying date.

Table 6. Statistics on incubation intervals by clutch size and egg-laying order.

A. CLSZ = 4	**EGG LAYING ORDER**			
Statistic	**A**	**B**	**C**	**D**
n	39	43	41	40
Median	23	21	20	20
% M [a]	38.5	34.9	61.0	60.0

B. CLSZ = 5	**EGG LAYING ORDER**				
Statistic	**A**	**B**	**C**	**D**	**E**
n	86	87	84	87	73
Median	23	22	21	20	20
% M [a]	31.4	35.6	41.7	70.1	61.6

[a] Percentage of cases with a value equal to the sample median

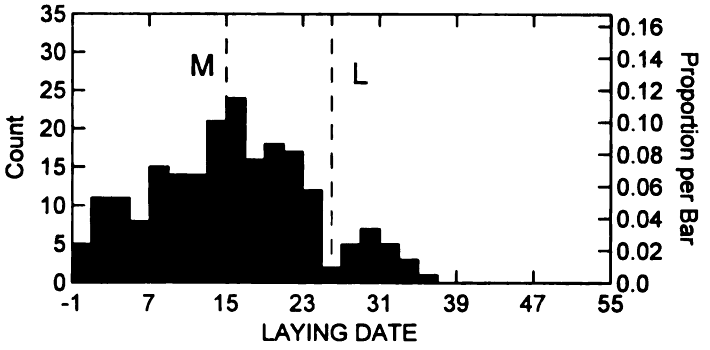
Figure 1 shows histograms of laying date for the 3 years of my study. In 1992 and in 1993, I kept searching for new nests until no more were constructed. In both of these years, there is a small group of delayed clutches, separated from those initiated during the main laying season by a number of days (1 in 1992 and 5 in 1993) when no new clutches were started (in histograms A and B of Figure 1, line L separates main from delayed nests). Such observations are common in ardeids, where late clutches represent either second attempts after the loss of the initial clutch, or first efforts by late-arriving pairs (Rodgers 1980a; Pratt and Winkler 1985; Campos and Fernandez-Cruz 1991).

In 1994, the workload associated with other aspects of my study prevented me from collecting data from clutches initiated after June 11 (indicated by line E in Figure 1C). Consequently, that year's sample does not include delayed nests, and may also lack some clutches initiated at the end of the main laying period. The latter are unlikely to be numerous, because I observed no more than 20 new nests after June 11, most of which were constructed after June 15, in a distinct cluster at the edge of the colony site, which was typical of delayed nests in the previous 2 years. Therefore, the median laying date of main clutches in 1994 is not likely to be underestimated by more than 1 day at the most. Because of these characteristics of the 1994 sample, I compared among years the median laying date of main clutches only (indicated by line M in all histograms of Figure 1). I made comparisons with non-parametric tests, because the distribution of this variable is not normal.

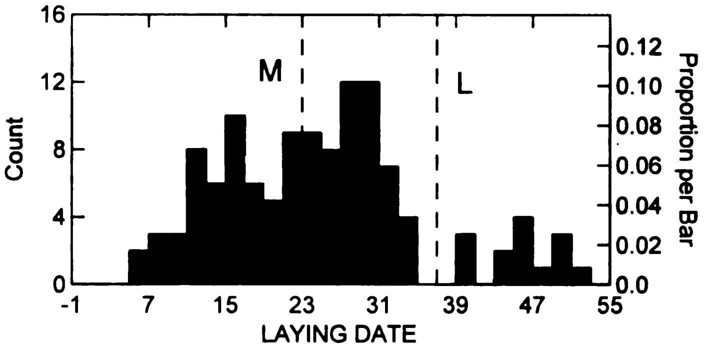
The Squacco Heron's main laying season at the Axios delta was not very long. It extended over 4-5 weeks in the 3 years, with 60-70% of all nests being initiated within 2 weeks only. In southern France, the species' main laying season seems to be 1-2 weeks longer, and delayed clutches may be laid throughout July (Hafner 1980).

The median laying date occurred progressively later over the 3 years of my study (Figure 1). In 1992, it was 8 d earlier than in 1993, and 13 d earlier than in 1994. These differences are not great in absolute terms, but they represent substantial parts of the full range of the main laying period (26-36 d). The variability in median laying date among all years, as well as all pairwise differences, are statistically significant (Table 7), the latter even with the application of the Bonferroni correction for multiple comparisons (critical $P = 0.017$). Moreover, since the 1994 median laying date may be slightly underestimated, the comparisons between 1994 and the other 2 years may be conservative.

A. 1992 (N=209)



B. 1993 (N=118)



C. 1994 (N=142)

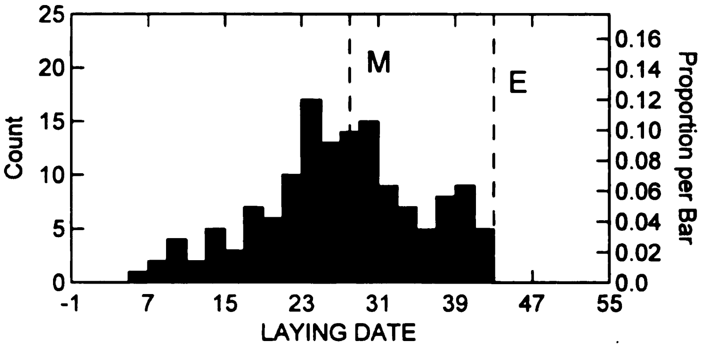


Figure 1. Laying curves by year. Line M: median laying date of main clutches. Line L (1992 and 1993): limit between main and delayed clutches. Line E (1994): end of observations. (See text for explanations.)

Table 7. Overall and pairwise statistical comparisons of laying date among years.

Years compared	Statistic	*P*-value
All 3	$H^{a} = 168.9$	< 0.001
1992 and 1993	$U^{b} = 4256$	< 0.001
1993 and 1994	$U = 4966$	< 0.001
1992 and 1994	$U = 2785$	< 0.001

[a] Kruskal-Wallis test statistic. [b] Mann-Whitney test statistic.

Arrival at the breeding grounds and the onset of laying seems to be associated with mean air temperature in various ardeids (Owen 1960; Pratt 1972a, 1972b; Campos and Fernandez-Cruz 1991), including the Squacco Heron (Sterbetz 1962). Data collected during my years of study at a nearby weather station are consistent with this, as daily mean air T° in the first 2 weeks of May was highest in 1992 and lowest in 1994 (Table 8). Moreover, the differences in the 2-week average T° between successive years are 1.56 °C for 1992-1993 and 0.94 °C for 1993-1994, and their ratio (1.56 / 0.94 = 1.67) is similar to that of the respective differences in median laying date (8d / 5d = 1.60).

Table 8. Daily mean air temperature (°C) during the first 2 weeks of May by year.

	PERIOD			
Statistic	**May 1-7**	**May 8-14**	**May 1-14**	**YEAR**
n	7	7	14	
Mean	16.37	18.93	17.65	1992
n	7	7	14	1993
Mean	15.89	16.29	16.09	
n	7	7	14	1994
Mean	14.89	15.41	15.15	

The timing of heron egg-laying can also be influenced by food availability (Butler 1993). Great Blue Herons (*Ardea herodias*) in areas where feeding grounds were covered by the 1993 Mississippi River flood laid eggs significantly later than in unaffected areas in the same year or the same areas in a year with normal river levels (Custer et al.1996). Food supplements have also hastened egg-laying in a variety of birds, including kestrels (Meijer et al.1988), owls (Korpimaki 1989), corvids (Dhinsa and Boag 1990; Soler and Soler 1996), and other passerines (Davies and Lundberg 1985; Arcese and Smith 1988; Clamens and Isenman 1989).

I was unable to estimate Squacco Heron food availability during egg-laying, but it is possible that it may have varied among the years of my study and contributed to the annual differences in median laying date. If the latter is true, mean egg size and clutch profile, as well as other aspects of breeding biology, may also differ among years. Therefore, in the following sections I will examine the annual variability of the data under analysis, albeit without *a priori* expectations.

In later sections, I will also examine the seasonal trends of certain variables. In order to do so, I grouped clutches initiated during the main laying period into *early* and *late* ones, using each year's median laying date as the dividing point. In each year, I put clutches started on the median date in the group with the fewer cases, in order to keep sample sizes as similar as possible. I kept the few delayed nests from 1992 and 1993 in a separate sample, in order to make comparisons between those and the main clutches of the same years. In some cases, I will compare laying dates among the levels of other factors, pooling data from all years. For this, I will use values that I standardized across years by subtracting from them their year's median.

3. CLUTCH SIZE

David Lack (1947) proposed that, in altricial birds, clutch size is ultimately determined by the number of nestlings parents can successfully raise under prevailing environmental conditions. This hypothesis predicts that a population's average clutch size should be the most productive one, taking into account the common trade-off between offspring number and quality (Smith and Fretwell 1974; Smith et al.1989). However, larger-than-average clutches can be the most productive (e.g. Perrins and Moss 1975), and a population's average clutch size may be evolutionarily constrained by the cost of reproduction, that is the parental trade-off between current breeding effort and future reproductive prospects (Williams 1966; Nur 1988). Parents are thus expected to maximize lifetime fitness by restraining their current effort in order to increase their future survival and fecundity (Charnov and Krebs 1974; Dijkstra et al.1990). Positive correlations between breeding success and parental survival (e.g. Coulson and Porter 1985), have led to the development of the individual optimization hypothesis of avian clutch size (Perrins and Moss 1975). According to this, females lay the clutches that best suit their individual circumstances, so that both experimental enlargements and reductions of their broods lower their fitness (Pettifor 1993; Pettifor et al.1988, 2001).

At the proximate level, the end of ovulation and clutch completion seem to be related to the abrupt onset of female incubation (Mead and Morton 1985), or the gradual development of that behavior beyond a threshold level (Haftorn 1981; Beukeboom et al. 1988; Meijer 1990). This probably results from a) the association between incubation and high plasma prolactin levels (Goldsmith 1990; Buntin 1996; Vleck 2002), and b) this hormone's inhibition of gonadotropin secretion (Lea et al.1981; Ramsey et al.1985), which c) terminates follicular growth, ovulation, and egg-laying (Opel and Proudman 1980; Youngren et al.1991). A seasonal increase in female prolactin levels, which may intensify the development of incubation of late nesters (Meijer et al.1990), could be responsible for the seasonal decline in clutch size seen in many single-brooded species (Klomp 1970), without any direct effect of female nutrition (Meijer et al.1988, 1990). Female nutrition and body reserves, however, directly influence clutch size in many anatids (Ankney and Afton 1988; Ankney and Alisauskas 1991; Erikstad et al.1993). Moreover, food supplements can increase clutch size beyond the levels expected by the hastening of laying in some raptors (Newton and Marquis 1981; Aparicio 1994b), gulls (Bolton et al.1992), crows (Soler and Soler 1996), and other passerines (Nilsson 1991).

The examination of clutch-size variability in my study population provides some necessary background information for my thesis. It also helped me identify certain groups of clutches which may have been laid under resource-limited conditions, and whose egg size may also have been nutritionally constrained (see later).

Historically, clutch size has been treated mostly as a continuous, normally-distributed variable: authors have been reporting sample means and variances, and subjecting data to parametric analyses (Lederle 1995). Avian clutch size, however, takes only a limited number of discrete values, so, strictly speaking, such a treatment of data is inappropriate. Below I will report average values of clutch size to illustrate differences among samples, but I will make statistical comparisons with contingency tables.

In order to examine the annual and seasonal variation in clutch size, I used data from nests initiated during the main laying period in each year. That is, I excluded from samples delayed clutches, and also nests where the first 1 or 2 eggs were lost before the onset of incubation (I will examine the clutch size of these 2 samples at the end of this section). Table 9 shows the frequencies of different clutch sizes during the main laying season in each year of my study.

Table 9. Clutch size (Clsz) frequencies during the main laying season by year.

	1992		**1993**		**1994**		**All Years**	
Clsz	**#**	**%**	**#**	**%**	**#**	**%**	**#**	**%**
3	8	4.6	1	1.0	4	3.1	13	3.3
4	56	32.4	27	27.3	50	39.4	133	33.3
5	96	55.5	68	68.7	72	56.7	236	59.1
6	13	7.5	3	3.0	1	0.8	17	4.3
All	**173**	100.0	**99**	100.0	**127**	100.0	**399**	100.0

The observed range of clutch size was 3-6 eggs (Table 9), but only clutches of 4 and 5 eggs were common (respectively, about 33 and 60% of all cases). Clutches of 3 and 6 eggs made up about 12% of observations in 1992, and 4% in the other 2 years. The overall average clutch size was 4.65 eggs (*n* = 399), which is very similar to the species' mean clutch size of 4.7 eggs in the Camargue region of southern France (Hafner 1980).

Median clutch size was 5 eggs in all years. The 3 successive year averages varied little, and were 4.66, 4.74 and 4.55 eggs, respectively (sample sizes in Table 9). I tested the significance of the annual variability in clutch size by comparing the numbers of small ($\leq$4 eggs) and large ($\geq$5 eggs) clutches with Pearson's X^2 test. I followed this grouping scheme, because, outside the common clutches of 4 and 5 eggs, observed frequencies were usually less than 5. The test's results approached, but did not achieve, statistical significance ($X^2 = 4.88$, df = 2, $P = 0.087$).

Weak fluctuations in clutch size have been observed in ardeids both over a few years (Rodgers 1980a; Moser 1986) and over much longer periods (Pratt and Winkler 1985; Campos and Fraile 1990). Significant variation among 16 years in Little Egret clutch size in Camargue seems to follow trends in food availability (Bennetts et al. 2000). Food supplements also increased Great White Heron (*Ardea herodias occidentalis*) clutch size, which seems to be currently constrained in Florida by habitat deterioration (Powell and Powell 1986). The latter 2 studies, however, did not examine the possible covariation of laying date with clutch size. Great Blue Heron clutches were smaller in areas adversely affected by the 1993 Mississippi River flood, but they were also laid later than in control areas (Custer et al.1996), so the effect of food availability may have been only indirect. Whether female nutrition can directly affect the size of heron clutches or not, the annual variation in clutch size that I observed is too low to imply any strong differences in Squacco Heron food availability among years, or to merit any special consideration in subsequent analyses of other clutch characteristics.

Table 10 shows the percentage of small and large clutches, together with sample size, mean and median clutch size, in the early and late parts of each year's *main* laying season. In all years there was a small seasonal decline in the percentage of large clutches and in average clutch size. I tested the significance of this trend with 2×2 contingency tables (again comparing numbers of small and large clutches). Two-tailed Fisher exact probabilities were > 0.05 in all successive years (0.349, 0.660 and 0.593, respectively), and in the pooled sample (0.253). Data from 1992 and 1993 indicate a much greater difference between *main* and *delayed* clutches (Table 11). The latter group had a lower percentage of large clutches, as well as a smaller mean and median number of eggs.

The difference in the relative frequencies of small and large clutches between the 2 samples is statistically significant (2-tailed Fisher exact $P = 0.004$).

Table 10. Percentage of small and large clutches, sample size, and mean and median clutch size in the early and late parts of each year's main laying season.

Clutch	1992		1993		1994		All Years	
Size	Early	Late	Early	Late	Early	Late	Early	Late
≤ 4	33.4	40.5	26.0	30.6	40.0	45.0	33.7	39.5
≥ 5	66.6	59.5	74.0	69.4	60.0	56.0	66.3	60.5
n	84	89	50	49	65	62	199	200
Mean	4.75	4.58	4.80	4.67	4.58	4.52	4.71	4.58
Mdn	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0

Table 11. Frequencies of small and large clutches, and mean and median clutch size of main and delayed nests from 1992 and 1993.

Clutch Size	Main Laying Period #	%	Delayed Clutches #	%
≤ 4	92	33.8	20	60.6
≥ 5	180	66.2	13	39.4
All	272	100	33	100
Mean	4.7		4.2	
Median	5.0		4.0	

The lack of a seasonal decline in Squacco Heron clutch size during the main egg-laying season may be partly due to this period's short span. Similar patterns of clutch-size variability (near constancy during most of the laying period and a drop towards its end) have also been observed in *A.ralloides* in southern France (Hafner 1980), and in other ardeids, mostly in cases where the laying curve shows a long right tail or a distinct group of delayed nesters (Jenni 1969; Custer et al.1983; Pratt and Winkler 1985).

Small delayed clutches may be laid by young females, who both tend to nest later, as in the Cattle Egret (McKilligan 1985), and lay fewer eggs than older ones, as in the Grey Heron (Fernandez-Cruz and Campos 1993). This combination of traits is common in many avian groups, including anatids (Afton 1984; Forslund and Larsson 1992), gulls (Sydeman et al.1991), owls (Korpimaki 1988), bee-eaters (Lessells and Krebs 1989), and passerines (Perrins and McCleery 1985; Jarvinen 1991; Desrochers and Magrath 1993). Delayed nests may also represent second attempts after the loss of the initial clutch (Wolford and Boag 1971; Pratt and Winkler 1985). Repeat clutches tend to be smaller than first attempts in night herons (Wolford and Boag 1971), as well as in oystercatchers (Nol et al.1984) and grouse (Erikstad et al.1985). In section 5, I will compare the average egg size of delayed and main-period clutches, with the expectation that it will be lower in the former group, due to increased nutritional constraints on the laying females.

In 4 nests in the early 1992 season, and in 2 in 1994, the first egg was lost before any others were laid. In half of these nests, a total of 5 eggs were laid and 4 remained after the initial loss. In the other half, 6 eggs were laid in total and 5 remained. In another 2 early 1992 nests, the first 2 eggs were lost sequentially, while they were the only ones present in the nest. In both of these nests the total and final clutch sizes were 7 and 5 eggs, respectively. These observations are summarized and compared with results from regular clutches from 1992 and 1994 in Table 12. No statistical tests can be run, because of the small number of nests where initial eggs were lost. However, comparisons of clutch size percentages, means and medians, all indicate that the *total* size of clutches with initial egg losses is greater than that of regular clutches, whereas their *final* size is very similar to that of regular clutches. Therefore, it seems likely that females were able to lay extra eggs in order to replace those lost early.

There is some evidence that other ardeids can also replace lost eggs (Jenni 1969; Maxwell and Kale 1977; McAloney 1973). Indeterminate egg-laying has been confirmed by egg-removal experiments in a wide array of avian taxa (reviews in Kennedy 1991, and Haywood 1993a), including raptors (Porter 1975; Beukeboom et al.1988), coots (Arnold 1992a), gulls (Weidmann 1956; Parsons 1976), and passerines (Meijer 1993; Haywood 1993 b,c). The above studies, as well as others (Kennedy 1991), have shown that the experimental manipulations must start from the beginning of egg-laying, so as to delay the onset of incubation and the associated increase in prolactin, which is involved in the termination of laying (see earlier in this section).

The laying of 1 or 2 extra eggs in the clutches where initial eggs were lost, must have increased the nutritional and/or energetic demands on the respective females, and may have thus constrained egg size in these clutches. Therefore, besides the delayed nests discussed earlier, this is another group whose average egg volume I will compare with that of regular clutches, expecting it to be lower (see section 5 in this chapter).

Table 12.- Frequencies, mean and median of total and final clutch size (Clsz) in cases where initial eggs were lost, and in regular clutches from 1992 and 1994.

Clutch Size	Clutches with early losses Total Clsz #	%	Final Clsz #	%	Regular Clutches #	%
≤ 4	0	0.0	3	37.5	118	39.3
≥ 5	8	100	5	62.5	182	60.7
All	8	100	8	100	300	100
Mean	5.88		4.63		4.61	
Median	6.0		5.0		5.0	

4. ESTIMATION OF EGG VOLUME

As I mentioned in the *General Methods*, I measured egg length and width with vernier calipers and 0.05-mm accuracy. From these measurements I calculated egg volume in cm^3 (ml) using a formula that I estimated from a sample of 40 eggs whose volume I also measured directly. To do the latter, I used the method of Evans (1969) and Hoyt (1979), which is based on the weight and volume of the water displaced by the immersion of eggs. I weighed each egg suspended in the air and in distilled water with a spring scale to the nearest 0.05 g. The difference equals the weight of the displaced volume of water, and the latter is the same as the immersed egg's volume. Within the range of ambient temperatures during data collection (15-20 °C), the specific gravity of water deviates from 1.0 g/ml by fractions far below my measurements' level of accuracy (Evans 1969). Therefore, the above difference between weight measurements in g equals egg volume in ml.

Figure 2 illustrates the relationship between egg volume and the product of the multiplication of egg length by the square of egg width. The linear regression of egg volume on this product ($n = 40$, $P < 0.001$, $R^2 = 0.958$) yielded the equation

$$\text{Volume} = 0.509 \times \text{Length} \times (\text{Width})^2 ,$$

which I used for the calculation of egg volume. The estimated slope is very close to the average (0.507) calculated for 26 avian species from various families by Hoyt (1979). The high R^2 value indicates that only about 4% of the variability in egg volume cannot be accounted for by this relationship. Nevertheless, since the values of egg volume I use are estimates, I examined the possible overlap of confidence intervals in clutches where differences in egg volume were less than 5% (see next chapter).

