Università degli Studi del Piemonte Orientale Amedeo Avogadro
Dipartimento di Scienze e Innovazione Tecnologica
Dottorato in Scienze Ambientali (Acque Interne e agroecosistemi)

Université Paris Ouest La Défense Nanterre
Laboratorie d'Éthologie et de Cognition Comparées
École doctorale 139 : Connaissance, Langage, Modélisation

PhD Thesis in "Scienze Ambientali"

and

"Neurosciences: Éthologie"

Maternal effects in birds: the role of some environmental stressors on egg quality

Presented and sustained by

Marco Grenna

March 22, 2013

Jury Members:

Michel Kreutzer	Examiner	Université Paris Ouest La Défense Nanterre, France
Leonida Fusani	Rapporteur	Università di Ferrara, Italy
Dominique Fresneau	Rapporteur	Université Paris Nord 13, France
Giorgio Malacarne	Research Director	Università degli Studi del Piemonte Orientale, Italy
Gérard Leboucher	Research Director	Université Paris Ouest La Défense Nanterre, France
Marco Cucco	Jury President	Università degli Studi del Piemonte Orientale, Italy

Ringraziamenti – Remerciement

Alla fine di questi tre anni di lavoro è doveroso ringraziare le persone che a vario titolo mi hanno aiutato in questo periodo.

In primo luogo Giorgio Malacarne e Marco Cucco, per avermi permesso di affrontare questo lavoro, et Gérard Leboucher, qui m'a accueilli dans son laboratoire et m'a toujours aidé, lors de mes séjours à Paris et aussi quand j'étais en Italie.

Grazie all'università italo-francese, che con la borsa che mi ha messo a disposizione ha permesso il finanziamento dei soggiorni a Parigi.

Un sentito grazie va ai miei colleghi (maestri) di laboratorio Stefano e Tiziano, che mi hanno iniziato con le due tesi di laurea alla ricerca e che hanno saputo creare nel laboratorio quell'atmosfera di serenità ed allegria che lo contraddistingue da anni e che ha permesso loro di ottenere tutti i risultati che costellano la loro carriera (spero, in minima parte, di poter raggiungere anche io queste capacità e questi traguardi).

Come non ringraziare Irene, che in questi tre anni è sempre stata presente nell'aiutarmi ogni qualvolta avessi bisogno, sia in laboratorio sia in campo, durante le varie analisi che nel tempo si sono rese necessarie. Senza di te questo lavoro sarebbe stato di sicuro molto più difficile e meno divertente. E grazie per aver cercato di insegnarmi qualche cosa di ornitologia, anche se sono stato un pessimo studente.

Grazie di cuore a Donata, che con il suo aiuto "tecnico" ha reso possibile la realizzazione dei lavori presentati in questa tesi e che mi ha supportato ed aiutato tantissimo a livello "morale" in ogni momento, anche quando le cose sembravano più difficili. I viaggi per l'allevamento avranno sempre un posto speciale fra i ricordi di questo periodo!

Grazie a quelle persone che hanno costituito la parte più importante del periodo di dottorato: i "miei" tesisti Giada, Roberto, Alessandro, Eric, Michele, Alessio, Francesco, Annalisa, Sabrina ed Anna. Siete stati la spina dorsale di questo lavoro di tesi che altrimenti non si sarebbe potuto realizzare; grazie a voi ho imparato molto! Di sicuro siete stati ciò che mi ha reso più orgoglioso in questi anni, la cosa per cui è valsa la pena lavorare e che costituirà il ricordo più bello di questa esperienza.

Desidero ringraziare anche le persone che mi hanno insegnato le tecniche ed i protocolli di laboratorio qui presentati: Lorena, Sara, Silvia, Elia e Valeria ed i professori con i quali collaborano, Simonetta Sampò, Elisabetta Carraro e Mauro Patrone.

Grazie a Maria Grazia, Gisella, Patrizia, Nadia e Rosanna per gli aiuti burocratico-operativi e per la disponibilità!

E grazie alla famiglia De Maria (Fabrizio, Aldo, Natalina, Barbara, Antonella) che mi ha permesso di svolgere i miei esperimenti nella più assoluta libertà, cercando di accontentare ogni mia richiesta (anche la più strana) e facendomi sempre sentire a casa. Grazie di cuore!!!

Pour la partie française, ils sont beaucoup les amis à remercier.

Merci à Guillaume, pour l'aide et pour les remplacements ! Et aussi pour le grand aide avec le statistiques.

Merci à Davy, pour le support au labo (aussi a Chizé), logistique et pour les statistiques !

Merci à Ophélie, pour tous les ‘’ bonjour ‘’ quand personne étais au labo à la matin tôt et pour tous les aides (télé, timbres, etc.).

Merci à Wim, pour son amitié et le soirée de CLASSE.

Merci à Alba pour les leçons de français et de musique française. Tu m'as tenu beaucoup de compagnie pendant mon séjour hivernal.

Merci à Philippe pour le journal tous les jours et pour la compagnie en début de matinée et pour s'occuper avec passion et application des animaux. Les expériences son possible grâce à vous !

Merci à Mathilde et Davy (encore lui), en particulier pour l'organisation des ‘’ soirées film ‘’!

Merci à Ahmed, pour les discussions sur tout et les conseils.

Merci à Tudor pour m'avoir permis de travailler avec lui et pour les conseils toujours très utiles.

Merci à Morgane, Mathieu et Sébastien pour les discussions sympas dans la cousine.

Merci à Josette qui m'a aidé avec tous les papiers, le logement et tous les problèmes bureaucratiques ! Vous avez été très gentille et disponible !

Et merci à tous les autres, Laurent, Nathalie, Frédérique, Dalila, Michel, Eric, Sophie, Joshua, Sylvie et le garçon de l'accueil du bâtiment L, pour l'accueil qu'ils m'ont donné ! Je me suis senti comme à la maison !

Enfin, je tiens également à remercier Charline Parentau et Colette Trouvé pour les analyses fait au CEBC de Chizé ; j'ai beaucoup appris pendant mon séjour chez vous.

A bientôt (et pardonnez-moi le français) !

Ed ora i ringraziamenti più importanti…

Grazie ai miei genitori, che con i loro sforzi e le loro rinunce mi hanno permesso di raggiungere questo traguardo altrimenti impossibile. In particolare grazie a mia mamma per l'aiuto che mi ha sempre dato in ogni istante, per l'incoraggiamento e per tutte quelle piccole cose che solo le mamme possono fare per i propri figli. E grazie a mio padre, che mi ha sempre supportato in ogni momento, sia moralmente sia "fisicamente" (con aiuti informatico-linguistici, anche per la stesura di questo elaborato). Le lauree e questo dottorato sono più vostri che miei: grazie di tutto!!!

Grazie a mio fratello Roberto, che sin dall'inizio ha ricoperto il ruolo di secondo padre! Tutto il mio percorso scolastico-universitario è stato supportato da grandi e piccoli aiuti da parte sua, senza i quali non sarei mai potuto arrivare fino a qui! Non potevo sperare in un fratello migliore! E grazie a Laura, che da poco si è unita a noi ma che già sta facendo molto per me e per tutti noi (non ultimo il tanto atteso "allargamento" della famiglia)!

Grazie a mia nonna Anna (ed ai nonni che non ci sono più: Carlo, Sandro e Nora). Se siamo qui è grazie a voi, ai vostri sforzi, alla vostra abnegazione e al vostro esempio. Non servono le parole per insegnare e voi me lo avete sempre dimostrato.

Grazie alla piccola Alisia che con il suo modo di fare, la sua allegria e la sua forza d'animo (che già così piccola ha dovuto dimostrare) mi aiuta sempre, anche nei momenti più difficili.

Ed infine grazie ai miei amici, che da più di vent'anni mi supportano/sopportano, ed a quelli che strada facendo si sono aggiunti o se ne sono andati. In particolare grazie a: Serena, Alberto, Alice, Luca, Andrea, Andrea (e quanti sono), Pier, Alessio, Ilaria, Chiara, Jessica, Valentina, Francesca, Alessandra, Nadide e Fede. Ognuno a suo modo mi è stato vicino e mi ha aiutato in tutti questi anni. Grazie per esserci stati e per esserci ancora!

Sicuramente avrò dimenticato qualcuno. Di questo mi scuso, ma tengo a precisare che è solo per un fatto di scarsa memoria! Grazie anche a chi doveva essere presente qui ma che mi sono scordato!

Ah, come è possibile?!? Mi ero quasi dimenticato dei protagonisti di questa tesi, senza i quali non si sarebbe potuta realizzare!!! Grazie alle starne, alle pernici rosse e ai canarini e scusate se per la nostra voglia di conoscere vi tormentiamo con esperimenti assurdi!!!

Dedicata ai miei genitori,

a Roberto e Laura,

alla nonna Anna ed agli altri nonni,

ad Alisia e "Fagiolino"

Note to readers

The PhD work presented here has been carried out in co-direction with an italian university (Università del Piemonte Orientale "A. Avogadro", Alessandria, Italy) and a french university (Université Paris Ouest Nanterre, Nanterre, France).

This PhD project has been possible by a grant of the "Università italo-francese".

English is the language chosen for the writing. Short summaries in italian and french appears at the beginning of each chapter.

My aim in writing this thesis is to summarize some knowledge acquired over many years by many researchers in ethology field and expose my finding.

I tried to be as objective as possible, but unfortunately this thesis is not free of anthropomorphism (inherent in ethology).

Enjoy the read!

Introduction

The costs of reproduction

At any particular age an organism is faced with two decisions. In the first place, should it reproduce or not? And second, if it should, is it better to commit so great a proportion of its resources to reproduction that it cannot survive to reproduce again, or to reproduce at some lower intensity (Bell, 1980)?

More obviously than at any other part of the life cycle, the allocation of resources during reproduction influences fecundity (*n*), survival (*S*) and life-cycle timing (*t*) (Sibly & Calow, 1986); indeed, life span can decrease due to energy employed in reproduction (Williams, 1994). *S* and *n* are likely to increase but *t* to reduce as more resources are invested in the processes that affect them. Yet, the resources available for allocation are limited and this is likely to lead to trade-offs between the various terms (Sibly & Calow, 1986).

Trade-offs

Trade-offs represent the costs paid in the currency of fitness when a beneficial change in one trait is linked to a detrimental change in another (Stearns, 1989). They have played a central role in the development of life-history theory, from Fisher (1930), Gadgil & Bossert (1970), Charnov & Krebs (1973), Schaffer (1972; 1974a; 1974b), Tinkle & Hadley (1975), Bell (1980), Townsend & Calow (1981), Dunham et al. (1989), Adolph & Porter (1993), Stearns (1992), Roff (1992a) e Zera et al. (1998) until today.

Classic examples include that between age and size to maturity, reproductive investment and survivor, etc. It has been observed in lines of *Drosophila melanogaster* selected for increased longevity a decrease in early fecundity (Rose 1984; Luckinbill et al., 1984; Partridge et al., 1999), and in lactating (reproductive) red deer, *Cervus elaphus*, a reduction in overwintering survivorship (Clutton-Brock et al., 1982).

Important point in the life cycle from the point of view of this classification is when propagules are released and become independent of parents. This point might be the release of gametes or of brooded offspring.

Costs of reproduction might be incurred before gamete release as a result of:

- increased risk associated with courtship and copulation due either to:
    - increases conspicuousness to predators;
    - aggressive behaviour to sexual competitors (e.g., Tinkle, 1969);
- increased foraging effort needed to obtain resources to make the gametes and/or to prepare nests etc.;
- morphological distensions and mechanical imbalances caused by the accumulation of gametes or they precursor or carrying the young, again causing increased metabolic requirements and mortality risks (e.g., if pregnant females are more easily caught by predators);

- the drain of resources from somatic to gametic tissues and process (reviewed in Calow, 1979).

Of all of these, the last is least likely as a direct cost since, at least in principle, it is reversible and there is considerable evidence for gamete and embryo resorption by parents under stress (e.g., reviewed in Calow, 1973).

Costs might be incurred after gamete release as a result of:

- the risks of parturition:
    - because individuals become more conspicuous or vulnerable to predation and disease at these times;
    - due to direct injury resulting from the release of gametes or young (the most extreme example of this is in some insect species where progeny eat their way out of their mothers' abdomens);
- the loss from the parent of resources locked into the reproductive products which are then no longer available as a fail-safe even under stress;
- the accumulation of the effects of metabolic stress arising from the events leading up to the release of gametes;
- care of eggs and offspring, after they are released involves considerable metabolic costs (Bryant, 1979) and survival risks (Nur, 1984a; 1984b; Hussell, 1972; Askenmo, 1979; Sibly & Calow, 1986).

Bell (1980) uses the term survival cost to mean the cost of reproduction in terms of diminished future survival. The cost of reproduction may be seen as a trade-off between current and future expected reproduction (Reznick, 1985; 1992; Petes et al., 2008). However this term requires special attention because it has been used in many different ways. For example, it can refer to either a price (e.g., amount of calories required to produce an egg) or penalty of reproduction, that is measured in physiological (i.e., calories), demographic (survivorship), or fitness units. Moreover, the cost of reproduction can refer to a direct penalty of current reproduction or to a penalty exacted in the future (Zera & Harshman, 2001). For example, if internal reserves allocated to current reproduction limit resources available for future reproduction, a trade-off between current and future reproduction exists for physiological reasons (Calow, 1979; Bell & Koufopanou, 1986). Trade-offs can occur between physiological traits expressed during the same or different times in the life cycle (Chippindale et al., 1996; Zera et al., 1998; Stevens et al., 1999).

Fitness

Fitness is perhaps one of the most difficult to understand and frequently misinterpreted concepts in evolution. Yet no single concept is more fundamental to understanding the evolutionary process (Hunt & Hodgson, 2010).

Contrary to popular belief, Darwin did not use the word *fitness* in early edition of "Origin of Species" (Darwin, 1859). Instead he used the verb *to fit* to describe how well the phenotype of an organism matched the environment in which it lives (Hunt & Hodgson, 2010). Using a lock-and-key analogy, environment is a lock and the organism (the key) must fit this lock if it want to survive, grow, and reproduce (Ariew & Lewontin, 2004). Fitness crept into later editions thanks to Herbert Spencer's coining of the phrase "survival of the fittest" (Spencer, 1866). Darwin and Wallace both enjoyed Spencer's metaphor because it avoided the supposed anthropomorphism associated with *selection.* Moreover "survival of the fittest" is often considered tautological because fitness is a function of survival; in other words, it is defined by some as "the ability to survive and reproduce" (Ariew & Lewontin, 2004; Bouchard & Rosenberg, 2004; Krimbas, 2004; Hunt & Hodgson, 2010).

Fitness, in its modern usage by evolutionary biologists, would ideally be a future of an organism that determines the spread of that organism's genes in a population, through time (Hunt & Hodgson, 2010). This could be seen like a deterministic processes, but in truth there are also stochastic characteristics; indeed, there are the mutations, random effects that effect, with natural disasters and demographic and environmental stochasticity, also the organisms most able in find food and fit with the environment. Hence the probability of future numerical spread of traits, driven by natural selection, is a statistical property that must be linked in some way to the propensity of organisms carrying those alleles or traits to survive and reproduce in the environments in which they and their descendants live (Bouchard & Rosenberg, 2004).

With this abstract model in mind, a diversity of fitness concepts has been proposed (Dobzhansky, 1968a; 1968b; Endler, 1986). For example, fitness has been defined as: (1) the propensity of an individual, or of all individuals conveying a trait or allele of interest, to survive and to produce viable offspring; (2) the rate at which an allele or trait spreads numerically; (3) the ability of individuals carrying alleles or traits to exploit resources and cope with environmental conditions to survive and reproduce; (4) the capacity of individuals carrying an allele or trait to adapt to all possible future environments (which we consider adaptability instead of fitness); (5) the long-term future dynamics, or persistence, of the allele or trait in a population.

…Continue

Each of these definitions captures some or all of the features required by the theory of natural selection, but they vary in timescale of measurement and in whether fitness should be ascribed to the current state of the entity or to its future possible states. So, none of them is satisfactory as written. The best formulation, usable both by population geneticists and behavioural ecologists, could be this: the fitness is a measurable feature of alleles, genotypes or traits of individuals that predicts their numerical representation in future generations (Hunt & Hodgson, 2010). For a population geneticist, fitness of an allele is best defined as a parameter that directly affects the change in frequency of that allele between one generation and the next. Instead, to a behavioural ecologist, fitness is usually defined for individual organisms (or the traits they express) rather than for alleles, and is defined by the relative numerical change in abundance of those individuals or traits through time. As can be seen, the definition before enunciated includes both the point of view (Hunt & Hodgson, 2010).

Indeed Speakman (2008) divides the costs of reproduction in physiological and ecological. The firsts can be direct (that include at their simplest level the energy and nutrients that the parental animal needs to acquire to successfully reproduce and also the physiological and anatomical modifications that are necessary for the animal to achieve these demands) or indirect ("compensatory physiological" costs, whereby the animal chooses to reduce investment in some other aspect of its physiology to maximize the input of resource to reproduction). Ecological costs are, for example, the increase of predation risk associated to foraging due to an increased demand of nutrients, like proteins and calcium, to allocate to the reproductive event (Speakman, 2008).

For what concerns physiological costs, a number of correlational and experimental studies indicate that a common effect of current reproduction may be the reduction of internal reserves, which impacts future reproduction either by reducing survivorship or by reducing nutrients required for future reproduction (Landwer, 1994; Schwarzkopf, 1994; Doughty & Shine, 1997; 1998; Wilson & Booth, 1998). Furthermore, field studies indicate that up to 90% of the energy used for egg production comes from fat stores rather than recently consumed food (Karasov & Anderson, 1998). Doughty & Shine (1998) also showed that variation in nutrient input affected variation in lipid stores and reproductive output during the next year (Zera & Harshman, 2001). Another trade-off in which reproduction is implicated is that with the growth of the reproductive organism; it has been suggested by two study. In the first it was seen an increase in the growth of some lizard species during years in which reproduction does not occur (Schwarzkopf, 1993). In the second, Landwer (1994) found that *Urosaurus* *ornatus*, in which egg production was experimentally reduced, showed greater growth in the field compared with unmanipulated controls.

Regarding birds, it has been demonstrated that its expend relatively high levels of energy during reproduction and thermoregulation (Weiner, 1992). Either from observational data or by experimental clutch manipulation, there is evidence for a cost of reproduction in birds (Linden & Möller, 1989; Dijkstra et al., 1990; Styrsky et al., 1999). This cost can be manifest in terms of parental survival, future reproduction and/or offspring condition-fitness (Zera & Harshman, 2001).

Fat storage could partially underlie the trade-off between reproduction and parental survival, especially in passerines where energy storage reserves tend to be quite limited. Increased fat storage could support reproduction, but there can be a survival cost (Lima, 1986; Witter & Cuthill, 1993). Specifically, stored fat might incur acquisition and maintenance costs (Houston et al., 1997) as well as flight energetics or agility costs (Rogers & Smith, 1993; Gosler et al., 1995).

Overall, metabolism and energy budgets have been the focus of physiological work on the cost of reproduction. Historically, evidence accumulated for an energetic cost of bird reproduction (King, 1973; Ricklefs, 1974; Drent & Daan, 1980). For many species, the preponderance of this cost is due to parental care rather than egg production (Trillmich, 1986; Ward, 1996; Zera & Harshman, 2001).

Some studies indicate oxidative stress like proximate mechanism of the trade-off between reproductive investment and decrease in survival and in future reproductive success (Alonso-Alvarez et al., 2004; 2006; Bertrand et al., 2006a); however, this does not show that they actually incurred more oxidative damage, as there were no parallel measurements of ROS production or oxidative damage (Monaghan et al., 2009).

Study on mammals [e.g., sheep (*Ovis aries* - Nussey et al., 2009), house mice (*Mus musculus domesticus* - Garrat et al., 2011), and bank vole (*Myodes glareolus* - Oldakowski et al., 2012)] seem not confirm the hypothesis that the oxidative damage of the tissues is the proximal mechanism of the reproduction costs.

Sexual selection and mate choice

Sexual selection is the process by which individuals compete for access to mates and fertilization opportunities (Andersson, 1994; Jones & Ratterman, 2009; Kuijper et al., 2012).

Darwin (1871) stated: "That kind of selection, which I have called *sexual selection*... depends on the advantage which certain individuals have over other individuals of the same sex and species, in exclusive relation to reproduction".

So Darwin (1871) defined sexual selection as (1) competition within one sex for members of the opposite sex and (2) differential choice by members of one sex for members of the opposite sex, and he pointed out that this usually meant males competing with each other for females and females choosing some males rather than others (Trivers, 1972). Darwin's original goal was to account for evolution of elaborate, exaggerated and flamboyant traits such as calls, odours, ornaments, and conspicuous behaviours that are present in one sex only and that could not be explained by natural selection for survival as adaptations to the ecological conditions of a species (Jennions & Kokko, 2010; Kuijper et al., 2012). Traits that are energetically wasteful, overly elaborate, and harmful to their bearer are difficult to reconcile with the view that natural selection continuously weeds out inferior, uneconomical variants (Cronin, 1991). Darwin noted that selection will favour traits that increase male mating success and allow a male to sire more offspring, even if this is at the expense of a reduced lifespan.

Sexual selection theory can explain not only why males (and sometimes females) bear traits that decrease their own lifespan through an increased risk of predation, parasitism, or susceptibility to disease, but also why they have evolved traits that damage the opposite sex (Jennions & Kokko, 2010). Now, mating is no longer seen as harmonious union. Instead, it is viewed as an arena for sexual conflict in which females are under selection to evolve traits to counter males' attempts to elevate their own reproductive success. In the last decade, empirical studies have confirmed that sexual conflict can elevate the rate of evolution of certain genes, biochemical, and morphological traits (e.g., Arnqvist & Rowe, 2002; Dorus et al., 2004; Andrès et al., 2006; Ramm et al., 2008). More generally, sexual selection theory explains why secondary traits, such as genitalic embellishments, courtship song, and breeding coloration, show greater variation both within species and among closely related species than do other traits (Arnqvist, 1998).

However, it is too simple to draw the arrow of causality from parental investment to sexual selection because it can also go in the reverse direction: sexual selection is itself an important causal factor driving the initial evolution of greater postfertilization care by females (Queller, 1997). Stronger sexual selection on males can therefore account for the taxonomically widespread pattern that females provide more parental care (Kokko & Jennions, 2008). The details of the coevolutionary process vary greatly among species, however, and understanding unique features of the breeding biology of some taxa can also explain why they do not obey this rule, such as *sex-role-reversed* species in which females compete for males and are sometimes more ornamental in appearance (e.g., Forsgren et al., 2004; Jones et al., 2005).

Darwin (1871) thought of sexual selection process as exclusively precopulatory because he assumed females to be sexually monogamous (Birkhead & Pizzari, 2002). Moreover, the discovery of females polyandry in the last 30 years changed a little the definition of the sexual selection. Female promiscuity has important biological implication: it means that sexual selection persists after copulation up to the point of fertilization, and in some case beyond (Birkhead & Pizzari, 2002). Postcopulatory sexual selection comprises both male-male interaction in the form of sperm competition, and cryptic female choice. Sperm competition is the competition between the sperm of different males to fertilize the ova of a female (Parker, 1970; Parker, 1998). Cryptic female choice is the ability of a female to bias the fertilization success of the males that copulate with and inseminate them (Eberhard, 1996). A more widely acceptable modern definition is therefore as follows: sexual selection favours investment in traits that improve the likelihood of fertilization given limited access to opposite sex gametes due to competition with members of the same sex. An early prediction of sperm competition theory was that, in species or situations in which sperm competition was intense, it would pay males to produce ejaculates that contain more sperm (Parker, 1970; Parker, 1984). The subsequent discovery that, across a wide range of taxa including mammals, birds, butterflies and fish, species that experience more sperm competition had relatively large testes is entirely consistent with this theory (Birkhead & Möller, 1998). Theory also predicted that because males of all species experience sperm depletion, they would be selected to allocate sperm to females in a strategic manner. The most obvious form of strategic sperm allocation is the allocation of ejaculates that contain more sperm in the presence of sperm competition, as occurs in crabs (Jivoff & Hines, 1998) and birds (Hunter et al, 2000; Nicholls et al., 2001). In addition, sperm completion selects for sperm form and function. Across species, sperm vary enormously in design and size (from few μm to >5 cm). In *Caenorhabditis* spp., larger sperm are more competitive (LaMunyon & Ward, 1999), and a comparative study of birds revealed that longer sperm occur in species with more intense sperm competition (Briskie et al., 1997). Traits favoured by sperm competition, such as increased ejaculate size or longer sperm, are also sexually selected (Jennions & Kokko, 2010).

One way of how sexual selection operates is mate choice. Broadly speaking, mate choice occurs when traits create mating biases that reduce the set of potential mates (Kokko et al., 2003). This does not necessarily involve direct rejection of a mate. For example, a preference for mating in a particular habitat can generate mate choice if only certain mates reach these locations (indirect mate choice – Wiley & Poston, 1996). Sexual selection and mate choice aren't synonymous, so we can do a clear theoretical distinction between them: the second generate sexual competition within the chosen sex, but it doesn't logically follow that competition for mates (sexual selection) must be associated with the other sex being choosy (Jennions & Kokko, 2010).

In many instance the evolution of mate choice means that the mating rate of the choosy sex is not maximized. If the Bateman Gradient (BG) is positive, fitness increases with each following mate, which selects against the reduction of the mating rate that follows from mate choice.

Choosiness will therefore more often evolve in females because they usually have a very low BG and consequently lower gains from mate choice are sufficient to compensate for a reduction in the mating rate. When the BG is negative, females directly benefit if they reduce their mating rate by rejecting males (a kind of mate choice). But it is incorrect simply to assume that the sex with the lower BG will be choosy. For examples, if the female BG is lower than that of males but both are positive, both the sexes still pay a mating rate cost by being choosy (Jennions & Kokko, 2010).

The Bateman Gradient

The *Bateman Gradient* (BG) is a direct measure of the benefit of an elevated mating rate and therefore a more explicit predictor of the current direction of sexual selection than the OSR (Operational *Sex Ratio*, the instantaneous ratio of sexually active males to sexually receptive females – Emlen & Oring, 1977). It is defined as the slope of the regression of offspring production on mating rate (Arnold, 1994), and its origins lie in experiments conducted on *Drosophila melanogaster* (Bateman, 1948), where it was counted how many offspring were produced by males and females that mated varying numbers of times when a small group of both sexes was housed together.

However, evidence for mutual mate choice in some species (Servedio & Lande, 2006) and male mate choice in others (Wedell et al., 2002), when combined with the fact that males almost always have a positive BG, suggests that BGs fail to capture some biologically relevant factors. So, the information missing from the BG is an explicit consideration of a key trade-off: choosiness typically changes both the number and the identity of actual mates (Jennions & Kokko, 2010).

Choosiness doesn't evolve simply because individuals of the opposite sex vary in the number and/or fitness of the offspring they produce. If mating incurs no cost in terms of future reproductive success and there is a current benefit to mating, then individuals should mate with every potential partner encountered. Mate choice evolves only if mating is costly: it might elevate predation or trade off with foraging, or, perhaps most important, indiscriminate mating might reduce the mating rate with individuals that confer greater benefits per mating. Male mate choice is a weaker force than females one because the time-out for sperm replenishment is short and, therefore, it is less likely that a male who mates will lose out on an opportunity to fertilize the eggs of a better quality female. In contrast, when females mate they often have a longer time-out for egg production. This means that they are more likely than males to lower their mating rate while waiting for better quality male. Anisogamy is therefore a driving force of sexual divergence in mate choice (Jennions & Kokko, 2010).

Anisogamy

Anisogamy (also called heterogamy) refers to a form of sexual reproduction involving the union or fusion of two dissimilar gametes (differing either in size alone or in size and form); these gametes take the name of anisogamous, anisogamic. The smaller gamete is considered to be male (sperm cell), whereas the larger gamete is regarded as female (egg cell).