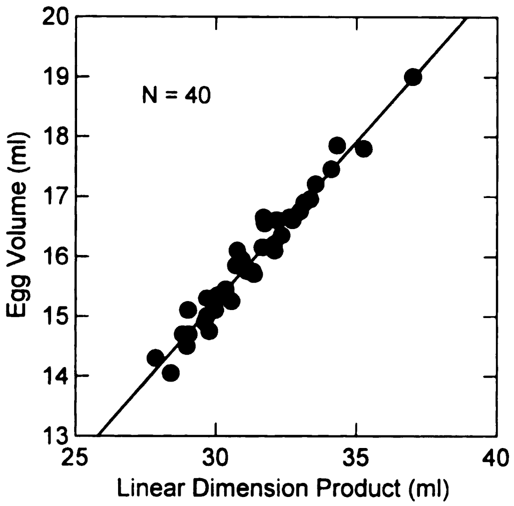


Figure 2. Regression of egg Volume on the product of egg Length × $(\text{Width})^2$.

5. AVERAGE EGG VOLUME OF CLUTCHES

As I mentioned earlier, I will use the average egg volume of clutches (AvVol) as an index of the amount of resources available to females for egg formation. The latter is postulated by the nutritional hypothesis to be the main factor affecting clutch profile (see *Introduction*). I will thus use AvVol as a factor in the next chapter's analyses to test this hypothesis' main prediction of a positive association between AvVol and the relative size of last (and first) eggs. In this section I will examine the variability in AvVol by year, season and clutch size, in order to derive secondary predictions about trends in clutch profile among the levels of these factors. I will also discuss whether the assumption that AvVol reflects female resource availability is justified, using information from the literature (in the following paragraphs), as well as some supporting data of my own (in the *Results and Discussion* part of this section).

Female resource availability and egg size

Egg production is a nutritionally and energetically demanding process for a wide variety of female birds (Meijer and Drent 1999), especially when one takes into account the costs of acquiring and metabolizing the resources that are invested in eggs (Perrins 1996). The physiological strain it exerts on females is illustrated by the fact that increased egg production can lower their future fitness (Nager et al. 2001). Many passerines invest in eggs about the same amount of protein they use for their own metabolism during the egg-formation period (Meijer and Drent 1999). In large non-passerines (e.g. arctic geese, Ankney and MacInnes 1978) the former may be >2 times the latter (Meijer and Drent 1999). The relative lipid requirements of eggs appear lower: 10-30 % of total needs in non-passerines (Meijer and Drent 1999), and 10% of the total in the Great Blue Heron (Butler 1993). However, the females' own energetic needs are augmented by egg production, as they need to forage for lipid-poor, protein- and calcium-rich foods, and the developing eggs increase their body weight and the cost of locomotion (Jones and Ward 1976,1979; Ojanen 1983; Alisauskas and Ankney 1985; Houston et al.1995; Perrins 1996). Given these high demands of egg formation, females may well face resource limitations during this process, which could affect their average egg size (see below). When this is true, the latter variable can be used as an index of female resource availability for egg production.

Under conditions of food plenty relative to egg-laying and metabolic requirements, most females in a population may be able to lay eggs of optimal size, and inter-clutch differences in egg size may mostly reflect the variation in female body size in the population. However, relatively few studies have demonstrated statistically significant (and mostly weak) correlations between uni- or multivariate indices of female body size and average egg size in shorebirds (Miller 1979; Nol et al.1984), passerines (Ojanen et al. 1979), and owls under high-food conditions (Hakkarainen and Korpimaki 1993). No significant relationship between mean egg size and multivariate indices of female size (which are more reliable than univariate ones, Freeman and Jackson 1990) has been found in studies of anatids (Leblanc 1989; Swennen and van der Meer 1992), kestrels (Wiebe and Bortolotti 1995), and Eastern Kingbirds *Tyrannus tyrannus* (Murphy 1986a).

The examination of female carcasses has shown a significant association between nutritional condition and egg size in gulls (Houston et al.1983) and kingbirds (Murphy 1986a). Statistically significant (but often weak) correlations between average egg size and female weight relative to body size (which reflects nutritional condition) have been found in anatids (Leblanc 1989; Hepp et al.1987), American Kestrels *Falco sparverius* (Wiebe and Bortolotti 1995), shorebirds (Galbraith 1988), and various passerine birds (Wiggins 1990; Smith et al.1993; Nager and Zandt 1994; Horak et al.1995).

Studies of the Pied Flycatcher (*Ficedula hypoleuca*) in Finland (Jarvinen and Vaisanen 1983, 1984), have indicated that the relative effects of female nutrition and body size on mean egg size depend on environmental (food) conditions. In years and areas with low temperatures and food (insect) abundance, egg size correlates with female nutritional condition, which presumably depends on individuals' foraging skills. When and where temperatures are high and food is plentiful (so that most females can meet their own and their clutches' demands), egg size merely reflects female body size.

Egg size correlates with food availability also in other passerines (Otto 1979; Murphy 1986b; Perrins and McCleery 1994), European Swifts *Apus apus* (O'Connor

1979), and American Kestrels (Wiebe and Bortolotti 1995). Egg size did not significantly vary between Great Blue Heron colonies adversely affected by the 1993 Mississippi River flood and control areas or years (Custer et al.1996), but the authors admit that their egg-sampling protocol may have been inadequate.

Food supplements have increased mean egg size in some passerines (Hogstedt 1981; Ramsay and Houston 1997), but not in others (Hochachka and Boag 1987; Arcese and Smith 1988; Arnold 1992b; Nilsson and Svensson 1993c). Neither did they affect the egg size of Tengmalm's Owls (*Aegolius funereus*) during a peak vole year (Korpimaki 1989). Extra food increased the mean egg size of American Coots (*Fulica americana*) in one study (Hill 1988), but not in another (Arnold 1994). Positive effects of supplemental food on egg size have also been found in kestrels (Wiebe and Bortolotti 1995) and gulls (Hiom et al.1991; Bolton et al. 1992).

Thus feeding experiments have yielded equivocal results regarding the effect of female nutrition on egg size, but they also have their limitations and must be carefully designed to produce meaningful results (Bolton et al.1992; Gehlbach and Roberts 1997). In some of the above studies, the lack of significant effects may be due to inappropriate feeding protocols (Arnold 1992b) or high natural food availability (Korpimaki 1989). Experiments with Lesser Black-backed Gulls have shown that extra food increases egg size only when females face natural food shortages (Hiom et al.1991), and that results also depend on supplemental food quality (Bolton et al.1992), which has also been observed in Blue Tits (Ramsay and Houston 1997).

Therefore, whether mean egg size a) can be used as an index of female resources for egg production, or b) it merely reflects female body size, seems largely to depend on the specific environmental circumstances of the focal population during the period it was studied. I was unable to assess Squacco Heron food availability or female nutritional condition during my study. However, I will present below some data and arguments that support the use of average egg volume as an index of female resources and as a factor in the analyses of clutch profile.

Results and Discussion

The average egg volume of clutches (AvVol) is a continuous quantitative variable, and sample distributions did not deviate significantly from normality (Lilliefors P-values were >0.05). Therefore, I used parametric models in data analyses. Year is a random factor with 3 levels. Season and clutch size are both fixed and have 2 levels each: early vs late (within the main laying period), and 4-egg vs 5-egg clutches. I was unable to test the effects of all 3 factors simultaneously with a 3-way ANOVA, because sample variances were heterogeneous (Bartlett $X^2 = 23.734$, df = 11, $P = 0.014$). Consequently, I performed 2 tests, using factor combinations that did not violate the model's assumptions.

I tested the effects of year and season on AvVol with a mixed-model 2-way ANOVA. Both factors' main effects, as well as their interaction were non-significant (Table 14). This is not surprising, as AvVol varied little among these factors' levels (Table 13). The largest inter-annual difference was 2% of the grand mean (16.134 cm^3), while the one between early and late clutches was only 0.6 % of that mean.

Assuming that AvVol reflects female resources for egg formation, these results indicate neither annual nor seasonal variation in the latter factor. Consequently, the nutritional hypothesis would predict no significant differences in clutch profile either among years or between early and late nests within the main laying season.

Table 13. Statistics on AvVol (cm^3) by year and season.

Statistic	YEAR 1992	YEAR 1993	YEAR 1994	SEASON Early	SEASON Late
n	90	69	88	132	115
Mean	15.975	16.152	16.282	16.186	16.074
S.E.	0.108	0.116	0.127	0.098	0.096

Table 14. ANOVA on AvVol by year (random factor) and season (fixed).

SOURCE	Sum Sq.	d.f.	Mean Sq.	*F*-ratio	*P*-value
YEAR	4.203	2	2.101	1.802	0.167
SEAS	0.830	1	0.830	4.716	> 0.400
Y * S	0.352	2	0.176	0.151	0.860
ERROR	280.995	241	1.166		

Average egg size did not vary significantly with clutch size in Purple Herons (*Ardea purpurea*) in Spain (Jover et al.1993), or in 3 ardeids in the U.S. (Custer and Frederick 1990). Egg size has also been found to remain essentially constant across clutches of different sizes in anatids (Rohwer 1988), grouse (Moss et al.1981), coots (Arnold 1992a), owls (Pietiainen et al.1986), corvids (Rofstad and Sandvik 1985; Heeb et al.1994), and other passerines (Howe 1978; Slagsvold 1982; Smith et al.1993). The lack of a possible trade-off (Smith and Fretwell 1974) between these 2 variables may be due to the proximate adjustment of clutch size to female nutritional circumstances (see section 3, *Clutch Size*). Given this information from herons and other birds, my tentative prediction was that AvVol would not vary between 4- and 5-egg Squacco Heron clutches (the only ones from which I had adequate data for egg-size analyses).

As I mentioned in section 3 (*Clutch Size*), there are 2 groups of clutches where I expected resource limitations that could constrain AvVol. The first one involves clutches (of a final size of 4 or 5) where extra eggs were laid to replace the loss of initial ones (see section 3). Protracted laying of replacement eggs negatively affects their size and quality in the Lesser Black-backed Gull (Nager et al. 2000). In my case, only 1 or 2 extra eggs were laid, but the additional demands on the laying females may have limited egg size.

The second group consists of delayed clutches, which are generally laid by highly resource-limited females (see section 2, *Laying Date*), who also tend to lay small eggs (see earlier in this section). Late nesters may also be young individuals, as in the Cattle Egret (McKilligan 1985). Young females lay smaller eggs, later than older breeders, in many avian taxa, including grouse (Willebrand 1992), shorebirds (Thompson and Hale 1991), gulls (Reid 1988), terns (Nisbet et al.1984), skuas (Ratcliffe et al.1998), and passerines (Crawford 1977; De Steven 1978). These traits of young breeders can be attributed to resource limitations due to inferior foraging skills (Jansen 1990; Desrochers 1992) or restrained reproductive effort (Pugesek 1983; Hamer and Furness 1991). In herons, delayed nests may also represent second attempts after the loss of initial clutches (Wolford and Boag 1971; Pratt and Winkler 1985). The physiological stress of laying 2 sets of eggs may be expected to constrain egg size in heron replacement clutches as in other birds (Mills 1979; Runde and Barrett 1981; Nol et al.1984).

In summary, my predictions were that a) AvVol would not differ between regular clutches of 4 and 5 eggs, but b) it would be lower in both extra-egg and delayed clutches of 4 or 5 eggs than in regular clutches. These predictions are supported by the observed differences in mean AvVol among these samples (Table 15).

Table 15. Statistics on AvVol (cm^3) by clutch size (Clsz) and in clutches laid under certain resource limitations (explanations in text).

Statistic	CLSZ = 4	CLSZ = 5	Delayed	Extra Eggs	D + E E
n	94	153	6	7	13
Mean	16.220	16.081	15.274	15.493	15.392
S.E.	0.097	0.093	0.284	0.388	0.239

I tested the statistical significance of these differences with a 1-way ANOVA, pooling extra-egg and delayed clutches in a single group (as sample sizes were small and expected trends in AvVol were similar). The overall variability in AvVol was significant ($F = 3.469$, df = 2, 257, $P = 0.033$), and this was due to the lower mean of the pooled sample of delayed and extra-egg clutches (D+EE). Tukey's probabilities indicate that the difference between regular 4-egg (C4) and 5-egg (C5) clutches was not significant ($P = 0.579$), while the one between C4 and D+EE was ($P = 0.024$), and the one between C5 and D+EE approached significance ($P = 0.066$).

These results support the assumption that, in my study, AvVol can be used as an index of female heron nutritional condition, since it was lower in the group of clutches expected to have been laid under increased resource limitations. The small size of the D+EE sample may weaken this conclusion, but it does not invalidate it, as results were statistically significant. Another possible shortcoming is the small difference in mean AvVol between the D+EE sample and the regular 4- and 5-egg clutches (about 5% of the grand mean). However, this difference need not be very large, as the comparison was between a group of nests (D+EE) where certain resource limitations of unknown severity were expected, and the population at large (regular C4 and C5 nests). Within the latter, resource availability for egg formation may well have varied, thus constraining the egg size of some females and decreasing the sample's mean AvVol. Besides, in species where food supplements had significant effects on egg size, the difference between experimental and control groups was mostly of a similar magnitude (but sometimes higher): 5% of the overall mean egg size in coots (Hill 1988); 6% in kestrels (Wiebe and Bortolotti 1995); 7% in tits (Ramsay and Houston 1997); 6-9% in magpies in 2 years (Hogstedt 1981); and 8-10% in gulls, depending on natural food availability and extra food quality (Hiom et al. 1991; Bolton et al.1992).

Squacco Heron average egg size may be influenced by female body size, as in some other species (see earlier), but the above results indicate that it also depends on female resource availability. Therefore, it can be used as an index of the latter, albeit an imperfect one. Body size may influence AvVol, but no authors have ever suggested that it can affect the allocation of resources among successive eggs and the profile of a clutch. Thus its interference may mask to some extent nutritional effects on clutch profile, but it is not expected to create any spurious association between AvVol and profile. As I will show in the next chapter, AvVol does have a significant influence on clutch profile, which, according to the above arguments, can only be effected through an association with female resource availability.

In conclusion, I believe that my use of AvVol as an index of female resources for egg formation is justified. As I showed in this section, AvVol did not vary significantly among years, seasonally, or between clutches of 4 and 5 eggs. Therefore, the nutritional hypothesis would predict no significant variability in clutch profile among the levels of any of these 3 factors.

6. HATCHING INTERVALS

As I mentioned earlier, I will use the hatch span (H-Span) of clutches, that is the time interval between the hatching of their first and last eggs, as an index of the female parents' incubation patterns. The early development of incubation behavior, through its hormone-mediated association with a depression of the function of reproductive organs is postulated by Parsons's (1976) hormonal hypothesis to be the main factor constraining last eggs to a relatively small size. I will thus use H-Span as a factor in the next chapter's analyses to test this hypothesis' main prediction of a negative association between hatch span and the relative size of last eggs. In this section, I will present the hatching intervals between successive eggs, whose sum equals H-Span. Then I will examine the variability in the latter by year, season and clutch size, in order to derive secondary predictions about trends in clutch profile among the levels of these factors. First, however, I will explain how I estimated sibling ages and hatching intervals, and I will discuss potential problems with the use of H-Span as an index of female incubation patterns.

Estimation of sibling ages and hatching intervals

In 1992 I checked nests in the hatching stage every day. I kept a regular time schedule, so successive visits to a nest were made approximately 24 h apart. I considered wet hatchlings as 0.0 d old, because under local climatic conditions natal down dried in 1-2 hours. I discovered wet hatchlings throughout the periods I visited nests (8:00-12:00 and 16:00-20:00), which indicated that hatching could occur at any time of the day (and, presumably, the night). Therefore, chicks who were found dry (the majority of cases) could have hatched at any point in the 24-h interval since my previous visit to their nest.

I assumed that they had hatched in the middle of that interval, and were thus 0.5 d old at the time of discovery. In a few cases where chicks were close to emerging from their eggs during a visit, but I first saw and measured them the next day, I assumed that they were 1.0 d old on the latter day. From the above chick age estimates I calculated sibling age differences, which correspond to their eggs' hatching intervals.

In the other 2 years I made hatch checks every other day, in order to minimize potential disturbance to the colony. I used the same ageing criteria as in 1992 for wet hatchlings and eggs in an advanced hatching stage, but I estimated the ages of dry nestlings from certain body measurements. In all years, I weighed hatchlings and I measured their culmen and tarsus lengths (see *General Methods*). I also calculated the ratio of body weight to egg volume (Wt/Vol) whenever I was certain which egg each chick had hatched from (majority of cases).

Several authors have suggested the use of multivariate techniques for the estimation of overall body size (Rising and Somers 1989; Freeman and Jackson 1990), nestling age (Gilliland and Ankney 1992), and adult gender (Phillips and Furness 1997) from morphometric data. Nevertheless, some studies have indicated that univariate metrics may be adequate for ageing nestlings (Mineau et al.1982; Coleman and Fraser 1989; Wiklund 1996). In many cases, regression models were employed for chick-age estimation, as the ages in question covered most of the nestling stage (Elowe and Payne 1979; Mineau et al.1982; Bortolotti 1984; Gilliland and Ankney 1992).

I was unable to employ multivariate methods, because time limitations prevented me from measuring some hatchlings' linear dimensions. This is true both for the 1992-sample, which I used for the development of ageing criteria, and especially in the other 2 years, when I applied these criteria. Thus I estimated and applied ageing criteria for each of the 4 morphometric variables independently.

Given my 2-d visits in 1993 and 1994, and using the same assumptions as in 1992, dry chicks could be aged as 0.5 - 1.5 d old. Within this age span, I had adequate 1992-data for the development of ageing criteria only from 0.5-d and 1.5-d nestlings (the sample of 1.0-d old chick measurements was very small). Since there were only 2 alternative ages (0.5 and 1.5 d), I did not employ a regression model. Instead, using 1992-data, I estimated for each variable the value that had the same probability of belonging to either age's distribution, and used it as an ageing criterion. Specifically, for each variable, using sample means ($\bar{x}_i$) and standard deviations (s_i), I estimated a value X that was associated in both ages' samples with *t*-values ($t_i = [X - \bar{x}_i] / s_i$) which, given their respective degrees of freedom ($df_i = n_i - 1$), had the same probability (*P*) of belonging to the *t*-distribution. Therefore, this X-value (referred to as *Criterion* in Table 16) had the same probability of belonging to either age's distribution, and chicks with measurements <X (or >X) were more likely to be 0.5 d (or 1.5 d) old.

Table 16 shows statistics on Wt/Vol, weight, and tarsus and culmen lengths of 0.5-d and 1.5-d old nestlings from 1992. It also shows, for each variable, the estimated criterion for making ageing decisions in the other 2 years, and its associated *P*-value. The latter is proportional to the degree of overlap between the 2 ages' distributions, so the lower it is the more reliably that variable can be used for ageing chicks. The best ageing variable by far was Wt/Vol ($P = 0.042$). I used nestling weight ($P = 0.091$) only when Wt/Vol was unavailable due to uncertain egg-chick matches. The 2 linear measurements were less reliable (their *P*-values were 0.120 and 0.171).

Table 16. Statistics on body measurements from nestlings at ages of 0.5 and 1.5 d in 1992, and values (*Criteria*) of equal probability (*P-value*) of belonging to either age's distribution.

	WT / VOL (g/cm^3)		WEIGHT (g)	
Statistic	**0.5 d**	**1.5 d**	**0.5 d**	**1.5 d**
n	57	32	87	42
Mean	8.156	12.297	13.350	20.045
S.D.	0.774	1.538	1.742	3.213
Lill *P* [a]	0.120	0.496	0.092	0.281
Criterion	9.540		15.725	
P-value	0.042		0.091	

	TARSUS (mm)		CULMEN (mm)	
Statistic	**0.5 d**	**1.5 d**	**0.5 d**	**1.5 d**
n	39	20	87	42
Mean	12.840	14.550	9.507	10.440
S.D.	0.442	0.852	0.475	0.450
Lill *P* [a]	0.142	0.654	0.805	0.495
Criterion	13.375		9.985	
P-value	0.120		0.171	

[a] Lilliefors probability of random deviation from the normal distribution

Therefore, in making ageing decisions, I employed the following rules:

1) Wt/Vol (or weight) took precedence and was corroborated by linear measurements.

2) When the latter were not taken, the decision was based entirely on Wt/Vol (or weight).

3) Agreement between Wt/Vol (or weight) and one linear measurement was decisive.

4) When there was strong disagreement between Wt/Vol (or weight) and both linear

measurements (well into the ranges of opposite ages), I made no ageing decision.

5) When there was weak disagreement between Wt/Vol (or weight) and the linear measurements (on opposite sides of their criteria, but close to them), the chick was assigned an age of 1.0 day.

Once I had estimated sibling ages on the dates on my visits to their nests, I could calculate their hatching dates and the intervals between the latter in 1993 and 1994.

Suitability of Hatch Span as an index of female incubation

Studies of larids (Parsons 1972; Nisbet and Cohen 1975), kestrels (Wiebe et al. 1998), and passerines (Magrath 1992a; Vega and Vinuela 1993) have shown that parents (mostly females) can control their eggs' hatching intervals through their incubation pattern (timing of onset and rate of development). Thus it seems reasonable to assume that a clutch's hatch span (H-Span) generally reflects parental (and in most of the above cases, female) incubation patterns.

In the Squacco Heron, however, both sexes incubate (Voisin 1980; personal observations), which is typical of ardeids (Blaker 1969; Pratt 1970; Rodgers 1980b; Ashkenazi and Yom-Tov 1997). Thus H-Span can be influenced by male incubation, whereas I would like to use it as an index of female incubation alone (because only the latter can affect clutch profile). Mates generally share incubation equally in the Little Bittern *Ixobrychus minutus* (Langley 1983) and in the Grey Heron (van Vessem and Draulans 1986a), and may begin to do so before the clutch is complete. It is therefore more likely that, in the Squacco Heron, male and female incubation patterns during egg-laying will resemble each other (with some random variation) rather than differ in a consistent way that would invalidate the aforementioned use of H-Span. Nevertheless, the male's involvement is likely to increase random error in H-Span and thus decrease the probability of detecting an existing relationship between H-Span and clutch profile.

Besides parental incubation, hatching patterns also depend on egg-laying intervals (Nisbet and Cohen 1975; Wiebe et al.1998). I showed in the first section of this chapter that laying intervals do not vary consistently among the levels of any factor used in the analysis of H-Span. In the next chapter I will also show that they do not vary among clutches of different profile types, either. Thus the variability in laying intervals may also add to the random error in H-Span, but it cannot bias results.

As I explained earlier, my hatching interval estimates are not very accurate. This measurement error in the data should occur randomly with respect to clutch profile and the other factors involved in the analysis of H-Span. Thus it is also likely to increase random error, but is not expected to confound analyses. All sources of error in H-Span I have so far discussed may mask its possible effects on clutch profile, but are not expected to create a spurious association between these 2 variables.

There is one factor which can affect H-Span in a consistent way with respect to clutch profile, but its bias will oppose the predictions of the hormonal-constraints hypothesis. This factor is egg size, which is positively associated with the length of the incubation period (Parsons 1972; Bollinger 1994). The hormonal hypothesis predicts that when incubation develops quickly (and thus H-Span is expected to be long) last eggs will be relatively small. Their shorter incubation period, however, will shorten H-Span and cause the rate of incubation development to be underestimated. The opposite is expected to happen in clutches with a delayed onset of incubation. (I will discuss this issue in more detail when I compare H-Span among profile types in the next chapter.)

Statistical Methods

In analyses, I used data from clutches of 4 and 5 eggs where complete sets of successive hatching intervals and the total H-Span were known. Hatching interval data were not normally distributed, so I performed non-parametric analyses. I compared the length of successive hatching intervals with Friedman's test, using each clutch as a block. I tested the effects of year, season and clutch size on H-Span with Kruskal-Wallis (for year) and Mann-Whitney U tests (for the other 2 factors).

If 2 or more factors are found to affect H-Span, their effects will be confounded, if they also happen to be inter-correlated. As I will show below, clutch size and season have significant effects on H-Span. To remove any confounding effects of clutch size, I tested the other 2 factors' effects in 4-egg (C4) and 5-egg (C5) clutches separately. In the 2nd case, I compared the standardized laying date of clutches (see section 2, *Laying Date*) among the levels of the other 2 factors to ascertain that it did not vary significantly.

Results and Discussion

Table 17 shows statistics on successive and cumulative hatching intervals by clutch size, pooling data from all years. These intervals are symbolized as *H(i-j)*, where *i* and *j* indicate the laying order of eggs that define each hatching interval. In 4-egg nests there is no E-egg, so there is no D-E interval and H(A-D) equals H-Span.

Hatching intervals between successive eggs increase in length as egg-laying order progresses. This trend is statistically significant both in 4-egg clutches (Friedman's $X_r^2 = 29.7$, df=2, $P < 0.001$) and in 5-egg nests ($X_r^2 = 171$, df=3, $P < 0.001$). Since egg-laying intervals did not increase with laying order (see section 1), this increasing length of successive H(i-j) must be due to the gradual development of parental incubation. In both

C4 and C5, the median A-B interval is 0.0 d, which indicates that incubation usually begins after the 2nd egg is laid. The last interval in C5 is the only one with a median length equal to the modal egg-laying interval (2.0 d). Thus full incubation may not often start before the 4th egg is laid. Parental incubation developed faster in C4, as their longer A-D interval indicates (3.0 d vs 2.5 d in C5; $U = 2019$, $P = 0.001$). Hatching patterns in other ardeids also indicate a gradual development of parental incubation during egg-laying (Maxwell and Kale 1977; Werschkul 1979; Inoue 1985; Mock 1985).

Table 17. Successive and cumulative hatching intervals (in d) by clutch size (Clsz). (Explanations in text.)

CLSZ	Statistic	H(A-B)	H(B-C)	H(C-D)	H(D-E)	H(A-D)	H-Span
	n	35	35	35	.	35	35
FOUR	Min	0.00	0.50	0.00	.	2.00	2.00
	Max	1.50	2.00	2.50	.	5.00	5.00
	Median	**0.00**	**1.00**	**1.50**	.	**3.00**	**3.00**
	n	73	73	73	73	73	73
FIVE	Min	0.00	0.00	0.00	0.50	0.50	1.50
	Max	1.00	2.00	3.00	3.00	4.50	6.50
	Median	**0.00**	**1.00**	**1.00**	**2.00**	**2.50**	**4.50**

Five-egg clutches have a 1.5-day (or 50%) longer hatch span than 4-egg nests (Table 17). This highly significant difference ($U = 465$, $P < 0.001$), is produced by the extra D-E interval of the larger clutches (I showed above that the A-D interval is longer in 4-egg nests). Nevertheless, the fact remains that last (5th) eggs in C5 are laid significantly later than last (4th) eggs in C4 relative to the onset of incubation. Thus the hormonal hypothesis would predict the former to be relatively smaller than the latter. Because of this difference in H-Span between C4 and C5, I tested the annual and seasonal variability in H-Span in each clutch size separately. H-Span also varied a lot within C4 and C5 nests (respective ranges were 2-3 d and 1.5 - 6.5 d). This variation, which presumably reflects differences in female incubation (with some random error, see earlier), allowed me to test the hormonal hypothesis' predictions concerning clutch profile (see later).

I will show below that there is a significant seasonal increase in H-Span in 5-egg clutches. The above comparisons between clutch sizes are not confounded by seasonal effects, as median standardized laying date did not vary significantly between samples ($U = 1398$, $P = 0.426$), and was actually *earlier* in C5 (0d vs 2d in C4). (I will further examine this issue at the end of this section.)

The annual variability in median H-Span was low in both clutch sizes (Figure 3). There was no significant year-effect either in C4 (Kruskal-Wallis $H = 0.656$, $P = 0.720$) or in C5 ($H = 0.826$, $P = 0.662$). The annual variability in the standardized laying date of 5-egg clutches was also low, statistically non-significant ($H = 2.452$, $P = 0.293$), and unlikely to have confounded results (see also later). These results indicate that, according to the hormonal hypothesis, there should be no variability in clutch profile among years in either 4- or 5-egg clutches.

In order to use H-Span as a factor in the next chapter's clutch profile analyses, I had to divide nests into those with short and long H-Span. I made this division in each year and clutch size separately, using the median H-Span values shown in Figure 3 as criteria : a) short H-Span < sample's median < long H-Span ; b) I put nests whose value equaled their sample median in the subset with the fewer cases, in order to keep sample sizes as similar as possible.

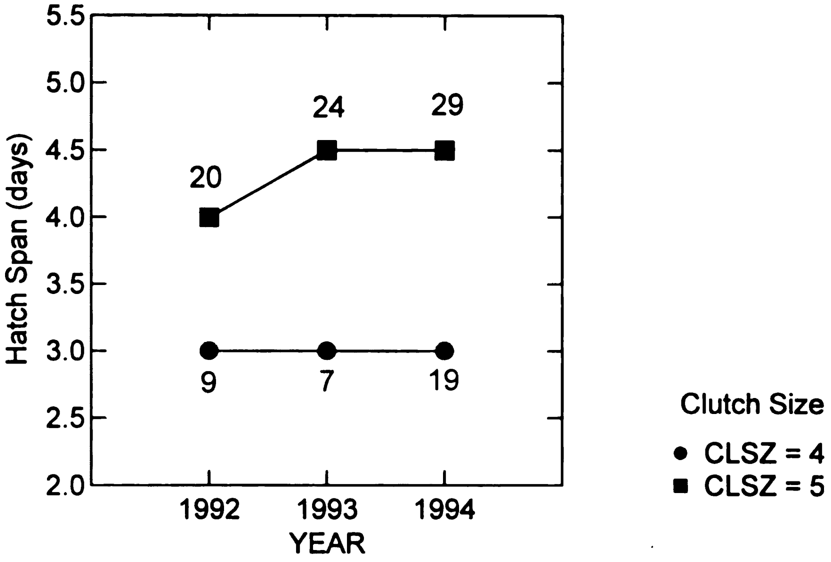


Figure 3. Median hatch span by clutch size and year.
Sample sizes indicated above or below data points.

Statistics on H-Span by clutch size and season are shown in Table 18. Using all available data (columns C4-All and C5-All), I found a significant seasonal increase in H-Span in C5 ($U = 395.5$, $P = 0.003$), but no seasonal trend in C4 ($U = 109.5$, $P = 0.166$). This apparent interaction between season and clutch size could be a mere artefact of the greater difference in median standardized laying date between early and late nests in C5 (12d) than in C4 (8d). In order to examine this possibility, I "trimmed" the C4-sample by excluding cases with laying dates close to the overall median value (results are shown in column C4-Trim of Table 18). Thus, by excluding the latest among early C4-nests, and the earliest among the late ones, I increased the difference in median laying date between the 2 trimmed sub-samples to the same level as in C5 (12d). The difference in median H-Span remained the same in magnitude (0.25 d) and statistically non-significant ($U = 64.5$, $P = 0.234$), despite the increased disparity in standardized laying date. I also applied the opposite trimming to the C5-sample, excluding the earliest among early clutches, and the latest among the late ones, in order to *reduce* the difference in median laying date to the same level as in the complete C4-sample (8d). In these trimmed samples, the difference in median H-Span retained its original magnitude (1d, see column C5-Trim1 in Table 18), and its statistical significance ($U = 238$, $P = 0.041$), despite the loss of power due to the decrease in sample sizes.

Table 18. Median hatch span (H-Span) and standardized laying date (Std L D) by season in various samples (explanations in text). Sample sizes are given in H-Span row.

Variable	Season	C4 - All	C4 -Trim	C5 - All	C5-Trim 1	C5-Trim 2
H-Span	Early	15	11	35	26	17
		3.00	**3.00**	**4.0**	**4.0**	**4.5**
	Late	20	16	38	27	19
		3.25	**3.25**	**5.0**	**5.0**	**4.5**
Std L D	Early	- 3.0	- 5.0	- 7.0	- 4.0	- 2.0
	Late	5.0	7.0	5.0	4.0	3.0

These results suggest an actual difference between 4-egg and 5-egg nests in the seasonal trend of H-Span. This could be related to parental reproductive strategies that involve hatching asynchrony and brood reduction (Lack 1947; Magrath 1990). Late-season nestlings gain less weight than earlier ones in various birds, including penguins (van Heezik et al.1993), skuas (Furness 1983), alcids (Birkhead and Nettleship 1982), and passerines (Slagsvold 1982; Magrath 1989). Late fledglings also suffer higher mortality in shearwaters (Perrins 1966), penguins (van Heezik et al.1993), cormorants (Harris et al.1994), gulls (Nisbet and Drury 1972), and passerines (Perrins 1965; Krementz et al.

1989; Naef-Daenzer et al. 2001). Thus late breeders may increase hatching asynchrony in order to improve the fitness of their senior offspring by limiting the competitive ability of their junior chicks (Mock and Forbes 1994), a strategy that would be more useful in larger, more demanding broods.

The possible adaptive significance of the seasonal trends in hatching asynchrony is beyond the scope of my dissertation. These results, however, suggest the following hormonal-hypothesis predictions about clutch profile: a) no seasonal variability in clutch profile in C4, and b) relatively smaller last eggs in late (relative to early) C5 nests. Since predictions differ between clutch sizes, in the next chapter I will test the effect of season on profile in each clutch size separately.

Since the H-Span of 5-egg clutches increases seasonally, other factors' effects on H-Span would be confounded by a significant variation in laying date among their levels. To investigate this possibility, I further constricted the C5-sample (excluding the earliest among early, and the latest among late nests), until the difference in median laying date between early and late nests was only 5d (column C5-Trim2 in Table 18). The new sub-samples did not differ in median H-Span statistically ($U = 153$, $P = 0.784$), which could be partly due to the loss of cases and power, or numerically (both early- and late-clutch medians converged to 4.5 d). It thus seems that this level of disparity in laying date (5d) is not enough to produce a difference in H-Span between samples. In 5-egg nests, median standardized laying date varied by a maximum of 4d among years and 3d among clutch profile types (see next chapter), and was 2d earlier than that of C4-clutches. Therefore, it is unlikely that the observed trends in H-Span among the levels of these factors could be confounded by seasonal effects.

7. RELATIONSHIP BETWEEN AV.VOL AND HATCH SPAN

Houston, Jones and Sibly (1983) proposed that, in gulls, incubation restricts female foraging time, which limits resource availability for egg formation, and thus constrains the size of eggs that are not fully-formed by the time incubation begins. Given the small clutch size of gulls (3 eggs) and the timing of relevant events, only the size of last eggs is expected to be negatively affected in these species.

As I showed in the previous section, hatch span (which depends on the timing of the onset and on the rate of development of incubation) varies a lot in the Squacco Heron, both in 4-egg clutches (range of 2-5 d) and in 5-egg nests (1.5 to 6.5 d). If incubation limits female heron foraging opportunities, the number of eggs that are affected, and the level of their size-limitation, should vary strongly across these ranges of H-Span values. Such strong variation should be reflected in the average egg size of clutches. Therefore, Houston, Jones and Sibly's (1983) hypothesis (*HJS-hypothesis*, for short) predicts a significant negative relationship between H-Span and AvVol.

I tested this predicted association with Spearman rank correlations (due to the non-normal distribution of H-Span) in each clutch size separately, because of the great difference in median H-Span between C4 and C5 nests. The 2 variables were independent both in C5 ($r_s = -0.002$, $n = 73$, $P > 0.990$) and in C4 ($r_s = 0.277$, $n = 35$, $P > 0.100$), where the correlation coefficient was actually positive (but not significantly so).

These results refute the validity of the HJS-hypothesis for *A. ralloides*. The most probable reason for this, is that incubation does not restrict the foraging opportunities of female herons by increasing the amount of time they spend at their nests. Both sexes tend to share breeding responsibilities equally in ardeids (van Vessem and Draulans 1986a ;

Voisin 1991), including the Squacco Heron (Voisin 1980). Competition for nesting sites (Burger 1981; Fasola and Alieri 1992) and materials (Jenni 1969; Burger 1978) may be intense in crowded heronries, where stealing of nest materials is common (Milstein et al. 1970; Burger 1978; Voisin 1991). Thus, species like the Little Egret need to constantly guard their nests, beginning well before the onset of incubation, to prevent them from being dismantled and their sites occupied by rival pairs (Voisin 1991). Actually, the mean duration of Grey Heron trips away from their nests is minimum before egg-laying and maximum during incubation (van Vessem and Draulans 1986 b), but the total time of absence may not vary in the same way, as the frequency of trips may be greater in the former period. Territory and nest defense seems to be especially pronounced in the small-bodied Squacco Heron (Voisin, personal communication; personal observations), and, for the above reasons, is likely to be fully developed from the early nesting stages. Therefore, the onset of incubation should not be expected to reduce female foraging time and nutritional levels, and, as I showed above, it does not seem to limit mean egg size.

Houston, Jones and Sibly's hypothesis may not fully account for the small size of final eggs even in larids. Nisbet's (1973) finding of a positive association between male feeding rates of females and last-egg size in Common Terns (*Sterna hirundo*) seemed to support it, until it was noticed that early eggs benefitted from extra feedings even more, so that last eggs were relatively *smaller* in clutches with higher mean egg size (Reid 1987). These results, however, do not conclusively refute the HJS-hypothesis, because male feeding rates dropped during laying, so final-egg albumen synthesis may have been resource-limited even in highly-provisioned females. Reid (1987) was able to increase the relative size of last (3rd) eggs in the Glaucus-winged Gull (*Larus glaucescens*) by providing females with additional food after they laid their 1st egg. This experiment, however, gave early eggs little or no opportunity to benefit from food supplements, so its results do not strongly support the HJS-hypothesis. Salzer and Larkin (1990) showed that egg-removal in the same species increased a) the period of female courtship-feeding by the male, b) clutch size, and c) the relative size of 3rd eggs (which were no longer last). These results are in agreement with the HJS-hypothesis, but are equally consistent with Parsons's (1976) hormonal hypothesis, which the authors overlooked. More recent experiments on Lesser Black-backed Gulls (Hiom et al.1991; Bolton et al.1992) have shown that food supplementation (from 2-3 weeks before the start, and until the end of egg-laying) can increase both the average egg size of clutches and the relative size of final eggs. The latter, however, is due to an increase in yolk, rather than albumen, protein (Bolton et al.1992). These results indicate that, at least in gulls, the yolk protein of last eggs may be nutritionally constrained, but their albumen is limited by some other factor, which could be the hormonal mechanism proposed by Parsons (1976).

In summary, the lack of a negative relationship between AvVol and H-Span (the indices of female nutrition and incubation, respectively), refutes Houston, Jones and Sibly's (1983) hypothesis of nutritional constraints on egg size associated with the onset of incubation in the Squacco Heron. In the next chapter, I will test the predictions of Parsons's (1976) hypotheses of independent nutritional and hormonal constraints on this heron's final-egg size. As AvVol and H-Span varied independently, there was no risk of their confounding each other's possible effects on clutch profile. Thus I was able to test the 2 hypotheses, and draw at least tentative conclusions about their validity, without experimentally controlling these variables.

IV. CLUTCH PROFILE ANALYSES

In this chapter I will address the main issue of my dissertation, the pattern of intraclutch egg-size variation by laying order in the Squacco Heron. In particular, I will a) test Parsons's (1976) nutritional and hormonal hypotheses' predictions about the effects of various factors on the relative size of final eggs, and b) examine hypotheses concerning the relative size (and shape) of first eggs.

For brevity, I will refer to the observed patterns of intraclutch egg-size variation as *clutch profiles*, and to their analyses as *clutch profile analyses*. The latter are not to be confused with the specific statistical methodology known as "Profile Analysis", which includes multivariate tests (von Ende 1993). As I will explain below, I used *univariate* Repeated Measures ANOVA models to test various factors' effects on clutch profile. For simplicity, I will refer to egg-laying order as egg *rank*. I will also capitalize the names of factors involved in analyses, and I will use abbreviations for some of them (the latter are presented below, and listed on page xii).

In the first section of this chapter, I will explain the statistical methods I used in my analyses. In the next, I will summarize the specific predictions of the nutritional and hormonal hypotheses I will be testing. The results of my analyses (section 3) concern the average egg-size and -shape profiles of all clutches, the different profile types observed, and the effects of various factors on clutch profile (tests of hypotheses' predictions). In the final section of this chapter, I will discuss these results and their implications for the proximate causation of intraclutch egg-size variation in *Ardeola ralloides*.

1. STATISTICAL METHODS

In the analysis of clutch profile, one must take into account the fact that the sizes of a clutch's eggs are not independent, but can be thought of as deviations from the clutch's average egg size (AvVol), which may vary significantly among females (Jover et al.1993). Therefore, testing the effect of egg Rank on clutch profile with a completely randomized ANOVA would violate the model's assumption of unit independence. Moreover, it would be inefficient, as the variability in AvVol among clutches would inflate the model's error term. An appropriate alternative, which I used in these analyses, is the Repeated Measures ANOVA (von Ende 1993; Zar 1996). This model allows for lack of egg independence within clutches, and removes the among-clutches variation in AvVol from the error term that tests the significance of clutch profile.

The main effect of the within-clutches factor, egg Rank, pertains to the average profile of the whole sample of clutches used. Among-clutches factors can also be incorporated, and their main effects represent differences in AvVol among their levels (subsets of the total sample). The Rank × among-factor interactions concern differences in clutch profile among the latter factors' levels. For brevity, I will refer to them as the among-factors' *effects on clutch profile*. They are the most important component of these analyses, because they are the actual tests of the different hypotheses' predictions. For example, the hormonal hypothesis predicts a difference in clutch profile between 4-egg and 5-egg nests (see next section), that is a significant Rank × Clutch Size (Clsz) interaction, or a *Clsz-effect on clutch profile*. Conversely, as AvVol did not vary between 4- and 5-egg nests, the nutritional hypothesis predicts no Clsz-effect on clutch profile (no Rank × Clsz interaction).