There are several types of anisogamy. Both gametes may be flagellated and thus motile. Alternatively, neither of the gametes may be flagellated. This situation occurs for example in some algae and plants. In the red alga *Polysiphonia*, large non-motile egg cells are fertilized by small, non-motilespermatia. In flowering plants, the gametes are non-motile cells within gametophytes.

The form of heterogamy that occurs in animals is oogamy. In oogamy, a large, non-motile egg cell (ovum) is fertilized by a small, motile sperm cell (spermatozoon). The large egg cell is optimized for longevity, whereas the small sperm cell for motility and speed. The size and resources of the egg cell allow for the production of pheromones, which attract the swimming sperm cells (Togashi & Cox, 2011).

So, anisogamy is the phenomenon of fertilization of small gametes (sperm) and big gametes (eggs). Gamete size difference is the fundamental difference between males and females. Anisogamy first evolved in multicellular haploid species after the differentiation of different mating types had already been established.

Two main theories have been proposed for the evolution of anisogamy: 1) one focuses on sperm competition and the selfishness of sperm (Parker et al., 1972; Parker, 1982); and 2) the other focuses on the cooperation of different mating types to deal with fertilization risks (Dusenbery, 2006; Yang, 2010).

Both theories assume that anisogamy originated through disruptive selection acting on an ancestral isogamous population, and that there is a trade-off between larger gamete number and smaller fitness of each gamete, because the total resource one individual can invest in reproduction is fixed (Bulmer & Parker, 2002).

The theory of sperm competition was the first theory to explain the evolution of anisogamy, which was proposed by Geoff Parker et al. (1972). He proposed that because the size of the zygote is determined by the sizes of both gametes and one gamete becoming smaller will decrease the zygote size to no less than half, so there is a large benefit to reduce gamete size in order to greatly increase gamete number. Then, the many smaller gametes can still "parasitize" the larger gametes to pass on their genes. Then, larger gametes have to increase their sizes to compensate the nutrient loss of the zygotes. So sperm competition, which was the selection force to greatly increase gamete number, was proposed to be the cause of size differentiation of different gametes, and eventually the evolution of sperm and eggs (Parker et al., 1972).

…Continue

However, competition not only exists among sperm, but also exists among eggs. Parker provided no explanations for why it was sperm that became smaller but not eggs, or what caused proto-sperm and proto-eggs to be unequal. In fact, a recent mathematical model (Yang, 2010) shows that sperm competition is neither necessary nor sufficient for the evolution of anisogamy. Sperm competition only caused sperm to become as small as possible (considering them still functional) after anisogamy and smaller sperm had already evolved. The real reason for the evolution of anisogamy is proposed to be fertilization risk. An important problem for the mating of multicellular organisms is the transport of gametes from place to place for fertilization, which should evolve for at least one mating type because gametes of different individuals usually do not have direct contact. However, the transport has a probability of failure and poses a fertilization risk. When the risk increases, gametes of one mating type need to become smaller to increase the number to overcome this risk, and gametes of the other mating type need to become larger to compensate for nutrient loss of the zygotes (Yang, 2010).

So it is fertilization risk and the cooperation of different mating types to deal with it that caused the evolution of anisogamy. Sperm evolve to increase fertilization events and eggs evolve to compensate the nutrient loss. Additionally, sperm can evolve even smaller when eggs become larger to ensure zygote nutrient, and eggs can evolve even larger when sperm become smaller to reduce fertilization risks. This cooperation may be the reason why many organisms have extremely small sperm and extremely large eggs (Yang, 2010).

In more species, females are congruent in their mate preference for a particular male, while in others, females are incongruent in their choice, with each preferring a different male (Brian & Pitcher, 2005). In the first case the response is quite easy: in these systems, males provide resources directly to females or offspring such as food, shelter, parental care and protection from predators. These facts could have a weighty impact on females fitness. Therefore, it was straightforward to posit that natural selection could lead to the evolution of male indicator traits that facilitated mate choice by advertising the quality or quantity of a male's resources (Møller & Jennions, 2001).

However, there are many other mating systems in which females receive no resources from males, yet females still express a preference among males. For example, in some taxa, males display at fixed courtship, territories known as leks and these males provide only genes (by sperm) to their mates. Females show congruence in their mating preference for males with the most elaborate trait

(Höglund & Alatalo, 1995). This congruence take the name of "paradox of lek" because a female only receives genes from the males she selects (Kirkpatrick & Ryan, 1991; Tomkins et al., 2004). The suggested response by these studies is that females receive an indirect benefit in the form of increased genetic quality of their offspring. Precisely, models have shown that the preferred male must provide genes that increase the survivorship or mating success of the offspring as compared to the genes provided by less desirable males (Kokko et al., 2003; Mead & Arnold, 2004). There are also empirical research on lek mating systems and other nonresource-based mating systems that have confirmed the association between mate preference and increased offspring viability, although the fitness effects appear small at only a few percent (Møller & Alatalo, 1999; Jennions et al., 2001; Kirkpatrick & Barton, 1997).

Components of genetic quality

We can say that genetic quality has two components: good genes and compatible genes.

Good gene: an allele that increases fitness independent of the architecture of the remaining genome, which, in diploid organisms, includes the homologue to the particular "good allele". Across the genome, good genes will show additive genetic variation. Thus, when variation in fitness exists as a result of good genes, the population will respond to directional selection.

Compatible gene: an allele that increases fitness when in a specific genotype; i.e. when paired with a specific homologue (overdominance) or allele at another gene locus (epistasis). Across the genome, compatible genes will show nonadditive genetic variation. Thus, when variation in fitness exists because of compatible genes, the population will not respond to directional selection, but the mechanisms of acquiring compatible genes (preference alleles) will respond to directional selection (Neff & Pitcher, 2005).

In nature we can also find mating systems in which females each prefer a different male and yet receive no resources from any of them. Researchers have postulated that they also must be selecting males that increase the genetic quality of their offspring; in these cases, we can think that the quality reflects interactions between paternal and maternal genomes. Based on these gene-gene interactions, the genetic compatibility hypothesis was proposed (Trivers, 1972; Zeh & Zeh, 1996; 1997): it suggests that favorable interactions between genes within an individual can lead to increased survivorship through, for example, heterozygote advantage (Neff & Pitcher, 2005).

Neff & Pitcher (2005) define quality based on the contribution a gene variant (allele) or genotype (alleles) makes to an individual's fitness; an individual is of higher genetic quality when it possesses an allele or genotype that increases its fitness relative to that of an individual with a different allele or genotype. Because genetic quality is defined based on an individual's fitness, it must have two components: additive genetic effects (good genes) and nonadditive genetic effects (compatible genes).

Parental investment and egg

Trivers (1972) divides the reproduction investment in sexual and parental investment. With sexual investment he means the energy implicated into the research and conquest of a partner or in the fight with a contender. Parental investment can include all those things that contribute to the care of the offspring: it corresponds to the acts done by an adult for the benefit of his descendants for increment the survivor possibility. Consequently, the parental investment reduces the individual capability to have a new offspring blunting his survivor probability (because he uses energy for the offspring).

This body of theory was formally initialized by Fisher (1930), who showed that the lifetime reproductive success should be estimated by taking into account how the current reproductive expenditure affects future reproductive success (Brommer, 2000). There are a lot of evidence that may suggest that organisms have been selected to allocate resources to reproduction over time in response to the costs and benefits of current and future reproductive opportunities (e.g., Houston & McNamara, 1999).

Life-history theory generally predicts that reproductive investment should be high when the expected returns in fitness are high; any factors that increase or decrease the expected returns on investment should have the potential to affect reproductive allocation decisions. This assumption implies that there should, optimally, be facultative adjustment of reproductive investment in response to biotic and abiotic factors, which can generate maternal effects on offspring development (Harris & Uller, 2009). We speak of maternal effects for all the things that concern non-genetic influence of the mother on the development of the future generation (Mousseau & Fox, 1998). One important factor that potentially could influence returns on a given reproductive investment is mate quality. Indeed, if the selection in female mate choice is based on male sexual traits it must be greater returns on investment generated by those males than others (Andersson, 1994), suggesting that differential reproductive investment in response to male quality should be widespread (Sheldon, 2000). This idea was published in 1986 by Nancy Burley (1986), who predicted that females should increase investment when paired with males of high quality as long as reproduction is costly, there is a trade-off between current and future reproductive success and mate quality influences reproductive returns on investment (e.g., by increasing offspring survival). This argument was developed by Burley (1986) on a model species where both males and females care for the young (zebra finches), but it is general and can be made for any iteroparous organism that faces variation in mate quality (Sheldon, 2000).

Life-History Theory

Life history theory posits that the schedule and duration of key events in an organism's lifetime are shaped by natural selection to produce the largest possible number of surviving offspring. These events, notably juvenile development, age of sexual maturity, first reproduction, number of offspring and level of parental investment, senescence and death, depend on the physical and ecological environment of the organism. Organisms have evolved a great variety of life histories, from pacific salmon, which produces thousands of eggs at one time and then die, to human beings, which produce a few offspring over the course of decades (Stearns, 1992). The theory depends on principles of evolutionary biology and ecology and is widely used in other areas of science.

Life history characteristics are traits that affect the life table of an organism, and can be imagined as various investments in growth, reproduction, and survivorship.

The goal of life history theory is to understand the variation in such life history strategies. This knowledge can be used to construct models to predict what kinds of traits will be favored in different environments. Without constraints, the highest fitness would belong to a Darwinian Demon, a hypothetical organism for whom such trade-offs do not exist. The key to life history theory is that there are limited resources available, and focusing on only a few life history characteristics is necessary (Stearns, 1992).

Examples of some major life history characteristics include:

- Age at first reproductive event;
- Reproductive lifespan and aging;
- Number and size of offspring.

Variations in these characteristics reflect different allocations of an individual's resources (i.e., time, effort, and energy expenditure) to competing life functions. For any given individual, available resources in any particular environment are finite. Time, effort, and energy used for one purpose diminishes the time, effort, and energy available for another (Stearns, 1992). For example, birds with larger broods are unable to afford more prominent secondary sexual characteristics (Gustafsson et al, 1995). Life history characteristics will, in some cases, change according to the population density, since genotypes with the highest fitness at high population densities will not have the highest fitness at low population densities (Müeller et al., 1991). Other conditions, such as the stability of the environment, will lead to selection for certain life history traits. Experiments by Michael R. Rose and Brian Charlesworth showed that unstable environments selected for flies with both shorter lifespans and higher fecundity (Rose & Charlesworth, 1980).

Hence, females are expected to invest more when they are paired with mates of high quality, thereby generating a positive relationship between partner quality and reproductive investment; Burley (1986) called this hypothesis the Differential Allocation Hypothesis (DAH). However, this hypothesis has been challenged in several papers (Gowaty et al., 2003; 2007; Bluhm & Gowaty, 2004; Gowaty 2008): here, the authors have suggested that, rather than increasing investment when paired with a high-quality partner, females should exhibit reproductive compensation (here-hence the name Reproductive Compensation Hypothesis, RSH) for poor-quality mates by boosting reproductive effort in order to counteract the negative effect that mate quality has on offspring fitness. In this way, the RCH predicts that females should invest relatively more when paired with males of low quality (Bluhm & Gowaty, 2004; Gowaty et al., 2007). Understandably, the two hypothesis bring to opposite prediction (Harris & Uller, 2009).

Differential investment in response to mate quality has implication not only in the study of optimal allocation of energetic resources to reproduction; in general, maternal effects can modify the rate and direction of evolution via indirect genetic effects (Kirkpatrick & Lande 1989; Cheverud & Moore 1994; Moore et al. 1997; Räsänen & Kruuk 2007), which is also true for maternal effects in relation to mate quality (Moore et al. 1997; Wolf et al. 1999; Sheldon 2000). More specifically, increased or reduced allocation to offspring of high-quality males could increase or decrease differences in male fitness, thereby affecting the strength of sexual selection, in particular in the context of genetic quality or benefits (Sheldon 2000; Qvarnström & Price 2001). For example, because there is a lot of evidence that maternal effects influence the attractiveness of sons in adulthood (e.g., Lindström 1999; Kotiaho et al. 2003; Forstmeier et al. 2004; Saino et al., 2007), DA can increase the similarity between fathers and sons caused by shared genes, even in species with no paternal resource investment, and thus lead to erroneous conclusions regarding the genetics of sexual traits (Sheldon 2000).

The extra resources can be allocated to increased egg size (Kolm 2001), egg number (Locatello & Neat 2005), more carotenoids (Surai et al. 2001), antibodies (Saino et al. 2003) or hormones (Schwabl et al. 1997; McCormick 1999; Lovern & Wade 2001).

In literature there are studies that support the DAH and others that sustain the RCH (Ratikainen & Kokko, 2010). For the first hypothesis we can find example in different groups: birds (e.g., Cunningham & Russell, 2000; Birkhead & Pizzari, 2002), arthropods (e.g., Thornhill, 1983; Kotiaho et al., 2003) and fishes (e.g., Kolm, 2001). Studies supporting RCH (even if not all speak clearly of compensation) have been conducted on barn swallows *Hirundo rustica* (Saino et al., 2002a), collared flycatchers *Ficedula albicollis* (Michl et al., 2005), house finches *Carpodacus mexicanus* (Navara et al., 2006a; 2006b), pronghorn *Antilocapra americana* (where female compensated by elevating rates of milk delivery to their young – Byers & Waits, 2006), zebra finches (Bolund et al., 2009), broad-nosed pipefish *Syngnathus typhle* (Goncalves et al., 2010), house mouse *Mus musculus* (Drickamer et al., 2000) and fruit fly *Drosophyla pseudoobscura* (Anderson et al., 2007).

In the majority of study the different or compensative allocation was made by the female, so we can speak of maternal effects. With this term we include all the non-genetic influences of the mother on the development of the future generation. These effects take account of all aspects of the maternal influence on the phenotype offspring. Mothers determine propagule size, where, when and how propagules are dispersed, protection of young from inclement conditions or predators, parental care and provisioning to developing young, as well as the attributes of the offspring's father if mate choice is operating (Räsänen & Kruuk, 2007). In addition, a mother's experience of the environment can lead to variation in her growth (i.e., body size), condition and physiological state that can be transmitted to offspring via cytoplasmic factors (e.g., yolk amount, hormones and mRNAs) in the egg that may directly (via maternal programming) or indirectly (via offspring sensitivity to maternally transmitted factors) influence offspring development. The extent to which maternal environment and behavior influence offspring phenotype and fitness will determine the likelihood that such maternal effects themselves will be shaped by the action of natural selection (Mousseau & Fox, 1998). Mousseau & Fox (1998) suggested that maternal effects often provide a mechanism for adaptive transgenerational phenotypic plasticity, in which the environment experienced by the mother is translated into phenotypic variation in the offspring, and that this relationship can be envisioned (and modeled) as a reaction norm. So the maternal experiences will be translate in variation in her descendants that will bring to a faster adaptation to environmental transformations than the genetic adaptations (Garcia-Fernandez, 2009).

The effects on the offspring change depending on the moment when the maternal effects are expressed: for example, Tanvez et al. (2009) investigated how mothers transfer their dietary carotenoid-related benefits to their offspring: either through the egg, or through the diet (during chicks' feeding). Female canaries were allowed to access β-carotene enriched food during egg formation and/or chicks' feeding. They sorted the chicks into four groups using the period when they assimilated the β-carotene as a variable. The four groups were: (1) before hatching (from yolk), (2) after hatching (from maternal feeding), (3) before and after hatching, or (4) never. The results show that benefits from maternal dietary carotenoids are transferred to the chicks, but according to the period when they are assimilated by the chicks, the physiological effects are different. It was found that the chicks' growth was enhanced when carotenoids were assimilated both before and after hatching. However an increase in cellular immunity efficiency only occurs when the assimilation takes place after hatching (Tanvez et al., 2009).

As we have seen, oviparous species are good models for the study of females investment because they must concentrate a great amount of their maternal effects into the egg in a little interval of days. After this time, they can't do any adjustment, contrary to the mammals that can regulate the resources during embryogenesis. Given that the egg contents represent all the resources available to the embryo, the study of maternal effects just before the laying take a lot of interest. Moreover, eggs are relatively voluminous, so easily to manipulate for do measurements or alteration of the contents.

Oviparity

Oviparous animals are animals that lay eggs, with little or no other embryonic development within the mother. This is the reproductive method of most fish, amphibians, reptiles, all birds, the monotremes, and most insects, some molluscs and arachnids (Lodé, 2001).

With more scientific rigor, five modes of reproduction can be differentiated based on relations between zygote and parents:

- Ovuliparity: fecundation is external (in arthropods and fishes, most of frogs);
- Oviparity: fecundation is internal, the female lays zygotes as eggs with important vitellus (typically birds);
- Ovo-viviparity: or oviparity with retention of zygotes in the female's body or in the male's body, but there are no trophic interactions between zygote and parents (e.g., *Anguis fragilis*). In the sea horse, zygotes are retained in the male's ventral "marsupium". In the frog *Rhinoderma darwinii*, the zygotes developed in the vocal sac. In the frog *Rheobatrachus*, zygotes developed in the stomach;
- Histotrophic viviparity: the zygotes developed in the female's oviducts, but find their nutriments by oophagy or adelphophagy (intra-uterine cannibalism in some sharks or in the black salamander *Salamandra atra*);
- Hemotrophic viviparity: nutriments are provided by the female, often through placenta. In the frog *Gastrotheca ovifera*, embryos are fed by the mother through specialized gills. The lizard *Pseudomoia pagenstecheri* and most mammals exhibit a hemotrophic viviparity (Lodé, 2001).

Land-dwelling animals that lay eggs, often protected by a shell, such as reptiles and insects, do so after having completed the process of internal fertilization. Water-dwelling animals, such as fish and amphibians, lay their eggs before fertilization, and the male lays its sperm on top of the newly laid eggs in a process called external fertilization.

…Continue

Almost all non-oviparous fish, amphibians and reptiles are ovoviviparous, i.e. the eggs are hatched inside the mother's body (or, in case of the sea horse, inside the father's). The true opposite of oviparity is placental viviparity, employed by almost all mammals (the exceptions being marsupials and monotremes).

There are only five known species of oviparous mammals (monotremes): four species of Echidna and the Platypus (Lodé, 2001).

The investment in the egg change with the character nidifuge or nidicole of the species. The offspring of the second type are very dependent by parents during the first days after the hatching: they born blind and without plumage, and they can just raise the head to demand food or heat. So, the parental care after the hatching are as essentials as the prenatal investment. In the nidifuge species, brood are relatively independent after the hatching: they step out to the egg already developed, with plumage and they are capable to find food by themselves. In these species the maternal investment before hatching are very essential for the growth of the bird (Garcia-Fernandez, 2009). It is interesting to know that more is precocious the species and more yolk is present (in proportion) into the egg. The yolk is considered an indicator of the quality of the eggs (Williams, 1994); in chicken, for example, it is present bounded to the intestine when the chick born and it assures nutrients until the self-feeding (Sotherland & Rahn, 1987).

Stress and environmental stressors

Life-history theory suggests that trade-offs exist between fitness components, with organisms balancing investment in reproduction against survival and future reproduction (Petes et al., 2008). Physiological trade-offs exist in all organisms when resources are limited, because each individual has a certain amount of energy available to maintain physiological processes, such as growth, reproduction, metabolism, and immune function (e.g., Roff, 1992b; Steams, 1992). Brett (1958) defined a physiological stress state as being induced "by any environmental factor which extends the normal adaptive responses of an animal, or which disturbs the normal functioning to such an extent that the chances of survival are significantly reduced".

For Selye (1963) "stress describes an animal's defense mechanisms, and thus a stress stimulus (stressor) is any situation that elicits defensive response".

Petes et al. (2008) defined stress as "any environmental factor (e.g., temperature, desiccation) leading to a physiological stress state". When a stress state occurs, organisms must reallocate energy away from these processes toward defense and repair mechanisms in order to survive. The cost of reproduction is the most prominent life-history trade-off, because costs are paid in terms of survival and future reproduction (Steams, 1992). Reproduction is energetically expensive (Williams, 1966), and reproductive processes may therefore be compromised under stressful conditions in an attempt to devote more energy toward survival (Wingfield & Sapolsky, 2003). Impaired or suppressed reproduction has large, negative consequences for population dynamics and, in the most extreme cases, for species persistence. The interactions between stress and reproduction have been widely documented in vertebrates, from fish (e.g., Schreck et al., 2001) to birds (e.g., Ots & Hörak, 1996), amphibians and reptiles (e.g., Moore & Jessop, 2003), and mammals (e.g., Moburg, 1991).

For what concern birds, since the environment is a composite of interacting stressors, a bird's success in coping with it depends on the severity of the stressor(s) and the bird's physiological ability to respond properly. In the broadest sense, the environment includes all combinations of conditions in which the bird lives-external (temperature, light, etc.) as well as internal (disease organisms, parasites). Significant changes in the environment signal regulatory processes to attempt to maintain or reestablish the equilibrium or homeostatic state (Siegel, 1980).

There are two general types of regulatory processes: (1) specific and (2) nonspecific.

The first type is a particular condition will elicit a specific response (e.g., when environment causes a bird's body temperature to rise). A nonspecific process occurs when, regardless of the environmental stimulus, the animal responds in a generalized manner, going into a state of general stress. The two types of regulatory responses are not mutually exclusive; they occur simultaneously, and one may have a dramatic effect on the other (Siegel, 1980).

Beyond this kind of stress named physiological stress we can also speak of social stress.

Social stress is common in many animal species and typically results from competition for resources such as space, access to a reproductive partner, food, or water (Tamashiro et al., 2005).

There are several models of social stress that are used in the laboratory and most can be categorized into one of two broadly defined groups: the resident-intruder and the social hierarchy or colony model. The resident-intruder paradigm of social defeat is a popular animal model of social stress typically utilized in rats and mice (Tamashiro et al., 2005).

For what concerns the social hierarchy, a number of animal models have been developed to capitalize on the natural tendency of different species to form social hierarchies when housed in groups, including sugar gliders (Jones et al., 1995; Mallick et al., 1994), rats (Barnett, 1958; Barnett et al., 1960; Taylor et al., 1987; Blanchard & Blanchard, 1989; De Goeij et al., 1992; Dijkastra et al., 1992; Blanchard et al., 1995; Fokkema et al., 1995; Stefanski et al., 2001), mice (Ely & Henry, 1978), and non-human primates (Kaplan et al., 1982; 1996; 2002; Sapolsky & Bennet, 1990; Fontenot et al., 1995; Shively et al., 1997a; 1997b; Virgin & Sapolsky, 1997; Shively, 1998). Establishing and maintaining dominance in a group setting is psychologically and physically stressful for all parties, including both the dominant and the subordinate animals (Tamashiro et al., 2005).

This work has investigated three potential source of stress for the females that can have consequence on the maternal effects: the male dominance, the anthropic noise and the immune challenge.

Dominance

Dominance can be defined as success in contests. By killing, driving away or using other means of intimidating individuals, dominant individuals exclude at least some of their rivals from access to mates or resources crucial for attracting mates. Dominance hierarchies are often settled by relative body size, aggressiveness, size of weaponry or signals of fighting ability (badges of status). Such morphological and behavioural traits are costly because they can cause increased predation risk, increased energy stress and/or increased disease susceptibility (Qvarnström & Forsgren, 1998). If it is only the individuals of relatively high quality that are able to bear the cost of dominance (Zahavi, 1975; Grafen, 1990), the position in the hierarchy *per se* or traits indicating dominance will reliably reflect certain aspects of mate quality.

However, because motivation (i.e. how much an individual values the contested resource) is also important, the best fighter will not always win a contest. Thus, dominance hierarchies could be unstable: an individual that is dominant in a social contest (e.g. successful in fights over food or resting sites) may not necessarily be successful in contests over mates. Furthermore, because individuals value their resources increasingly with their time of ownership, previous site knowledge and order of occupancy will affect the outcome of territorial disputes (Qvarnström & Forsgren, 1998).

There are several direct costs of copulation that can vary in magnitude according to the copulatory partner. Females might be expected to try to minimize such costs when selecting their partner, and the question then becomes what are the relationships between these costs and male dominance? These costs include the following:

- Risk of injury or death caused by males. In species where males are larger than females or where the *sex ratio* is strongly male biased, females can be injured and occasionally killed during mating, typically by several competing males (Le Boeuf & Mesnick, 1990). Thus, by selecting a dominant male, who by definition is successful at excluding rivals, a female could reduce the risk of being injured;
- Disease transmission. It has been suggested that the outcome of male–male competition reliably reflects a male's disease state because infection will prevent a male from becoming dominant, as observed in mice (Freeland, 1981). Consequently, females could avoid infection by parasites by mating with dominant males. However, high androgen levels not only increase male dominance, but also suppress the immune system, which means that dominant males could actually be more susceptible to diseases and parasites. However, dominant males might be able to bear the cost of this immunocompetence handicap (Folstad & Karter, 1992). Consequently, there is no *a priori* expectation for the relationship between dominance and health state in any particular species. Sexually transmitted diseases are exclusively transferred during copulation. For species with mating systems in which male mating success is strongly skewed, such as in lekking species or in species where single males defend harems of females, top-ranking males are likely to be highly exposed to such diseases. Because the costs of exposure to these diseases are higher for females than for males (Sheldon, 1993), a sexual conflict could arise. In the presence of a virulent sexually transmitted disease, a dominant male might still benefit from monopolizing several females (by excluding other males from mating),

whereas it may be in the females' interest to seek private mates even if that would mean accepting subdominant individuals. Thus, whether females face an increased or a reduced risk of disease transmission by mating with dominant males is still unclear;

- Fertilization failure caused by sperm depletion. Given that males do not have a superabundance of sperm, increased mating frequency will lead to reduced sperm provision per mating (Pitnick & Markow, 1994), which might lower female fertilization probabilities. Consequently, if dominant males monopolize many females, their fertilization efficiency may be reduced (Qvarnström & Forsgren, 1998).

But some studies seem to demonstrate that can be problems also when a female mate with a dominant male. Ophir & Galef (2003; 2004) have found that females quail *Coturnix Japonica* chose subordinate male; in this way, they loss direct and indirect benefits deriving by the mate with best male, but they avoid possible aggressive behaviour. Also a study on the canary *Serinus canaria* has showed a similar behaviour. Females canary have chosen male different depending on the type of competition: if it was a singing competition females have chosen the dominant male, while in a competition for food they have preferred the dominated one (Amy et al., 2008).

So, female can encountered different type of stress both she chooses a dominant male or a subordinate one.

Anthropic noise

Noise is generally understood as any sound that interferes with the proper transmission of an acoustic signal, from sender to receiver (Brumm & Slabbekoorn, 2005).

Anthropogenic noise currently affects large areas of natural habitat worldwide (Forman, 2000; Barber et al., 2009).

Growth in transportation networks, resource extraction, motorized recreation and urban development is responsible for chronic noise exposure in most terrestrial areas, including remote wilderness sites. Increased noise levels reduce the distance and area over which acoustic signals can be perceived by animals (Barber et al., 2009).

In natural environments, acoustic signals have to be isolated from other sounds to extract the relevant information (Pohl et al, 2012). A reduction in signal detection caused by the acoustic energy of another sound is known as masking. Masking depends on the relative amplitude of the signal and the background noise, but also on their similarity in frequency distribution (e.g. Klump, 1996). Masking sounds may, for example, be produced by other birds or insects (so we can speak of biotic source; e.g., Staicer et al., 1996; Poesel et al., 2007; Kirschel et al., 2009) or by wind and water (abiotic sources; e.g., Douglas & Conner, 1999; Schomer & Beck, 2010). A ubiquitous masking sound of modern times is provided by the relatively low-frequency noise caused by human traffic (e.g., Skiba, 2000; Slabbekoorn & Peet, 2003; Warren et al., 2006). Increasing urbanization worldwide is creating novel environments that animals must either adapt to or abandon (Warren et al., 2006).