The Repeated Measures ANOVA (*RMA*) makes the usual assumptions of normal data distribution and homogeneity of sample variances, which I continued to test. It also assumes a) circularity of the variance-covariance matrix for all levels of the within-factor, and b) homogeneity of such matrices for the differences among the levels of Rank across all levels of the among-factors (von Ende 1993). I will report test result probabilities, generated by SYSTAT (1996), that have been corrected by the Huynh-Feldt (1976) estimator for violations of the circularity assumption. There is no method for correcting test results for violations of assumption (b), but RMA is robust to such violations as long as sample sizes are similar (von Ende 1993). Moreover, I ran orthogonal contrasts for the Rank main effect and its interactions with the among-clutches factors, which are free from assumptions (a) and (b) above, and thus more reliable (SYSTAT 1996). Contrasts are very useful also because they concisely describe the effect of Rank on egg size (the shape of clutch profile) and the way the latter is affected by the among-nests factors. SYSTAT also runs multivariate RMAs, which some authors prefer (e.g. Jover et al.1993), but such analyses also have their limitations (James and McCullogh 1990 ; von Ende 1993). In my case, uni- and multivariate analyses always produced similar results, and I will present only the former.

As I mentioned above, the among-clutches factors' main effects refer to trends in AvVol among their levels. I have already presented the results of such analyses, run on a sample of 247 nests, in the *Preliminary Analyses* chapter. I ran those tests in advance, in order to derive predictions (from the nutritional hypothesis) about various factors' effects on clutch profile. In this chapter's RMAs I used only 216 (or fewer) of the above 247 nests, because I excluded 31 clutches where egg-laying order was uncertain. I checked the among-factors' main effects in the RMAs in order to ascertain that the same trends in AvVol (and the same nutritional-hypothesis predictions) still held. As this was always the case, I will not present these tests' results in this chapter, but I include them in complete RMA tables that can be found in the *Appendix*.

For the sake of simplicity, I will also present only pertinent orthogonal contrasts. SYSTAT (1996) runs linear, quadratic and cubic contrasts. These refer, respectively, to a) the clutch profile's overall linear trend, b) its curvature (relationship between middle and marginal eggs), and c) irregularities (zig-zags) in the previous trends. I never found significant cubic trends, so I will not present the results of such contrasts. Moreover, I will present results of the other 2 contrasts only when the respective overall Rank-effect or interaction with among-clutches factors was statistically significant.

All clutches used in the same RMA must have the same number of eggs, because the within-factor (Rank) must always have the same number of levels. These differ between clutches of 4 and 5 eggs. Thus, in analyses that required the simultaneous use of clutches of both sizes (e.g. when Clsz was an among-factor), I reduced the levels of Rank in the larger clutches, by averaging the volumes of 2nd and 3rd eggs, as suggested by Jover et al.(1993). I will refer to the resulting 4 egg ranks as *First*, *Intermediate*, *Penultimate*, and *Last*, and to all eggs except the first and the last as *middle* ones.

I tested the effects of 5 among-nests factors on clutch profile. Two of them are expected by the nutritional and hormonal hypotheses to directly affect clutch profile :
1) Average egg size (AvVol), with 2 levels (small $\leq$ Year × Clsz median < large). I will refer to this factor as *Egg Size* (*Egsz*), to avoid confusion when "AvVol" denotes data.
2) Hatch span (H-Span), with 2 levels (short and long; for nest assignment see p.56).

Another 3 factors may be expected by these hypotheses to indirectly affect clutch profile, depending on whether AvVol and H-Span vary significantly among their levels :

a) Year, which in this case a is fixed factor, because I have specific predictions (see next section) about the variation in clutch profile among its levels (the 3 years of my study).

b) Season, with 2 levels (early and late; for nest assignment to these categories see p.30).

c) Clutch size (Clsz), with 2 levels, 4-egg (C4) and 5-egg (C5) clutches. All predictions about these 5 factors' effects on clutch profile are reviewed in the following section.

As I mentioned earlier, I have egg-size data from 216 nests (86 C4 and 130 C5) where egg ranks were certain. The levels of 4 of the above 5 factors are known for all of these nests, but I have hatching data from only 108 of them (35 C4 and 73 C5). Thus I tested the effect of H-Span on clutch profile in the latter (smaller) sample, and the effects of the other 4 factors in the former (bigger) one. I could incorporate all of these 4 factors in a single model, but this would result in the division of nests among 24 sub-samples (equal to the number of all Year × Season × Egsz × Clsz level combinations), each including 3-16 nests. Model assumptions would not be met under such conditions, so I ran one RMA with Clsz and Egsz as among-nests factors, and a different one with Year and Season. I performed the latter analysis in each clutch size separately, because the seasonal effect on profile was expected to differ between clutch sizes (see next section), using the original C5-data (from 5 egg-ranks). I used the 4 modified ranks in the other 2 RMAs, because Clsz was a factor in both, and C4- and C5-data had to be compatible. The total number of cases were thus divided into fewer sub-samples, with larger and less disparate sizes (Table 19). Sample variances were homogeneous in all cases, and distributions did not significantly deviate from normality.

The data I used in RMAs are egg volumes. In figures depicting clutch profiles, however, I will use a relative egg-size variable, the % deviation (%D) of each egg-rank's value from the clutch's AvVol. This should facilitate visual comparisons of patterns, as whole-clutch means (the equivalent of AvVol) will equal 0.0 in all samples compared. Figures will indicate each rank's mean %D and its 95% confidence interval.

Table 19. Sizes of samples used in clutch profile analyses (RMAs).
Factor levels and name abbreviations are explained in the text.

A. RMA by Hatch Span and Clucth Size

Short H-Span		Long H-Span		All
C 4	C 5	C 4	C 5	Nests
18	36	17	37	108

B. Separate RMAs by Year and Season in each Clutch Size

	1992		1993		1994		All
CL SZ	Early	Late	Early	Late	Early	Late	Nests
C 4	13	17	11	7	18	20	86
C 5	26	22	21	15	25	21	130

C. RMA by Egg Size and Clucth Size

Low AvVol		High AvVol		All
C 4	C 5	C 4	C 5	Nests
43	65	43	65	216

2. PREDICTIONS TESTED

In this section I review the predictions of the nutritional and hormonal hypotheses concerning the effects of certain factors on the relative size of final eggs. These are based on the results of the preliminary analyses I presented in Chapter III, and are summarized in Table 20. At the end of this section, I will also present predictions concerning the relative size and shape of first eggs.

The nutritional hypothesis postulates that the relative size of last eggs depends on the amount of resources available to females for egg formation, which I infer from AvVol. This hypothesis predicts a positive association between AvVol and %D(L), which should appear in the respective RMA as a significant linear Rank × Egsz interaction. According to the hormonal hypothesis, the size of last eggs is constrained by the early onset and development of female incubation, which is inversely proportional to a clutch's hatch span. This hypothesis predicts a negative association between H-Span and %D(L), and a significant linear Rank × H-Span interaction in the respective RMA. Since AvVol and H-Span are not correlated (see last section of the *Preliminary Analyses*), their effects on clutch profile are not expected to confound each other: each hypothesis' main factor is not predicted by the alternative hypothesis to affect clutch profile (Table 20A).

The 2 hypotheses' secondary predictions about the effects of Year, Season, and Clutch Size (Clsz) on clutch profile depend on the variability in AvVol and H-Span among the levels of these factors. As I showed in the respective section of the previous chapter, none of these factors affected AvVol significantly. Therefore, neither are any of them expected to affect clutch profile according to the nutritional hypothesis. The results of the analyses of H-Span (see section 6 in last chapter) suggest the following hormonal hypothesis predictions: a) no inter-annual variability in clutch profile in either Clsz; b) no seasonal variability in profile in C4, but relatively smaller last eggs in 5-egg clutches late in the season (significant linear Rank × Season interaction); c) significant Clsz-effect on profile (linear Rank × Clsz interaction), due to lower %D(L) in C5. These predictions are summarized in part B of Table 20.

The 2 hypotheses' secondary predictions are not as strong as their primary ones for 2 reasons. First, AvVol and H-Span, even when they vary significantly among the levels of the secondary factors, may not do so at a level sufficient to produce a detectable effect on clutch profile. Second, there may be additional factors which also vary yearly, seasonally, or between clutch sizes, but affect clutch profile in ways that oppose the expected effects of AvVol or H-Span (though no such factors are readily suggested by the literature). Nevertheless, it is still worthwhile to test these predictions, because they provide additional tests of the nutritional and hormonal hypotheses.

Both hypotheses predict a lack of variability in clutch profile among years, and between early and late C4 nests. Therefore, the respective tests are not diagnostic (results will support either both hypotheses or neither). Nevertheless, Table 20 indicates that both hypotheses' main predictions, and half of their secondary ones, differ and thus lead to diagnostic tests. I should mention, however, that the 2 hypotheses are not mutually exclusive. Resource limitations and hormonal constraints may both affect clutch profile, either independently or interactively (see next section).

The hypothesis of nutritional constraints on the size of *first* eggs (Slagsvold and Lifjeld 1989) predicts a positive association between AvVol and %D(F), which can be evaluated by the same analysis that examines the effect of AvVol on %D(L). Since no other factor had a significant effect on AvVol, this hypothesis predicts no variation in %D(F) by Year, Season, or Clsz. The hypothesis of anatomical constraints on first-egg size (Robertson and Cooke 1993) predicts more elongate first eggs than subsequent ones, and I tested it with RMAs on egg shape (width/length ratio) in C4 and C5 clutches.

Table 20. Summary of nutritional and hormonal hypotheses' predictions (descriptive and referring to RMA results).

A. Primary Factors and Predictions

Factor	**Nutritional Hypothesis**	**Hormonal Hypothesis**
Egg Size (AvVol)	Positive association between AvVol and %D(L)[a] Linear Rank × Egsz Interact.	No effect on Profile
H-Span	No effect on Profile	Negative association between H-Span and %D(L) Linear Rank × H-Span Interact.

B. Secondary Factors and Predictions

Factor	**AvVol**	**H-Span**	**Nutritional Hypoth.**	**Hormonal Hypoth.**
Year	Constant	Constant	No effect on Profile	No effect on Profile
Season	Constant	Greater Late in C5 only	No effect on Profile in either clutch size	Lower %D(L) Late Lin. Rank × Seas Int. in C5 only
Clutch Size	Constant	Greater in C5	No effect on Profile	Lower %D(L) in C5 Lin. Rank × Clsz Int.

[a] Last egg's relative size, expressed as percent deviation from clutch's AvVol

3. RESULTS

A. AVERAGE CLUTCH PROFILE

The average egg-volume profiles of 4- and 5-egg clutches are shown in Figures 4A and 4B, respectively. I tested their significance in each clutch size separately, using the original 5 egg ranks (A-E) in C5, with RMA-models that also included 2 among-nests factors (Year and Season). Here I will present the main effect of Rank (laying order) on egg size, and I will discuss its interactions with the 2 among-factors later.

The significance of the main Rank effect, and its linear and quadratic components, are shown in Table 21 (A and B, for C4 and C5). For simplicity, I present only the *F*-ratio, d.f., and *P*-value (complete ANOVA tables can be found in the *Appendix*). The number of error d.f. differs between the test of the overall Rank-effect and the 2 contrasts. This is because, in the latter, it depends on the number of clutches in the sample, whereas in the former it depends on the number of eggs (ranks × clutches).

In both clutch sizes, the average profile is "arched", that is marginal eggs (first and last) are smaller than middle ones (Figure 4). This trend is statistically significant in both samples, as quadratic contrasts indicate (Table 21). In C5 there was also an overall decline in egg size with laying order, while there was a positive, but non-significant linear trend in C4 (see Figure 4, and linear contrasts in Table 21).

The clutch profiles shown in Figure 4 seem to indicate that, on the average, egg size does not vary greatly within clutches. The mean difference between the largest and smallest rank is 4.3% of AvVol in C4 and 6.1% of AvVol in C5. As I will show later, however, largest and smallest eggs vary in rank among clutches. When the maximum difference is calculated *within* clutches, and then is averaged, its mean value is 8.7% of

AvVol in C4 (twice as big as before), and 10.8 % in C5 (almost twice as big). Such egg-size differences within clutches may affect sibling growth and survival (see *Discussion*).

Table 21. RMA on egg volume by egg Rank in C4 (A) and C5 (B).
(Original 5 ranks used in C5.)

	Source	*F*-ratio	D.F.	*P*-value
A. C 4	Rank	26.697	3, 240	< 0.001
	Linear	1.228	1, 80	0.271
	Quadratic	97.814	1, 80	< 0.001
B. C 5	Rank	69.482	4, 496	< 0.001
	Linear	5.353	1, 124	0.022
	Quadratic	301.526	1, 124	< 0.001

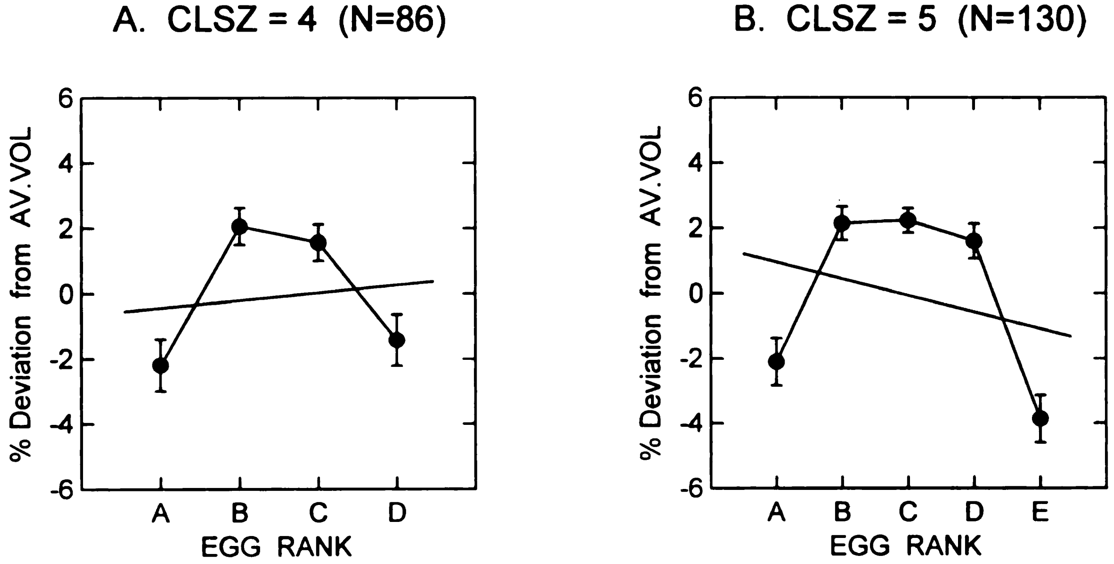


Figure 4. Average egg-size profile by clutch size (Clsz).
Error bars represent 95% confidence intervals.
Regression lines indicate each profile's linear trend.

The intraclutch trend in egg shape (width/length) was very weak in both C4 and C5 nests (Figures 5A and 5B), and non-significant in the former ($F = 1.897$, df = 3,255, $P = 0.139$). Despite the statistical significance of the egg-shape profile in C5 ($F = 3.565$, df = 4,516, $P = 0.008$), and its quadratic term ($F = 14.130$, df = 1,129, $P < 0.001$), this pattern of variation can hardly account for the 5 times greater differences in volume between marginal and middle eggs (Figure 4B). Moreover, variation among females in the postulated initial oviduct inelasticity (which is expected to constrain the size of first eggs by limiting their width) should produce a positive association between relative first-egg volume and shape (% deviations from clutch means). These 2 variables, however, were not correlated in C5 nests (Pearson $r = -0.064$, $n = 130$, $P = 0.794$), and were *negatively* associated (though weakly so) in C4 clutches ($r = -0.221$, $n = 86$, $P = 0.041$). Thus, first-egg size does not seem to be anatomically constrained in the Squacco Heron.

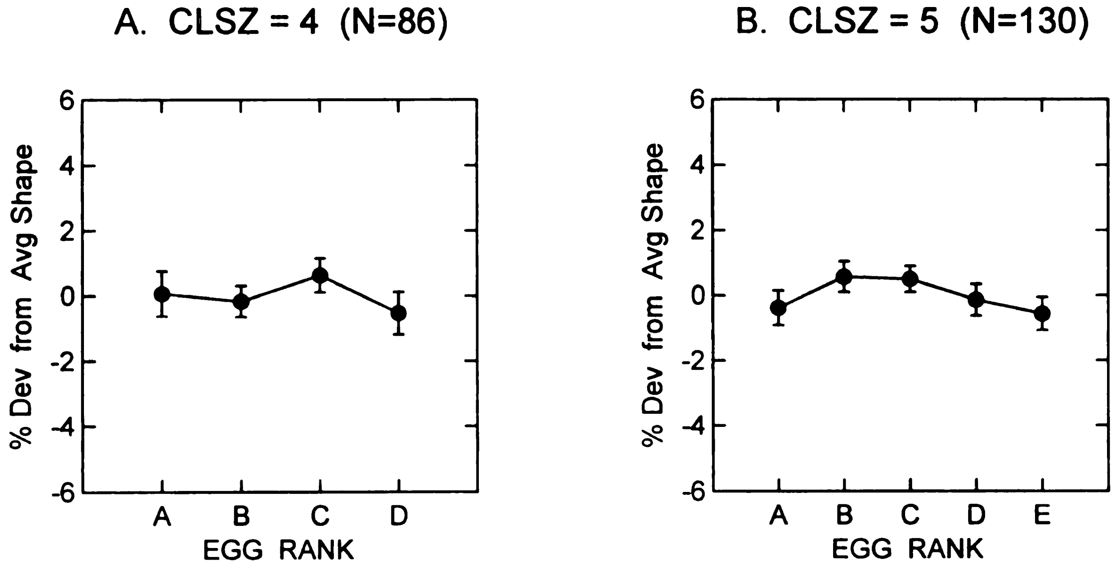


Figure 5. Average egg-shape (width/length) profile by clutch size (Clsz). Error bars represent 95% confidence intervals.

B. CLUTCH PROFILE ANALYSES

I described above the main effect of laying order (Rank) on egg size, which affects the average appearance of clutch profile. In this section, I will examine profile differences among the levels of certain factors, represented by RMA interactions between Rank and these among-nests factors. These are the only terms I will present in RMA tables here (complete ANOVAs can be found in the *Appendix*). I used the original 5 egg ranks in C5 in the analysis by Year and Season (which was performed in each clutch size separately), and the modified 4 ranks (first, intermediate, penultimate, last) in the other RMAs (in which I made comparisons between 4- and 5-egg clutches). The error d.f. for orthogonal contrasts will again differ from those of the RMA error terms (see earlier).

Analysis by Hatch Span and Clutch Size

I tested the main prediction of the hormonal hypothesis, which concerns the effect of H-Span on clutch profile, on the sample of 108 nests (35 C4 and 73 C5) from which hatching data are available. I also incorporated Clsz as an among-nests factor, in order to examine whether the effect of H-Span on profile varied between clutch sizes (by looking at the Rank × H-Span × Clsz interaction). I will not examine the Clsz-effect on profile here, because I tested it in the larger sample of 216 nests from which egg-size data are available (I present the results of that analysis later).

The results of the relevant RMA (Table 22) show a non-significant 3-way interaction, indicating that the effect of H-Span on profile is similar in both clutch sizes. This is not surprising, because, according to the hormonal hypothesis, Clsz is expected to affect clutch profile only due to the difference in H-Span between its levels (see earlier), that is for the same reason as the other among-nests factor in the model.

Table 22. RMA on egg volume by Rank (within-clutches factor), and by H-Span and Clsz (among-clutches factors).

Source	*F*-ratio	D.F.	*P*-value
Rank × H-Span	5.811	3, 312	**0.002**
Linear	9.673	1, 104	**0.002**
Quadratic	0.032	1, 104	0.858
R × H-Sp × Clsz	0.530	3, 312	**0.620**

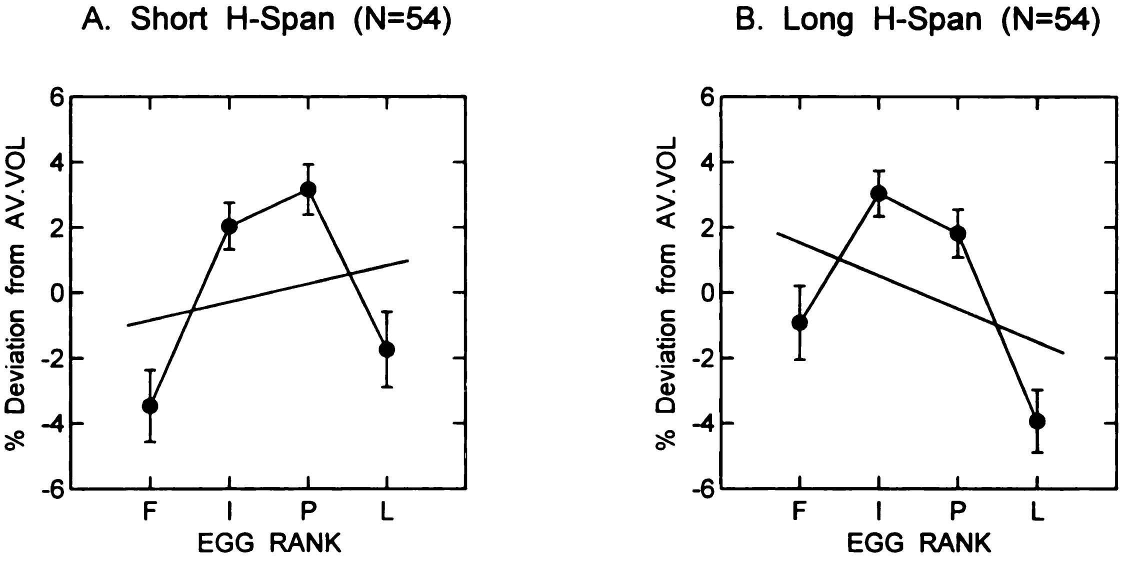


Figure 6. Clutch profile by H-Span (pooling clutch sizes). Error bars and regression lines as in Figure 4.

In nests with delayed incubation development, indicated by a short H-Span, egg size increases from the first until the penultimate egg, last eggs are larger than first ones, and the overall linear trend of clutch profile is positive (Figure 6A). When incubation develops early in the laying period (and H-Span is consequently long), the linear trend of clutch profile becomes negative, as penultimate eggs become smaller than intermediate, and final eggs smaller than first (Figure 6B). Thus, as the hormonal hypothesis predicted, H-Span (the index of female incubation) had a significant (Table 22) negative effect on the relative size of last eggs and, more generally, the linear trend of clutch profile. The change in the relative sizes of intermediate and penultimate eggs is also statistically significant. When only these 2 ranks are used in an RMA (first and last excluded), the Rank × H-Span interaction is still significant ($F = 8.544$, d.f.=1,106, $P = 0.004$). This shift of the profile's peak to an earlier position when H-Span increases is also consistent with the hormonal hypothesis. Constraints on egg size, induced by hormonal changes associated with the onset of incubation, should affect all eggs that are not fully formed at the time of the latter (Arnold 1991). Thus, when incubation starts early in the laying sequence (and H-Span is long), there may be time for these constraints to exert their influence on penultimate eggs. This may not be the case when incubation starts later (and H-Span is short), and last eggs may be the only ones affected.

The quadratic component of the Rank × H-Span interaction was non-significant (Table 22), indicating that H-Span did not affect that trend of clutch profile. Indeed, both profiles depicted in Figures 5A and 5B are arched, and vary little in the overall difference between marginal (first and last) and middle (intermediate and penultimate) eggs, even though there were differences in the relative sizes of eggs *within* these two groups (see above). A significant quadratic Rank × among-factor interaction was not expected in this case, neither is it predicted by either hypothesis in any of the following analyses. Thus, I will not discuss such terms, unless they happen to be significant against expectations.

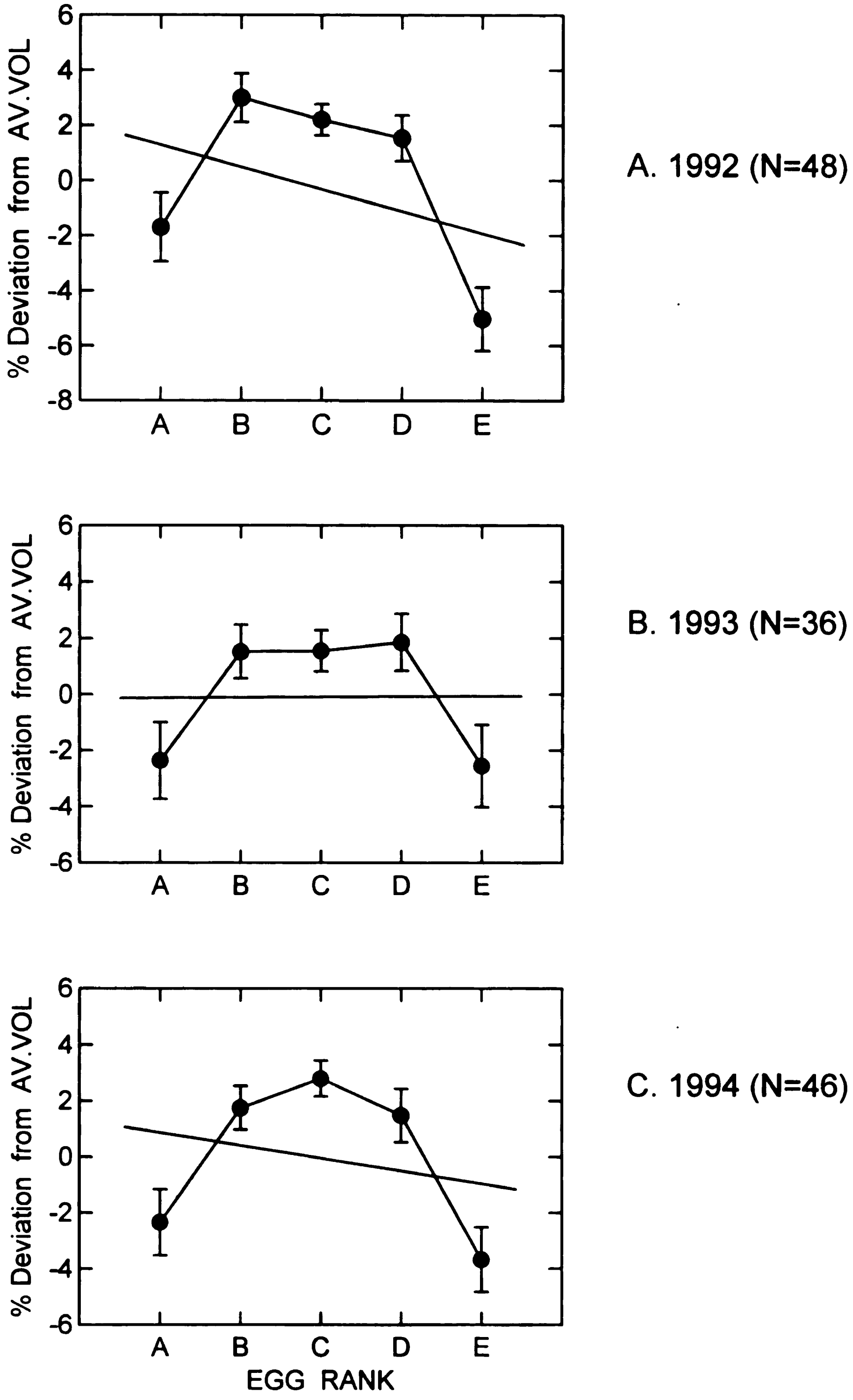
Analysis by Year and Season

I tested the effects of Year and Season on the profiles of 4- and 5-egg clutches separately, because of the difference between the 2 groups in the hormonal hypothesis' predictions concerning the seasonal variability in profile (see earlier). Since the 2 samples were not pooled or compared, I used the original 5 egg ranks (A-E) in C5 nests.

The profile of 4-egg clutches did not vary significantly either among years or between early and late nests (Table 23A). This is as both the nutritional and the hormonal hypotheses predicted, so results are consistent with both. There was no seasonal variation in the profile of C5 nests (Table 23B), even though the hormonal hypothesis predicted relatively smaller final eggs late, due to the seasonal increase in H-Span. On the contrary, the annual variation in the profile of C5 nests approached statistical significance (Table 23B), something that neither the hormonal nor the nutritional hypotheses predicted.

Table 23. RMAs on egg volume by Year and Season (among-nests factors), and by Rank (within-clutches factor) in each clutch size (C4 and C5).

	Source	*F*-ratio	D.F.	*P*-value
	Rank × Year	0.380	6, 240	0.867
A. C 4	Rank × Season	0.072	3, 240	0.961
	R × Y × S	1.528	6, 240	0.181
	Rank × Year	2.079	8, 496	**0.060**
	Linear	2.069	2, 124	0.080
B. C 5	*Quadratic*	0.544	2, 124	0.181
	Rank × Season	1.046	4, 496	**0.369**
	R × Y × S	1.149	8, 496	0.334



A. 1992 (N=48)

B. 1993 (N=36)

C. 1994 (N=46)

Figure 7. Clutch profile in 5-egg nests by Year.
Error bars and regression lines as in Figure 4.

Figure 7 shows that the annual variation in the profile of 5-egg clutches was not great (besides not being highly significant statistically), and mainly involves the pattern's linear trend. The latter appeared to differ the most between 1992 (negative) and 1993 (horizontal), but not as much as between nests with short and long H-Span (positive vs negative; Figure 6). This variation may indicate that additional, unidentified factors could weakly affect clutch profile in ways that do not involve H-Span or AvVol, but it does not argue against the hormonal and nutritional hypotheses.

The lack of a seasonal trend in the profile of 5-egg clutches is intriguing, given the significant seasonal increase in the H-Span of C5 nests. The latter, however, was found in a sample of 73 nests of known hatching intervals (see section 6 in *Preliminary Analyses*). For this RMA, I used a larger sample (130 clutches), where H-Span may have varied less between early and late nests. This is unlikely, though, as the difference in median laying date between early and late clutches is the same (12 d) in both samples.

A possible explanation for the disagreement between the hormonal hypothesis' prediction and the observed results is that the seasonal variability in H-Span is not strong enough to affect clutch profile. In Table 24, I compare the difference in H-Span between early and late nests with that between clutches with short and long H-Span (which, as I showed earlier, was sufficient to affect profile). In both cases, the difference between medians is the same (4.0 vs 5.0 d), but this may be misleading, as these were the modal H-Span values in the respective samples. As minimum and maximum values show, the ranges of short- and long-H-Span nests barely overlap (because this is how the 2 groups were divided), while the H-Span range of early nests completely envelops that of late clutches. In agreement with this, the difference in mean H-Span is much greater in the former (1.6 vs 0.7 d). Therefore, the seasonal variation in H-Span may be statistically significant, but inadequate to produce an observable effect on clutch profile. It is also possible that some other, unknown seasonal factor opposes the effect of H-Span on profile, but neither my data nor information from the literature suggest any such factor.

Table 24. Statistics on H-Span (d) by Season and by Hatch Span in 5-egg clutches.

	Season		Hatch Span	
Statistic	**Early**	**Late**	**Short**	**Long**
N	35	38	36	37
Min	1.50	2.00	1.50	4.50
Max	6.50	6.00	4.50	6.50
Mean	4.0	4.7	3.6	5.2
Median	4.0	5.0	4.0	5.0

Analysis by Egg Size (Egsz) and Clutch Size (Clsz)

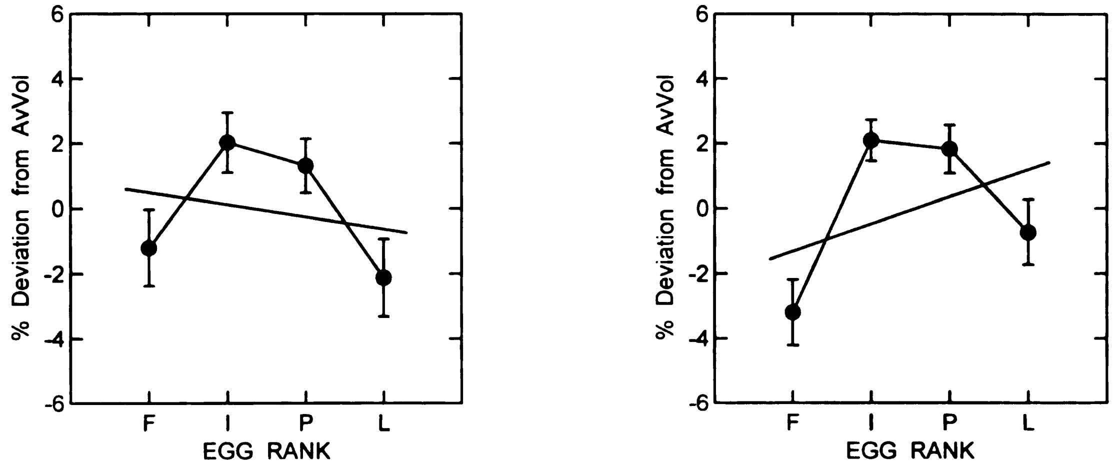
The effect of Egsz (which reflects AvVol, the mean egg volume of clutches) on clutch profile tests the main prediction of the nutritional hypothesis of a positive association between AvVol and the relative size of last eggs. The effect of Clsz tests indirectly the hormonal hypothesis, as H-Span (and the time between the onset of incubation and the laying of the last egg) is longer in the larger clutches.

The results of an RMA by Egsz and Clsz on the total sample of 216 nests (86 C4 and 130 C5) are shown in Table 25. The *among*-nests part of the table shows that Clsz had no effect on AvVol (as the preliminary analyses also showed). Thus its possible effect on clutch profile will not be confounded by any variability in AvVol between its levels. The significant among-nests effect of Egsz is not surprising, as this factor's levels represent low- and high-AvVol clutches. The significant Clsz × Egsz interaction indicates that the magnitude of the difference in AvVol between small- and big-egg nests varies between clutch sizes (I will refer back to this later).

The Rank × Egsz × Clsz interaction in the *within*-clutches part of Table 25 approached statistical significance. This indicates that the Egsz-effect on profile may vary between clutch sizes, and vice versa. Therefore, I tested each factor within each level of the other, thus running 4 separate RMAs, each with 1 among-nests factor. These are essentially 4 pairwise comparisons among the 4 different sub-samples (Egsz × Clsz level combinations). Therefore, I applied the Bonferroni correction to the critical *P*-level, which I reduced to 0.0125 (0.05 divided by 4). The average clutch profiles of the 4 samples are illustrated in Figure 8 (A-D).

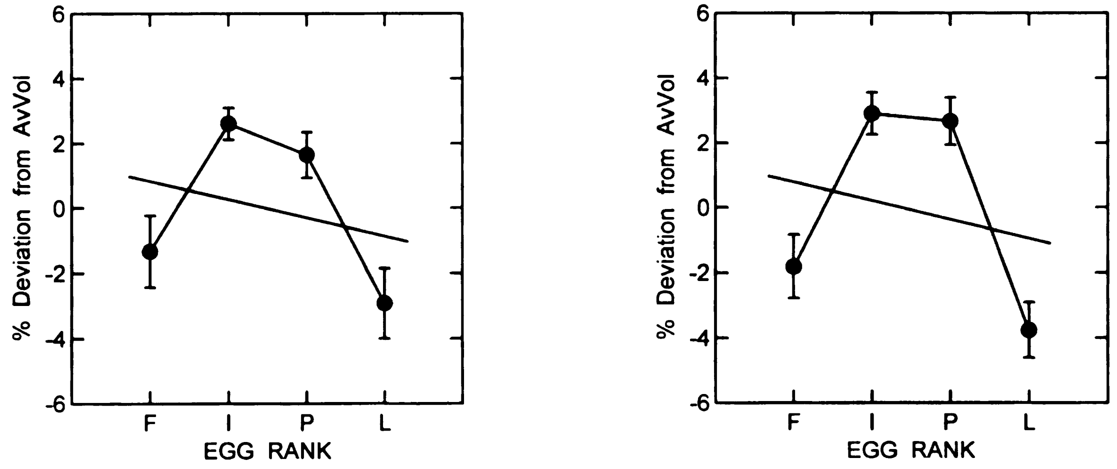
Table 25. RMA on egg volume by Rank (within-clutches factor), and by Egsz and Clsz (among-clutches factors).

Source	***F*-ratio**	**D.F.**	***P*-value**
Among Clutches			
Clsz	1.058	1, 212	0.305
Egsz	329.256	1, 212	< 0.001
Clsz × Egsz	5.024	1, 212	**0.026**
Within Clutches			
Rank	89.695	3, 636	< 0.001
Rank × Clsz	6.011	3, 636	0.002
Rank × Egsz	3.989	3, 636	0.015
R × C × E	2.702	3, 636	**0.061**



A. CLSZ = 4, Low AvVol (N=43)

B. CLSZ = 4, High AvVol (N=43)



C. CLSZ = 5, Low AvVol (N=65)

D. CLSZ = 5, High AvVol (N=65)

Figure 8. Clutch profile by clutch size (Clsz) and mean egg size (AvVol). Error bars and regression lines as in Figure 4.

Egsz had a significant effect on the profile of 4-egg clutches (Table 26A), mainly due to its influence on the latter's linear trend (whose *P*-value was slightly greater than the corrected critical level). Clutch profile varied between samples in the direction predicted by the nutritional hypothesis: a) in low-AvVol nests (Figure 8A), there was a weak overall decline in egg size with laying order, while b) high-AvVol clutches (Figure 8B) showed an overall increase in egg size with Rank, and a higher relative last-egg size. The quadratic effect of Egsz on the profile of 5-egg clutches appears to be statistically significant, but is not protected by the significance of the overall Egsz-effect (Table 26B). In agreement with this, the differences between the respective samples' profiles appear to be very small (Figures 8C and 8D).

The absence of the expected Egsz-effect on the profile of C5 nests could be due to a smaller difference in mean AvVol between the levels of this factor than in C4 clutches. However, this difference was actually greater in 5-egg nests (17.06 - 15.17 = 1.89 cm^3) than in C4 nests (16.95 -15.48 = 1.47 cm^3), and the trend is statistically significant, as the Clsz × Egsz interaction in the *among-clutches* part of the RMA of Table 25 indicated. Alternatively, the difference in the response of clutch profile to Egsz between the 2 clutch sizes (which also differ in H-Span) can be interpreted as evidence that both AvVol and H-Span can influence clutch profile in an interactive way. It seems that last eggs can benefit from extra resources (as the nutritional hypothesis predicts) only in C4, where H-Span is short, and hormonal constraints on final-egg size are presumably weak. In C5, the stronger hormonal constraints (inferred from the longer H-Span) apparently prevent additional resources (indicated by higher AvVol) from being invested in final eggs.

These results refute the hypothesis that the size of *first* eggs is nutritionally constrained: a) in C5 nests, overall clutch profile, and the relative size of first eggs, were not affected by Egsz (AvVol), while b) in C4, first eggs were actually relatively *smaller* in high-AvVol clutches (where more resources were presumably available for eggs).

Table 26. Separate RMAs on egg volume by Egsz (among-clutches factor), and by Rank (within-clutches factor), in each clutch size (Clsz). Bonferroni-corrected critical *P*-level: 0.0125.

Source	***F*-ratio**	**D.F.**	***P*-value**
A. Clsz = 4			
Rank × Egsz	4.168	3, 252	**0.010**
Linear	6.285	1, 84	**0.014**
Quadratic	2.312	1, 84	0.132
B. Clsz = 5			
Rank × Egsz	2.757	3, 384	**0.062**
Linear	0.040	1, 128	0.842
Quadratic	10.458	1, 128	**0.002**

Table 27. Separate RMAs on egg volume by Clsz (among-clutches factor), and by Rank (within-clutches factor), in each level of factor Egsz. Bonferroni-corrected critical *P*-level: 0.0125.

Source	***F*-ratio**	**D.F.**	***P*-value**
A. Low Egsz (Av Vol)			
Rank × Clsz	0.501	3, 318	0.627
B. High Egsz (Av Vol)			
Rank × Clsz	8.228	3, 318	< 0.001
Linear	9.788	1, 106	0.002
Quadratic	6.826	1, 106	0.010

When AvVol is low, profile does not vary between clutch sizes (Figures 8A and 8C; Table 27A), despite their difference in H-Span, and against the hormonal hypothesis' prediction. In high-AvVol nests, however, Clsz had a significant overall and linear effect on clutch profile (Table 27B), in the direction predicted by the hormonal hypothesis. In C4 nests (Figure 8B) egg size increased overall with laying order, while in C5 the linear trend was negative, and last eggs were relatively smaller (Figure 8D). The 2 profiles also differ in their quadratic components (Table 27B), but this is only due to the greater drop from the penultimate to the last egg in C5 (which is also responsible for the linear Clsz-effect). The relative sizes of the first 3 eggs are similar in both clutch sizes (Figures 8B and 8D; n.s. Rank × Clsz interaction in RMA using only these 3 ranks: $F = 0.220$, d.f.= 1,212, $P = 0.803$). Thus it seems that last eggs a) can benefit in the smaller clutches from weaker hormonal constraints on their size (inferred from their shorter H-Span), as the hormonal hypothesis predicts, only when b) their size is not nutritionally constrained (only in high-AvVol clutches).

These results confirm the implication of the Rank × Egsz × Clsz interaction in Table 25: profile's response to Egsz does differ between clutch sizes, and its response to Clsz does depend on the level of AvVol. Therefore, the splitting of a single RMA with 2 among-nests factors into the 4 single-factor analyses presented above was appropriate.

The partial confirmation of the nutritional and hormonal hypotheses' predictions indicates that both AvVol and H-Span (the profile-relevant factor that varies between clutch sizes) may affect clutch profile in an interactive way. Moreover, the specific cases where each factor's effect on profile did not meet the respective hypothesis' prediction can be attributed to interference from the other profile-relevant factor.