It's known that birds, primates, cetaceans and a sciurid rodent shift their vocalizations to reduce the masking effects of noise (Brumm & Slabbekoorn, 2005; Slabbekoorn & Ripmeester, 2008). However, compromised hearing affects not only acoustical communication. Comparative evolutionary patterns attest to the alerting function of hearing:

1) Auditory organs evolved before the capacity to produce sounds intentionally (Fay & Popper, 2000);
2) Species commonly hear a broader range of sounds than they are capable of producing (Fay, 1988);
3) Vocal activity does not predict hearing performance across taxa (Fay, 1988; Bradbury & Vehrencamp, 1998);
4) Hearing continues to function in sleeping (Rabat, 2007) and hibernating (Lyman & Chatfield, 1955) animals;
5) Secondary loss of vision is more common than is loss of hearing (Fong et al, 1995).

Deleterious physiological responses to noise exposure in humans and other animals include hearing loss (Dooling & Popper, 2007), elevated stress hormone levels (Babisch, 2003) and hypertension (Jarup et al., 2008).

And what are the ecological problems given by the noise on the animal communication?

Intentional communications, such as song, are often processed by multiple receivers (Barber et al., 2009). Birds may use vocalization (i.e., calls or songs) to interact with their offspring (Kilner & Johnston, 1997), maintain group cohesion (Ritchison, 1983) and pair bonds (Swaddle & Page, 2007), to attract a mate (Catchpole & Slater, 2008), stimulate it for reproduction (Kroodsma, 1976), and compete with other individuals of the same sex for a territory (Catchpole & Slater, 2008), a prerequisite in many species before males can attract a mate (Ríos-Chelén, 2009). These communication networks enable female and male songbirds, for example, to assess multiple individuals simultaneously for mate choice, extra-pair copulations and rival assessment (McGregor, 2005). Acoustic masking resulting from increasing background sound levels will reduce the number of individuals that comprise these communication networks and have consequences for reproductive processes very little known (Habib et al., 2007; Swaddle & Page, 2007; Halfwerk et al., 2011a; 2011b; Huet des Aunay 2011; Schroeder et al., 2012). All the above mentioned processes can have a direct or indirect effect on fitness and it thus becomes relevant to study the possible effects of disrupting factors (such as urban noise) both on acustic signals and on attributes of populations or communities (e.g., population density, diversity – Ríos-Chelén, 2009).

Because acoustic communication is a critical component of both male-male competition and female mate choice in many taxa including anurans, crickets and birds (Andersson, 1994), the effects of urban ambient noise on signal transmission might have significant consequences for mate choice and resource defence across a diversity of taxa (Luther & Derryberry, 2012). Extensive research suggests that both anurans and birds adapt their vocalization behaviours to the urban acoustic environment (Brumm & Todt, 2002; Slabbekoorn & Peet, 2003; Brumm, 2004; Wood & Yezerinac, 2006; Parris et al., 2009; Pohl et al., 2009; Verzijden et al., 2010), and recent studies suggest that receivers respond to differences in signals between urban and nonurban areas (Mockford & Marshall 2009; Ripmeester et al., 2010a).

Immune challenge

The immune system is the animal's defense mechanism to fight or control any parasitic or pathogenic infection (Lochmiller, 1996; Sheldon & Verhulst, 1996; Zuk, 1996). In addition, immunity is one of the major physiological mechanisms regulating host survival (Lochmiller & Deerenberg, 2000).

The concept of inducible defence includes a large range of protective responses to attacks by parasites, predators or herbivores, including toxic compounds in plants, morphological changes in rotifers, and the vertebrate immune system (Tollrian & Harvell, 1999). A common assumption when we speak about defence is that there should be a trade-off between cost and benefit of the response (Harvell, 1990).

Mounting an immune response, and even maintaining a competent immune system, is thought by many ecologists to be a nutritionally demanding process that necessitates trade-off decisions among competing nutrient demands for growth, reproduction, temperature, work, and immunity (Sheldon & Verhulst, 1996).

For example, mounting a cell-mediated immune response in house sparrows *Passer domesticus*, or an antibody response in great tits *Parus major*, significantly elevates metabolic rate (Ots et al., 2001; Martin et al., 2003). In cabbage butterfly pupae *Pieris brassicae L.* metabolic rate is significantly elevated following an immune challenge (Freitak et al., 2003).

And what about the effects of an immune challenge? Is-it stressfully or not?

One of the first study that investigated the costs of immune defence in natural population was that of Williams et al. (1999): they immunized female starlings *Sturnus vulgaris* with sheep erythrocytes or saline, removed their clutches, and compared performance of the two groups during laying of a replacement clutch. These authors found no effects of immunisation on laying interval, clutch size or egg mass, and concluded that there was no evidence for a cost of the immune response. But in several avian species, females forced to invest in mounting an immune response while breeding exhibit changes in reproductive behaviour, particularly decreased feeding rate of nestlings, resulting in decreased fledging, and ultimately lowering breeding success (Ilmonen et al., 2000; 2002; Bonneaud et al.; 2003). In particular, Ilmonen et al. (2000) found that female pied flycatchers *Ficedula hypoleuca* given diphtheria-tetanus vaccination during the nestling-feeding period had lower reproductive success than saline-injected controls. Råberg et al. (2000) found that vaccinated females reduced their nestling feeding rate, thus demonstrating a cost of the immune response in the currency of parental effort. Additionally, elevated humoral activity reduces ovarian protein content in the mosquito *Anopheles gambiae* (Ahmed et al., 2002). Collectively, these descriptive and experimental studies provide support for energetic trade-offs between reproduction and immune function such that increased investment in one process leads to decreased investment in other processes (French et al., 2009).

In his paper, Hanssen (2006) divided the effects on an individual's reproductive investment of an induced immune challenge in two:

1) The resource demand could increase to "fuel" the immunologic reaction, which in turn can lead to an adaptive decrease in investment in resource-costly activities, such as reproduction;
2) The individual could assume that the immune activity it experiences is indicative of a serious infection. The latter can lead to an adaptive increase in reproductive investment in response to the reduced prospects of survival and future reproduction, so called "terminal investment".

His experiment, done on common eider *Somateria mollissima*, showed that an immune challenge lead to increased incubation cost measured as larger mass loss and longer incubation period. Despite these increased costs, immune-challenged common eider females increased their investment in reproduction after hatching, as a higher percentage of these birds cared for their ducklings compared to sham-injected controls. This increase in costs or in investment due to an immune challenge might even have long-term consequences: the reduced return rate of the immune-challenged group indicated abstention from breeding or perhaps reduced survival (Hanssen, 2006).

In conclusion, here is a brief overview on the short and long-term costs of immune system activation. Generally speaking, the short-term costs are more transient and can be (at least partly) compensated for after the end of the immune challenge, whereas the harmful effects of long-term costs accumulate and become more severe over time (Hasselquist & Nilsson, 2012).

Immediate costs in the form of reduced current reproductive success have been demonstrated experimentally by immunizing parents during breeding, resulting in a prolonged incubation period, reduced feeding effort and/or reduced fledging success (Williams et al. 1999 ; Ilmonen et al. 2000; Råberg et al. 2000; Bonneaud et al. 2003; Faivre et al. 2003; Alonso-Alvarez et al. 2004; Hanssen et al. 2004; Peters et al. 2004; Verhulst et al. 2005; Garamszegi et al. 2006; Hanssen 2006; Fitze et al. 2007; Marzal et al. 2007; Torres & Velando 2007; Gasparini et al. 2009). A substantial long-term cost associated with immune system activation has been found in terms of considerably reduced between-year return rate in eiders, *S. mollissima*. Incubating and hence fasting females that mounted antibody responses to two antigens simultaneously apparently suffered from impaired survival (Hanssen et al. 2004). Furthermore, induced immune responses commonly reduce growth rate in chicks (Fair et al. 1999; Soler et al. 2003; Brommer 2004; Grindstaff 2008), impair postfledging survival (Eraud et al. 2009) and reduce testosterone levels in adults (Boonekamp et al. 2008).

Finally, the analyses made by Hasselquist & Nilsson (2012) give results that don't support the hypothesis that energy is the key proximate currency mediating the costs of immunity. Nutrient savings from immunosuppression seem to be even less beneficial as this constitutes only a minor part of the daily nutrient turnover in the body. They say that there are some indications that oxidative stress can be an important currency that could mediate both short-term and long-term costs of immune system activation, although direct evidence is so far limited (Hasselquist & Nilsson, 2012).

Deposition quality

As already seen in chapter concerning the parental investment, the resources allocation into the egg is influenced by maternal environment before and during the egg formation (Schwabl, 1996a).

Sheldon (2000) speak about differential allocation when the characteristics of the partner influence the female investment into the eggs. Usually, in literature the differential allocation is interpreted as a greater maternal investment when female is coupled with a good male. The selective valour, already favourite by the "good genes" potentially transmitted by the father, is maximized (Burley, 1988; Sheldon, 2000).

There is also another theory that says that exists a compensation of the poor quality of the male with a greater investment of the mother (Bluhm & Gowaty, 2004; Burley, 1988).

The theories has been observed in concentration of antioxidants, essentials for a good embryo development and for the offspring survivor. The concentration augmented with the partner attractiveness in zebra finch *Taeniopygia guttata* (Williamson et al., 2006) while in the house finches *Carpocadus mexicanus* there are more antioxidant in the eggs produced with the less attractive male (Navara et al., 2006c).

The most utilized indices for the determination of the deposition quality are clutch and egg mass (Garcia-Fernandez, 2009).

Clutch size

In oviparous animals, clutch production represents a huge investment in terms of energy and reserves allocated to eggs in a limited period of time (Nager, 2006).

Difference can be find between species in the modulation of the clutch size: some species (i.e., blue-winged teal *Anas discors*) can produce a precise number of eggs because they have an exact number of ovarian follicles developed at the same time. They are species with specific clutch size that can't modulate the number of the eggs (Kennedy, 1991). Other species, such as the domestic fowl *Gallus gallus domesticus*, have brood size highly variable and adaptable; they are the species that are indeterminate number of eggs (Kennedy, 1991). Various factors may modulate the number of eggs laid, as the physical condition of the female or food available (Christians, 2002). Female canary (*S. canaria*) heaviest are those that lay the largest number of eggs (Müller et al., 2008). In the house wrens *Troglodytes aedon*, the females compensate the decrease in the number of insects at the end of season reproduction by laying less heavier eggs. The offspring, less numerous and in better physical condition to the hatching, have, despite the small amount of food, equal possibility of survival than the chicks born earlier in the season (Styrsky et al., 1999).

Male characteristics appear to have little influence; thus, the quality of male song in the canary (Tanvez et al., 2004) and in the eastern kingbird *Tyrannus tyrannus* (Murphy et al. 2008) does not change the number of the egg.

Egg mass

In avian species, it has been shown that egg mass is highly repeatable and heritable at the individual level (Christians, 2002), suggesting a strong genetic component. Environmental components, for example food availability, and health conditions of the laying female can also play a role in intra-clutch variation in mass and egg composition (Ardia et al., 2006).

When the within-clutch variation is considered, in several species the well-known phenomenon of hatching asynchrony occurs (Magrath, 1990), and, frequently, the last-laid egg can be smaller and with fewer resources than the first-laid eggs (Lack, 1968; Sockman, 2008). Also, in precocial synchronous species, a pattern of egg size variation with laying order can exist, with either an increase, a decrease or a tendency to increase up to a threshold and to decrease thereafter (Cabezas-Diaz et al., 2005). Egg size can have important long-term consequences for the survival of offspring (Starck & Ricklefs, 1998; Forbes & Wiebe, 2010) because larger eggs tend to have higher hatching success (Perrins, 1996) and lead to larger hatchlings (Christians, 2002).

Nidifugous, Nidicolous and Altricial

Nidifugous organisms are those that leave the nest shortly after hatching or birth. It is derived from Latin *nidus* for "nest" and *fugere* meaning "to flee". The terminology is most often used to describe birds and was introduced by Lorenz Oken in 1816 (Starck, 1998). The chicks of birds in many families such as the waders, waterfowl and gamebirds are usually nidifugous.

The term "nidifugous" is sometimes used synonymously with "precocial", as all nifidugous species are precocial (that is, born with open eyes and capable of independent locomotion). However, not all precocial birds leave the nest; some may stay at the nest, and are thus nidicolous instead (Ehrlich et al., 1988).

…Continue

Nidicolous animals are those that stay at their nest or birthplace for a long time after birth, due to their dependence on the parents for feeding, protection and learning survival skills. It is the opposite of nidifugous where the animal is able to leave the nest very quickly. The great majority of nidicolous animals are altricial, in that an animal born helpless, blind, without feathers, etc. simply is unable to fend for itself. Examples of nidicolous animals are most mammals, marsupials and many species of birds. However, the concepts of altricial and nidicolous are not identical. All altricial animals are nidicolous by necessity, however, an animal may be nidicolous (i.e. staying at the nest) even if they are precocial and fully capable of leaving if needed (Starck, 1998).

Altricial, meaning "requiring nourishment", refers to a pattern of growth and development in organisms which are incapable of moving around on their own soon after hatching or being born. The word is derived from the Latin root *alere* meaning "to nurse, to rear, or to nourish", and refers to the need for young to be fed and taken care of for a long duration (Ehrlich et al., 1988). In bird and mammal biology, altricial species are those whose newly-hatched or born young are relatively immobile, lack hair or down, not able to obtain food on their own, and must be cared for by adults; closed eyes are common, though not ubiquitous. Altricial young are born helpless and require care for a specific amount of time. Among birds, these include, for example, herons, hawks, woodpeckers, owls and most passerines. Among mammals, marsupials and most rodents are altricial. Cats, dogs, and humans are some of the best-known altricial organisms. At the opposite end of the spectrum are precocial animals in which the young have open eyes, have hair or down, have large brains, and are immediately mobile and somewhat able to flee from, or defend themselves against, predators. For example, with ground-nesting birds such as ducks or turkeys, the young are ready to leave the nest in one or two days. Among mammals, most ungulates are precocial, being able to walk almost immediately after birth. Beyond the precocial are the superprecocial animals, such as the megapode birds, which hatch with full flight feathers. In birds, the terms *"Aves altrices"* and *"Aves precoces"* was introduced by Carl Jakob Sundevall (1836) and the terms "nidifugous" and "nidicolous" by Lorenz Oken in 1816. The two classifications were considered identical in early times, but the meanings are slightly different, in that "altricial" and "precocial" refer to developmental stage, while "nidifugous" and "nidicolous" refer to leaving or staying at the nest (Starck, 1998). The two strategies result in different brain sizes of the newborns compared to adults. Precocial animals' brains are large at birth relative to their body size, hence their ability to fend for themselves. However, as adults, their brains are not much bigger or more able. Altricial animals' brains are relatively small at birth, thus their need for care and protection, but their brains continue to grow. As adults, altricial animals end up with comparatively larger brains than their precocial counterparts. Thus the altricial species have a wider skill set at maturity (Ehrlich et al., 1988).

Normally, egg mass isn't correlated with clutch size (Christians, 2002), but with the dimension of the skeleton of the chick and the yolk mass. The egg mass is a good index of the survivorship of the offspring (Bolton, 1991; Williams, 1994). Only some species (i.e., house wren *T. aedon*) produce eggs heavier when they reduce the clutch size (Styrsky et al., 1999).

Male characteristics influence more the egg size than the clutch size: female of mallard *Anas platyrhynchos* laying egg bigger when coupled with an attractive male than an imposed one (Cunningham & Russel, 2000).

The egg components have different and specific roles in the embryo and chick development. An augmentation of the egg size (or mass) can be isometric or only of a part of the egg (Garcia-Fernandez, 2009).

Egg components

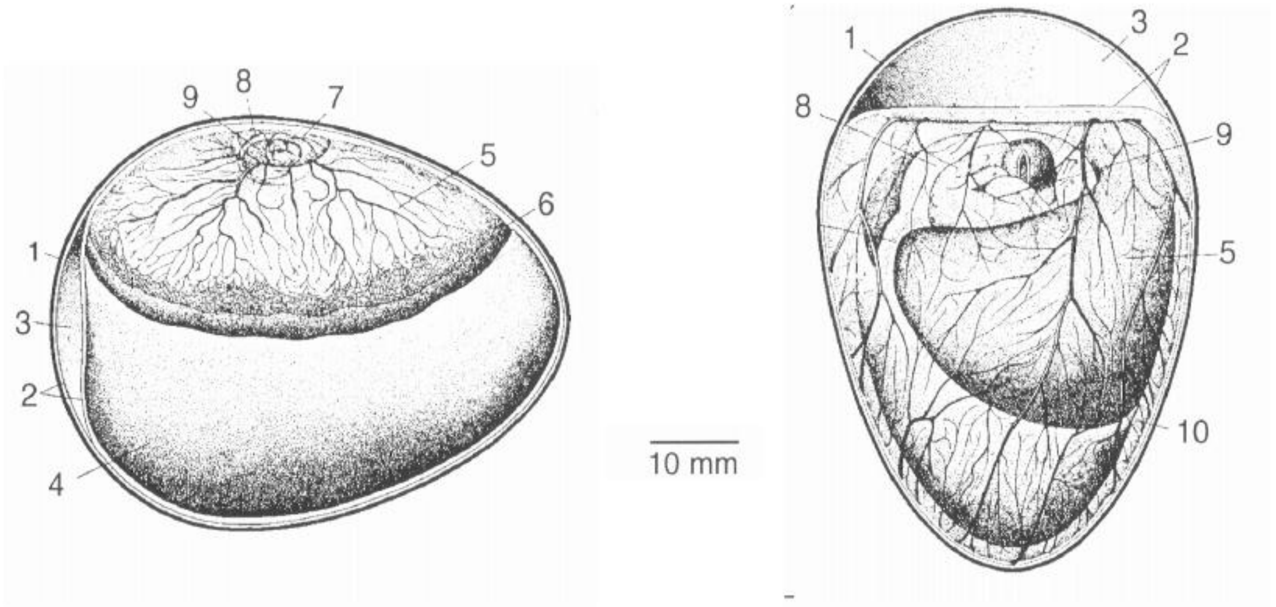


Figure 1 - Bird egg. Legend: 1) Eggshell; 2) Inner and outer membranes; 3) Air cell; 4) Yolk; 5) Vitelline membrane; 6) Bloody marginal sinus; 7) Corion membrane; 8) Amnion; 9) Embryo; 10) Chorioallantoic membranes. Taken from Guasco, 2006.

The eggs of birds are defined:

- Telolecithic, since there is an accumulation of yolk to the vegetative pole;
- Endoidhric, since the females allocate sufficient water for support embryogenesis (Wallace et al., 2006);
- Cleidoic type, because they can be considered more or less as self-sufficient systems that provide protection to the embryo in the process of development, by means of extraembryonic membranes contained within a protective shell (Guasco, 2006).

The eggs appear to be protected by a calcareous shell that allows the gas exchange (oxygen, carbon dioxide and water vapor) with the outside during ontogeny. Changes in the composition of the eggs of birds occur between species and within the same species (Carey et al., 1980; Sotherland & Rahn, 1987).

Into the egg, we can distinguish some main structures:

- The yolk;
- The albumen;
- The shell, which also includes the inner and outer membranes (Guasco, 2006).

Yolk

The yolk is formed in the follicular sack, through the deposition of successive layers of material, prior to ovulation. The follicles are produced sequentially so as to prevent their simultaneous maturation. The egg yolk is the essential component from which the embryo develops itself and presents a subspherical shape. The period of yolk formation lasts 4-5 days in the Passeriformes, 6-8 days in Anseriformes and Columbiformes, up to a maximum of 16 days in some species of penguins (Gill, 2007).

The yellow coloration which the yolk often assumes is due to the presence of pigments of carotenoid nature, like the xanthophylls, arising from nutrition (Blount et al., 2000; Møller et al., 2000). The egg yolk is not homogeneous, and is rich in lipids and white material, poor in pigments and fats (Campebell & Lack, 1985; Carey et al., 1980). The yolk is the main source of lipids and is involved mainly in the weight increase rather than in the development of body size.

In the yolk, there is also a complex of proteins, among which predominates a low-density lipoprotein which represents 65% of the solids of the supernatant and 95% of lipids. 10% of proteins consists of levitine, which have close similarities with some blood proteins (globulins, glycoproteins and albumin).

The eggs of birds vary considerably in composition, especially in the amount of yolk that they may contain in relation to their size (Sotherland & Rahn, 1987). In seabirds eggs of large size generally contain more albumen and less yolk, relationship exactly inverse in aquatic species (Williams, 1994; Vieira, 2007). A higher content of albumen usually corresponds to a higher percentage of water than proteins, while an increase of yolk reflects a higher percentage of lipids *in ovo* (Williams, 1994). The eggs of early development species, have a large yolk (about 35% of egg weight) compared with eggs of inept offspring birds (about 20% of egg weight – Ricklefs, 1977).

The different percentages of yolk vary according to the maturity of the chicks to hatch, depending on whether you are in front of an inept offspring or of an early development (Gill, 2007).

Albumen

The albumen is produced in the front part of the oviduct, called magnum, where are secreted four layers of egg white.

We distinguish:

- a chalazipher layer, in contact with the vitelline membrane of the egg cell; from this layer depart the chalazae, one to the obtuse pole, two twisted on tip with the function of maintaining the yolk to the center of the egg;
- a thin layer inside and one outside (fluids);
- a gelatinous layer (dense), which extends itself at each end by the ligaments of the albumen, very rich of ovomucin that gives the appearance of thick gel.

The albumen appears as a viscous, transparent substance without a homogeneous constitution. It consists primarily of water (90%) and several proteins (approximately 10% – Sotherland & Rahn, 1987) some of which are bactericidal in an aqueous medium, immunologically different in different species of birds. The most represented proteins are albumin (90%) that provides protein stores necessary for embryonic development (Finkler et al., 1998), mucins and globulin (to 5% – Gill, 1996). Dissolved in the aqueous component of the albumen can also be found sugars and mineral ions; many of these are bound to proteins.

Lipids and minerals are present in small quantities (Romanoff & Romanoff, 1949). Albumen provides protection to yolk and to developing embryo in two ways, a mechanical and a chemical:

1) From a mechanical point of view, the albumen sustains the yolk preventing contact with membranes of the shell; moreover, for its viscous consistency acts as a barrier against bacteria capable of crossing the shell. The ovomucin is responsible for this high viscosity (Robinson, 1987), which is further increased by the lysozyme complex (Hayakawa et al., 1983).
2) From a chemical point of view, the albumen can prevent infections of microbiological origin by killing the bacteria or by creating an environment unfavourable to their development. The lysozyme is the only albumen protein known to have a direct effect on the bacteria, because it is able to hydrolyse the β(1-4) glycosidic bonds, a component of the cells constituting the walls of certain bacteria.

As mentioned above, the albumen is a source of water and proteins and, in particular, appears to be a key resource for the development of the embryo as suggested by Nisbet (1978).

Shell and membrane of the egg

These two structures are formed during the descent of the embryo along the ducts of the mother's reproductive system. The outer and inner membrane are composed of two keratin layers, whitish and elastic, necked between them, which are not united at the obtuse end of the egg, leaving between them a space called "air chamber", containing a liquid. This air chamber, after the deposition, as a result of the rapid cooling of the egg, causes a reserve of air with the evaporation of the liquid and the detachment of the two layers, extending (volume increase) during incubation.

The shell ensures the survival of the embryo allowing the passage of oxygen, carbon dioxide and water vapour, also provides structural support and protection from microbes and soil invertebrates; it is also the main source of calcium for embryo development (Board, 1982). The calcium carbonate, in form of crystals of calcite ($CaCO_3$) constitutes its main component (Campbell & Lack, 1985). The shell consists of inorganic calcium and magnesium salts (carbonates and phosphates) compacted into a lattice of fiber's collagen (Johnson, 2000; Gill, 2007).

The shell typically constitutes 11 to 15% of the total weight of an egg (Gill, 2007). The shell formation takes about 20 hours. The structure of the shell may limit the geographical and altitudinal distribution of birds. Considerable losses of water occur in dry habitats where the relative humidity is low or at high altitudes where the barometric pressure is low. A similar loss of water may limit the ability of hatching of the eggs (Guasco, 2006).

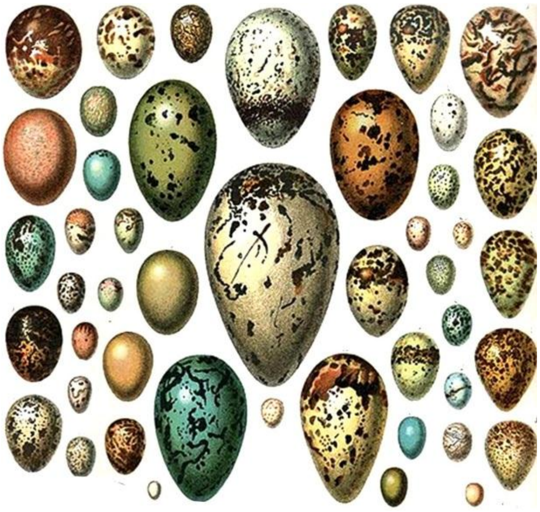


Figure 2 - Different types of eggs.

Lysozyme

Lysozyme has a fundamental role in the study of substances transferred from the mother to the egg and then to the offspring, and consequently in the studies on maternal effects.

It is an antibacterial enzyme capable of digesting the bacterial cell walls, it is also considered a reliable indicator of maternal effects healthy for the survival of chick. It also affects the hatchability of eggs, the antiparasitic defense and the viability of the offspring; moreover, it has a direct bactericidal action via the hydrolysis of glycosidic bonds of the bacterial cellular walls (Bonisoli-Alquati et al., 2010).

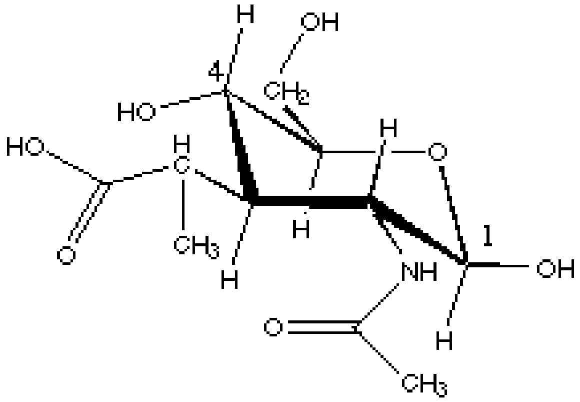


Figure 3 - Lysozyme structure.

Lysozyme is a globular protein, consisting of 129 amino acids and weighs 14.6 kd, discovered by chance in 1922 by Alexander Flemming, when a few drops of nasal mucus ended up in a Petri dish, causing the death of bacteria. This enzyme was later found in saliva and tears, in breast milk, in plants, and very abundant in egg albumen (Rasmussen, 1999). In humans it is found in neutrophils and macrophages. The enzyme is contained in tears and nasal secretion in a quantity greater than in serum (Caligaris, 1997).

Lysozyme is extracted and purified from albumen since it has been shown that it possesses antimicrobial activity and is useful against food-borne microorganisms (*Listeria monocytogenes* and *Clostridium botulinu* - Hughey & Johnson, 1987); for this reason it can be used as a preservative of foods that naturally haven't it.

Lysozyme destroys the bacterial walls, but is totally innocuous to humans (Hughey & Johnson, 1987); it's also particularly effective against oral infections, caused by cariogenic bacteria, such as *Streptococcus mutans*, and bacteria associated with paradentite, because it's also present in saliva (Anton et al., 2005).