These results also reinforce my earlier statement (in *Predictions Tested*) that the nutritional and hormonal hypotheses concerning clutch profile need not be mutually exclusive. I have shown here that both factors postulated by these hypotheses to affect clutch profile can actually influence it, and that their effects are not independent.

C. CLUTCH PROFILE TYPES

The results I have presented so far indicate that, on the average, the Squacco Heron's clutch profile is arched, as middle eggs differ little among themselves, but are significantly larger than marginal ones (first and last). However, the profiles of individual clutches vary, and most of them can be classified in the following 4 types, which are also illustrated in Figure 9 (in which 4- and 5-egg clutches are pooled):

a) *Decline* (Dcln), where egg size decreases from the first egg to the last (panel C);

b) *I-max*, where the profile is arched and the intermediate egg is the largest (panel A);

c) *P-max*, where the profile is arched and the penultimate egg is the largest (panel B);

d) *Rise*, where egg size increases from the first egg to the last (panel D).

Since the clutches making up each of these groups were specifically selected to fit a certain profile type, there was no reason to test the statistical significance of the pattern of egg-size variability in each sample. The average difference between the smallest and largest eggs varies from 8% of AvVol in I-max to 12% of AvVol in Decline.

There were also 22 other clutches (10% of total sample), whose profile did not fit the above patterns, and which I designated as *Irregular*. Approximately half of them showed erratic fluctuations in egg size, while differences among egg ranks in the rest were very small and statistically non-significant (overlap of confidence intervals set on

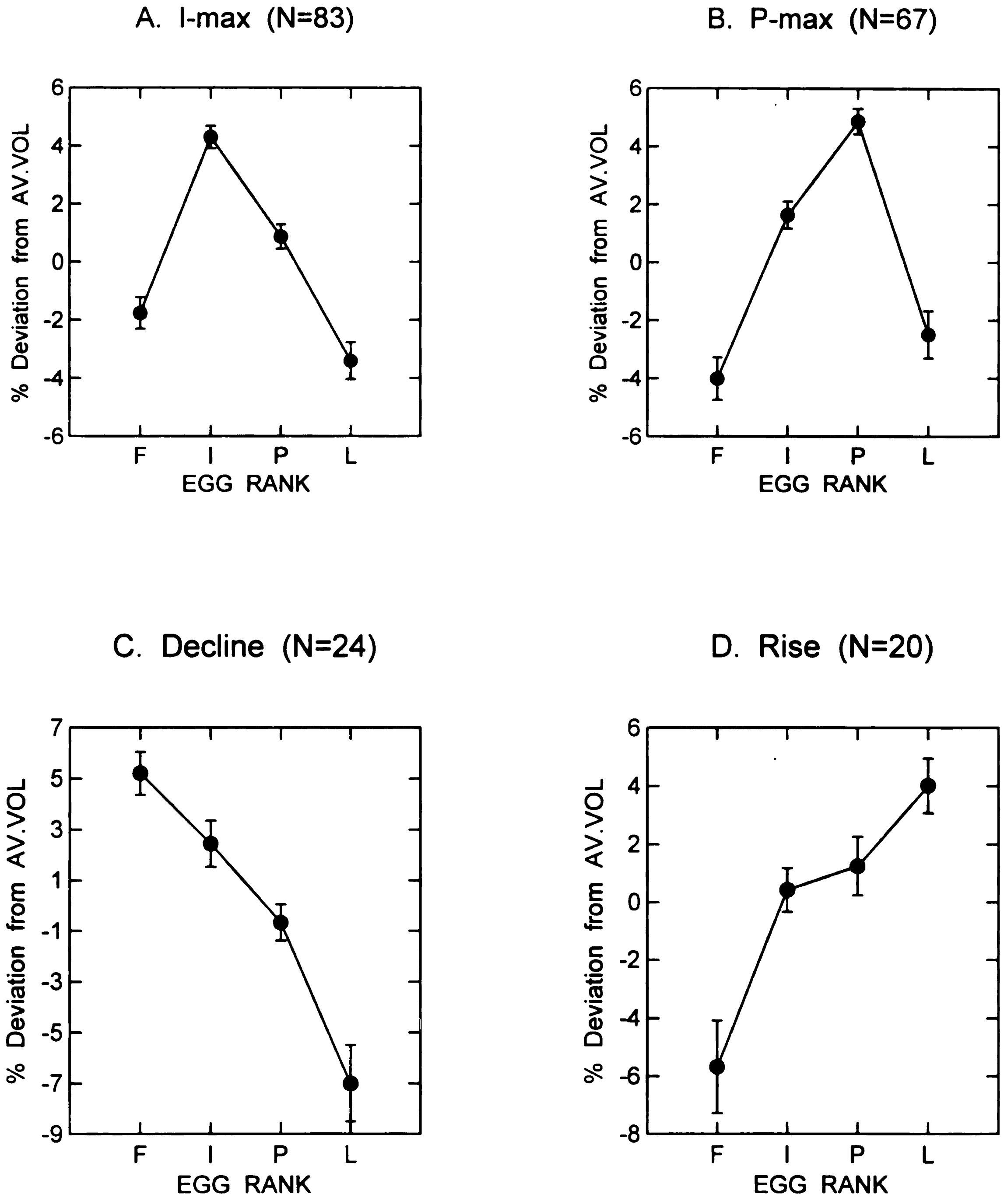


Figure 9. Clutch profile types. Error bars represent 95% confidence intervals.

egg-size estimates based on the regression of egg volume on linear egg measurements presented in the *Preliminary Analyses*). The average profile of Irregular clutches shows very low variability in egg size: last eggs are smaller than the previous ones (whose sizes are very similar) by less than 1.5 % of AvVol (average profile not shown in Figure 9).

Table 28 shows the frequencies of the different profile types in each clutch size, and in the pooled sample. In both C4 and C5, the 2 arched profiles (I-max and P-max) are the only common ones, and together they make up almost 70% of the pooled sample. Each of the other 3 profile types represents approximately 10% of all clutches. The difference in relative frequencies between clutch sizes did not seem to be great, and it only approached statistical significance ($X^2 = 8.167$, d.f.= 4, $P = 0.086$). Apparently, the most striking difference involves Rise, which is overall uncommon, but particularly so in C5, where its percentage is only about 1/3 as among 4-egg clutches. This is consistent with the hormonal hypothesis, as the stronger hormonal constraints on the size of last eggs in 5-egg clutches (inferred from their longer H-Span) should make this rare profile type even less common there than among C4 nests.

Table 28. Numbers and percentages of clutches with different profile types.

Clutch		**Decline**		**I-max**		**P-max**		**Rise**		**Irregular**	
Size	**N**	**#**	**%**	**#**	**%**	**#**	**%**	**#**	**%**	**#**	**%**
C 4	86	9	10.5	27	31.5	26	30.0	13	15.0	11	13.0
C 5	130	15	11.5	56	43.0	41	31.5	7	5.5	11	8.5
Both	216	24	**11.0**	83	**38.5**	67	**31.0**	20	**9.5**	22	**10.0**

The 4 "regular" profile types, in the order in which I presented them above (Decline, I-max, P-max, Rise), form a gradient along which the rank of the largest egg shifts to a progressively later position in the laying order, and the relative size of the last egg increases (Figure 9). According to the hormonal hypothesis, these phenomena mainly depend on the timing of the onset of female incubation, which I infer from hatch span values. Therefore, this hypothesis would predict a gradual shortening in H-Span (negative linear trend), along this profile gradient. The nutritional hypothesis postulates that the relative size of last eggs is positively associated with female resource availability for egg formation, which I infer from clutch AvVol. Therefore, this hypothesis would predict an increase in AvVol (positive linear trend) along this profile gradient.

As I explained above, nests with Irregular profiles are a heterogeneous group, comprising clutches whose profile did not fit any of the 4 other types. Thus I was unable to derive predictions from the 2 hypotheses about the values of H-Span and AvVol in this group relative to the other profile types. Nevertheless, I included Irregular clutches in a *post hoc* analysis of AvVol (see below). I was unable to do so in the analysis of H-Span, due to the lack of adequate hatching data from such clutches.

The above tests of the 2 hypotheses involve the reversal of the positions of the dependent variable (clutch profile) and certain independent factors (H-Span and AvVol) of last section's RMAs. The benefit of this approach is that the new independent factor, clutch profile type, has more levels (4), across which I can test the predicted linear trends in H-Span and AvVol. These corroborative analyses are not merely redundant, especially in the case of AvVol, where they provide additional insight to its proximate effects on clutch profile (see below).

Figure 10 shows the trends in median H-Span among profile types in 4- and 5-egg clutches. As the hormonal hypothesis predicted, there is an overall decrease in H-Span along the profile gradient in both cases. Separate Kruskal-Wallis tests in each clutch size indicated that the observed trend approached statistical significance in both samples (C4: $H = 6.703$, d.f.= 3, $P = 0.082$; C5: $H = 7.134$, d.f.= 3, $P = 0.068$). I used non-parametric tests, because, as I mentioned earlier, H-Span is not normally distributed. I did not pool 4- and 5-egg clutches together, despite the fact that the same trend was predicted in both, because differences in the relative frequencies of Decline and Rise would bias results in a way favorable to the hormonal hypothesis' prediction. Decline, whose median H-Span was expected to be the longest, is more common in C5, where H-Span is generally longer than in C4. The opposite is true for Rise, whose H-Span was expected to be the shortest.

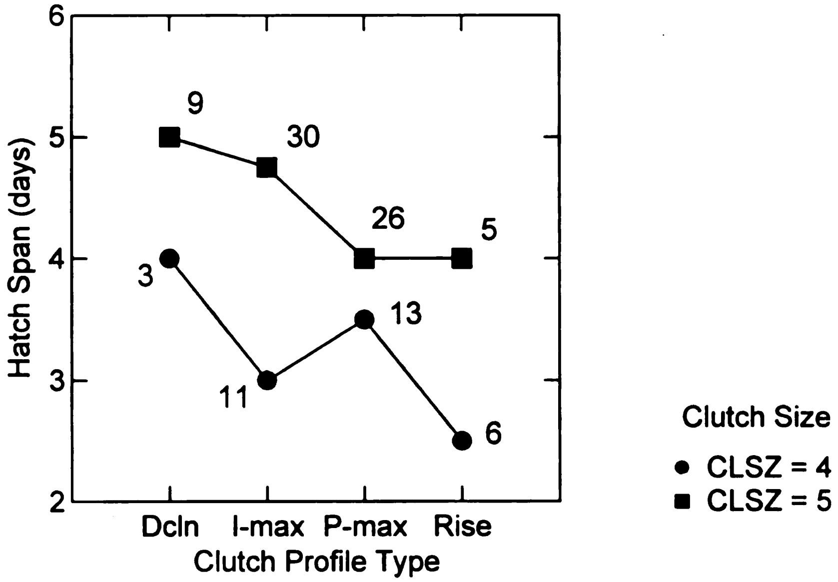


Figure 10. Median H-Span by profile type and clutch size. Sample sizes are indicated next to data points.

The variation in median H-Span among profile types shown in Figure 10 appears substantial in both clutch sizes, and especially so among C4 nests, where the difference in median H-Span between Decline and Rise is 1.5 d, or 50% of the overall median (in C5 this difference is 1.0 d). Therefore, the apparent lack of statistical significance in these trends may in good part be due to the expected low power of the respective tests. The reasons for the latter are: a) the small size of the Decline and Rise samples (see Figure 9), and b) the high random error in H-Span due to low data accuracy, the male's contribution in incubation, and the possible variability in egg-laying intervals (details can be found in the *Preliminary Analyses* section on hatching intervals). The latter variability is not likely to have biased results, because laying intervals did not differ significantly between profile types that were expected to have a long H-Span (Decline and I-max) and those predicted to have a short H-Span (P-max and Rise), as shown in Table 29. (The pooling of these groups was necessitated by the small samples of Decline and Rise. Laying intervals were inversely estimated from the number of eggs laid during 4-day intervals between nest visits, as in the respective section of the *Preliminary Analyses*.)

Table 29. Frequencies of cases where 2 or 3 eggs were laid in 4 d for pairs of profile types, and result of statistical comparison.

	Dcln + I-max		**P-max + Rise**	
	#	**%**	**#**	**%**
2 eggs in 4 d	77	86.5	72	80.0
3 eggs in 4 d	12	13.5	18	20.0
Total	89	100	90	100
2-tailed Fisher exact $P = 0.317$ n.s.				

In 5-egg clutches, where H-Span increases seasonally (see earlier), results could be confounded by a strong variation in laying date among samples. Differences in median standardized laying date, however, were not statistically significant (Kruskal-Wallis $H = 0.085$, $P = 0.994$), and of a magnitude (the largest such difference was 3.0 d) that is not expected to affect H-Span (see section 6 in *Preliminary Analyses*).

Results, however, may be biased *against* the predictions of the hormonal hypothesis by a positive association between egg size and the length of the incubation period (Parsons 1972; Bollinger 1994). Such a relationship has not been demonstrated in herons, but, if present, it will have the following consequences. In Decline, the relatively large first eggs will take longer than average to hatch, while the small last eggs will hatch relatively quickly. This will shorten the H-Span of clutches expected to have the longest H-Span. The opposite is true in Rise, the profile type expected to have the shortest hatch span, where the small size of the first eggs and the large size of the last eggs will tend to lengthen it. The hatch spans of I-max and P-max clutches should not be affected, as both first and last eggs are similarly small in these groups.

In summary, the observed trend in H-Span among profile types agrees with the hormonal hypothesis' prediction, despite the possible bias against the expected results described above. This trend appears to be strong in both clutch sizes, and its lack of statistical significance may be attributed to low power in the respective tests (see earlier). Overall, these findings corroborate the results of last section's analyses of clutch profile (RMAs), and further support the association between H-Span and clutch profile predicted by the hormonal hypothesis.

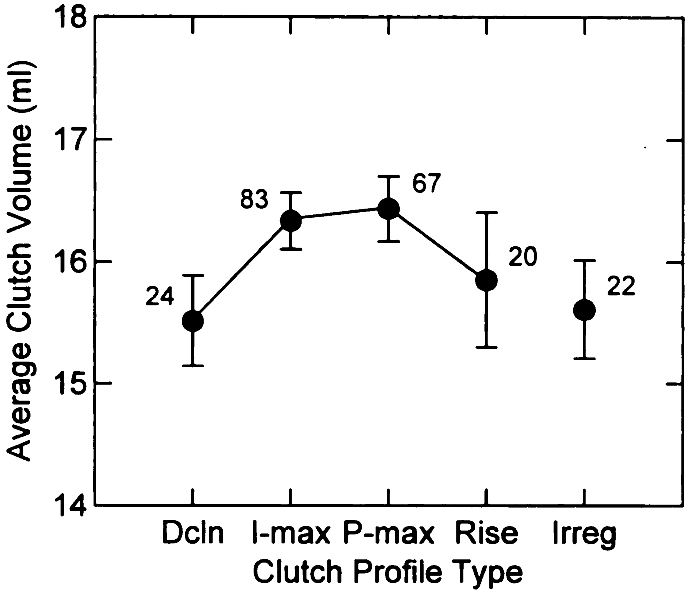


Figure 11. AvVol by profile type (means with 95% confidence intervals). Sample sizes are indicated next to data points.

The variability in AvVol among the 4 regular profile types did not follow the pattern that was predicted by the nutritional hypothesis (Figure 11; data points for these 4 groups are connected with solid lines; I will refer to Irregular clutches later). Instead of a linear increase in AvVol along this profile gradient, the predominant (and statistically significant) trend was quadratic (Table 30), as mean AvVol in I-max and P-max was greater than in clutches with linear profiles (Figure 11). The non-significant interaction between Clsz and profile type (Table 30) indicates that the pattern of variation in AvVol among profile types does not differ between clutch sizes.

Figure 11 indicates that Irregular clutches also have a relatively low mean AvVol. A 1-way ANOVA on AvVol (pooling clutches of both sizes) by profile type including Irregular nests (among all 5 profile groups) yielded significant results ($F = 5.943$, d.f.= 4,211, $P < 0.001$). Tukey's pairwise-comparison probabilities confirmed that the mean

AvVol of Irregular clutches is similar to those of Decline ($P = 0.998$) and Rise ($P = 0.946$), but significantly smaller than those of I-max ($P = 0.032$) and P-max ($P = 0.012$).

In summary, AvVol, which I use as an index of female resource availability for egg production, seems to be higher among clutches with the common, arched profiles, than in the 3 other groups, which are relatively rare. This suggests the possibility that Decline, Rise, and Irregular profiles represent deviations from the normal, arched pattern, produced by the combination of hormonal factors and nutritional constraints on the size of various, but mainly middle, eggs. For instance, the delayed onset of incubation (inferred from short H-Span) may be necessary, but not always sufficient to produce an increase in egg size with laying order until the last egg. The size of the usually large middle eggs may also need to be constrained by resource limitations. (I will discuss these issues in greater detail in the following section.)

Table 30. ANOVA on AvVol by clutch size and profile type (excluding Irregular).

SOURCE	Sum Sq.	d.f.	Mean Sq.	*F*-ratio	*P*-value
Clsz	1.395	1	1.395	1.220	0.271
Profile	18.292	3	6.097	5.332	**0.002**
Linear	0.728	1	0.728	0.636	0.426
Quadratic	16.826	1	16.826	14.715	**< 0.001**
Cubic	0.002	1	0.002	0.002	0.968
Clsz × Prof	0.511	3	0.170	0.149	0.930
Error	212.684	186	1.143		

4. DISCUSSION

In the Squacco Heron, the average egg-size profile of both 4-egg (C4) and 5-egg (C5) clutches is arched (Figure 4), as marginal eggs (first and last) are significantly smaller than middle ones (Table 21). In these average profiles, the greatest difference between egg ranks (laying-order positions) is 4.3% of AvVol in C4 (between A and B), and 6% of AvVol in C5 (between C and E). Arched profiles (Figures 9 A-B) are also the commonest, both in C4 (>60% of cases) and in C5 (almost 75% of cases). However, egg size sometimes increases or decreases with laying order (Figures 9 C-D), or it may vary irregularly. Each of the latter 3 profile types represents about 10% of the total sample (pooling C4 and C5; Table 28). Due to this variation in the ranks of smallest and largest eggs, when the maximum egg-size difference is calculated *within* clutches, and then averaged, its mean value is about 9% of AvVol in C4 and 11% of AvVol in C5.

The above patterns are not unique among ardeids. Data I collected from a few Little Egret nests at the Axios river delta, suggest that this species' average profile is similar to the Squacco Heron's, both in pattern (though first eggs appear relatively bigger) and in the level of variation among egg ranks (Figure 12). In the Purple Heron in Spain, the mean profile of C4 and C5 clutches was arched in one year, but showed a significant negative linear trend in another (Jover et al.1993). The largest difference among egg-rank means appeared greater than in *A.ralloides* in C5 (9-12% of AvVol by year), but this may be partly due to a lack of significant variation in profile type among clutches. In 3 North American species, who lay only 3 or 2 eggs, egg size decreased with laying order, on the average, but profile type also varied among clutches, especially 3-egg ones (Custer and Frederick 1990).

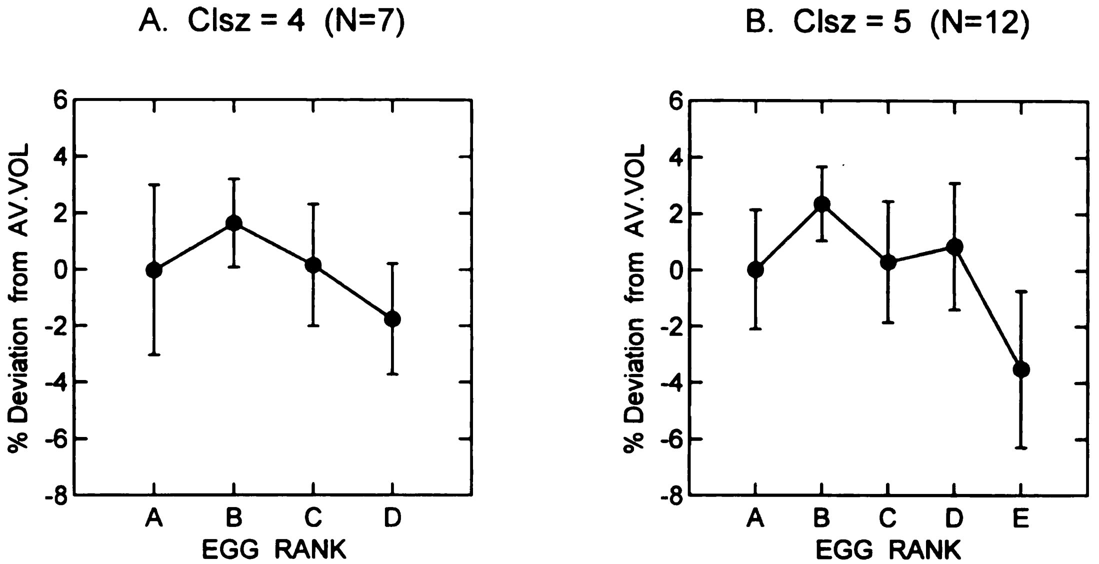


Figure 12. Average egg-size profiles of 4- and 5-egg Little Egret clutches. Error bars represent 95% confidence intervals.