The size of a molecule of lysozyme, however, is too high and, therefore, it isn't usable as a medicinal product; it can be applied topically, but is not able to free the whole body from infection, precisely because, due to its size, it can't move between the cells. The enzyme acts on the cell wall of gram positive bacteria, which have a thick coating of carbohydrate chains (linked transversely by small peptide chains), which surrounds their membrane to protect it from the strong osmotic pressure inside the cell.

Lysozyme breaks these carbohydrate chains and the cell wall loses its structural integrity and yields, due to the internal pressure; this is because this molecule cleaves the glucosidic linkages β (1-4) between residues of N-acetilmuraminic acid and N-acetilglicosamine and has the ability to attack and demolish the nitrogenous polysaccharides which constitute the outermost cell layers of different bacteria, both saprophytic and pathogens. Its protein molecule has a polypeptide structure, rich in basic and cyclic amino acids, with a spectrum of maximum ultraviolet absorption between the 2700 and 2800 Å (Caligaris, 1997).

Lysozyme causes to sensitive germs:

1) lysis;
2) agglutination;
3) changes in their dyeing characteristics.

When the polysaccharides are located in an accessible position of the cell wall, they may be attacked by lysozyme and lysis occurs directly; when, instead, they can't be attacked, because covered by protein, lipid or polysaccharide stratifications, bacteria are resistant or become sensitive only after treatment with other enzymes (protease, lipase).

After the bacteriolysis made by lysozyme, energy and plastic metabolites, such as polysaccharides, proteins, ribonucleic acid, nucleotides, nucleosides, and peroxidases, respiratory and glycolytic enzymes, are disintegrated in the bacterial protoplasm.

The action is maximum on *Micrococcus lysodeickticus*, but it's also present on pathogenic bacteria such as staphylococci and pneumococci.

Probably the lysozyme also acts indirectly through immune phenomena, it significantly stimulates phagocytosis of staphylococci and also seems the complementary activity of sera, acting synergistically with other antibacterial agents such as antibodies. Lysozyme has also an antiviral activity by intervening in the production of interferon (Caligaris, 1997).

Ovotransferrin

Transferrins are 80kDa glycoproteins composed of two domains that each reversibly bind one molecule of iron (Valenti et al., 1981a; Wu & Acero-Lopez, 2012). These proteins are present in different concentrations in the cells and biological fluids of mammals and birds. Transferrins play a role in iron transport and inhibit the growth of micro-organisms at pH 6 to 10 by limiting the amount of free iron available (Phelps & Antonini, 1975). Transferrins bind a number of other transition metals but the affinity of the protein is always higher for iron than the various other metals (Valenti et al., 1987).

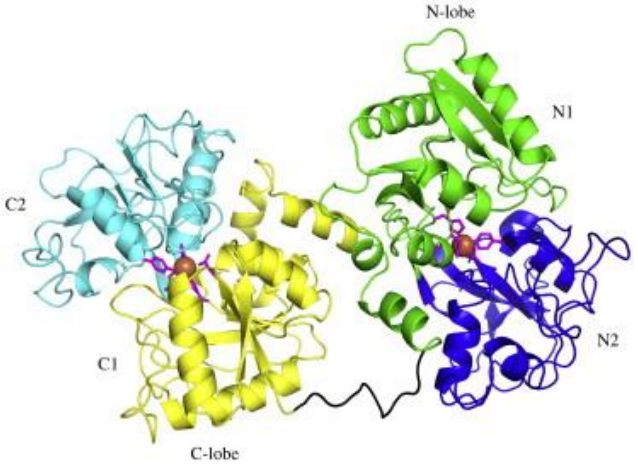


Figure 4 - Transferrin whole molecule. Taken from Mizutani et al., 2012.

The transferrin family of proteins is divided into two branches: soluble glycoproteins and membrane melanotransferrins (Giansanti et al., 2012). The amino acid composition has been found to be similar across transferrins while the number and composition of carbohydrate groups attached to different transferrins has been shown to be variable (Graham & Williams, 1975). Transferrins are present in mammals as serum transferrin and lactoferrin while ovotransferrin and serum transferrin are the forms present in birds (Wellman-Labadie et al., 2007). Serum transferrin, also known as siderophilin, has been found to play a role in iron transport in the plasma of vertebrates including birds (Rawas et al., 1989). In the chicken, the protein moieties of serum transferrin and ovotransferrin appear to be identical while the carbohydrate groups differ (Graham & Williams, 1975).

Hen egg white ovotransferrin was first characterized by Schade & Caroline (1944) who called it conalbumin. It was renamed ovotransferrin when it was recognized as an iron-binding protein and a member of the transferrin family (Williams, 1968). It is a major egg white protein and represents about 12-13% of chicken albumen protein (Burley & Vadehra, 1989). The unavailability of iron through chelation by ovotransferrin is the principle impediment to the growth of bacteria in the albumen of the hen's egg (Seviour & Board, 1972). In *Escherichia coli* and other Gramnegative micro-organisms which possess an iron transport system mediated by citrate, it was demonstrated that bicarbonate enhanced the antimicrobial action of ovotransferrin while citrate had an antagonizing effect (Valenti et al., 1981a; Valenti et al., 1985). The addition of citrate and bicarbonate to ovotransferrin inhibits the growth of *Staphylococcus aureus* by 50% (Valenti et al., 1981a). *Pseudomonas sp.*, *E. coli* and *S. mutans* were most sensitive while *S. aureus*, *Proteus sp.* and *Klebsiella sp.* were most resistant to ovotransferrin (Valenti et al., 1983). In a study by von Hunolstein et al. (1992), iron-binding proteins, such as ovotransferrin and lactoferrin, failed to show any activity towards *Streptococcal* species such as *Streptococcus bovis*, *S. mutans* and *Enterococcus faecalis*. Valenti et al. (1981b) noted that almost 100% of the strains tested from the genus *Proteus* were capable of multiplying in the presence of 5mg/ml of ovotransferrin.

Ovotransferrin has also been found to show antiviral and antifungal activity. Giansanti et al. (2002) reported antiviral activity against infection by Marek's Disease Virus. Valenti et al. (1985) reported antifungal activity of ovotransferrin towards *Candida albicans* and noted that the activity did not appear to depend only on iron chelation but involved a more complex mechanism such as an interaction of protein and fungal cells (Wellman-Labadie et al., 2007). Of the 100 strains within the *Candida* genus tested by Valenti et al. (1985), only *C. krusei* showed noticeable resistance to ovotransferrin. Fungus was found to be more sensitive to ovotransferrin than bacteria and retained activity even during iron saturation suggesting that there is a direct interaction of ovotransferrin and fungal cells (Valenti et al., 1985).

Ovotransferrin is also responsible for the transfer of ferric ions from the hen oviduct to the developing embryo (Huopalahti et al., 2007).

Testosterone

Hormones, mainly in the form of testosterone, appear to be a key component for the development of the hatchlings (Lipar & Ketterson, 2000; Eising et al., 2001).

Testosterone acts on target tissues, also being at very low concentrations, and influences behaviour, physiology and morphology of the individual (Wingfield, 1984). It is a steroid hormone containing 19 carbon atoms.

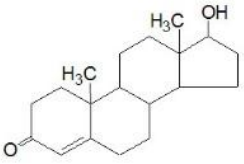


Figure 5 - Testosterone structure.

The eggs of all bird species, when analyzed, show in their yolk significant concentrations of hormones such as testosterone, 5-alpha-dihydrotestosterone and androstenedione. The concentrations of these hormones appear to be much higher in the yolk, than in the embryonic or adult plasma (Groothuis et al., 2005). Testosterone is transmitted to the offspring via the egg yolk and its levels *in ovo* vary greatly within the same brood (Lipar et al., 1999).

In the yolk of the eggs are present variable amounts of maternal hormones (Schwabl, 1993), which are generally associated with a positive influence on offspring (Lipar & Ketterson, 2000; Eising et al., 2001). Lipar & Ketterson (2000) have also shown that testosterone influences the development of the musculoskeletal system in vertebrates and stimulates the growth of muscles important for the process of hatching and demand of food (begging); also the transfer *in ovo* of these molecules can affect the competitiveness between brothers and affect the rate of growth (Schwabl, 1993; 1996b; Eising et al., 2001). However, high levels of testosterone may result in adverse effects on the physiology of the chick (Andersson et al., 2004), or even a reduction of the probability of survival (Ketterson & Nolan, 1992; Navara et al., 2005). In addition, prenatal exposure to these substances may not only influence the youth traits of an individual, but it can have an impact on the development of secondary sexual characteristics and on the reproductive success (Rubolini et al., 2006; Clark & Galef, 1995). Schwabl (1993) found that the social status of young canaries was positively correlated with the concentrations of testosterone in the yolk of the eggs from which they hatched. In the zebra finch, females can deposit high concentrations of testosterone in their eggs when they mate with males more attractive than when they mate with males less attractive; thus due to the positive effect of the expression of testosterone on the expression of secondary sexual traits, there is also a potential effect on the growth of children, their survival and future reproductive success (Gil et al., 1999).

Testosterone determines the development of primary sexual characteristics in the embryo and the appearance and maintenance of secondary sexual characteristics after birth. Generally, in fact, the steroid hormones, particularly testosterone, influence sexual dimorphism in plumage, in voice and in body size (Gill, 1996): for example in the Starling (*S. vulgaris*), this hormone causes a color change in its beak, which becomes bright yellow during the breeding season; also the red ornaments on the head of the domesticated fowl (wattles and ridges) and the formation of ornaments on the beak of the other birds during the breeding season depend on the testosterone.

We must not overlook the role of testosterone in determining the sex of the offspring. Experimental manipulations of this hormone in the eggs of birds and reptiles can change the sex of the unborn (Wade et al., 1997; Freedberg et al., 2006). In domestic canary (*S. canaria*) and in zebra finch the control of the *sex* *ratio* is carried by the female; the variation of concentrations of plasmatic testosterone, in fact, would influence the follicle during the preovulatory phase and consequently the sex of the embryo (Schawbl, 1993). Müller et al. (2002) have shown that changes in the level of plasmatic hormones, in particular of testosterone, depends on the social status of the female. The dominant females (whose eggs containing male embryos produce a higher concentration of testosterone) have a predominantly male offspring than females dominated. Therefore, there is an adaptive mechanism thanks to which the females favour an increase in the concentration of testosterone in eggs containing embryos of one or the other sex.

The grey partridge, *Perdix perdix*

Systematic classification

The grey partridge, originally classified as *Tetrao perdix* by Linnaeus in 1758, is currently defined *P. perdix* (L).

It belongs to the order Galliformes, family Phasianidae, subfamily Perdicinae, genre Perdix. Also the tibetan partridge (*Perdix hodgsoniae*) and daurian partridge (*Perdix daurica*) belong to this type.

In Europe they can be divided into two groups: the first, present in the west, is recognizable by the red-brown color of the plumage, while the second, located in the east, is lighter colored and with more gray (Potts, 1986).

It is a polytypic species in Eurasian chorology, spread with 8 subspecies:

- *P. p. hispaniensis*, present in the Iberian Peninsula (Reichenow, 1982);
- *P. p. armoricana*, resides in Brittany, Normandy, north-western and central France (Harter, 1917);
- *P. p. sphagnetorum*, found in the north-east of the Netherlands and on the border with Germany (Altum, 1984);
- *P. p. perdix*, found in Scandinavia, Ireland, the British Isles and in an area that extends from southern and central Europe up to the Alps and the Balkans (Linnaeus, 1758);
- *P. p. lucida*, distributed between Finland and the Urals up to the Black Sea and the Caucasus (Altum, 1984);
- *P. p. canascens*, in Turkey, Caucasus and Iran (Buturlin, 1906);
- *P. p. robusta*, who lives on east of the lower stream of the Ural River, in south-western Siberia and in Kazakhstan (Homeyer & Tancrè, 1883);
- *P. p. italica*, present in Italy (Harter, 1917).

Morphology

The grey partridge is a Galliforme of small-medium size, with a compact body, rounded and relatively small head, short neck and short tail, rather broad wings (Brichetti & Fracasso, 2004). Its total length is approximately 29-31 cm, wingspan is between 45-48 cm. Its wing is 15-16 cm long and tail, which has 18 rectrices, is round and 6 to 8 cm long.

The length of its tarsus is about 4 cm; the fourth finger, small and rear, is located in a higher position than the other fingers (Leporati, 1983). Its beak is sturdy and curved downward and measures 1.3 to 1.8 cm (Cramp & Simmons, 1980).

Its weight is between 325 and 445 g in males and between 310 and 450 g in females.

Grey partridge shows a marked sexual dimorphism, especially with regard to some colour differences. Its coloration is due to melanin pigments. Cheeks, throat, and sides of its head are of a colour ranging from orange and rusty brown, more pronounced in males. The upper part of its chest is vermiculated gray bluish, its sides are reddish, belly and undertail are white and rectrices are brown. Its beak is cream-colored, legs are gray-bluish. The head of the male has a uniform grayish and reddish colour, the female shows a dark brown colour. The brown stain on its chest, shaped as a horseshoe open at the bottom (Beani & Dessì, 1995), can't be used as a discriminating sign between sexes, because it is present in both males and older females.

The nude periorbital area of the male exhibits a red colour very evident during the mating season in the spring (Leporati, 1983), while in the rest of the year it's less intense.

A sign for discriminating the two sexes is the presence or absence of light bars on the wing coverts, as in the male there's only one longitudinal light streak, while in females there's the presence of transverse streaks.

Annual cycle

Grey partridge has a gregarious behaviour in the period following the reproduction (early June). In the dispersion phase, characteristic of the period that precedes the reproduction, when pairs are formed, it presents a territorial behaviour.

The medium size of groups, defined brigades, varies from 5 to 15 individuals, while the composition is variable: it can be formed by one or more broods to which they can be added not-reproductive pairs or isolated individuals.

Pairs are formed in late winter and spawning occurs near the end of May (Mussa & Boano, 1990): produced eggs are about 15 (in breeding conditions they can go up to 30) and are deposited with an interval of 1-2 days between each other. Brooding lasts about 24-25 days and doesn't begin until the female has laid the last egg. Hatching is synchronous and occurs in late May and early June. Chicks leave the egg already formed and have little need of maternal care (nidifugous offspring), they stay with their parents until at least the end of January, when the brigade is dissolved (dispersed phase); new pairs are formed in February.

The domestic canary, *Serinus canaria*

The domestic canary (*S. canaria*, Linnaeus, 1758 - BirdLife International, 2013a) belongs to the order Passeriformes, family Fringillidae and genre Serinus. The strain of canary domestic has been selected by man from the wild strain.

Morphology

Individuals of both sexes measure about 15 cm, have a wingspan of 25 cm and a weigh between 18 and 28 g; there isn't sexual dimorphism.

Wild strain has a plumage with green as the main colour. The domestic canary has instead developed a variety of colours ranging from dark green to black, passing through the famous "canary yellow", shows also liveliest colours, acquired with particular nutritional uses.

The species takes its name from original islands, the Canary Islands, located off the coast of Morocco.

The canary is granivorous and varies its feeding with legumes and fruits.

Figure 6 - Different phenotypes of *S. canaria*. The type on left is the closer to the wild species. Taken from Garcia-Fernandez, 2009.

Reproduction

S. canaria is social and monogamous: its pairs last at least one breeding season. The female builds the nest and broods alone from two to six eggs in 14 days (Pomarède, 1992); sometimes it is fed by the male during this period. It's possible that a pair may have three reproductive cycles during the same breeding season (Voigt & Leitner, 1998).

As a nidicolous species, offspring born blind and without an own effective temperature control: they depend entirely by the parents, that cover and feed them until they leave the nest (about 10-15 days after hatching, when they formed the feathers). In the successive week, they often return to the nest to be fed.

The breeding season is in the spring. For individuals in captivity, coupling predisposition can be induced by increasing the hours of light (Hinde, 1958; Leboucher et al., 1994). The photoperiod extension leads to the development of gonads (Storey & Nicholls, 1976; Nicholls & Storey, 1977; Pohl, 1994), to the construction of the nest (Hinde, 1958; Hinde & Steel, 1975; Steel et al., 1975), to the Copulation Solicitation Display in the female (Nagle et al., 1993; Leboucher et al., 1994 – **Errore. L'origine riferimento non è stata trovata.**) and even to the reorganization of a part of the brain architecture, that allows the male to produce its song (Follett et al., 1973). In the canary, in fact, this practice assumes a great importance in social life, in particular as regards the period of courtship (Warren & Hinde, 1961; Leboucher et al., 1994; Bentley et al., 2000; Garcia-Fernandez, 2009).

Figure 7 - Copulation Solicitation Display (CSD) in female canary.

Red-legged partridge, *Alectoris rufa*

Geographical distribution

The red-legged partridge is a polytypic species in European distribution, of which three subspecies have been recognized:

- *A. rufa rufa*: present in the central and southern France, in the Northern Apennines, in Corsica. It was also introduced and is well established in the Balearic Islands and in southern England;
- *A. rufa hispanica*: widespread in northern and western Iberian Peninsula, differs from *A. rufa rufa* for the slightly darker and lively color; the rump is grayish with a more massive beak;
- *A. rufa intercedens*: typical of the north-western and south-central Spain, has duller colors of *A. hispanica* and is, therefore, more similar to *A. rufa rufa* (Brichetti & Fracasso, 2004).

Morphology

The red-legged partridge is a galliforme, medium-sized, of family of phasianidae (Birdlife, 2013b): it reaches a length of 290-340 mm, a wingspan of 470-500 mm and a weight between 400-500 g. The sexual dimorphism is not very evident even if the male is slightly bigger and can present the spurs, that in some cases are also present in older females, so making difficult the differentiation (Brichetti & Fracasso, 2004).

It's characterized by a white color plumage on the throat, that seems a gag, surrounded by a well-defined black ring, that expands on the neck sides and on goiter in a series of dark spots and streaks, that thins as we move toward the chest.

Regarding the head, it has a clear and well defined eyebrow on the sides, while the streak behind the eye is bluish.

The lower part of the body is characterized by an intense cinnamon color on belly, that is separated from a black spotted bib by a sharp-cut bluish gray streak; finally the legs and the beak are bright red (Brichetti & Fracasso, 2004).

When in flight, it may be distinguished from *P. perdix* for the size that are slightly higher and for the homogeneous colouring of the back and of the wings.

The young red-legged partridges are smaller and already have a slight coat under the throat and on the sides.

Instead, the chicks, in their lower part, are of fawn whitish color slightly darker on the chest. The top of their head and nape are reddish with a paler streak above eyes. The back is reddish marked by three streaks passing through the wings (Brichetti & Fracasso, 2004).

The reproduction

The breeding season begins in late April with the formation of pairs, but already before the males take possession of the territory and defend it against possible competitors.

The encroachment of a male in the territory of another, leads to a challenge that, even if ends in a head-on, resolves without damage to the contenders.

The nest is prepared in a depression in the ground at the edge of the woods and of the bushy zones.

The female lays 12 to 16 eggs, which broods for 23-24 days, while the male watches over the territory in the closeness of the nest, although sometimes it cooperate in brooding. The eggs measure, on average, 38 x 29 mm and have a weigh about 18-19 g. They have an elliptical shape and are smooth and shiny. The color ranges from yellowish white to pale yellowish brown mottled or stippled.

The chicks leave the nest soon after birth and are able to fly at a few weeks of age.

The red-legged partridge lays eggs usually once a year.

Chapters plan

Animal behavior and its study has always been, since the first university courses, a topic very interesting to me. In particular, the sexual selection and the subsequent parental investment, already studied by Darwin (1871), have still many unclear points that stimulates my curiosity.

For this reason, I focalized my PhD work on the study of the maternal effects, in particular on how they relate with certain situations. Therefore, I focused my studies on the investment of females birds in the eggs. I chose this group because the great amount of the maternal effects are concentrated in the eggs by the mother, and so it's easier evaluate the maternal effort than other groups. I work on different characteristics of the eggs such as number, total mass, mass of the major constituents (albumen, yolk, and eggshell), and also as concentration of some molecules, namely lysozyme and ovotransferrin (in the albumen) and testosterone (in the yolk). I carry out these measures in four different situation.

In first chapter, I focused my attention on the different allocation in the eggs of grey partridge females in relation to the hierarchical rank of the partner. I performed test to realize a rank of males, and after I let the females to see the competition between two males and to choose one of them. After this, I paired some females with the winner of the competition (and chosen by the female) and others with the loser.

The second chapter is about the allocation of canary females faced with a physic competition between two males for the access to food. The first thing was to understand which male females prefer in this type of competition, and after see if they different allocate the resources in the eggs.

The third chapter is focused on the effects of an anthropic noisy environment on the sexual selection and the reproduction success in canary females. These females were exposed to different situations in which the male's song was presented masked by noise or in a condition of silence. After this, I evaluated the sexual behaviour and the subsequent investment of the females in the eggs.

The fourth chapter investigates the effects of an immune challenge and supernormal clutch on egg quality in the red-legged partridge. Females were immunized two weeks before the laying period and after the egg characteristics were analyzed.

These four chapter are related to different experiments conducted on three species of bird, two nidifugus (the grey and the red-legged partridges), and one nidicolous (the canary). In the firsts the prenatal investment is fundamental, in the canary it is important but is partially compensated by parental care after the hatching in the early days of life of the birds.

The last chapter (Overall discussion) summarizes the results of the four experiences and tries to find general tendencies. Finally, it proposes some indications for future works of interest to continue the study of maternal effects.

Materials and Methods: Common Aspects

To make the work clearer, a briefly explanation of the conditions of the animals and the technical protocols and descriptions of the experiences that are in common in the majority of the following experiences are here introduced.

For what concerns procedures that are specifically of a single study, the descriptions can be find in the specific material and methods.

So, the recommendation is to see the section "Materials and Methods: Common Aspects" for details missing in each chapter.

The general idea of the experiences is to put the females into specific situations (i.e., pairing with dominant/subordinate male; different situation of anthropic noise; immune challenge) and then to observe the consequences on the characteristics of their eggs.

The grey partridge, *Perdix perdix*

The experience on the grey partridge was performed to the Dipartimento di Scienze ed Innovazione Tecnologica (DiSIT) of the Università del Piemonte Orientale "Amedeo Avogadro" to Alessandria, Italy. All the females and males were at the first year of reproduction when the experience was realized. The breeding named "Allevamento De Maria" was situated in Ovada, near Alessandria. All the aviaries were to the exterior and the animals were exposed to natural light and temperature; for this reason the experiment was performed in the months between February and June, when the days was longer and the animals were ready for the coupling.

The food provided was a mix in powder compact, normally used by the farmers; this kind of food is indicated for the females in the laying period and for the offspring. Water and food were provided *ad libitum*. In addition, grass and insects were naturally present on the soil.

Before the breeding season, birds were separated in little groups of individuals of the same sex. Each groups were housed in a middle aviary. In this way all contacts between individuals of the different sex were impossible; this precaution was essentially to avoiding possible pairing before the experience.

The common canary, *Serinus canaria*

All the canaries were from the breeding of the Laboratoire d'Éthologie et Cognition Comparées (LECC) of the Université de Paris Ouest – Nanterre La Défense, to Nanterre, France.

For what concerns the birds utilized for the experiment on the dominance (Chapter II), all the individuals had already lived at least a reproductive season. They were from 2 to 6 years old.

For the birds implicated in the experience on the anthropic noise (Chapter III), the individuals had never lived a reproductive season and they were 1-2 years old.

Before the start of each experience, the birds were housed in batteries (120 x 50 x 50 cm) and they were subjected to a photoperiod called of "Short Day" (SD: 8h with light and 16h of darkness); this trick simulated the winter season.

The batteries could contain up till 7 individuals, all of the same sex. Males and females were bred in separated batteries.

To the beginning of an experience, all the birds were passed to the condition of "Long Day" (LD: 16h with light and 8h of darkness); in this way it was possible to simulate the conditions that excite the reproductive behaviour (Follet et al., 1973).

The females of both experiments were placed into individual cages (40 x 30 x 24 cm); the females of the anthropic noise experience (Chapter III) were housed also into a soundproof cage (see below). Males remained into batteries.

The feeding of the birds was *ad libitum*, with "pâtée" (brand Cédé®, type Cédé for eggs for canaries and exotic birds or indigenous, composition: wheat flour, eggs, honey, hemp, Niger seed, shell grind oats, blue poppy, vitamins, minerals, amino acids: Lysine, Methionine, Yeast) and a mix of seed for SD (brand Beyers®, type diet for canaries, composition: 85% alpiste, 6% oats, 5% navette, 2% Niger and 2% hemp) or a mix enriched in hemp for LD (brand Beyers®, it's the same mix for the SD, but with 5% of hemp). Each day the water was changed. In the reproduction period, the females had cuttlefish bone (to provide calcium indispensable for the formation of the eggshell), apple, "sharpie" (cotton, Sharpie Benelux®) and a nest (in plastic for the experience of the Chapter II and in wicker for the experiment of the Chapter III) as support for the nest built by them.

The red-legged partridge, *Alectoris rufa*

The experience on the red-legged partridge was done to the Dipartimento di Scienze ed Innovazione Tecnologica (DiSIT) of the Università del Piemonte Orientale "Amedeo Avogadro" to Alessandria, Italy. All the females and males were at the first year of reproduction when the experience was carried out. The breeding was situated in San Giuliano, near Alessandria. All the aviaries were to the exterior and the animals were exposed to natural light and temperature; for this reason the experiment was performed in the months between February and June, when the days was longer and the animals were ready for the coupling.

The food provided was a mix in powder compact, normally used by the farmers; this kind of food is indicated for the females in the laying period and for the offspring. Water and food were provided *ad libitum*. In addition, grass and insects were naturally present on the soil.

Before the breeding season, birds were separated in little groups of individuals of the same sex. Each groups were housed in a middle aviary. In this way all contacts between individuals of the different sex were impossible; this precaution was essentially to avoiding possible pairing before the experience.

The experimental precaution

The laboratory analyses were performed into the laboratories of the DiSIT or in the structure of the Centre d'Études Biologiques de Chizé (CNRS-CEBC) without taking into account the origin of the samples.

All the canaries exploited in the experiments were between 1 and 6 years old. The females of the experiment of the Chapter II already had a reproduction experience (deposition and brooding); the ones implicated in the study of the Chapter III never had a sexual experience.

For what concerns grey partridges and red-legged partridges utilized in the experiences performed in Alessandria, all the individuals (both males and females) were at first reproduction experience and so they were 1 years old. This fact is important especially for the study on the influence of male dominance on the maternal investment (Chapter I), because age could influenced the ranking and the female choice.

Both grey partridges, red-legged partridges and canaries can had a strictly family relationship; this fact has been taken into account at the time of formation of the experimental groups (i.e., two sisters were separated between the experimental groups).

The sound isolation of the canaries females

For the experience of the Chapter III was fundamental that females didn't hear and see each other during the diffusion of the stimulus. So, a single soundproof caissons (68 x 51 x 51 cm) was utilized (**Errore. L'origine riferimento non è stata trovata.**); each caisson was provided with a digital recorder (Marantz PMD670, bandwidth: 20 Hz – 20 kHz ± 1 dB) connected to a stereo amplifier (Pioneer A-209-R) and a diffuser of 60 watts (Elipson Mini Horus, frequency: 80 Hz – 20 Hz) placed to the bottom of the caisson at 20 cm from the middle of the cage containing the bird. This equipment diffused to each female particular stimulus (see Chapter III for the details).