Arched profiles, where first and final eggs are significantly smaller than middle ones, have been observed in birds from taxonomically and ecologically diverse families, and in clutches of widely varying sizes (Table 31). In most species, both first and last eggs were similarly small, but there are also cases with an overall increase or decline in egg size with laying order (but where last and first eggs are still smaller than middle ones). Maximum differences among egg-rank means (*Max %D* in Table 31) appear to vary moderately around my values from Squacco Heron clutches. The lowest (1.7% of AvVol), but still statistically significant value has been observed in the Kentish Plover *Charadrius alexandrinus* (Amat et al. 2001), and the highest in the Purple Heron (12% of AvVol in one year, 9% in another; Jover et al.1993).

On the other hand, clutch profile type shows strong variations within taxonomic groups (from Orders to species). Passerine profiles can range from Rise (Howe 1976) to

Table 31. Avian species with arched clutch profiles (marginal eggs smaller than middle).

Family and Species	Profile [a]	Clsz	Max %D [b]	Source
Phalacrocoracidae				
Shag *Phalacrocorax aristotelis*	A	3	3.0	Stokland and Amundsen 1988
Ardeidae				
Purple Heron *Ardea purpurea*	A [c]	4,5	5.0 - 12	Jover et al.1993
Little Egret *Egretta garzetta*	D	4,5	3.4 - 5.9	This study
Squacco Heron *Ardeola ralloides*	A	4,5	4.3 - 6.1	This study
Anatidae				
Canada Goose *Branta canadensis*	A	4-7	3.5 - 6.5	Leblanc 1987
Barnacle Goose *Branta leucopsis*	D	3,4	5.5 - 8.0	Williams et al.1996
Wood Duck *Aix sponsa*	A	6-11	5.0 - 6.0	Kennamer et al.1997
Rallidae				
American Coot *Fulica americana*	A	7-13	4.0 - 8.0	Arnold 1991
Haematopodidae				
American Oystercatcher *Haematopus palliatus*	R	3	3.6	Nol et al.1984
Charadriidae				
Kentish Plover *Charadrius alexandrinus*	A	3	1.7	Amat et al. 2001
Sturnidae				
European Starling *Sturnus vulgaris*	A	5,6	3.0 - 6.0	Ojanen et al.1981
Turdidae				
European Blackbird *Turdus merula*	R	3-5	2.5 - 4.5	Magrath 1992 a
Corvidae				
Northwestern Crow *Corvus caurinus*	D	4,5	6.0 - 8.0	Verbeek 1990

[a] A: True Arch (first and last eggs are of similar, and small, size).
D: Arched Decline (first eggs smaller than intermediate, but greater than last).
R: Arched Rise (increase in egg size from first to penultimate eggs; last < penultimate).

[b] Difference between egg-ranks of highest and least mean size, as % of clutch average. In all cases, intraspecific variation is among clutches of different sizes.

[c] In 1 year only. In another year, only the profile's negative linear trend was significant.

Decline (Rofstad and Sandvik 1985), through a number of intermediate patterns (Ojanen et al.1981), and may even vary within populations (Mead and Morton 1985). Profile type also varies among shorebird (Charadrii) species (Vaisanen et al.1972), and within the genus *Fulica*: it is arched in *F. americana* (Arnold 1991), but egg size increases with rank in the European Coot *F. atra* (Horsfall 1984). In gulls (genus *Larus*), egg size usually decreases with laying order (Parsons 1976; Reid 1987; Sydeman and Emslie 1992), but clutch profiles can sometimes appear arched (Meathrel et al.1987), or flat (show little egg size variation), which may be the result of abundant food (Pierotti and Bellrose 1986).

This variability in profile type, especially within populations (Mead and Morton 1985; Custer and Frederick 1990; this study) suggests that clutch profile is amenable to the influence of proximate factors, which I examined in this study. Whether this also reflects adaptive adjustments of reproductive strategy to environmental conditions and/or parental attributes (Slagsvold et al.1984), is beyond the scope of my thesis. Nevertheless, the level of egg-size variation within Squacco Heron clutches may potentially affect sibling fitness (see below), and thus has additional ecological interest, whether it may be an adaptation for or a constraint on parental reproduction.

Data I collected from unfed hatchlings, indicate that their weight increases in direct proportion to egg volume, but their bill+head length increases less than proportionately (log-log regression slopes were, respectively, similar to and less than 1; Table 32). Thus egg size seems to influence Squacco hatchling body condition, possibly by affecting the amount of remaining yolk (which I could discern below young nestlings' abdominal skin, but was unable to quantify), as has also been found in anatids (Ankney 1980; Pelayo and Clark 2002), quail (Ricklefs et al.1978), shorebirds (Amat et al. 2001), gulls (Parsons

1970), and crows (Rofstad and Sandvik 1987). In synchronous broods, egg size can affect nestling growth (Howe 1976; Smith et al.1995; Anderson and Alisauskas 2002) and survival up to fledging (Blomqvist et al.1997; Styrsky et al. 2000; Amat et al. 2001). In asynchronous broods, where junior siblings are in a competitive disadvantage, egg size variation was deemed to have no impact on sibling hierarchies in the Shag (Stokland and Amundsen 1988) and the European Blackbird (Magrath 1992a), where it was low (Table 31), or in the Purple Heron, where it was higher (Jover et al.1993). In the Squacco Heron, even though a chick's competitive status may be unaffected by relative egg size, the latter may still influence early nestling survival through its effect on yolk reserves and the chick's ability to withstand starvation (Parsons 1970,1975a; Howe 1978; Williams 1994). (The consequences of clutch profile on Squacco sibling fitness are currently under study, but will be published elsewhere.)

Table 32. Logarithmic regression of hatchling weight (g) and bill+head length (mm) on egg volume (cm^3). *P*-levels pertain to slope comparisons with values of 0 and 1.

Variable	***n***	**Slope**	**S.E.**	***P* / 0.0**	***P* / 1.0**	R^2
Weight	46	1.073	0.068	< 0.001	n.s.	0.873
Bill + Head	46	0.215	0.026	< 0.001	< 0.001	0.615

The main focus of my thesis is the examination of certain proximate factors that may influence clutch profile, and especially the relative size of final eggs, in *A.ralloides*. The causes for small final eggs, according to the nutritional and hormonal hypotheses, are female resource limitations and the early onset of incubation (Parsons 1976). However, as I was unable to directly measure these variables, I used as indices of their values the average egg volume (AvVol) and the hatching span (H-Span) of clutches. As I explained in the respective sections of the *Preliminary Analyses* chapter, the use of these indices is supported both by the literature and by my own data. Moreover, potential associations between AvVol or H-Span and other variables may increase the random error in the relationship between each index and the profile-relevant factor it represents, but are not expected to produce any spurious associations between these indices and clutch profile. Thus, since I did find that both H-Span and AvVol can affect clutch profile (mostly in ways predicted by the respective hypotheses), I was able to reach some (at least tentative) conclusions about the validity of these hypotheses in the Squacco Heron. The lack of a correlation between AvVol and H-Span (see last section in *Preliminary Analyses*), also allowed me to reach such conclusions without experimentally controlling these variables, as there was no risk of each confounding the other's effects.

Parsons's (1976) hormonal hypothesis postulates that the development of female incubation is associated with an increase in circulating prolactin, which depresses the function of the oviduct, and thus limits the size of subsequently-laid eggs (only last ones in the case of gulls, and due to a deficiency in albumen). In populations where the timing of incubation onset varies among females (as in *A.ralloides*, see section 6 in *Preliminary Analyses*), this hypothesis would predict that in clutches with a longer H-Span a) final eggs would be relatively smaller, and b) the decline in egg size might begin at an earlier point in the laying order (Arnold 1991).

Both predictions were confirmed by a comparison of the egg-size profiles of nests with short (< median) and long (> median) H-Span (Figure 6). The profile's overall linear trend changed from positive in the former sample to negative in the latter, where final eggs were relatively smaller and the profile's arch peaked at an earlier egg-rank (differances are statistically significant; see Table 22 and associated text). Hatch span (and the onset of incubation relative to the laying of the last egg) is much longer in C5 than in C4 nests (4.5 vs 3.0 d ; Table 17). Therefore, the hormonal hypothesis would predict smaller final eggs in the larger clutches. This was confirmed when the Clsz-effect on profile was tested using high-AvVol nests only (Table 27B): the profile's linear trend changed from positive in C4, where last eggs were relatively large (Figure 8B), to negative in C5, where last eggs were relatively smaller (Figure 8D). However, no Clsz-effect on clutch profile was detected when only low-AvVol nests were involved in the comparison (Table 27A; Figures 8A and 8C). This could be the result of interference by resource limitations (inferred from the low AvVol-level) on last-egg size (as predicted by the nutritional hypothesis): if last eggs are nutritionally constrained to a small size in both C4 and C5 nests, variation in the intensity of the incubation-related hormonal constraints may have no further effect on their relative size. This is an indication that the final eggs in Squacco Heron clutches may be under the influence of both nutritional and hormonal factors.

Despite the statistically significant seasonal increase in H-Span in C5 (Table 18), there was no corresponding variation in the profile of 5-egg clutches (Table 23B). This is probably due to the fact that the seasonal trend in H-Span is of insufficient magnitude to produce a measurable effect on clutch profile (see page 80). It could also stem from interference by some additional, unknown factor (not AvVol, which showed no seasonal variation; see Table 14), though no such factor is suggested in the literature. The profile of C5 clutches showed some annual variation (Figure 7), which was not predicted by either hypothesis tested (see Table 20). This variation, however, was weak (Figure 7), and only approached significance (Table 23B), so it does not refute either hypothesis.

The hormonal hypothesis is also supported by the overall decrease in H-Span along the profile type gradient from Decline to Rise (Figure 10). Along this gradient, the largest egg in the clutch is of a progressively later rank, and the relative size of the final egg increases (Figure 9). If these phenomena mostly depend on the timing of the onset of incubation (as the hormonal hypothesis postulates), then this should occur progressively later, and H-Span should consequently become shorter, along this series of profile types. Observations from both C4 and C5 clutches confirmed this prediction (Figure 10), but results only approached statistical significance. The latter, though, may merely stem from a) low test power (due to small sample sizes and high random error; see section 6 in the *Preliminary Analyses*) and b) the fact that a positive relationship between egg size and the length of incubation period would tend to limit H-Span values in Decline and increase them in Rise (see page 93). A final piece of supporting evidence for the hormonal hypothesis is that the uncommon profile type *Rise* was particularly rare in C5 nests (Table 28), where the longer H-Span should make it even less likely for final eggs to attain maximum size within clutches (though the overall variation in profile type frequencies between C4 and C5 nests only approached statistical significance).

In summary, the direct test of the hormonal hypothesis (examination of H-Span influence on clutch profile) showed a strong and statistically highly significant effect on clutch profile in the predicted direction, and most secondary tests corroborated its results. In the 2 exceptions, the lack of the expected seasonal trend in profile is probably due to insufficient variation in H-Span, while the lack of the predicted Clsz-effect in low-AvVol nests can be attributed to interfering nutritional constraints. Thus results suggest that the onset of female incubation (inferred from H-Span values) can affect the point in the laying sequence at which egg size begins to decline, and especially the relative size of final eggs in Squacco Heron clutches.

A direct association between the onset of female incubation and the relative size of last eggs was demonstrated by Parsons (1976) in Herring gulls. In the American Coot, where females lay clutches of 7-13 eggs with arched profiles (Table 31), the points in the laying sequence where egg size peaks and incubation begins are closely associated, and occur progressively later as clutch size increases (Arnold 1991). Moreover, several lines of evidence suggest that nutritional limitations are not responsible for the decline in egg size after the onset of incubation in these coots (Arnold 1991), or in some geese (Leblanc 1987; Williams et al.1996), where authors have attributed this aspect of clutch profile to the hormonal constraints proposed by Parsons (1976).

Given the small clutch size of gulls (3 eggs) and the timing of relevant events, 3rd eggs are smaller than earlier ones, and this is because they lack albumen (Parsons 1976; Houston et al.1983). This deficiency was not alleviated by food supplements in Lesser Black-backed Gulls (though yolk protein did increase in all eggs, and especially in last ones; Bolton et al.1992), and thus seems to result from hormonal constraints on the function of the oviduct. Additional support for a depression of oviduct functions comes from the examination of eggshell-color profiles. Eggshell pigments are synthesized and deposited in the oviduct (in its shell gland area; Burley and Vadehra 1989). Therefore, factors that can constrain albumen synthesis may also limit eggshell pigmentation. Shell color profiles match those of egg size in the Glaucous-winged Gull *Larus glaucescens* (Verbeek 1988) and the Northwestern Crow *Corvus caurinus* (Verbeek 1990). Moreover, they did not seem to stem from resource limitations in either species (Verbeek 1988, 1990), while initial egg removals in the former suggested that only last eggs have lighter shells, irrespectively of absolute laying order (Verbeek 1990). In species that lay larger clutches, though, and where females begin low-intensity incubation early in the laying sequence, both ovarian and oviductal functions could be hormonally constrained (without being completely suppressed), so that egg size could decline after the onset of incubation due to deficits in both yolk and albumen, and with little change in egg composition (Arnold 1991; Williams et al.1996). Whether this is true in *A.ralloides* or other herons is not known, but can be the object of future studies.

In my study, I did not examine the detailed physiological pathways through which female incubation may affect the egg-size profile of clutches. Nevertheless, information from the literature suggests certain mechanisms. First, the onset of incubation seems to be closely associated with an increase in prolactin secretion in many avian taxa (Buntin 1996; Vleck 2002), including both domesticated anatids (Hall and Goldsmith 1983), fowl (Proudman and Opel 1981), doves (Ramsey et al.1985) and canaries (Goldsmith et al. 1984), as well as wild albatrosses (Hector and Goldsmith 1983), kestrels (Sockman et al. 2000), grouse (Etches et al.1979), shorebirds (Oring et al.1988) and passerines (Silverin and Goldsmith 1983). Experimental studies have confirmed the association between these traits in various birds (El Halawani et al.1986; Sharp et al.1989; Youngren et al.1991; March et al.1994), but the precise causal relationships remain uncertain (Goldsmith 1991; Vleck 2002). Nevertheless, this information supports the first step of Parsons's (1976) hormonal hypothesis, that is the connection between the onset of incubation and high levels of circulating prolactin.

The second step in Parsons's (1976) hypothesis concerns the negative effects of increased prolactin secretion on the female's reproductive organs. There is evidence (Buntin et al.1998) that high prolactin levels inhibit the release of the hypothalamic neurotransmitter Gn-RH that stimulates gonadotropin (FSH and LH) secretion by the adenohypophysis (Scanes 1986; Etches 1996), and thus reduce the plasma levels of both FSH (Dawson and Goldsmith 1982; Silverin and Goldsmith 1983) and LH (Lea et al. 1981; El Halawani et al.1991). Both gonadotropins are necessary for the development of the ovary and its gametogenic and endocrine functions (Murton and Westwood 1977; Scanes 1986), while cyclic LH surges induce ovulation (Johnson 1986; Etches 1996). The prolactin-related drop in FSH and LH levels leads to a decrease in ovarian estradiol secretion (Zadworny et al.1989; Sockman and Schwabl 1999), and to follicular atresia (Opel and Proudman 1980). Estradiol stimulates oviduct growth (Murton and Westwood 1977) and albumen synthesis (Johnson 1986), whereas high progesterone levels (as those released by atretic follicles) elicit oviduct regression (Murton and Westwood 1977). Thus, the series of hormonal changes set in motion by the onset of incubation and its concomitant increase in plasma prolactin, results in the suppression of ovarian and oviductal functions (Buntin and Tesch 1985), which may initially limit egg size (Williams et al.1996), and eventually terminate egg-laying (Youngren et al.1991).

I tested Parsons's (1976) hypothesis that the size of last eggs is nutritionally constrained by comparing the profiles of clutches with low and high AvVol (the index of female resource availability for egg formation). I tested the effect of Egsz (the factor whose levels represent the variation in AvVol) on profile in each clutch size separately, because an RMA with both Egsz and Clsz as among-nests factors indicated that this effect might differ between C4 and C5 clutches (the Rank × Clsz × Egsz interaction was marginally significant; see Table 25). In C4 nests, where H-Span is shorter (and hormonal constraints on last-egg size are expected to be weaker), Egsz affected clutch profile in the way predicted by the nutritional hypothesis: a) in low-AvVol clutches (Figure 8A) the profile's linear trend was weakly negative and last eggs had the lowest mean size, while b) in high-AvVol nests (Figure 8B) the overall linear trend was positive and last eggs were relatively bigger, and c) the difference in clutch profile between the 2 samples was statistically significant (Table 26A). In C5 nests, however, where H-Span is longer, and hormonal constraints on final-egg size are expected to be stronger, the effect of Egsz on clutch profile appeared to be very weak (Figures 8C and 8D) and was not statistically significant (Table 26B). Therefore, female resource availability (as inferred from AvVol) seems to be able to affect the size of last eggs only when the latter is not strongly limited by hormonal constraints associated with the onset of incubation. These results, together with the fact that the Clsz-effect on profile depends on the level of AvVol (see earlier), suggest that the egg-size profile of Squacco Heron clutches can be influenced both by hormonal and nutritional factors in an interactive way: each factor's effect becomes apparent only when the other factor's impact is weak.

According to the nutritional hypothesis, no annual or seasonal variation in clutch profile was expected (Table 20B), as these factors did not affect AvVol (Table 14), and none was found (Table 23). The trend in AvVol along the Decline-to-Rise profile-type gradient did not match this hypothesis' prediction of a linear increase, as it was quadratic in shape (Figure 11), and significantly so, statistically (Table 30). Clutches with Irregular profiles also appeared to have a low mean AvVol, which was similar to those of Decline and Rise, but significantly smaller than those of the arched profiles (I-max and P-max), as Tukey's probabilities indicated (see page 95). Thus AvVol tends to be low in all clutches whose profile type deviates from the common, arched types. In Decline, a low AvVol was expected by the nutritional hypothesis, but could also be imposed on clutches of well-provisioned females who begin to incubate very early in the laying sequence, and are thus prevented from investing their abundant resources in their eggs. The negative effect of H-Span on relative final-egg size, together with the low AvVol of clutches with Rise-type profiles, suggest that a delayed onset of female incubation may be necessary, but not sufficient for last eggs to attain maximum size in their clutches: the size of the (normally large) middle eggs may also need to be constrained. This joint requirement may help explain why Rise is so rare (15% in C4, 5.5% in C5), even though last eggs are similar to or greater than AvVol in 45% of C4 and 22% of C5 nests. The existence of clutches where egg size fluctuates strongly and irregularly (zig-zag profiles) indicates that acute (possibly nutritional) constraints may occasionally affect single eggs in the middle of the laying sequence. Such patterns are more likely to be produced by limitations of albumen synthesis and/or deposition, as this occurs separately in each egg (as it passes through the oviduct), whereas yolk is deposited simultaneously in multiple eggs (e.g. in cormorants, Grau 1996; anatids, Alisauskas and Ankney 1994; kestrels, Meijer et al.1989; coots, Alisauskas and Ankney 1985; larids, Moore et al. 2000; passerines, Krementz and Ankney 1985). However, since I did not analyze the composition of Squacco Heron eggs, this possibility remains unverified.

Parsons (1976) rejected the hypothesis that the size of normal last (3rd) eggs of Herring Gulls was nutritionally constrained, because 3rd and later eggs in clutches where early eggs were removed (and the onset of incubation was delayed) were not significantly smaller than 1st and 2nd ones, as long as they were not last. Experiments on other gulls, however, showed that all, and especially last eggs can benefit from food supplements, but that this is due to an increase in yolk (rather than albumen) protein (Bolton et al.1992). The decline in egg size between intermediate and final eggs seen in some coots (Arnold 1991) and geese (Leblanc 1987; Williams et al.1996) cannot be explained by resource limitations, but nutrient availability can affect the relative size of last eggs in the European Blackbird (Magrath 1992b). Species whose final-egg size is particularly susceptible to nutritional limitations, may have evolved Rise-type profiles, because these reduce the risk of last eggs falling below a minimum functional size (Magrath 1992b).