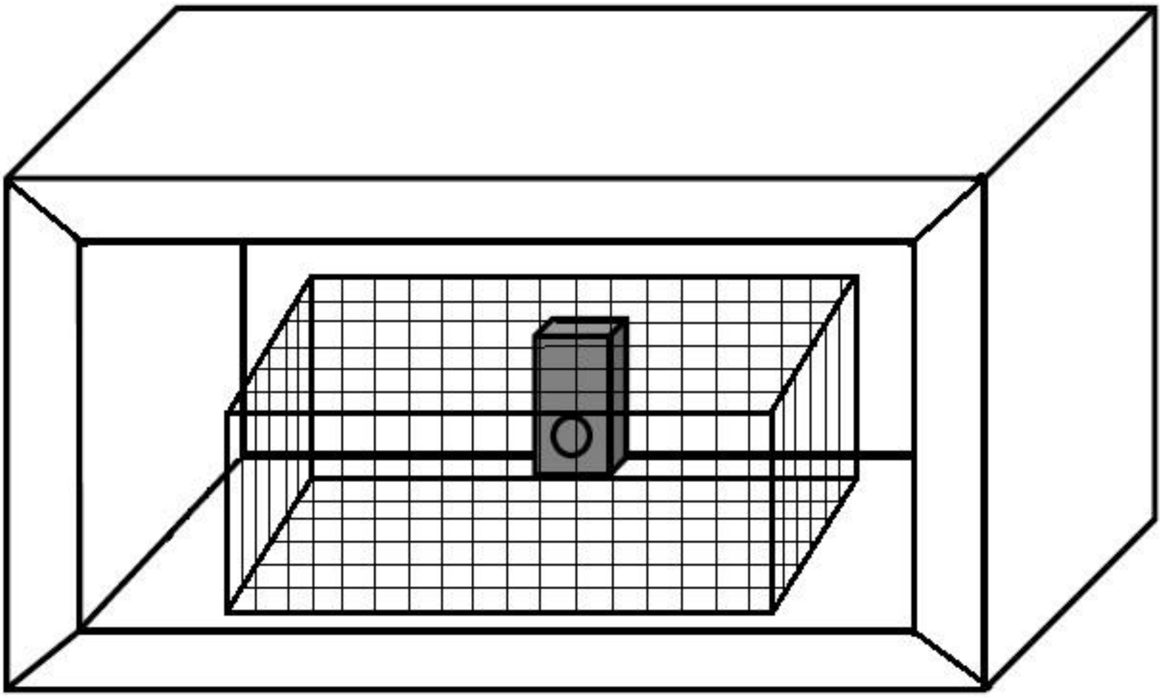


Figure 8 - Soundproof caissons (68 x 51 x 51 cm) provided with a speaker to diffuse the stimuli.

The clutch characteristics

For the grey partridge the latency period is calculated between the day of the couple formation and the day of first egg production; for the canary in the Chapter III between the first day of the stimulus presentation and the first day of laying.

For what concerns the number of the eggs, for the canary it has been counted; for the experience on the dominance the clutch of only one cycle of reproduction has been utilized, while for the second on the anthropic noise two cycles have been considered.

For the partridges, since they was farm animals and they produced a lot of eggs (more than in natural condition), a threshold of 30 eggs have been established; after the 30th the eggs were no longer analysed.

Egg characteristics

All the day the eggs were collected (for all experiences). For the canaries, to simulate natural conditions, the real egg have been replaced with a plastic one.

Each egg was marked, weighed with an electronic balance (precision of 0.01 g for the canary eggs and 0.1 for the partridges ones) and measured by means a calibre (precision of 0.1 mm). The last measure has not be done on the canary eggs because they were very fragile and, to prevent breaking, the values could be misrepresent. The measures has been taken by means of standard photos utilising the image processing software ImageJ (available at http://rsb.info.nih.gov/ij).

For the works on the canary all the eggs were taken for the laboratories analyses. For the partridges, for every experience some eggs have been collected for the laboratory analyses.

The remaining eggs (in the partridge experiments) were incubated to see the hatching rate.

To the laboratories of the DiSIT, after have separated into is constituents part every eggs (albumen, yolk and shell), the weight of each have been taken and the yolk has been homogenized with an equal part of NaCl solution (0.9%); the albumen has been sonicated. Finally, all the albumens and the yolks were frozen for subsequent laboratory analyses. The precision of the balance was of 0.01 g.

To the LECC, the components of the canary eggs have been separated, weighted (balance precision 0.01 g) and frozen for future analyses.

Lysozyme: protocol Osserman & Lawlor, (1966)

Lysozyme activity in samples of grey partridge and red-legged partridge eggs was measured using the lyso-plate method (Osserman & Lawlor, 1966): an agar gel with a dried strain of *M. lysodeikticus* (Sigma Aldrich®), which is particularly sensitive to lysozyme activity, was inoculated with 25 µL of albumen. Standard dilutions of crystalline hen egg-white lysozyme [(Sigma Aldrich®) 250, 500, 1000, 2000, and 4000 µg/ml] were run with each group of test samples.

The plates were incubated at 25°C for 18h, during which bacterial growth was inhibited in the area of the gel surrounding the albumen inoculation site. The diameters of the cleared zones were proportional to the logarithm of the lysozyme concentration. This area was measured from a photographic image using the image processing software ImageJ (available at http://rsb.info.nih.gov/ij), and converted on a semi-logarithmic plot into hen egg lysozyme equivalents (HELML equivalents, expressed in µg/ml) according to the standard curve.

Lysozyme: classical antibiogram protocol

The Osserman & Lawlor protocol (1966) has proven to be poorly sensitive with the canary samples.

So, a common method of analysis in microbiology have been applied: the classical antibiogram.

The first step was to isolate the bacterium to obtain a certain strain to utilize for the antibiogram. As culture medium was utilized the Trypticase Soy Agar (Becton, Dickinson and Company®) to isolate the *M. lysodeikticus*. After have reached a very probable strain of the bacterium the gram coloration has been performed to be sure of the microorganism nature.

Once obtained the strain the out-and-out protocol started. The first passage was the preparation of the Mueller Hinton Agar (Becton, Dickinson and Company®). Then the bottle with the medium have been sterilized into autoclave at 121°C for 15 minutes. After have cooled down the medium to 50°C, 20 ml of the gel (still liquid) were put into a Petri plate (Ø 90 mm). So, after the resuspension of the microorganism into a magnesium sulphate solution, it was distributed with a sterile tampon on the medium in the Petri plate (in the meanwhile solidificated).

After 20 minutes, four diskette of blotting paper have been positioned (Ø 0.5 mm) in each plate and then 20 minutes have been waited to allow the drying. Finally, 10 µl of albumen sample or standard dilutions of crystalline hen egg-white lysozyme (previously prepared – Sigma Aldrich®) were inoculated in triplicate. The standards have been prepared in PBS 1% with the following concentration: 500 µg/ml, 250 µg/ml, 125 µg/ml, 62,5 µg/ml, 31,3 µg/ml, 15,6 µg/ml, 7,8 µg/ml, 3,9 µg/ml, 1,9 µg/ml, 0,97 µg/ml. When all the samples have been inoculated, the plates were incubated at 27°C for 48 h, during which bacterial growth was inhibited in the area of the gel surrounding the albumen inoculation site. The diameters of the cleared zones were proportional to the logarithm of the lysozyme concentration. This area was measured from a photographic image using the image processing software ImageJ (available at http://rsb.info.nih.gov/ij) and converted on a semi-logarithmic plot into hen egg lysozyme equivalents (HELML equivalents, expressed in µg/ml) according to the standard curve.

Ovotransferrin

A modified version of the total iron-binding capacity assay described and verified by Yamanishi et al. (2002) was used to measure concentration of ovotransferrin in each egg (Shawkey et al., 2008; D'Alba et al., 2010; Horrocks et al., 2011).

Each well of a 96-well microplate was filled with 50 µl of reagent one [300 mm Tris (ICN Biomedicals®), 150 mm sodium hydrogen carbonate (Sigma Aldrich®), 4.2 g/l Triton X-100 (Sigma Aldrich®), pH 8.4] containing a 1:250 dilution of iron-standard solution (1000 mg/l – Sigma Aldrich®). 10 µl of plasma samples or ovotransferrin standards (conalbumin from chicken egg white – Sigma Aldrich®) was added in triplicate into the wells in the plate. The standards were prepared in reagent one (without iron-standard solution) for a standard curve ranging from 1.25 to 20.0 mg/ml. The assay plate was placed in a spectrophotometric microplate reader (Tecan, Infinite 200Pro, Männedorf, Schweiz). In the reader, the plate was shaken for 10 s to mix the well contents and then incubated for 5 min at 36°C. Following incubation, initial 'preread' absorbance was recorded at 570 nm (primary wavelength) and 660 nm (reference wavelength) to later account for any differences among the plasma samples and between the coloured plasma samples and the colourless standards. 10 µl of reagent two [50 mm Tris, 32.6 mm L-ascorbic acid (Sigma Aldrich®), 10 mm FerroZine (Sigma Aldrich®), pH 4.0] was then added to each well. The contents were mixed again for 10 s and left to incubate for 5 min at 36°C in the plate reader. Finally, 20 µL of reagent three [600 mm citric acid (Sigma Aldrich®), 25.6 mm thiourea (Sigma Aldrich®)] was added to each well, the contents were mixed for 3 s and absorbance was first recorded immediately after mixing (t = 0). Absorbance at 570 and 660 nm was recorded again at 6 min (t = 6). The microplate reader and all reagents were warmed to 36°C prior to use in the assay.

Absorbance values were used to calculate ovotransferrin concentrations. First, a correction was done for initial differences in absorbance values among samples and the standards. Well-specific 'preread' absorbance at 570 and 660 nm were subtracted from both the t = 0 and the t = 6 read at the corresponding wavelength. Then, all absorbance values were normalized by subtracting the reference wavelength (660 nm) absorbance from the primary wavelength (570 nm) absorbance at both time points. Finally, the change in absorbance (ΔA) owing to release of Fe^{3+} from the ovotransferrin and additional formation of the coloured Fe^{2+}–FerroZine complex was determined. For each well, the normalized absorbance value at the start of the assay (t = 0) was subtracted from the normalized absorbance value at the end of the assay (t = 6; i.e. $\Delta A = A_{570–660}end – A_{570–660}start$). A standard curve, which related ΔA and ovotransferrin concentration of the standards, was plotted. This curve was used to calculate ovotransferrin concentration of the samples (in mg/ml) based on their ΔA. A sample was run in triplicate, and the mean concentration was used in further analyses.

Testosterone

The testosterone concentration in the egg yolk was estimated by radioimmunoassay at CEBC (Centre d'Études Biologiques de Chizé, CNRS-UPR1934, Villiersen-Bois, France). These dosages are carried out in two phases. First, the egg yolk testosterone was purified and after its concentration was measured by competition with specific antibodies and radioactively labelled testosterone. Previously prepared samples were first defrosted. They were before worked in the same way for both species of birds. 100 µl of samples were diluted in 400 µl of PBS buffer (0.01 mol/l phosphate buffered saline, pH 7.4) and after 3 ml of ethyl ether (Sigma Aldrich®) were added. To homogenize the mixture, the tubes were vortexed 2 times for 1 minute. Centrifugation for 5 min at 2000 rev/min at 4°C separates the sample in two phases: the organic phase, in which diluted the testosterone rises to the surface of the tube, leaving the aqueous phase at the bottom. The tubes were then placed in an alcohol bath at -40°C to freeze the aqueous phase only. It was then possible to recover the organic phase containing the hormone by simply pouring the liquid into a new tube. This tube was placed in hot water bath in a fume hood to evaporate the ether. The tube appeared to be empty, but the bottom of the tube was totally covered with steroids. All the precedent passages were repeated one time to be sure of the sample purification.

The steroids were recovered in 300 µl of PBS and then vortexed 2 times for 1 minute. At 100 µl of the new mixture were added 100 µl of a solution of antiserum (rabbit polyclonal antibody specific to T) and 100 µl of a solution containing radioactive testosterone, T*, [1,2,6,7,3H]-Testosterone (Amersham, France). Incubation lasted 12 hours at 4°C, during which the T* will competed with testosterone in the sample to bind the antibodies. This step gave a fraction bounded to the antibody (B) and a fraction unbounded (F), which were separated with the method of carbon dextran: it was carried out at 0°C.

Each tube received 500 µl of charbon dextran and was mixed using a vortex for 10 min. Centrifugation for 10 min at 3500 rev/min at 4°C allowed to separate the charbon, that fell to the bottom of the tube bringing with it-self the fraction F, from the aqueous phase containing fraction B, which we dosed. 500 µl of supernatant was carefully collected and placed in polypropylene tubes. It must be added 1 ml of scintillation fluid before to put a cap and mixed the two phases. Each tube was then read by a scintillator (Packard-1600) that measured the radioactivity, that is the proportion of antibody bound to the T* and not to the testosterone of the sample.

A standard curve was performed in parallel with concentrations ranging from 1000 µg/100µl to 7.8 µg/100µl that allowed, through a semi-logarithmic graph, to transform the data provided by the scintillator in concentration (ng hormone/mg of egg yolk).

Statistical analysis

The majority of the analyses were performed by statistical software Systat 12® (SYSTAT Software Inc., San José, California). The analyses of the chapter III on the effect of noise were performed both with Systat 12® and Statistica 10® (StatSoft Inc., Tulsa, Oklahoma).

Chapter I: Dominant males influence egg allocation of female grey partridges.

Chapter I – Abstract

This chapter is focused on the effects of the male social rank on the resources allocation in eggs.

Literature presents different studies where has been seen that the female preference for an attractive male could increase maternal investment (Burley, 1988; Schwabl, 1996a; 1996b; Sheldon, 2000). In red-legged partridge, for example, an increase of clutch number in relation to the choice of the reproductive partner has been found (Bottoni et al., 1993). For what concerns androgens, some paper have been shown that females can vary the androgens concentration as answer to visual male stimuli (Gil et al., 1999; Saino et al., 2002b; Gowati et al., 2007) or in relation to song characteristics (Gil et al., 2004a).

Dominance is a key concept in the study of the social organization. Some studies have been focused on the relation between this social characteristic of the male and sexual selection operates by female; collected results are in contrast, because sometimes choice has rewarded the dominant, while in other situations was in favor of subordinate male (Cox & Le Boeuf, 1977; Alatalo et al., 1991; Horne & Ylönen, 1996; Qvanström & Forsgren, 1998; Johnsen et al., 2001; Ophir & Galef, 2003; 2004; Amy et al., 2008).

This study is carried out on 20 males and 20 females of grey partridge. After a pre-test (that helped to understand the social ranking of the 20 males) ten groups of two males in which the difference in terms of dominance was evident were formed. Every group was shown to two different females, that could see males characteristics and make their choice. At the end of these tests, 10 females were paired with the dominant (and chosen) male, and 10 with the subordinate. All the eggs were weighted and measured, some of these were taken for laboratory analyses on main components and on lysozyme, ovotransferrin, and testosterone concentration, while the others were put into the incubator to evaluate the hatching rate.

Hypothesis was that females paired with dominant males allocated a greater amount of resources into the eggs.

Main findings of this work are that the females paired with dominant and chosen males, compared with others who were paired with the dominated ones, laid eggs with more testosterone and with a higher hatching rate. No correlations were found with egg, albumen, yolk, eggshell mass, and lysozyme and ovotransferrin concentration.

Minor results of this study are on the quantity of egg components in relation to the laying order (decrease of yolk and eggshell mass, and increase of albumen

and testosterone), and a higher hatching rate of the eggs with an intermediate elongation index.

In conclusion, the social ranking of the male, associated with the female preference for the dominant males, modified the allocation of hormonal substances into the eggs and the subsequent hatching rate.

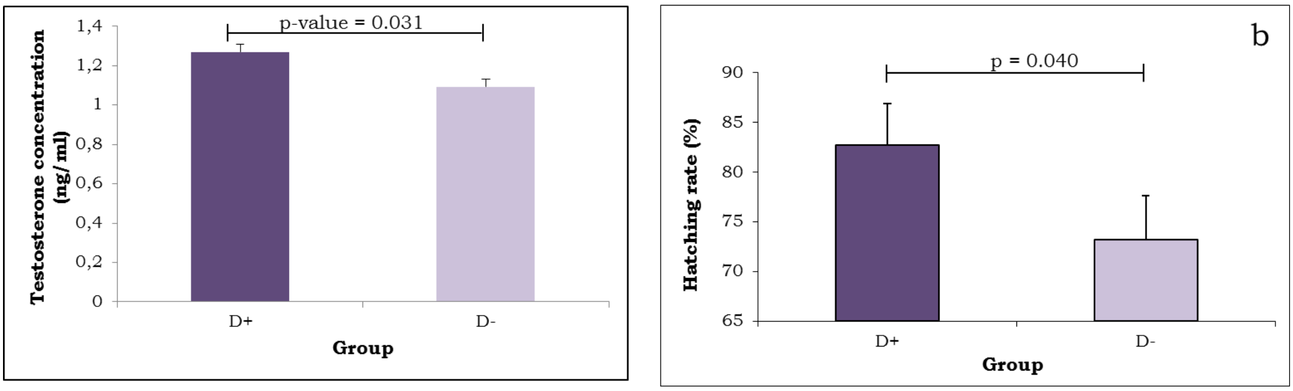


Figure 9 - a) Testosterone concentration related to laying order; b) hatching rate related to laying order.

Key-words: Male dominance, female choice, maternal effects, testosterone concentration, hatching rate.

Capitolo I – Riassunto

Questo capitolo si concentra sugli effetti del rango sociale del maschio sull'allocazione di risorse nell'uovo.

In letteratura vi sono diversi studi che indicano come la preferenza della femmina per un maschio attrattivo possa far aumentare l'investimento materno (Burley, 1988; Schwabl, 1996a; 1996b; Sheldon, 2000). Nella pernice rossa, per esempio, è stato visto come aumenti la quantità delle uova deposte quando la femmina può scegliere il proprio partner riproduttivo (Bottoni et al., 1993). Per quanto riguarda gli androgeni, alcuni lavori hanno dimostrato come le femmine possano variare la concentrazione di essi in risposta a stimoli visuali del maschio (Gil et al., 1999; Saino et al., 2002b; Gowati et al., 2007) o a caratteristiche del canto (Gil et al., 2004a).

La dominanza è un concetto fondamentale nello studio dell'organizzazione sociale. Alcuni lavori si sono concentrati sulla relazione fra questa caratteristica del maschio e la selezione sessuale della femmina; i risultati raccolti sono contrastanti, in quanto a volte la scelta premia il dominante mentre altre è a favore del dominato (Cox & Le Boeuf, 1977; Alatalo et al., 1991; Horne & Ylönen, 1996; Qvanström & Forsgren, 1998; Johnsen et al., 2001; Ophir & Galef, 2003; 2004; Amy et al., 2008).

Questo studio è stato eseguito su 20 maschi e 20 femmine di starna. Dopo un pre-test che è servito per comprendere il ranking sociale di questi 20 maschi, sono stati formati dieci gruppi di due nei quali la differenza in termini di dominanza era evidente. Ogni gruppo è stato fatto vedere a due femmine differenti, che hanno potuto apprezzare le caratteristiche dei maschi e mostrare la loro preferenza. Al termine di questi test, 10 femmine sono state accoppiate con il maschio dominante (e scelto) e 10 con il dominato. Tutte le uova sono state pesate e misurate, alcune sono state portate in laboratorio per l'analisi delle componenti principali e della concentrazione di lisozima, ovotransferrina e testosterone, mentre le altre messe ad incubare per valutare il tasso di schiusa.

L'ipotesi era che le femmine accoppiate con maschi dominanti allocassero una maggior quantità di risorse nelle loro uova.

I risultati principali di questo lavoro sono che femmine accoppiate con maschi dominanti e scelti, comparate con altre ai quali è stato dato un maschio dominato, hanno deposto uova con più testosterone e con un più alto tasso di schiusa. Nessuna correlazione è stata invece trovata con il peso dell'uovo, dell'albume, del tuorlo e del guscio e con la concentrazione di lisozima ed ovotransferrina.

Risultati minori dello studio sono quelli riguardanti le quantità delle componenti delle uova in relazione all'ordine di deposizione (diminuzione del tuorlo e del guscio ed aumento di albume e testosterone) e una maggior schiusa di uova con un indice di *elongation* intermedio.

In conclusione, il rango sociale del maschio associato alla preferenza della femmina per maschi dominanti ha modificato l'allocazione di sostanze ormonali nelle uova e la successiva percentuale di schiusa.

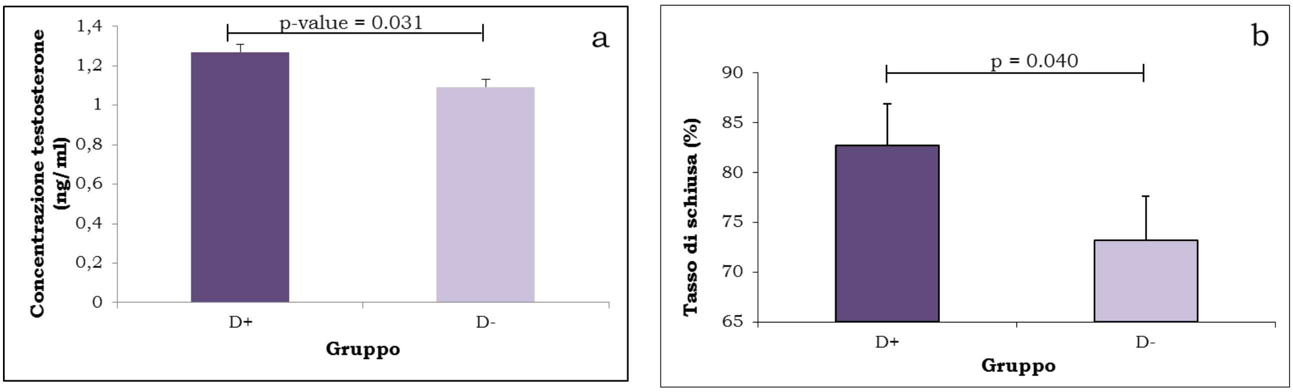


Figure 10 - a) Concentrazione di testosterone in relazione al gruppo sperimentale; b) tasso di schiusa in relazione al gruppo sperimentale.

Parole-chiave: Dominanza maschile, scelta della femmina, effetti materni, concentrazione di testosterone, tasso di schiusa.

Chapitre I – Résumé

Ce chapitre se concentre sur les effets du statut social du mâle sur l'allocation des ressources dans l'œuf.

Dans la littérature, plusieurs études existent qui indiquent que la préférence des femelles pour un mâle attractif peut accroître les investissements maternelle (Burley, 1988 ; Schwabl, 1996a ; 1996b ; Sheldon, 2000). Dans la perdrix rouge, par exemple, a été vu une augmentation du nombre d'œufs pondus quand la femelle peut se reproduire avec le partenaire choisi (Bottoni et al., 1993). En ce qui concerne les androgènes, des travaux ont montré comment les femmes peuvent faire varier la concentration d'androgènes en réponse à des stimuli visuels du mâle (Gil et al., 1999 ; Saino et al., 2002b ; Gowati et al., 2007) ou aux caractéristiques du chant (Gil et al., 2004a).

La dominance est un concept fondamental dans l'étude de l'organisation sociale. Certaines études ont été portés sur la relation entre cette caractéristique sociale du mâle et la sélection sexuelle fait par la femelle ; les résultats obtenus sont contradictoires : quelque fois le choix récompense le dominante tandis que d'autres est en faveur du dominé (Cox & Le Boeuf, 1977 ; Alatalo et al., 1991 ; Horne & Ylönen, 1996 ; Qvanström & Forsgren, 1998 ; Johnsen et al., 2001 ; Ophir & Galef, 2003 ; 2004 ; Amy et al., 2008).

Cette étude a été réalisée sur 20 mâles et 20 femelles de perdrix grise. Après un pré-test qui est servi à comprendre le rang social de ces 20 mâles, dix groupes de deux, dans lesquels la différence en termes de dominance était évident, ont été formés. Chaque groupe a été montré à deux femelles différentes, qui ont pu apprécier les caractéristiques des mâles et montrer leur préférence. A la fin de ces tests, 10 femelles ont été accouplées avec le mâle dominant (et choisi) et 10 avec le dominé. Tous les œuf ont été pesés et mesurés, certains ont été emmenés au laboratoire pour analyser les composantes principales et la concentration de lysozyme, ovotransferrine et testostérone ; les autres ont été laissés à incuber pour l'évaluation du taux d'éclosion.

Notre hypothèse était que les femelles accouplées avec des mâles dominants allouent une plus grande quantité de ressources dans leurs œufs.

Les principaux résultats de ce travail ont été que les femelles accouplées avec des mâles dominants et choisi, par rapport à d'autres qui ont reçu une mâle dominé, ont pondu œufs avec plus de testostérone et un taux plus élevé d'éclosion. Aucune corrélation a été trouvée avec le poids de l' œuf, de l'albumen, du jaune et de la coquille et la concentration de lysozyme et ovotransferrine.

Autres résultats de l'étude ont été celles concernant les quantités des composants des œufs par rapport à l'ordre de dépôt (diminution du jaune et de la coquille et augmentation du blanc et de la testostérone) et une plus grande éclosion des œufs avec un indice d'allongement intermédiaire.

En conclusion, le rang social du mâle liée à la préférence de la femelle a changé la répartition des substances hormonales dans les œufs et la pourcentage du taux d'éclosion.

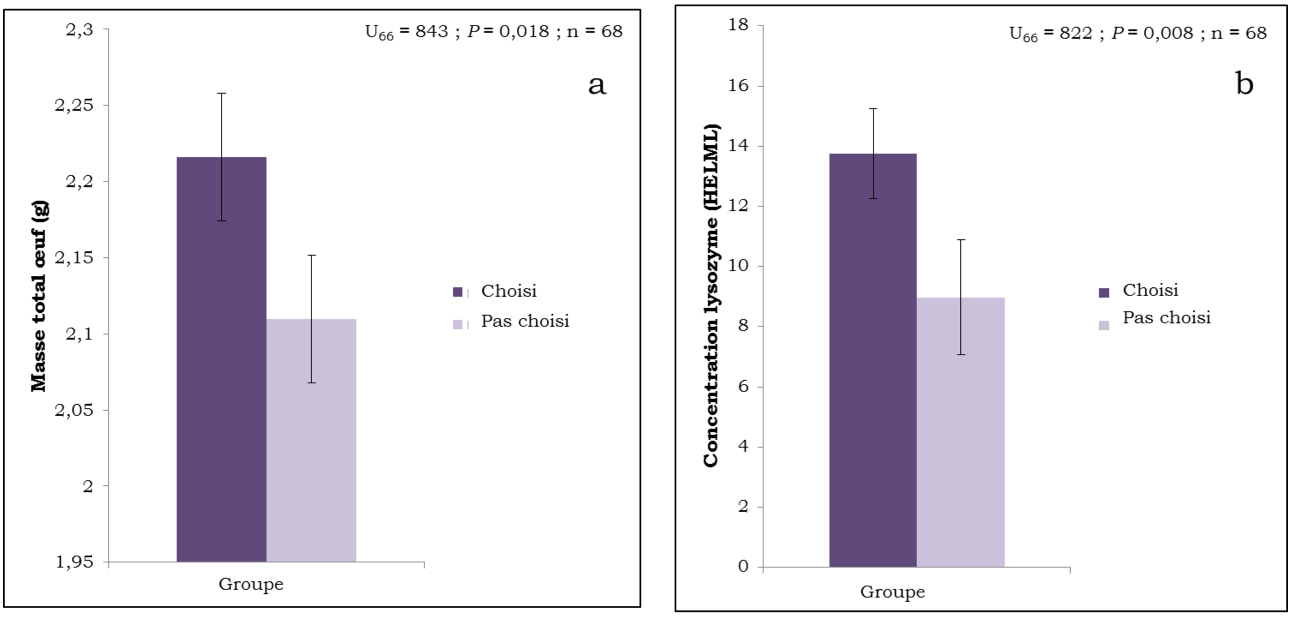


Figure 11 - a) Concentration de la testostérone par rapport au groupe expérimental ; b) taux d'éclosion par rapport au groupe expérimental.

Mots clés: Dominance du mâle, choix de la femelle, effets maternels, concentration de testostérone, taux d'éclosion.

~

Introduction

~

In maternal effects can be inserted the mechanisms non-genetic that intervene when the mother's phenotype or the environment influence the progeny's phenotype; in the viviparous animals, for example, it has been demonstrated that they effect birth date, birth mass, mortality, etc. (Mousseau & Fox, 1998).

In contrast with viviparous species, in oviparous vertebrates, the female must include all the resources required by the embryo in the egg laid in a limited window of time; she can't do adjustments to their components after the deposition, so maternal resource allocation is limited. For this, the pre-laying parental effort holds a fundamental role in the embryo's development. These resources into the eggs could potentially influence genes' expression and offspring's fitness (Bolton, 1991; Clutton-Brock, 1991; Lovern & Wade, 2003; Mansour et al., 2007; Pilz et al., 2004; Williams, 1994). The allocation of these resources is costly for the female (Gil et al., 1999; Pilz et al., 2003); for this it's possible to observe a differential allocation associated to the expected fitness of the young (Rutstein et al., 2004; Stearns, 1992). The environmental conditions encounter by the mother before and during egg formation and the characteristics of the partner influence the quality of the eggs (Burley, 1988; Schwabl, 1996a; 1996b; Sheldon, 2000). The male attractiveness, in particular, can increase, in oviparous species, an important aspect of maternal allocation: the egg androgens. Females can vary yolk androgen concentration in response to male visual stimuli (Gil et al., 1999; Saino et al., 2002b; Gowati et al., 2007) or to male song (Gil et al., 2004a). In a few cases, however, male choice did not affect (Cucco et al., 2011a) or even negatively influenced androgen concentration in eggs (Gowati, 2008).

Yolk androgens have been shown to affect various traits both in the short and long-term (Ruuskanen et al., 2012). The long lasting effects of egg yolk androgens may result in positive or negative fitness consequences. On the negative side, a suppressive effect of high androgen levels on the immune system has been shown (Gil, 2008), and yolk androgens may also increase the predation risk via an enhancement of dangerous behaviors (Ruuskanen & Laaksonen, 2010). On the other hand, androgens may positively affect chick physiology, making easier the hatching process (Lipar & Ketterson, 2000) or enhancing begging behavior (Saino et al., 2002c). On the long term, adults born from eggs with high testosterone levels can be more attractive and have a high mating success (Partecke & Schwabl, 2008).

In particular, testosterone is a steroid hormone commonly implicated in aggressive behaviour (Baptista et al., 1987; Wingfield et al., 1990; 2003; Van Duyse et al., 2002; Mazuc et al., 2003a; Poisbleau et al., 2005); because aggressiveness plays a key role in dominance interactions (Wilson, 1975; Beaugrand, 1983), many studies have focused on the relationship between testosterone levels and dominance (Tanvez et al., 2008). Testosterone-implanted males of white-throated sparrows, *Zonotrichia albicollis*, showed an increase in aggressiveness and social status in the presence of unfamiliar opponents (Archawaranon et al., 1991). Similarly, testosterone implants enhanced the social rank of female spotless starlings, *Sturnus unicolor* (Veiga et al., 2004). Nevertheless, it is worth noting that, in male and female canaries, *S. canaria*, no correlation between natural plasma testosterone levels and social status was found (Parisot et al., 2005).

Females do not limit maternal effects to androgen deposition, but other egg components and substances can be allocated differentially in response to male quality (Cucco et al., 2011a). Carotenoids and other yolk antioxidants, immunoglobulins and antibacterial substances have been examined in detail. In particular, lysozyme and ovotransferrin have been investigated. They are two of the best characterized and most abundant proteins in egg albumen that are thought to play an important role in defense against microbial infection (Tranter & Board, 1982). Lysozyme can break down the polysaccharide walls of a broad spectrum of Gram-positive bacteria (Rogers & Perkins, 1968). Ovotransferrin sequesters iron that is essential for bacterial growth and it is a major egg white protein, representing 12% of chicken albumen protein (Li-Chan et al., 1995). These antimicrobials protect the egg from infectious bacteria entering through the shell and, after being swallowed with the rest of the albumen at hatching, can be used in immune defense by the hatchling for several days (Saino et al., 2002b). Similarly to testosterone, results are heterogeneous for the others components and, aside studies that detected a positive effect, others did not. According to Horváthová et al. (2012) female generally invest more into reproduction when paired with attractive males, but investment reflects the context-dependent costs and benefits of egg compounds to females and offspring.

Aside male attractiveness, male dominance can influence female choice: females frequently choose dominant males, even if the opposite could be found (Berglund et al., 1996; Pradhan & Van Schaik, 2009).

The dominance is a key concept in studies of social behaviour and is usually associated with social organization. It was first developed by Schjelderup-Ebbe (1935) who coined the term 'pecking order' when studying fowl *G. gallus* (Hoeschele et al., 2010). Many definitions have been created to explicate this concept (Drews, 1993): most of these indicate the dominance as a relative concept often used to define competitive relationships between individuals within a social group (Bernstein, 1981; Piper, 1997). Dominance hierarchies come out from dyadic relationships between dominant and subordinate individuals in a social group (Drew, 1993; Neumann et al., 2011). Hierarchies can be presented in most animal taxa, e.g. insects (Kolmer & Heinze, 2000), birds (Amy et al., 2008; Kurvers et al., 2009; Tanvez et al., 2008), and mammals (Keiper & Receveur, 1992).

The capability of a bird to become dominant can depend on several factors; certain may be fixed, while others are variable (Piper, 1997). Fixed attributes can be genetic factors: males tend to dominate females (Fugle et al., 1984; Richner, 1989). For the variable attributes age can be cited: for example, the oldest individuals tend to dominate the younger ones (Richner, 1989; Teather & Weatherhead, 1995).

Also the bird's hormonal status is an important factor. Hormones can influence the behaviour: a variation in their concentration can change the social status of an individual (Archawaranon et al., 1991; Creel, 2001; Parker et al., 2002).

Empirical and theoretical evidences stressed that intra and intersexual selection may work in opposing directions (male dominance and related high aggressiveness may not be a desired feature for females). These traits may not be a desired feature for females of polygynous species if, for example, several females have to share the male territory (Qvanström & Forsgren, 1998). Accordingly, females *G. gallus* preferred to mate with subordinate males provided that they have large crests and wattles (Johnsen et al., 2001). In the japanese quail *Coturnix japonica*, females avoided the dominant male and preferred the subordinate one if they assessed the presence of an aggressive male (Ophir & Galef, 2004).

In this study on the grey partridge *P. perdix* it was tested if females paired with a dominant males allocate differentially egg substances. Females were paired with males of different rank in a dominance hierarchy, and then measures on testosterone and antibacterial substances (lysozyme and ovotransferrin) concentration in the eggs were performed. A previous work showed that females paired to a preferred male laid more testosterone in their eggs (Garcia-Fernandez et al., 2010a). This result was in accord with the differential allocation hypothesis DAH [females should allocate more when mating with preferred males (Sheldon, 2000)], while did not agree with the compensatory hypothesis [females should allocate more when paired with males of lower quality (Gowaty et al., 2007)]. Aim of this work was to verify the relationship between male dominance and female preference, and to test if male dominance is a feature that can have consequences on female investment.

~

Materials and Methods

~

Experimental groups

The study was conducted on grey partridges (*P. perdix*), reared in 2011 at a breeding farm in Ovada, Alessandria, NW Italy. In total, 20 breeding pairs were housed in individual outdoor reproduction cages (4 m long × 1 m wide × 0.5 m high). The birds experienced natural light and temperature conditions throughout the year, and all of them were of the same age (first year), to avoid possible age effects. Each pair had food and water available *ad libitum*. From April to June, the hens laid a total of 569 eggs.

The experimental groups were two: one composed by females paired with subordinated males and the other by females paired with dominant ones.

Dominance test

The tests was performed in March, at start of the mating season. Trials were run in an outdoor aviary measuring 10 × 12 m. Social hierarchy was evaluated using a method of dyadic competition for access to a female (Drews, 1993). To determine the male ranking 190 competitions for the access to a female were conducted between 20 males: every male had 19 fights with the others. The test was composed by 2 minutes for the acclimatization to the enclosure and 7 minutes for the competition. At the end a score to the two competitors was gave. After the 190 fights the scores were added and a ranking of the 20 males was obtained. The females utilized as stimulus didn't see the same male for two times and they haven't been used for the formation of the couples. After this, the males were paired following this principle: the first male of the ranking with the eleventh, the second with the twelfth etc. This procedure was followed to have a couple where the difference between the males was considerable. Hereafter, the pair of males were shown to two females separately for 5 minutes and after this the dominant male was given to the first one and the subordinate to the other. At the end, 10 couples with females paired with a dominant male (group D+) and 10 with females paired with a subordinate (group D-) were formed.

Egg Collecting and Sampling

When females started laying, all aviary was inspected daily to collect eggs. Using a non-toxic marker pen, each egg was marked with the female's code, the position in the laying order and date of laying. Just after collection, the egg length (L) and breadth (B) were measured by a calibre (0.05 mm accuracy) and the eggs were weighted by an electronic balance (± 0.01 g accuracy – Cucco et al., 2012). From April to June, each female laid a mean of 28.5 eggs, yielding a total of 569 eggs.

Most of the eggs laid were incubated for 26 days in a commercial incubator at 37.5 °C and 60% humidity, while 146 were brought to the laboratory for the analyses. For every female the 1st, 2nd, 5th, 11th, 14th, and 20th egg were taken; for three females of the two group also the 25th egg was collected. After the separation of the yolk from the albumen and the weighing of all components, the yolk was homogenized with an equal part of NaCl solution (0.9%) and the albumen was sonicated; after this, the two components were frozen at -20 °C.

Testosterone assay

The testosterone concentration in the egg yolk was estimated by radioimmunoassay at CEBC (Centre d'Études Biologiques de Chizé, CNRS-UPR1934, Villiersen-Bois, France).

This protocol was based on a simple principle: the hormone to dose (hormone cold H) was in competition with the same marked hormone (hormone hot or tracker H*) for an H-specific antibody whose concentration was limited (failing antibody dosage). After incubation at equilibrium, a bound fraction (H-Ac and H*-Ac) and a free fraction (H and H*) were obtained. Immune complexes were then separated from the free hormone and the radioactivity was counted.

For the exact protocol see the "Materials and Methods: Common Aspects" above.

Lysozyme Assay

Lysozyme activity was measured using the lyso-plate method (Osserman & Lawlor, 1966). Brief, the diameters of the cleared zones (inhibition zones) that the samples formed around the site of inoculation into a Petri plate containing *M. lysodeiktikus* was an indicator of the lysozyme concentration. These diameters were proportional to the logarithm of the lysozyme concentration and, compared with the standard curve, allowed to obtain values in hen egg lysozyme equivalents (HELML equivalents, expressed in µg/ml).

For the exact protocol see the "Materials and Methods: Common Aspects – Lysozyme: protocol Osserman & Lawlor, (1966)" above.

Ovotransferrin Assay

A modified version of the assay described and verified by Yamanishi et al. (2002) was used. This assay measured total iron-binding capacity – the maximum amount of iron necessary to saturate all the ovotransferrin in a sample. It correlated very well with ovotransferrin concentration, as determined by comparison with immunological measurement of serum transferrin (Gambino et al., 1997; Yamanishi et al., 2002). A similar version of the assay has been used previously to measure concentrations of ovotransferrin in egg albumen (Shawkey et al., 2008; D'Alba et al., 2010). The assay consisted of three reaction steps. First, ovotransferrin in the sample was saturated with ferric iron (Fe^{3+}) under alkaline conditions. Then, the unbound excess iron was reduced to Fe^{2+} by addition of ascorbic acid, and this Fe^{2+} became inactivated by forming coloured complexes with the chromogen FerroZine. Finally, the ovotransferrin-bound Fe^{3+} was dissociated under acidic conditions. This newly released Fe^{3+} allowed further formation of the coloured Fe^{2+}–FerroZine complex. The associated increased in absorbance of the reaction mixture owing to this additional formation of the coloured complex was monitored over time (Horrocks et al., 2011).

For the exact protocol see the "Materials and Methods: Common Aspects" above.

Statistical analysis

First, the differences in egg components between the females were analysed using an ANOVA test.

The difference between two groups in the delay between the pairs formation and the first egg laid and in the day of laying were performed with a General Linear Model.

Egg characteristics were analysed using multivariate mixed models with egg mass and egg characteristics as dependent variables. Dominance was inserted as a fixed effect, position in the laying order as covariate, and female identity was included as a random effect to control for among-female variation.

A logistic regression model was used to determine if any of these variables (dominance, position in the laying order, elongation and $elongation^2$) predicted which eggs would hatch. Elongation was inserted both as a linear and a quadratic term to allow a U-shaped pattern of variation.

Statistical analyses were performed using SYSTAT 12 (SYSTAT Software Inc., San José, CA).

~

Results

~

Descriptive statistics

Variable	Mean	±SD	CV	ANOVA		
				F	d.f.	P
Egg mass	14.18	± 1.19	8.4	41.64	19	0.001***
Yolk mass	4.94	± 0.50	10.2	5.68	19	0.001***
Albumen mass	6.47	± 0.81	12.5	6.26	19	0.001***
Eggshell mass	2.41	± 0.40	16.6	2.62	19	0.001***
Elongation	1.31	± 0.05	3.5	13.53	19	0.001***
Lysozyme conc.	1669.76	±627.01	37.6	0.60	19	0.90
Ovotransferrin conc.	8.86	± 6.06	68.4	0.83	19	0.66
Testosterone conc.	1.18	± 0,26	22.4	5.48	19	0.001***

Table 1: Egg mass, macro-components of the eggs, elongation, lysozyme, testosterone and ovotransferrin concentration and statistical differences among laying females in the grey partridge *P. perdix*. Legend: ANOVA, analysis of variance; CV, coefficient of variation; d.f., degrees of freedom; ***, P < 0.001.

There were statistically significant differences in egg mass, egg macro-components and testosterone concentration in relation to female identity, i.e. each female laid eggs that differed in mass and shape from other females (Table 1; all eggs laid by each female were included). No differences were found in lysozyme and ovotransferrin concentration (Table 1). A small variation in egg mass, eggshell, yolk and albumen mass and elongation [coefficient of variation (CV) = 3.5–16.6%] and a medium variation in lysozyme, ovotransferrin and testosterone concentration [CV = 22.4–68.4 (Table 1)] were found.

No statistically significant difference was found in days between couple formation and first egg laid (D+ = 21.8 ± 1.4 days, D- = 19.5 ± 1.4 days; p-value = 0.25) and in duration of the laying period (D+ = 49.5 ± 2.9 days, D- = 43.4 ± 2.9 days; p-value = 0.20).

	Coefficient (SE)	F	P
Egg mass (n = 567)			
Dominance	-0.238 (0.405)	0.343	0.56
Laying order	0.001 (0.005)	0.001	0.97
Yolk mass (n = 146)			
Dominance	-0.222 (0.152)	2.132	0.15
Laying order	-0.011 (0.005)	6.085	**0.015**
Albumen mass (n = 146)			
Dominance	-0.115 (0.262)	0.194	0.66
Laying order	0.032 (0.007)	22.210	**0.001**
Eggshell mass (n = 146)			
Dominance	0.023 (0.100)	0.052	0.82
Laying order	-0.023 (0.004)	38.537	**0.001**
Lysozyme concentration (n = 140)			
Dominance	35.004 (106.605)	0.108	0.74
Laying order	4.815 (7.594)	0.402	0.53
Ovotransferrin concentration (n = 77)			
Dominance	-0.528 (1.428)	0.137	0.71
Laying order	-0.066 (0.118)	0.314	0.58

Testosterone concentration (n = 86)			
Dominance	0.196 (0.089)	4.846	**0.031**
Laying order	0.008 (0.002)	9. 289	**0.003**

Table 2: Multivariate mixed-model analysis of egg mass and egg macro-components, with dominance as factor, and position in the laying order of the focal egg as covariate. In the models, the female identity was inserted as a random factor.

Table 2 shows the results of a linear mixed models with egg mass, egg components, lysozyme, ovotransferrin and testosterone concentration as dependent variables.

Yolk, albumen and eggshell mass were related to laying order (see Table 2 and Figure 15) but not to the dominance (see Table 2 and Figure 12). Lysozyme and ovotransferrin concentration were no significantly related to any independent variable.

Testosterone concentration was related to both dominance and laying order (Table 2, Figure 14 and Figure 17).

Dominance effects

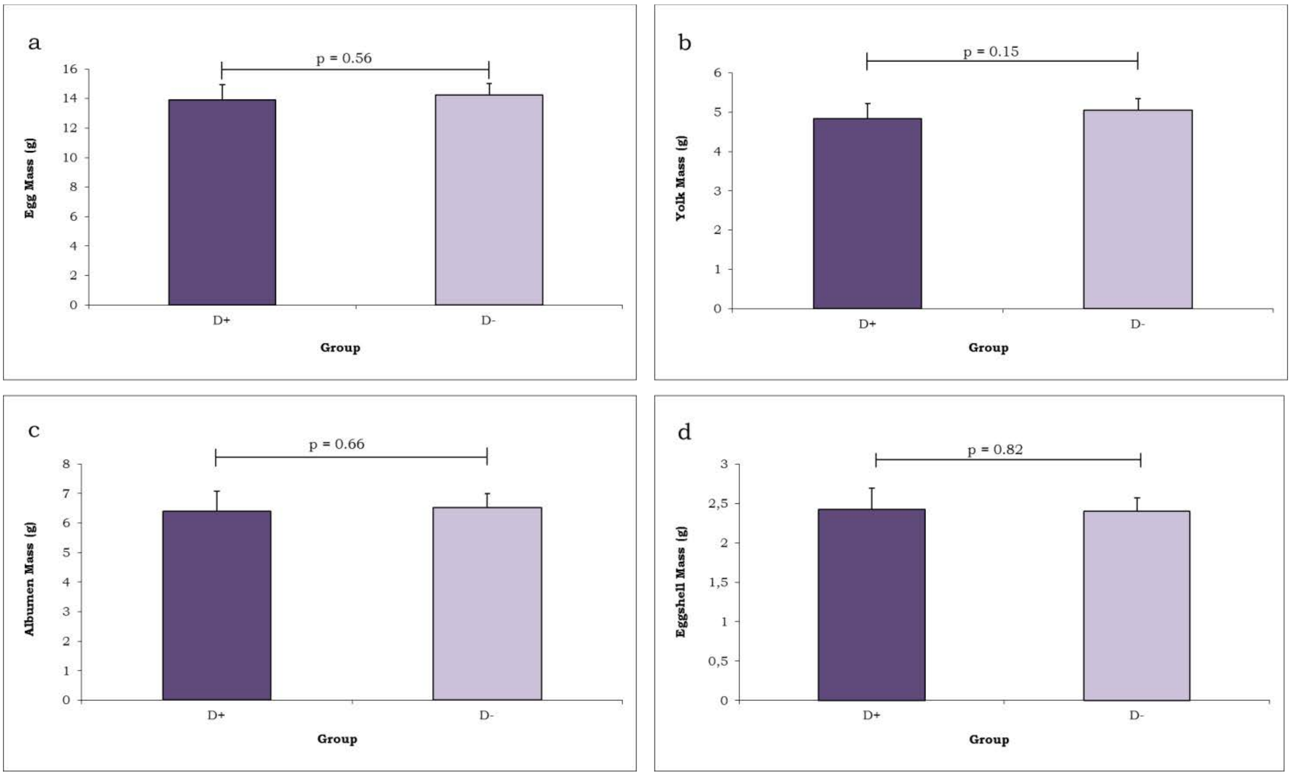
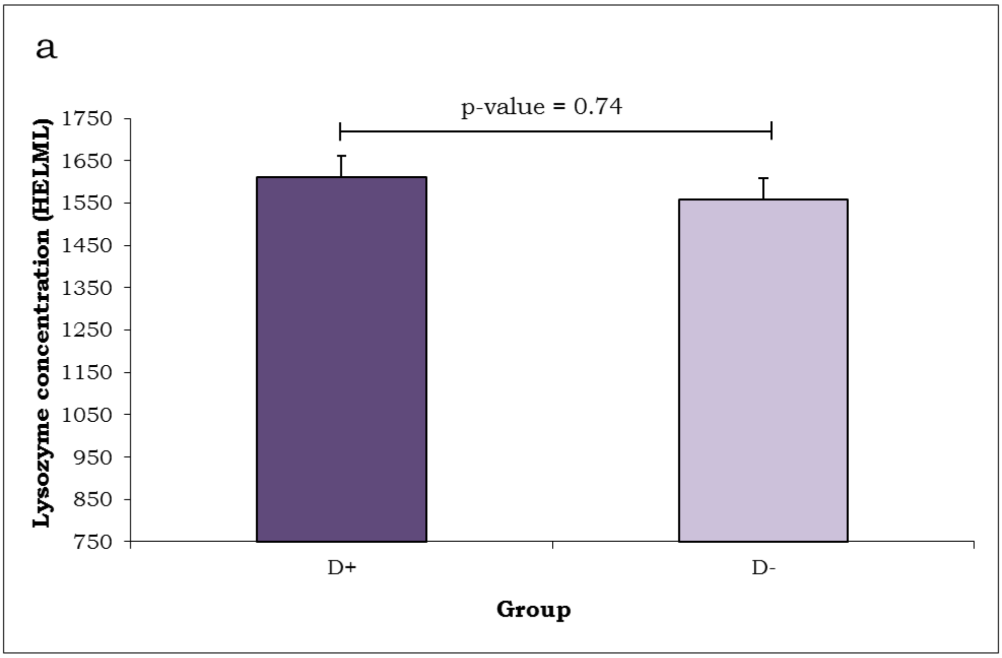


Figure 12 - Histograms where the two experimental groups are correlated with the macro-components of the eggs.

In the Figure 12 there are four histograms where the macro-components of the eggs are correlated with the two experimental groups: females paired with dominant males (D+) and females paired with dominated males (D-).

No statistically significant difference were found between the two groups [a) egg mass: D+ = 13.92 ± 1.04 g, D- = 14.26 ± 0.74 g; p-value = 0,56; b) yolk mass: D+ = 4.83 ± 0.39 g, D- = 5.06 ± 0.29 g; p-value = 0,15; c) albumen mass: D+ = 6.40 ± 0.68 g, D- = 6.52 ± 0.49 g; p-value = 0,66; d) eggshell mass: D+ = 2.42 ± 0.27 g, D- = 2.40 ± 0.17 g; p-value = 0,82 – Table 2].



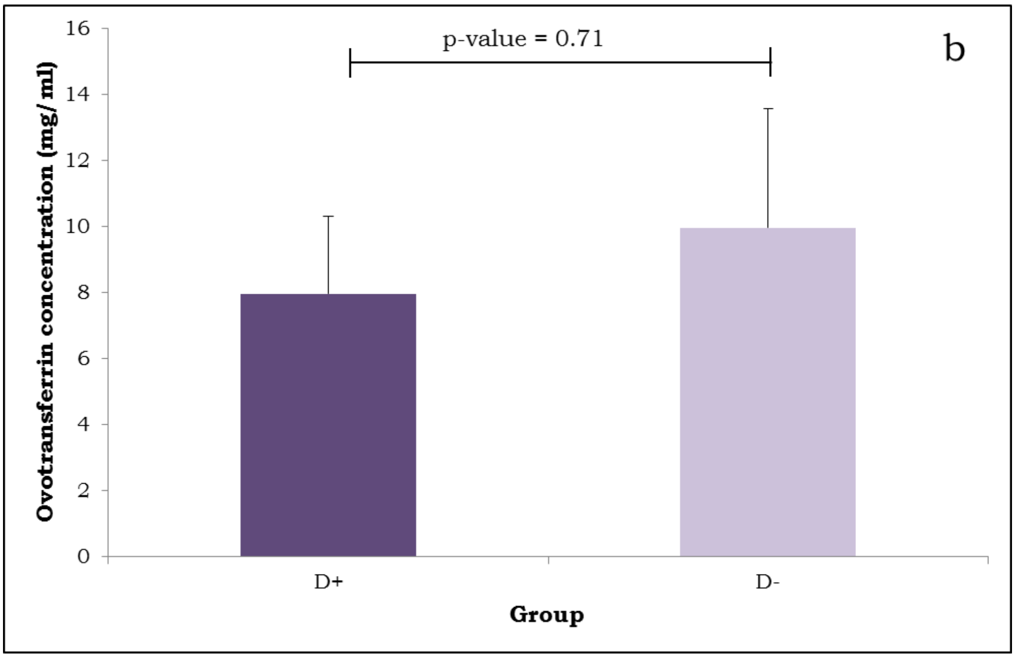


Figure 13 - Histograms where the two experimental groups and two albumen components [lysozyme (a) and ovotransferrin (b)] are correlated.

In the Figure 13 two of the major antimicrobial components is correlated with the two experimental groups.

Both for lysozyme and for ovotransferrin, no statistically significant differences were found [a) lysozyme: D+ = 1611.65 ± 48.70 HELML, D- = 1557.96 ± 49.65 HELML; p-value = 0,74; b) ovotransferrin: D+ = 7.97 ± 2.35 mg/ml, D- = 9.97 ± 3.61 mg/ml; p-value = 0,71 – Table 2].

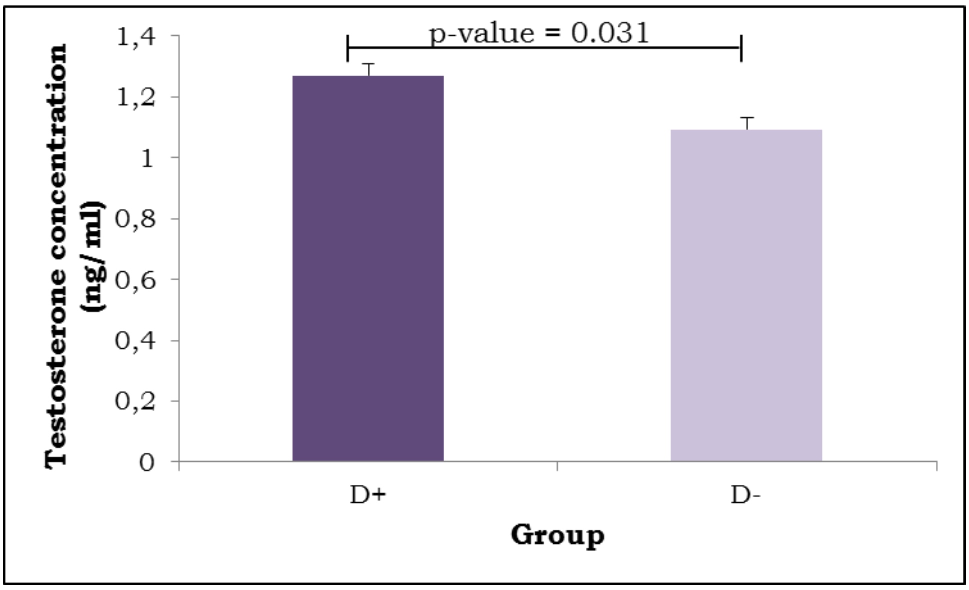


Figure 14 - Histogram where the two experimental groups are correlated with testosterone concentration.

In the histogram of Figure 14 it's shown the comparison between the testosterone concentration of the eggs laid by the females of the groups D+ and D-.

Females of the group D+ laid eggs with a higher concentration of testosterone than the females of the group D- (D+ = 1.29 ± 0.05 ng/ml, D- = 1.11 ± 0.04 ng/ml; p-value = 0.031 – Table 2).