The size of first eggs, and the factors that may influence it, have received a lot less attention. One hypothesis, proposed for small, insectivorous passerines, claims that first-egg size may be nutritionally constrained, because females start forming eggs in a season when ambient temperature and food availability are low, but tend to improve significantly from day to day (Slagsvold and Lifjeld 1989; Nilsson and Svensson 1993a). This hypothesis does not seem to apply to the Squacco Heron, as relative first-egg size a) was not affected by AvVol (the index of female resources for eggs) in C5 clutches (Figures 8C and 8D; Table 26B), while b) it was *negatively* affected by AvVol in C4 nests (Figures 8A and 8B; Table 26A), which is the opposite of this hypothesis' prediction. Another hypothesis, proposed for anatids, postulates that the initial inelasticity of the oviduct limits first-egg width, but poses no such constraints on later eggs, as the passage of the first egg expands this organ (Robertson and Cooke 1993). This anatomical constraint on the width of first eggs may also limit their volume, because birds may have evolved a mechanism that prevents the extreme elongation of eggs (which could be effected by the deposition of extra albumen along the egg's longitudinal axis), in order to avoid abnormal embryonic development and low hatching success (Robertson et al.1994). This hypothesis was also refuted, both by the very low variation in mean egg shape (width/length ratio) among ranks (Figure 5), and by the lack of a positive correlation between first-egg shape and volume (see page 74). Finally, some authors have suggested that first-egg size may be limited by an initially low efficiency of the ovary (Parsons 1976) and the oviduct (Leblanc 1987; Williams et al.1993), which may improve as egg production progresses. However, no specific mechanism or relevant predictions have been proposed for this physiological "inertia", so I was unable to examine this hypothesis critically. Therefore, in summary, the relative size of first eggs in Squacco Heron clutches does not seem to be either nutritionally or anatomically constrained, but my data could not help me identify any of the factors that do limit it.

A summary of my results and the conclusions that can be drawn from them can be found in the following chapter (*Summary and Conclusions*).

V. SUMMARY AND CONCLUSIONS

I studied certain aspects of the breeding biology of the Squacco Heron (*Ardeola ralloides*), a poorly known species, and I focused on factors that may proximately affect this heron's intraclutch egg-size variation (clutch profile), an issue that has rarely been rigorously addressed in wild birds, and never before in ardeids.

I found that the mean egg-size profiles of both 4-egg (C4) and 5-egg (C5) clutches were arched, that is first and last (marginal) eggs were significantly smaller than middle ones. To investigate the proximate causes of these trends, I tested Parson's (1976) hypotheses of independent nutritional and hormonal constraints on last-egg size. The former attributes the lower relative size of last eggs to female nutritional resource limitations. Being unable to assess female resource availability directly, I inferred its level from the average egg volume of clutches (AvVol), and predicted a positive relationship between AvVol and relative last-egg size. The latter hypothesis postulates that the high levels of plasma prolactin that are associated with the onset of female incubation limit the size of last (or late) eggs through their depressive effects on the function of the oviduct (and also, possibly, the ovary; Arnold 1991). I was not able to assess female incubation directly, so I inferred its rate of development during egg-laying from the hatch span (H-Span) of clutches, and I predicted a negative relationship between H-Span and the relative size of final eggs. I also examined Houston, Jones and Sibly's (1983) hypothesis that the onset of incubation restricts female foraging time and food ingestion, and thus creates resource shortages that limit egg size. This hypotheses makes both of the above predictions, and also forecasts a negative correlation between AvVol and H-Span.

With respect to first eggs, I examined whether their size may be resource-limited (Slagsvold and Lifjeld 1989), in which case it should be positively associated with AvVol (Nilsson and Svensson 1993a). This hypothesis was refuted, as relative first-egg size was actually lower in high-AvVol 4-egg clutches, and was not affected by AvVol in C5 nests. First eggs might also be anatomically constrained by an initial oviduct inelasticity, which could restrict their width (Robertson and Cooke 1993) and size (Robertson et al.1994). This hypothesis predicts that first eggs should be thinner than later ones, but I did not find them significantly so. Variability among females in the intensity of this constraint should produce a positive correlation between first-egg volume and shape (width/length ratio), but neither this prediction was confirmed. Thus the size of first eggs in Squacco Heron clutches does not seem to be either nutritionally or anatomically constrained, but my data did not permit any further examination of its proximate control.

Before I tested the hypotheses concerning the relative size of final eggs, I did some preliminary data analyses that provided necessary background information. The median laying date of clutches occurred progressively later in each of the 3 years of my study (1992-1994), a trend that seems to be related to annual differences in mean ambient temperature. Clutch size did not vary substantially among years or within each main laying season, but was significantly lower in delayed nests. Females whose initial eggs were lost soon after they were laid, apparently replaced them by laying 1 or 2 extra ones. These females, as well as those laying delayed clutches, could have faced increased nutritional limitations when producing eggs, and were thus expected to lay clutches of lower AvVol than females who laid regular 4- and 5-egg clutches. This was confirmed, but the low-AvVol sample of nests was small and the difference between sample means was low (about 5% of the grand mean). Nevertheless, these results support, at least weakly, the use of AvVol as an index of female resource availability for egg formation. Besides, extraneous influences (e.g. that of female body size) may increase the random error in the relationship between these 2 variables, but are not expected to create any spurious association between AvVol and clutch profile. AvVol did not vary annually, seasonally (within the main laying period) or between C4 and C5 nests. Therefore, the nutritional hypothesis would predict no significant variation in clutch profile among the levels of any of these 3 factors.

Parents can control the hatching patterns of their clutches through incubation in many birds (e.g. Parsons 1972; Magrath 1992a; Wiebe et al.1998). In my study, male involvement in this activity, measurement error in H-Span data, and possible variation in egg-laying intervals, may have increased the random error in the relationship between female incubation during egg-laying and H-Span. Thus they may have made the latter an imperfect index of the former, but none of them were expected to create any spurious association between H-Span and clutch profile. However, a possible correlation between egg size and the length of required incubation (Parsons 1972; Bollinger 1994), may have biased results against the predictions of the hormonal hypothesis. Hatch span was significantly longer in C5 (median of 4.5 d) than in C4 (3.0 d), which is not surprising, given the extra egg and hatching interval in the larger clutches. The hormonal hypothesis would therefore predict relatively smaller last eggs in C5 nests. The testing of annual and seasonal effects on H-Span revealed only a significant positive seasonal trend within C5 clutches. The hormonal hypothesis would thus predict a seasonal decline in relative final-egg size in C5, but no other seasonal or annual variation in clutch profile.

AvVol and H-Span were not correlated in either clutch size. This result refuted Houston, Jones and Sibly's (1983) hypothesis of nutritional constraints on final-egg size due to female feeding restrictions imposed by the onset of incubation. It also allowed me to test these 2 factors' effects on clutch profile without risk of mutual confounding, even in the absence of experimental manipulations.

The primary prediction of the hormonal hypothesis was confirmed: between nests with short and long H-Span (late and early incubation onset), the linear trend of clutch profile changed from positive to negative, and the relative size of last eggs decreased. The profile's arch also peaked earlier in the latter sample, which is also consistent with this hypothesis. There was a weak annual variation in the profile of 5-egg clutches, which approached statistical significance, but does not constitute strong evidence against either hypothesis (neither of which predicted it). The expected (by the hormonal hypothesis) seasonal decrease in relative last-egg size in C5 was not observed. This probably occurred because the seasonal increase in H-Span, though statistically significant, was not of sufficient magnitude to produce a response in clutch profile. Therefore, it does not offer strong evidence against the hormonal hypothesis. Since this prediction concerned only a secondary effect of H-Span, it is also possible that some other, seasonally varying factor may have opposed H-Span's influence on clutch profile, but no such factor is suggested in the literature.

The hormonal hypothesis' secondary prediction of relatively smaller final eggs in C5 (than in C4) was also confirmed, but only among high-AvVol nests. When AvVol was low (and resource limitations were presumably high), there was no difference in profile between clutch sizes. Therefore, it seems that last eggs in C4 clutches can attain a greater relative size (presumably due to weaker hormonal constraints) only when female resources are abundant (AvVol is high). When AvVol is low (and nutritional resources are poor), the alleviation of hormonal constraints has no apparent effect on last-egg size. Similarly, the nutritional hypothesis' primary prediction of a positive effect of AvVol on relative final-egg size and the profile's linear trend, was confirmed only in 4-egg clutches. Apparently, the longer H-Span (and stronger hormonal constraints) of 5-egg nests did not allow last eggs in high-AvVol clutches to benefit from the higher resource availability of the laying females, but kept them as small (relatively) as in low-AvVol nests. These results suggest that both H-Span and AvVol (and the inferred hormonal and nutritional factors) can influence the Squacco Heron's clutch profile in an interactive way: each factor's effects can become apparent only in the absence of strong interference from the other. Other studies of the proximate causes of avian clutch profile have so far produced evidence supporting one or the other of the above hypotheses (e.g. Parsons 1976; Arnold 1991). My results are the first to indicate that both hormonal and nutritional factors may interactively affect the relative size of birds' final eggs.

Trends in AvVol and H-Span among the different clutch profile types also offer some insight to the phenomena under study. H-Span decreased along the Decline-to-Rise gradient (where the relative size of last eggs progressively increases), as was predicted by the hormonal hypothesis. The nutritional hypothesis' prediction of a linear increase in AvVol along this gradient was not confirmed. AvVol was high only in nests with the common, arched profiles, and significantly lower in all others. The short H-Span and low mean AvVol of Rise suggest that a delayed onset of incubation may be necessary, but not sufficient for last eggs to attain maximum size: the size of the (normally large) middle eggs may also need to be constrained (probably nutritionally). The existence of nests with Irregular profiles also indicates that the size of middle eggs may sometimes be limited (again, probably nutritionally). Size constraints on single eggs are more likely to result from albumen deficiencies, as this is deposited separately in each egg, whereas several eggs' yolks grow simultaneously (e.g. Meijer et al.1989; Grau 1996; Moore et al. 2000), but this issue cannot be resolved until egg composition is examined.

In conclusion, my study makes the first detailed examination of clutch profile in ardeids, and has indicated certain proximate factors that seem to influence it (or not), thus suggesting further investigations of its proximate causes. The size of first eggs in heron clutches does not seem to be either nutritionally or anatomically constrained. Some authors have hypothesized that their size may be limited by an initially low physiological efficiency of the female reproductive organs (Parsons 1976; Leblanc 1987). No specific mechanisms have been proposed, however, and no predictions about the variation in relative first-egg size have been made, so this hypothesis needs theoretical development.

The size of last eggs in Squacco Heron clutches is negatively associated with their clutches' hatch span, which reflects the early development of parental (and female) incubation. This relationship may be mediated by a) an association between the onset of incubation and an increase in prolactin secretion (Buntin 1996; Vleck 2002), and b) the latter's involvement in the suppression of the female reproductive organs' activities (Buntin and Tesch 1985; Youngren et al.1991). These behavioral and physiological links can be examined with measurements, and especially manipulation, of female prolactin levels during egg-laying, with and without removal of initial eggs, whose presence stimulates incubation (Williams et al.1996). Such experiments, however, may not be tolerated by ardeids, who are sensitive to disturbances at their nesting sites (Tremblay and Ellison 1979; Frederick and Collopy 1989).

The size of last eggs in Squacco Heron clutches may also be subject to nutritional constraints, as its positive association with the mean egg size of clutches indicates. The latter, however, becomes apparent only in 4-egg clutches, where the interval between the onset of incubation and the laying of last eggs is shorter, and the hormonal constraints on the size of the latter are presumably weaker. Females acquire most of their resources for egg production from their daily diet in diverse avian taxa (Meijer and Drent 1999), so this may also be true in ardeids. However, my data indicated that the onset of incubation does not restrict female feeding opportunities (as proposed by Houston et al.1983), which agrees with the existing information about heron activity patterns during nesting (see last section in *Preliminary Analyses*). The effects of female nutrition on mean egg size and clutch profile can be examined a) with appropriate food supplementation at times of low natural resource availability (Hiom et al.1991; Bolton et al.1992), or b) with repeated early-egg removals, which can increase nutritional demands in females who respond by laying extra eggs (Nager et al. 2000).

The examination of egg composition (which requires collection and destruction of eggs) can indicate whether herons' last eggs mostly lack albumen, as those of gulls (Parsons 1976; Houston et al.1983), or whether they are deficient in both albumen and yolk, as those of coots (Arnold 1991) and geese (Williams et al.1996). This information pertains to the question of whether hormonal changes lower (before suppressing) only the oviduct's function, or if they also affect the ovary. Moreover, resource limitations may sometimes have small effects on last eggs' size, but significantly lower their quality, both in terms of lipid and protein content, and as inferred from hatchling survival and fledging success (Nager et al. 2000).

Finally, in a separate (and still incomplete) study, I examine the ecological effects of clutch profile in *A.ralloides*, that is the possible influence of egg-size variation on the growth and survival of siblings who differ in age (due to hatching asynchrony) and in hierarchical status in their competition for finite parental care.

APPENDIX

Table A-1 RMA on egg volume by Rank (within-clutches factor), and by Year and Season (among-clutches factors), using 4-egg nests. Results are used in text Tables 21A and 23A.

Source	Sum of Sqs	D.F.	Mean Sq	*F*-ratio	*P*-value
Among Clutches					
Year	13.29	2	6.65	1.952	0.149
Season	0.05	1	0.05	0.013	0.909
Y × S	16.35	2	8.17	2.400	0.097
Error	272.44	80	3.41		
Within Clutches					
Rank	28.47	3	9.49	26.697	**< 0.001**
R × Y	0.81	6	0.14	0.380	0.867
R × S	0.08	3	0.03	0.072	0.961
R × Y × S	3.26	6	0.54	1.528	0.181
Error	85.31	240	0.36		
Linear Contrast					
Rank	0.75	1	0.75	1.228	0.271
R × Y	0.69	2	0.35	0.565	0.570
R × S	0.00	1	0.00	0.002	0.968
R × Y × S	1.08	2	0.54	0.886	0.416
Error	48.92	80	0.61		
Quadratic Contr.					
Rank	27.41	1	27.41	97.814	**< 0.001**
R × Y	0.11	2	0.06	0.195	0.823
R × S	0.00	1	0.00	0.001	0.979
R × Y × S	1.04	2	0.52	1.851	0.164
Error	22.42	80	0.28		

Table A-2 RMA on egg volume by Rank (within-clutches factor), and by Year and Season (among-clutches factors), using 5-egg nests. Results are used in text Tables 21B and 23B.

Source	Sum of Sqs	D.F.	Mean Sq	*F*-ratio	*P*-value
Among Clutches					
Year	3.66	2	1.83	0.257	0.773
Season	9.57	1	9.57	1.394	0.248
Y × S	12.48	2	6.24	0.879	0.418
Error	880.00	124	7.10		
Within Clutches					
Rank	101.92	4	25.48	69.482	**< 0.001**
R × Y	6.10	8	0.76	2.079	0.060
R × S	1.53	4	0.38	1.046	0.369
R × Y × S	3.37	8	0.42	1.149	0.334
Error	181.89	496	0.37		
Linear Contrast					
Rank	4.31	1	4.31	5.353	**0.022**
R × Y	4.14	2	2.07	2.572	0.080
R × S	0.04	1	0.04	0.053	0.818
R × Y × S	1.33	2	0.66	0.825	0.441
Error	99.75	124	0.80		
Quadratic Contr.					
Rank	94.58	1	94.58	301.526	**< 0.001**
R × Y	1.09	2	0.54	1.734	0.181
R × S	0.00	1	0.00	0.000	0.993
R × Y × S	1.82	2	0.91	2.898	0.059
Error	38.89	124	0.31		

Table A-3 RMA on egg volume by Rank (within-clutches factor), and by clutch size (Clsz) and H-Span (among-clutches factors). Clsz included only in order to examine the R×H×C interaction. Results are used in text Table 22.

Source	**Sum of Sqs**	**D.F.**	**Mean Sq**	***F*-ratio**	***P*-value**
Among Clutches					
H-Span	6.46	1	6.46	1.253	0.266
Clsz	10.39	1	10.39	2.015	0.159
H × C	7.68	1	7.68	1.491	0.225
Error	535.94	104	5.15		
Within Clutches					
Rank	56.36	3	18.79	47.385	< 0.001
R × H	6.91	3	2.30	5.811	**0.002**
R × C	5.97	3	1.99	5.019	0.005
R × H × C	0.63	3	0.21	0.530	**0.620**
Error	123.70	312	0.40		
Linear Contrast					
Rank	0.00	1	0.00	0.000	0.990
R × H	6.73	1	6.73	9.673	**0.002**
R × C	3.28	1	3.28	4.713	0.032
R × H × C	0.36	1	0.36	0.511	0.476
Error	72.32	104	0.70		
Quadratic Contr.					
Rank	56.36	1	56.36	173.013	< 0.001
R × H	0.01	1	0.01	0.032	0.858
R × C	2.40	1	2.40	7.369	0.008
R × H × C	0.27	1	0.27	0.835	0.363
Error	33.88	104	0.33		

Table A-4 RMA on egg volume by Rank (within-clutches factor), and by Egsz and Clsz (among-clutches factors). Contrasts were not performed, because R×H×C interaction approached significance. Results are used in text Table 25.

Source	Sum of Sqs	D.F.	Mean Sq	*F*-ratio	*P*-value
Among Clutches					
Egsz	586.78	1	586.78	329.256	< 0.001
Clsz	1.89	1	1.89	1.058	0.305
E × C	8.95	1	8.95	0.024	**0.026**
Error	377.82	212	1.78		
Within Clutches					
Rank	101.78	3	33.93	89.695	< 0.001
R × E	4.53	3	1.51	3.989	0.015
R × C	6.82	3	2.27	6.011	0.002
R × E × C	3.07	3	1.02	2.702	**0.061**
Error	240.55	636	0.38		

Table A-5 RMA on egg volume by Rank (within-clutches factor), and by Egsz (among-clutches factors), in 4-egg clutches. Bonferroni-corrected critical *P*-level: 0.0125. Results are used in text Table 26A.

Source	Sum of Sqs	D.F.	Mean Sq	*F*-ratio	*P*-value
Among Clutches					
Egsz	188.15	1	188.15	135.15	< 0.001
Error	116.94	84	1.39		
Within Clutches					
Rank	31.66	3	10.55	31.144	< 0.001
R × E	4.24	3	1.41	4.168	**0.010**
Error	85.39	252	0.34		
Linear Contrast					
Rank	0.55	1	0.55	0.972	0.327
R × E	3.54	1	3.54	6.285	**0.014**
Error	47.27	84	0.56		
Quadratic Contr.					
Rank	30.52	1	30.52	111.163	< 0.001
R × E	0.64	1	0.64	2.312	0.132
Error	23.06	84	0.28		

Table A-6 RMA on egg volume by Rank (within-clutches factor), and by Egsz (among-clutches factors), in 5-egg clutches. Bonferroni-corrected critical *P*-level: 0.0125. Results are used in text Table 26B.

Source	Sum of Sqs	D.F.	Mean Sq	*F*-ratio	*P*-value
Among Clutches					
Egsz	465.09	1	465.09	229.00	< 0.001
Error	259.96	128	2.03		
Within Clutches					
Rank	88.91	3	29.64	73.412	< 0.001
R × E	3.34	3	1.11	2.757	**0.062**
Error	155.03	384	0.40		
Linear Contrast					
Rank	5.89	1	5.89	7.702	0.006
R × E	0.03	1	0.03	0.040	0.842
Error	97.86	128	0.76		
Quadratic Contr.					
Rank	83.02	1	83.02	284.189	< 0.001
R × E	3.06	1	3.06	10.458	**0.002**
Error	37.40	128	0.29		

Table A-7 RMA on egg volume by Rank (within-clutches factor), and by Clsz (among-clutches factors), in Low-Egsz nests. Bonferroni-corrected critical *P*-level: 0.0125. Results are used in text Table 27A.

Source	**Sum of Sqs**	**D.F.**	**Mean Sq**	***F*-ratio**	***P*-value**
Among Clutches					
Clsz	9.53	1	9.53	5.732	0.018
Error	176.18	106	1.66		
Within Clutches					
Rank	37.84	3	12.62	33.282	< 0.001
R × C	0.57	3	0.19	0.501	0.627
Error	120.53	318	0.38		
Linear Contrast					
Rank	2.69	1	2.69	3.846	0.052
R × C	0.14	1	0.14	0.194	0.660
Error	74.22	106	0.70		
Quadratic Contr.					
Rank	34.96	1	34.96	128.537	< 0.001
R × C	0.43	1	0.43	1.595	0.209
Error	28.83	106	0.27		

Table A-8 RMA on egg volume by Rank (within-clutches factor), and by Clsz (among-clutches factors), in High-Egsz nests. Bonferroni-corrected critical *P*-level: 0.0125. Results are used in text Table 27B.

Source	Sum of Sqs	D.F.	Mean Sq	*F*-ratio	*P*-value
Among Clutches					
Clsz	1.31	1	1.31	0.689	0.408
Error	201.64	106	1.90		
Within Clutches					
Rank	68.46	3	22.82	60.460	< 0.001
R × C	9.32	3	3.11	8.228	**< 0.001**
Error	120.02	318	0.38		
Linear Contrast					
Rank	0.06	1	0.06	0.086	0.770
R × C	6.56	1	6.56	9.788	**0.002**
Error	71.00	106	0.67		
Quadratic Contr.					
Rank	68.24	1	68.24	227.854	< 0.001
R × C	2.04	1	2.04	6.826	**0.010**
Error	31.75	106	0.30		

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