Laying order effects

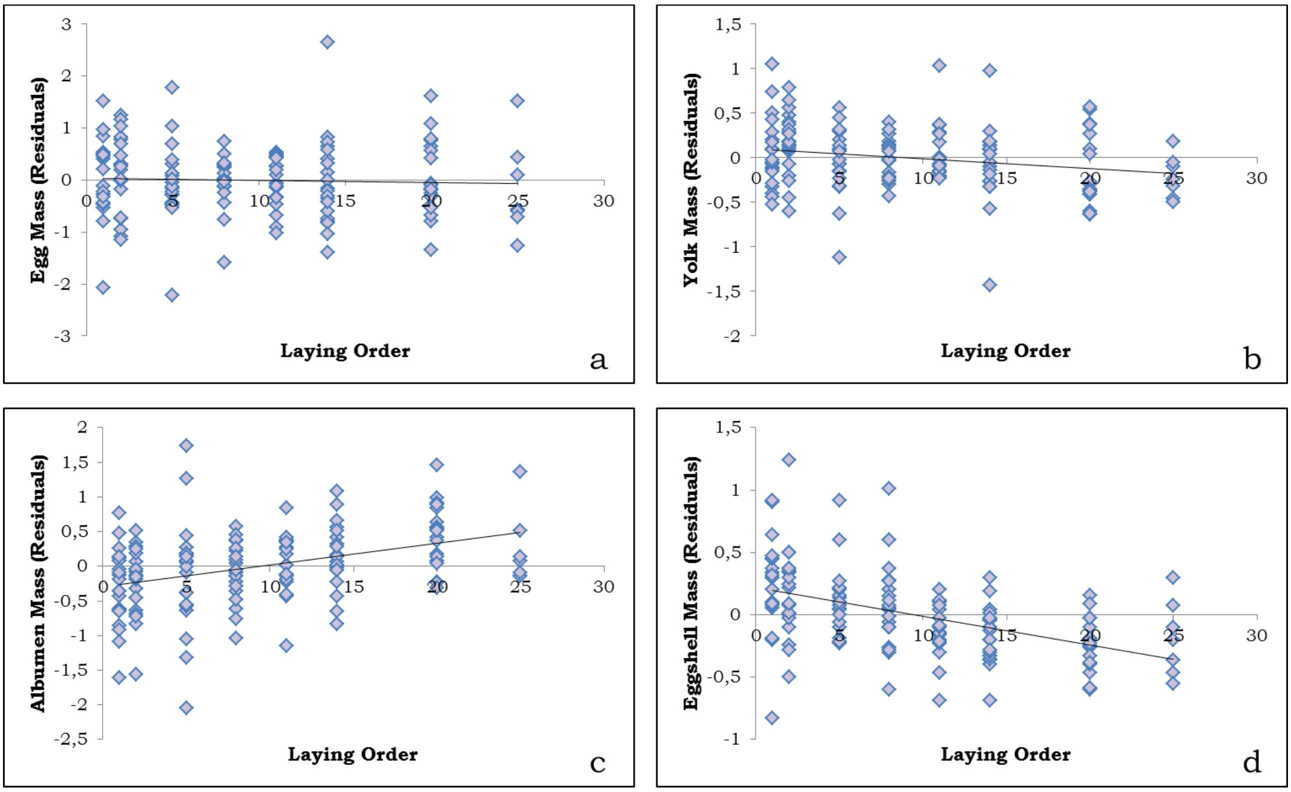


Figure 15 - Correlations between the residuals of the egg macro-components and the deposition order.

Figure 15 presents four scatter plots where is shown how the various egg macro-components varying in relation to the order of deposition.

Significant patterns through the laying order were found in yolk mass [b) p-value = 0.015, that decreased], albumen mass [c) p-value < 0.001, that increased], and eggshell mass [d) p-value < 0.001, that decreased]; for what concerns egg mass, no pattern was present [a) p-value = 0.97 – Table 2].

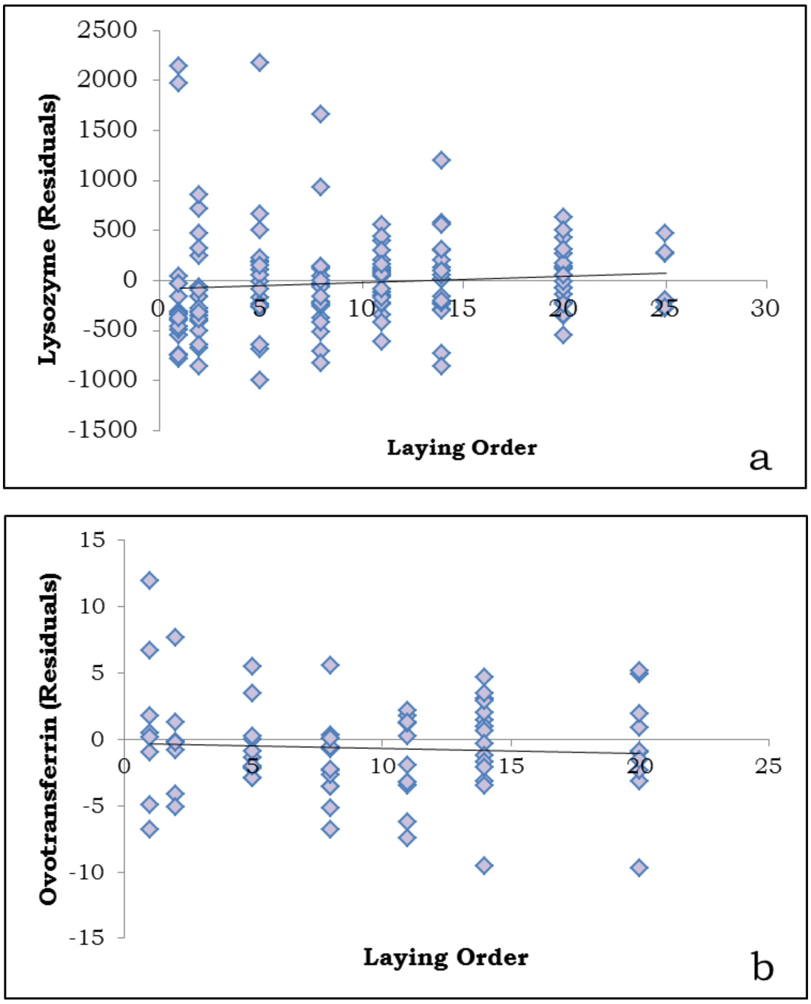


Figure 16 - Correlations between the residuals of lysozyme and ovotransferrin concentration and the deposition order.

In Figure 16, for both the antimicrobial albumen components (lysozyme and ovotransferrin) no significant results were found: lysozyme [a) p-value = 0.53] and ovotransferrin [b) p-value = 0.58 – Table 2] didn't vary in relation with the order of deposition.

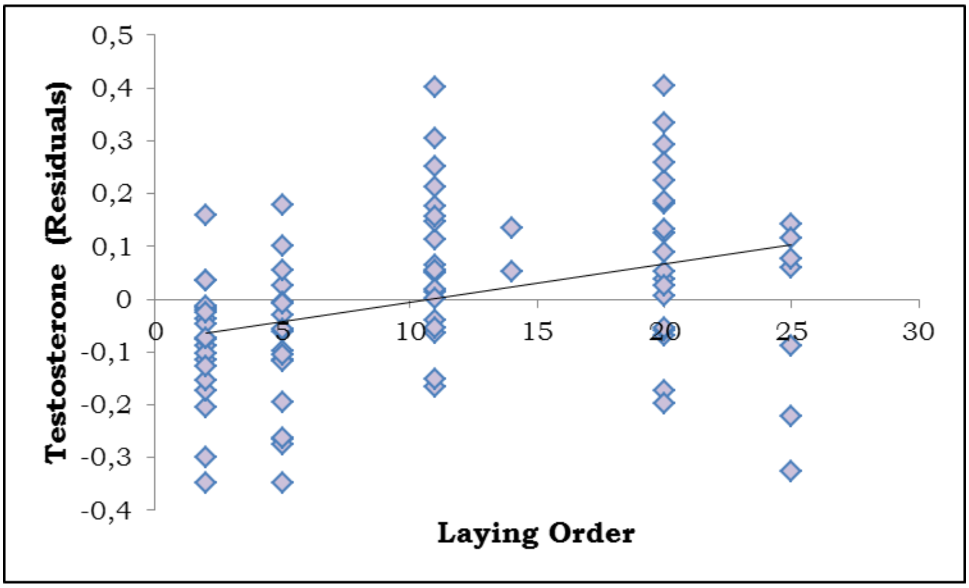


Figure 17 - Correlations between the residuals of testosterone concentration and the deposition order.

In Figure 17 is shown a scatter plot with the residuals of the testosterone concentration plotted with the order of egg deposition.

In this case, a significant pattern of variation in the testosterone concentration was present: it increased with the laying order (p-value = 0.003 – Table 2).

Hatchability

	Coefficient (SE)	P
Constant	-166.549 (69.805)	0.017
Dominance	2.641 (1.225)	**0.040**
Laying order	-0.002 (0.017)	0.894
Elongation	248.923 (107.044)	**0.020**
Elongation^2	-92.594 (40.932)	**0.024**

Table 3: Logistic regression analysis of hatching success in relation to dominance, position in the laying order, egg elongation and egg elongation^2.

Logistic regression indicated that egg elongation and the group of the female significantly contributed to the model predicting hatching success (Table 3). The negative quadratic coefficient determined an inverse U-shaped pattern of variation (Figure 18), with higher hatchability for the intermediate egg elongations (index = 1.30–1.33).

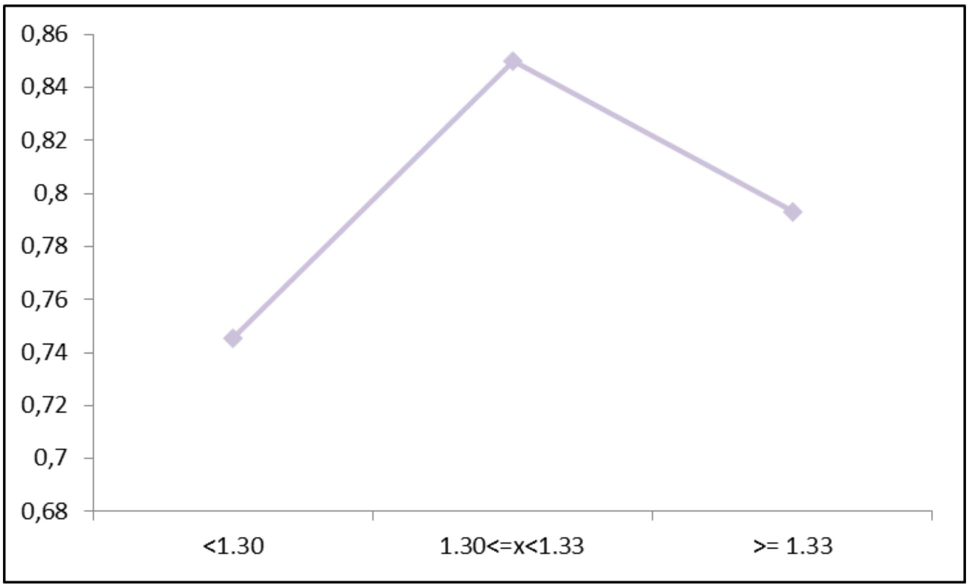


Figure 18 - Relationship between hatchability and egg elongation (length/breadth).

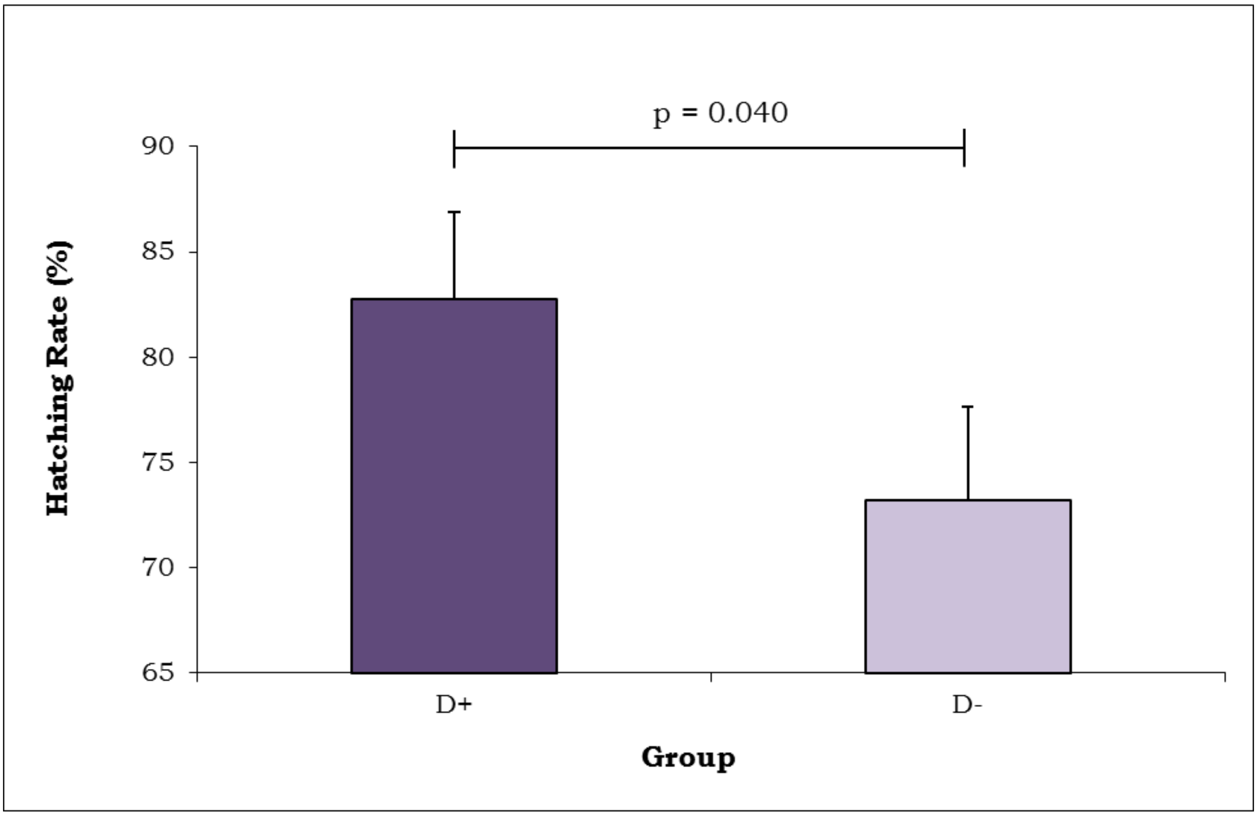


Figure 19 - Relationship between hatchability and experimental groups.

A statistically significant difference in hatchability was found between the two experimental groups (D+ = 0.828 ± 0.032, D- = 0.732 ± 0.029; p-value = 0.040); so, the eggs laid by females paired with dominant males hatched more than eggs laid by females coupled with subordinate males (see Table 3 and Figure 19).

~

Discussion

~

This experiment has been designed to evaluate: 1) if grey partridge female chose or not a male that resulted dominant after a male-male physic competition and, 2) if the female differently assessed maternal investment and testosterone, lysozyme and ovotransferrin deposition in eggs when paired with a dominant (and chosen) male. To do this, females saw interactions between two male, one dominant and one subordinate; after this, the female choice was noted and finally the maternal investment was valued.

This work shows that females paired with dominant males (and compared with females coupled with dominated ones) laid eggs with more testosterone and that hatched more; no correlations have been found with egg, yolk, shell and albumen mass and lysozyme and ovotransferrin concentration. Moreover, a different allocation regarding the laying order has been found: yolk and eggshell mass decreased, while albumen and testosterone increased with the laying order. Despite this, the egg mass remained unvaried. Finally, this study has shown also an inverse U-shaped pattern of variation for what concerns the hatchability of the eggs compared with the elongation index, with higher hatchability for the intermediate egg elongations.

This experiment shows that in the grey partridge dominance has been related to female preference: the preferred males were dominant males. The preference for dominant male was found in different animal taxa (Qvarnström & Forsgren, 1998): mammals (e.g., Cox & Le Boeuf, 1977; Horne & Ylönen, 1996), insects (e.g., Breed et al., 1980), fishes (e.g., Bisazza & Marin, 1991) and birds (e.g., Alatalo et al., 1991). Instead, other studies have proved the opposite (Ophir & Galef, 2003; 2004; Amy et al., 2008). Zahavi (1975) suggested that males should use their ornaments both to attract females and to intimidate their opponents. Borgia (1979) proposed the "war propaganda model": in lek and territorial species females should prefer the display of an owner male, an individual that honestly communicate dominance over competitor males. Indeed in many species females are known to choose the winner in contests (Trail, 1985 in cock of the rock, *Rupicola rupicola*; Graves et al., 1985 in domestic fowl, *G. gallus*). In the domestic cock the female even incite such competitive contests (Thornhill, 1988). Pizzarri & Birkhead (2000; 2002) demonstrated also a post-copulatory selection: female of domestic fowl favor sperm of dominant males than sperm of subordinate ones.

In the grey partridge, a complementary effect of female preference and male dominance on egg characteristics has been found. First, the active choice of females (and, consequently, the male dominance) affected subsequent maternal investment in terms of testosterone concentration. Significantly differences have been shown between the two experimental groups: females paired with the preferred and dominant male invested more in testosterone compared with females coupled with the male not preferred.

This result doesn't match those obtained in the study on female preference for male song by Garcia-Fernandez et al. (2010b) in the canary, but it's agree with studies on other species showing that females paired with attractive males deposit more testosterone in their eggs (reviewed in Kingma et al., 2009). Female barn swallows increased yolk androgen concentration when mated to males with experimentally elongated tails (Gil et al., 2006) or to more colourful males (Safran et al., 2008); female canaries deposited significantly more testosterone when exposed to song with attractive repertoires than when exposed to unattractive ones or maintained in acoustic isolation (Gil et al., 2004a; Tanvez et al., 2004); blue tit, *Cyanistes caeruleus,* females laid eggs with higher testosterone concentration when mated with control males than with males with an artificial reduction of the crown UV coloration (Kingma et al., 2006); zebra finches, peafowl and grey partridge increased yolk testosterone when paired with attractive males (Gil et al., 1999; Loyau et al., 2007; Garcia-Fernandez et al., 2010b). The positive effect of male rank on female investment has been found in two other birds, the starling *S. vulgaris* and the canary *S. canaria*. In the starling, females paired with dominant bigamous males laid eggs with a higher concentration of testosterone than those paired with monogamous males (Gwinner & Schwabl, 2005). In the canary, females laid eggs with more yolk (but not testosterone) when have listened a dominant male song (Garcia Fernandez et al., 2010b). To my knowledge, the effect of dominance on egg maternal investment has concerned only T and egg components (Gwinner & Schwabl, 2005; Garcia Fernandez et al., 2010b).

Previous studies have reported that breeding density (Schwabl, 1997; Reed & Vleck, 2001; Groothuis & Schwabl, 2002; Mazuc et al., 2003a; Pilz & Smith, 2004), frequency of intrusion into the territory (Whittingham & Schwabl, 2002; Navara et al., 2006d), and maternal social status (Müller et al., 2002; Tanvez et al., 2008) could influence the androgen concentration of the egg yolk (Hargitai et al., 2009). Some researches revealed several positive short and long-lasting effects of maternally derived yolk testosterone, such as increased growth rate ranging from the developing embryo (Schwabl, 1996b; Eising et al., 2001; Navara et al., 2005; Lipar & Ketterson, 2000) to the newborn chicks (Carere & Balthazart, 2007) and to adults, in a long-term perspective (Ruuskanen et al., 2012), better competitive ability and begging vigour (Schwabl, 1993; 1996b; Eising & Groothuis, 2003), enhanced development of hatching muscle (Lipar & Ketterson, 2000), higher dominance rank (Schwabl, 1993; Strasser & Schwabl, 2004), and enhanced sexual and aggressive displays and success in competition later in life (Eising et al., 2006). In some species, however, negative effects have been found, mostly related to increased metabolism, immunosuppressive effect, dangerous aggressive behaviour of chicks (Uller et al., 2005; Boncoraglio et al., 2006). In grey partridge, experimental injections of T in the egg improved growth and immune reaction in chicks (Cucco et al., 2008). This suggests that pairing with dominant males can have positive consequences for offspring, through the effect of increased egg testosterone concentration.

A theory that would be interesting to investigate (but unverifiable due to a lack of data regarding the sex of the offspring) is that illustrated in some works (Janzen et al., 1998; Bowden et al., 2000; Pike & Petrie, 2003) concerning the *sex ratio* of the hatchlings. These studies have shown that maternal yolk hormones might be involved in sex determination.

No correlation between the preference (and male dominance) and egg, albumen, yolk and eggshell mass and with lysozyme and ovotransferrin concentration have been found. This result is agree with the theory that the egg mass doesn't change due to the high repeatability of egg mass as a reproductive trait (Christians, 2002). The result of present study is in contrast with studies on the mallard, *A. platyrhynchos*, the chinese quail, *Coturnix chinensis*, the peafowl, *Pavo cristatus*, and the zebra finch, *T. guttata*, which laid larger eggs when mated with more attractive males (Cunningham & Russell, 2000; Uller et al., 2005; Gilbert et al., 2006; Loyau et al., 2007), but is in agreement with data obtained on the collared flycatcher, *F. hypoleuca*, the domesticated canary, and the house sparrow, *Passer domesticus* (Mazuc et al., 2003a; Tanvez et al., 2004; Michl et al., 2005). The same could be think for the other components (yolk, albumen and eggshell mass), that is to say that these characteristics don't change due to their high repeatability.

Another more interesting founding of the present study is the correlation between the male dominance and the hatching rate: females coupled with a dominant male laid eggs with a higher hatchability than females paired with a subordinate one. The same result has been found in a previous study on the female preference in grey partridge (Garcia-Fernandez, 2009). This fact could find a justification in what before said: a high concentration of testosterone (here found in correlation with male dominance) enhance development of hatching muscle (Lipar & Ketterson, 2000). More studies should be done to confirm the theory that male dominance may increase the hatching rate, but this first finding is an important tile of this mosaic.

In this work an influence of the laying order on different components of the egg has been also found. Variations with laying order of egg mass, egg components (shell, yolk, and albumen) and egg substances (macronutrients, hormones, vitamins) have been reported in a variety of species (Kennamer et al., 1997; Lessells et al., 2002). In the red-legged partridge, it was found an increase of egg size along the laying sequence (Cucco et al., 2011a) in agreement with Cabezas-Díaz et al. (2005), who also found differences between first-year and older breeding females (older females laid smaller eggs). In both studies, the variation was significant but very small in magnitude (a few percentage points of total egg mass). This suggests that intra-clutch variation in egg size can be related to external factors but such factors only have an influence within the confines of a large heritable component of egg size (Ojanen et al., 1979; Van Noordwijk et al., 1980). This is the reason that could justify no variation in the egg mass verified in this study.

In addition, a slight increase of albumen mass, and slight decrease of yolk mass and shell mass with laying order have been found. A decrease of shell mass along the laying order has been observed in several species. Eggshell provides both a protective enclosure for the egg contents and essential minerals for the developing embryo (Romanoff & Romanoff, 1949). The reduction in shell material suggests that females may be limited in their acquisition of sufficient dietary minerals or by the rate of mobilisation of body mineral reserves. However, it has been shown that some species do not rely on mineral reserves for clutch development (Drobney, 1980; Ankney & Alisauskas, 1991), and in present study the food was provided *ad libitum*; hence, the acquisition of mineral substances from food should not have greatly limited the females' ability to obtain minerals for forming eggs. The decrease of shell mass was significant but very small in magnitude (a few percentage points of total eggshell mass). It is unlikely that this slight variation compromised the mechanical and mineral properties of the eggs.

In the grey partridge, a progressive increase of albumen mass with laying sequence has been shown. This pattern of deposition is similar to that reported in the red-legged partridge (Cucco et al., 2011b) and it confirms a previous study on the grey partridge (Cucco et al., 2007), but, in contrast with them, it is associated with an increase of lysozyme with laying sequence. The intra-clutch pattern of lysozyme deposition varies among species. In the barn swallow *Hirundo rustica*, as well as in the two partridge species, the first eggs contain more lysozyme than the last-laid ones (Saino et al., 2002b), while in the yellow-legged gull *Larus michahellis* (a bird laying only two or three eggs), the last egg has higher lysozyme titers than the first (Bonisoli-Alquati et al., 2010). In the blue tit, lysozyme concentration does not change with laying order (D'Alba et al., 2010). The possible adaptive value of these patterns is still an open question, which deserves more studies and experimental designs. Probably, this variation also in the same species in different experiments could be related to external environmental factors and individual differences.

The negative relationship between position in the laying order and yolk mass is in line with the theory of a natural depletion of the mother resources with the progress of deposition: females have limited resources and the decline could simply reflects exhaustion of maternal resources.

Finally, a correlation between hatchability of the eggs and the elongation index has also been recognized. In present study, eggs with higher or lower elongation hatched at a lower rate. This result is in accord with the findings in the turkey (*Meleagris gallopavo*), where hatchability was highest in intermediate egg shape elongation (Erisir & Ozbey, 2005) and with another study on grey partridge (Cucco et al., 2012). Also a study on japanese quail (*C. japonica*) showed the same thing (Baspinar et al., 1997).

In sum, it is conceivable that female grey partridges allocate egg resources according to DAH and that female choice coincides for the most extent to the preference of a dominant male.

Further research is needed to clarify if this preference for a dominant male influence also the post-hatching survival of the offspring. Moreover, it would be interesting to see if the offspring of dominant males is dominant in turn and if the testosterone concentration influences or not the *sex ratio* of the hatchlings.

Chapter II: What determines the preference of a canary female faced to a male-male physical interaction? And how does an eventually preference influence egg allocation?

Chapter II – Abstract

The aim of the study presented in this chapter is to understand if there is a preference for a dominant male in female canary and if it influences maternal investment.

In several articles on communication networks has been shown as females use public information (Valone, 1989), such as the interaction between different males, to evaluate the quality of them and to determine its choice (e.g., Doutrelant & McGregor, 2001; Leboucher & Pallot, 2004) or to set its extrapair copulatory behaviours (e.g., Otter et al., 1999; Mennill et al., 2002).

The types of interaction between males from which females can obtain information on their quality are mainly three: competition based on visual characteristics; singing competition and physical competition. This work, in particular, has been focused on the study of the third type of interaction.

An experiment has been carried out in which a female assisted to a competition between two males for access to food. After these competitions the female choices were evaluated and eggs were collected to provide the laboratory analyses of its key components and of certain molecules in the albumen and in the yolk.

In this study it has been discovered as the female performed its choice based on a male behavior: the females spent more time (index of choice) in front of males that had attacked for several times the other contender for first.

The coupling with the chosen male had led to different results: the eggs produced by the group of females mated with the preferred male laid heavier eggs, with a heavier shell, an elongation index higher and with a higher concentration of lysozyme.

The greater weight of the eggs produced mating with the preferred male is in agreement with previous studies (Cunningham & Russell, 2000; Uller et al., 2005; Gilbert et al., 2006; Loyau et al., 2007) and it is considered an index of good quality of the eggs (Magrath, 1992; Perrins, 1996; Blomqvist et al., 1997; Starck & Ricklefs, 1998; Styrsky et al., 1999; Christians, 2002; Silva et al., 2008; Forbes & Wiebe, 2010).

Instead, lysozyme is considered an important antimicrobial agent and its greater concentration in the eggs produced by females mated with more aggressive males may be an attempt to counteract the negative effects on health made from high concentrations of testosterone.

In this study no statistically significant difference between the two groups with regard to the concentration of testosterone has been found; other previous

studies have shown, however, that the sons of aggressive males were aggressive in turn and had a larger amount of testosterone. The highest concentration of lysozyme could find explanation in this fact.

In conclusion, females mated with the preferred male (resulting by viewing physical interactions between males) laid eggs of best quality in terms of total mass of the egg and concentration of lysozyme.

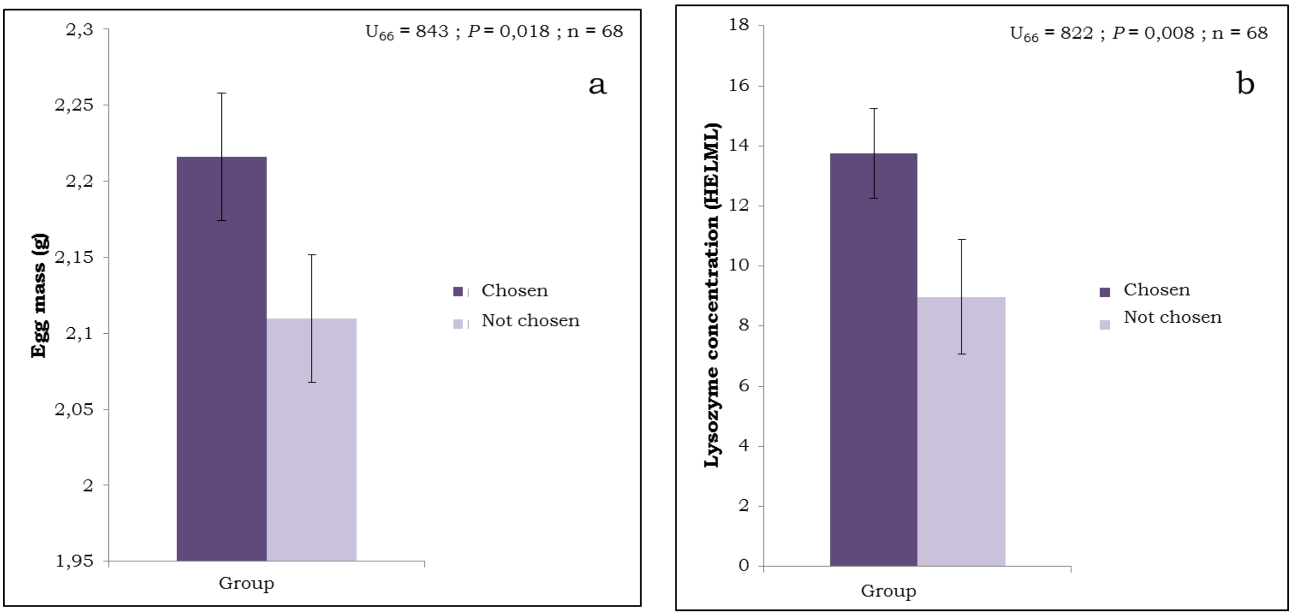


Figure 20 - a) Histogram that correlates the total mass of the eggs and the experimental groups; b) histogram that correlates lysozyme concentration and the experimental groups.

Key-words: Male-male physic interactions, female choice, maternal effects, lysozyme concentration, egg quality.

Capitolo I – Riassunto

Lo scopo dello studio presentato in questo capitolo è di comprendere se esiste una preferenza per un maschio dominante nelle femmine di canarino e se questa influenza l'investimento materno.

In diversi articoli sulle reti di comunicazione è stato visto come le femmine utilizzino informazioni pubbliche (Valone, 1989), come ad esempio l'interazione fra diversi maschi, per valutare la qualità di essi e determinare così la propria scelta (es., Doutrelant & McGregor, 2001; Leboucher & Pallot, 2004) o per impostare i propri comportamenti copulatori al di fuori della coppia (es., Otter et al., 1999; Mennill et al., 2002).

I tipi di interazione fra individui di sesso maschile dai quali si possono ricavare informazioni sulla loro qualità sono sostanzialmente tre: competizione basata su caratteristiche visuali; competizione canora e competizione fisica. Questo lavoro, in particolare, si è concentrato sullo studio del terzo tipo di interazione.

Per fare questo è stato predisposto un esperimento in cui viene mostrata ad una femmina la competizione fra due maschi per l'accesso al cibo. In seguito a questi scontri si è valutata la scelta attuata dalle femmine e si sono raccolte le uova per svolgere delle analisi di laboratorio sulle sue componenti principali e su alcune molecole presenti nell'albume e nel tuorlo.

In questo studio si è scoperto come la femmina svolga la propria scelta in base ad un comportamento del maschio: le femmine utilizzate hanno infatti passato più tempo (indice di scelta) davanti a maschi che per più volte avevano attaccato per primi l'altro.

L'accoppiamento con il maschio prescelto ha portato a diversi risultati: le uova prodotte dal gruppo di femmine accoppiate con il maschio preferito hanno infatti deposto uova più massive, con un guscio più pesante, con un indice di *elongation* più elevato e con una maggior concentrazione di lisozima.

Il maggior peso delle uova prodotte accoppiandosi con il maschio preferito è in accordo con studi precedenti (Cunningham & Russell 2000; Uller et al., 2005; Gilbert et al., 2006; Loyau et al., 2007) ed è ritenuto un indice di buona qualità delle uova (Magrath, 1992; Perrins, 1996; Blomqvist et al., 1997; Starck & Ricklefs, 1998; Styrsky et al., 1999; Christians, 2002; Silva et al., 2008; Forbes & Wiebe, 2010).

Il lisozima è invece considerato un importante agente dell'azione antimicrobica e la sua maggior concentrazione nelle uova prodotte da femmine accoppiate con maschi più aggressivi potrebbe essere un tentativo di contrastare gli effetti negativi sulla salute dati da alte concentrazioni di testosterone.

In questo studio non è stata trovata una differenza statisticamente significativa fra i due gruppi per quanto riguarda la concentrazione di testosterone; altri studi precedenti hanno però dimostrato che i figli di maschi aggressivi lo sono a loro volta ed hanno una quantità maggiore di testosterone. La concentrazione più alta di lisozima potrebbe trovare spiegazione in questo fatto.

In conclusione, femmine accoppiate con il maschio risultato preferito dopo aver visualizzato interazioni di tipo fisico con altri depongono uova di miglior qualità in termine di massa totale dell'uovo e di concentrazione di lisozima.

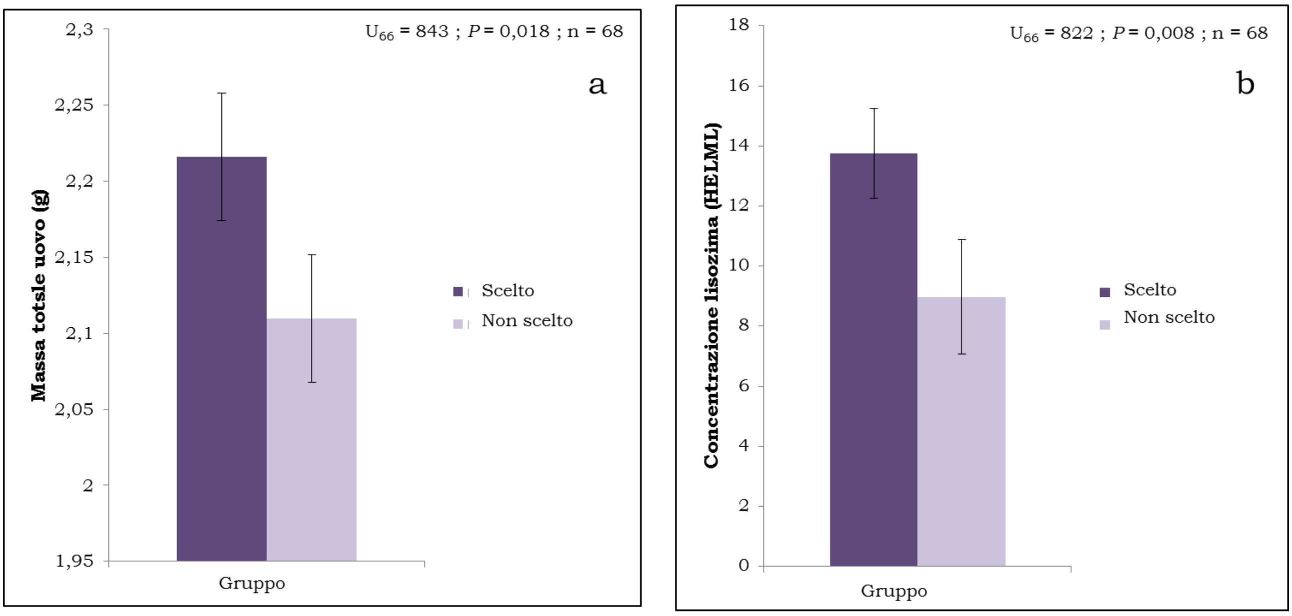


Figure 21 - a) Istogramma che mette in relazione la massa totale delle uova ed il gruppo sperimentale; b) istogramma che mette in relazione la concentrazione di lisozima ed il gruppo sperimentale.

Parole-chiave: Interazioni fisiche maschio-maschio, scelta della femmina, effetti materni, concentrazione di lisozima, qualità dell'uovo.

Chapitre II – Résumé

Le but de l'étude présentée dans ce chapitre a été de comprendre s'il y a une préférence pour un mâle dominant chez les femelles de canari et si ce facteur influence l'investissement maternel.

Dans plusieurs articles sur les réseaux de communication a été considéré comme les femelles utilisent les informations publiques (Valone, 1989), telles que l'interaction entre différents mâles, afin d'évaluer la qualité et déterminer ainsi ses choix (par exemple, Doutrelant & McGregor, 2001 ; Leboucher & Pallot, 2004) ou pour régler ses comportements de copulation à l'extérieur de la paire (par exemple, Otter et al., 1999 ; Mennill et al., 2002).

Les types d'interaction entre les mâles à partir de lesquelles on peut obtenir des informations sur leur qualité sont essentiellement trois : concurrence fondée sur les caractéristiques visuelles; concurrence avec le chant et concurrence physique. Ce travail, en particulier, a mis l'accent sur l'étude du troisième type d'interaction.

Pour faire ça, une expérience a été mis en place où un compétition entre deux mâles pour l'accès à la nourriture a été montré à une femelle. Après ces affrontements, la choix des femelles a été évaluée et les œufs ont été prélevés pour effectuer les analyses en laboratoire des principaux éléments et des certaines molécules dans l'albumen et dans le jaune.

Dans cette étude, on a découvert que la femelle effectue son choix en se basant sur un comportement particulier du mâle : les femelles utilisées ont passé plus de temps (indice de choix) devant les mâles qui avaient attaqué à plusieurs reprises l'autre pour premier.

Le couplage avec le mâle choisi a conduit à des résultats différents : les œufs produits par le groupe de femelles accouplées avec le mâle préféré ont pondu œufs plus lourds, dont la coquille était plus lourde, avec un indice d'allongement plus élevé et avec une concentration plus élevée du lysozyme.

Le poids plus grande des œufs produits par femelles couplées avec le mâle préféré est en accord avec des études précédentes (Cunningham & Russell, 2000 ; Uller et al., 2005 ; Gilbert et al., 2006 ; Loyau et al, 2007) et il est considéré comme un indice de bonne qualité des œufs (Magrath, 1992 ; Perrins, 1996 ; Blomqvist et al., 1997 ; Starck & Ricklefs, 1998 ; Styrsky et al., 1999 ; Christians, 2002 ; Silva et al., 2008 ; Forbes & Wiebe, 2010).

Le lysozyme est plutôt considéré comme un agent antimicrobien importante et sa plus grande concentration dans les œufs produits par les femelles accouplées avec des mâles les plus agressifs peut être une tentative pour contrer les effets négatifs sur la santé des concentrations élevées de testostérone.

Dans cette étude des différences statistiquement significatives n'ont pas été trouvé entre les deux groupes en ce qui concerne la concentration de testostérone ; autres études antérieures ont montré, cependant, que les fils des mâles agressifs sont agressifs eux-mêmes et ont une plus grande quantité de testostérone. La concentration plus élevée du lysozyme pourrait trouver une explication dans ce fait.

En conclusion, les femelles accouplées avec des mâles préféré après avoir visionné des interactions de type physique avec des autres pondent des œufs de meilleure qualité en termes de masse totale de l'œuf et de la concentration de lysozyme.

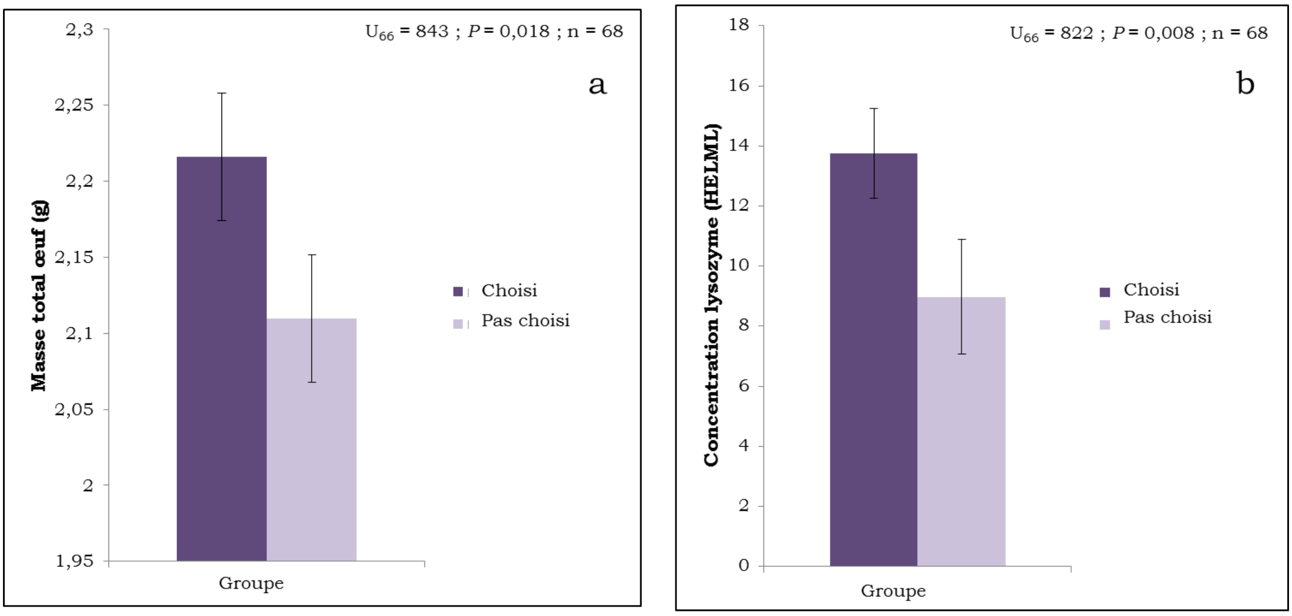


Figure 22 - a) Histogramme qui corrèle la masse de l'œuf et le groupe expérimental. b) histogramme qui corrèle la concentration de lysozyme et le groupe expérimental.

Mots clés : Interactions physiques mâle-mâle, choix de la femelle, effets maternels, concentration de lysozyme, qualité de l' œuf.

~

Introduction

~

In species living in group, animals can often be ordered according to a dominance hierarchy that is based on the proportion of conflicts that an individual wins against each group member and/or on the number of submissive displays he/she receives (e.g. Drews, 1993; Ellis, 1995). Dominance hierarchies can vary significantly between or within species depending on how consistently one animal wins fights with, or receives submission from another individual across different contexts and over time (e.g., Thierry, 2000; Silk, 2007). A number of factors (Ellis, 1995; Silk, 2007; Clutton-Brock, 2009) such as habitat characteristics (e.g. food abundance), social system, group size and socionomic *sex ratio* affected such variation in the steepness of the dominance hierarchy. Animals fight to obtain a dominant position within the group, so dominant individuals are expected to gain various benefits from this fact. However, there is contrasting evidence in the literature on the possibility that dominant male gains fitness advantage over subordinate ones; for example, not always the female chooses the dominant one (e.g., Ophir & Galef, 2003). This is probably due, at least for a part, to the difficulty of obtaining reliable measures of fitness because, for example, we need long-term studies to obtain data on lifetime reproductive success in species with a long lifespan. Moreover, dominance may provide benefits (e.g., increased feeding efficiency) that do not always, or not automatically, translate into fitness benefits such as a greater number of offspring (Majolo et al., 2012). Various meta-analyses on the effect of dominance in different taxa have been recently conducted, providing overall support for the benefits of dominance in terms of reduced stress and associated diseases and of increased mating and reproductive success (Fiske et al., 1998; Brown & Silk, 2002; West & Sheldon, 2002; Abbott et al., 2003; Schino, 2004; Sheldon & West, 2004; Silk et al., 2005; Kaplan et al., 2009; Rodriguez-Llanes et al., 2009).

The dominance doesn't affect only reproductive success, but it can also modulate the *sex ratio* of the offspring. Trivers & Willard (1973) hypothesised that, if maternal physical condition differentially affects the reproductive success of male and female offspring, mothers should invest more in the sex that provides the highest fitness return. This is usually named the “Trivers-Willard effect”. Among mammals, females in good physical conditions (that can provide the best parental care) are generally expected to produce more male, cause they usually have a larger variance in reproductive success (Clutton-Brock, 1990). However, in female phylopatric species the reverse pattern can be predicted if females in better condition or higher in dominance rank can provide better protection from competition for their phylopatric daughter than for their emigrating sons, or if daughters inherit their mother's rank (Silk, 1983).

In a meta-analysis on ungulate mammals, Sheldon & West (2004) provided overall evidence for the “Trivers-Willard effect”; in contrast, three meta-analytical studies conducted so far on primates (Brown & Silk, 2002; Schino, 2004; Silk et al., 2005) didn't find conclusive support for an effect of dominance on birth *sex ratio*. The lack of overall support for the "Trivers-Willard hypothesis" in primates may derive from the influence of modulating variables or from the presence of contrasting selection pressures (van Schaik & Hrdy, 1991; Schino, 2004). In fact, a meta-analysis by West & Sheldon (2002) showed how the possibility for parents to predict the environmental conditions encountered by their offspring constraints their ability to adaptively manipulate offspring *sex ratio* (Majolo et al., 2012).

In a series of papers on communication networks, it was revealed that females use public information (Valone, 1989) as male-male interactions to assess the relative quality of males and to determine mate choice (e.g., Doutrelant & McGregor, 2000; McGregor & Peake, 2000; Leboucher & Pallot, 2004) or to direct their extrapair copulatory behaviour (e.g., Otter et al., 1999; Mennill et al., 2002). This process of gathering information seeing the interactions of others individuals is called eavesdropping (McGregor & Dabelsteen, 1996) and it allows females to assess directly the relative quality of the two interacting males, as well as it permit to an individual to evaluate potential opponents in future aggressive encounters (e.g., Naguib & Todt, 1997). In theory, this operation is very useful because females can potentially identify mates of higher quality faster than by sequential sampling, reducing time, energy and other costs associated with mate searching (Otter & Ratcliffe, 2005).

The studies on the use of male-male interaction can be analyzed to evaluate relative male quality in light of the specific type of interaction; so we can have:

1) Male-male visual displays: for example, female fish obtain information about relative male quality from these types of interaction (Doutrelant & McGregor, 2000);

2) Male-male singing interactions: for birds especially, studies have shown that females assess male quality mainly from discrimination between the song of the different competitor (e.g., Mennill et al., 2002; Leboucher & Pallot, 2004);

3) Male-male physical interactions: still in birds, there is little evidence that female birds measure male quality by observing physical interactions between two or more individuals. Good example is the japanese quail, *C. japonica*, where females use previously observed aggressive interactions between two males to determine their sexual preferences (Ophir & Galef, 2003; Ophir et al., 2005).

Several previous studies have focused on the relation between male dominance and female sexual selection. Preference for dominant males (Qvarnström &

Forsgren, 1998) has been found in different taxa: mammals (Cox & Le Boeuf, 1977; Horne & Ylönen, 1996), insects (Breed et al., 1980), fishes (Bisazza & Marin, 1991), and birds (Alatalo et al., 1991), although other studies prove just the reverse (Amy et al., 2008; Forsgren, 1997; Ophir & Galef, 2003; 2004; Ophir et al., 2005).

Studies mentioned above showed that it is more difficult to make predictions about the influence of visually observing male-male contests on female preferences. Threat displays used during agonistic interactions give to the eavesdropper information about aggressive motivation (Hurd & Enquist, 2001) and could be used to assess dominance (Bayly et al., 2006). However, is universally established that females doesn't use threat displays to assess male status even if it is commonly admitted that winners of male-male competition are of superior quality and that females would benefit by mating with them (Berglund et al., 1996). As seen above, some studies reveal that females do not always prefer dominant males, especially when the cost of mating with potentially harmful males could be greater than the expected benefit (Qvarnström & Forsgren, 1998). For example, studies of Ophir and collaborator on japanese quail indicate that females that observe physical aggressive interactions between males prefer to pair with the subordinate male to avoid "contact with males more likely to engage in potentially injurious behaviour when courting and mating" (Ophir & Galef, 2003), even if this means losing any potential direct or indirect benefits of affiliating with a dominant male (Ophir & Galef, 2003; Ophir et al., 2005). Furthermore, different studies indicate that males may develop coercive behaviours against mates (Clutton-Brock & Parker, 1995; Valera et al., 2003) and this tendency is clearly expected to be more developed in aggressive males (Amy et al., 2008).

For what concerns male dominance correlates with the parental cares, only few studies have been performed (Bisazza et al., 1989; Forsgren, 1997) and, to my knowledge, anyone pertain to birds, except for that lead by Garcia-Fernandez et al. (2010b), where was investigated the relation between the male dominance established by singing male-male interaction and the allocation of some components into the eggs. For this fact the present study has concentrated on possible relationships between male dominance and maternal effects in the common canary (*S. canaria*).

Maternal effects represent a particular environmental source of phenotypic variance in early-life traits (Mousseau & Fox, 1998); indeed, they influence birth mass, birth date, and natal litter size in many mammalian species (Wilson et al., 2005). Oviparous animals show a specific early-maternal effect in the form of substances that accumulate in the egg. Apart from the macronutrients (proteins and lipids) necessary for growth, micronutrients and molecules such as hormones, vitamins, and carotenoids play an important role in the physiological and behavioural maturation of individuals (Minvielle & Oguz, 2002; Royle et al., 2003; Williams, 2005). Proteins linked to innate immunity (immunoglobulin, lysozyme, ovotransferrin, etc.) are other substances transferred from mother to egg that influence the future prospects of chick survival (Saino et al., 2002c; D'Alba et al., 2010). With components insert by the female into the eggs researchers generally refer to the differential allocation theory (Burley, 1986; Sheldon, 2000). Several studies have shown that female birds can allocate their resources to eggs differentially according to the quality of their partner (e.g., Burley, 1988; Petrie & Williams, 1993; Sheldon, 2000). A differential allocation has been shown for several characteristics as the number of eggs laid, their size and the concentration of the hormones or other components deposited (Garcia-Fernandez et al., 2010b). So, features of the mother's environment may be translated into the differential deposition of several substances in eggs, such as corticosterone (Pike & Petrie, 2005; Rubolini et al., 2005), androgens (Schwabl, 1993; Gil et al., 1999), carotenoids (Blount et al., 2002; Williamson et al., 2006) and lysozymes (important albumen antimicrobial components; Saino et al., 2002c; Cucco et al., 2007; Garcia-Fernandez et al., 2010a).

The canary is a highly amenable model system for studies across a wide range of behavioural, genetic and developmental questions (e.g., Leboucher & Pallot, 2004; Amy et al., 2008; Garcia-Fernandez et al., 2010b), and it is well suited to studies of maternal effects because it's willingness to breed in laboratory conditions under intensive scrutiny (e.g., Tanvez et al., 2004; Garcia-Fernandez et al., 2010b). So, there are several studies analysing a range of maternally derived resources and their consequences to offspring and even grand-offspring (Leboucher et al., 2012).

Aim of this experiment is to investigate 1) which male female domestic canary, *S. canaria*, choses when she can use information gathered from a visual male-male interaction and 2) if she invests differently in eggs for what concerns egg mass, macro- (yolk, albumen and shell) and micro-components (signally lysozyme and testosterone) when paired with the chosen male or not.

~

Materials and Methods

~

Experimental groups

The experiment was performed under proper legislation by the French law.

28 female and 28 male canaries (*S. canaria*) randomly selected from the breeding stock of the LECC (Laboratoire d'Ethologie et Cognition Comparées, Université Paris Ouest, Nanterre-La Défense, France) were studied. All individual already had a reproduction experience at least.

At first, all animals were housed in batteries (120 cm length x 50 cm width x 50 cm height) in rooms under controlled conditions (22 ± 1 ° C) with a maximum of 6 individuals per battery. All animals begun the experience in short photoperiod (light/dark cycle: 8/16h). Successively, the conditions were switched to long photoperiod (light/dark cycle: 15/9h).

Females were housed individually in small cages (40 cm length x 30 cm width x 24 cm height). Food (classical mix of seed, Beyers®, containing all necessary nutrients for breeding females, and an egg-based mix, Cédé®) and water were provided *ad libitum*. Each cage was provided with a piece of cuttlebone for the calcium, a nest bowl and nesting material (cotton string). The nesting material was renewed every day (see "Materials and Methods: Common Aspects" for more details). Females had the first cycle of reproduction, replacing real eggs with plastic ones. This first cycle was utilized to synchronize reproductive cycles of females. 7 days after that the last egg was laid, the nest was removed (this procedure marked the beginning of the second reproductive cycle). So, the out-and-out experiment started.

Pre-test

For the pre-test, a certain number of batteries of 6 males have been prepared; these males have been kept for two hours without food in order to increase the stimulus to competition. After this time, some food with a quarter of apple (which had not been given in earlier days to make it more appetizing to the contenders) were placed in the cage, and male behaviours were observed for a quarter of an hour. Threats and attacks, succeeded and failed, and time spent on the feeder have been assessed.

At the end of the pre-test, the collected data have been analyzed in order to obtain a ranking for each battery.

Test

In regard to the results of pre-test, from each battery 3 couples of males was formed in the following way: the first male was paired with the fourth, the second with the fifth and the third with the sixth. This procedure was utilized to obtain couple of males with a good difference in competitive behaviour in a physic contest.

Regarding the effective test, the day before its execution, all the content was removed from female cage (including the nest) and two feeders and two water containers were placed inside, arranged symmetrically (so to avoid possible errors due to the disposition of objects). Three perches were also placed, one on the right, one in the middle and one on the left of the cage.

The experiment day, two hours before the beginning, two males were taken from a battery and placed inside a cage, which presented a transparent separation in half and which contained only two water containers, one in each partition. Individuals have been chosen in the way above descripted. After an hour and a half, the cage with the males and the one with the female were transported for conditioning into the room used for the test, where there was a two-way mirror, that allowed to see the behaviour of canaries without being seen; the cage of males was covered with a large partition (G), to avoid that female was aware of their presence. At the end of the two hours, the separation G and the transparent one of the cage of males were removed and a feeder was positioned inside the cage; for a quarter of an hour, the competition between the two males was shown to the female and threats and attacks, succeeded and failed, and time spent by the two contenders to feed themselves were noted (session of clash).

At the end of this time, an opaque partition (M) was positioned inside the cage of males, thus separating the two, and the female was allowed to see them for five minutes. At the end, a small opaque separation (P) was put between the cage of the female and that of the males; this had the task of allowing the female to see only a male, if was standing on right or left perch, and both the males, if was standing on the middle one. At this point, for fifteen minutes, time elapsed from the female in the three zones of the cage was noted (session of choice).

At the end, the partition P was removed and the partition G was placed to prevent the female to see the two males. After this, the first female was carried out of the room and the second was positioned to repeat the same procedure with the same males.

All part of the experience was recorded with a camera Sony DSC W50 (Sony®, Berlin, Germany) placed to 1 meter from the cage to allow to reanalyze the experiments and individual behaviour (both males and females).

So, each pair of males participated in two tests of this kind with two different females. At the end, each female was paired randomly with one of the competitors and they were put in the cage of the female (40 cm length x 30 cm width x 24 cm height) to start the mating period.

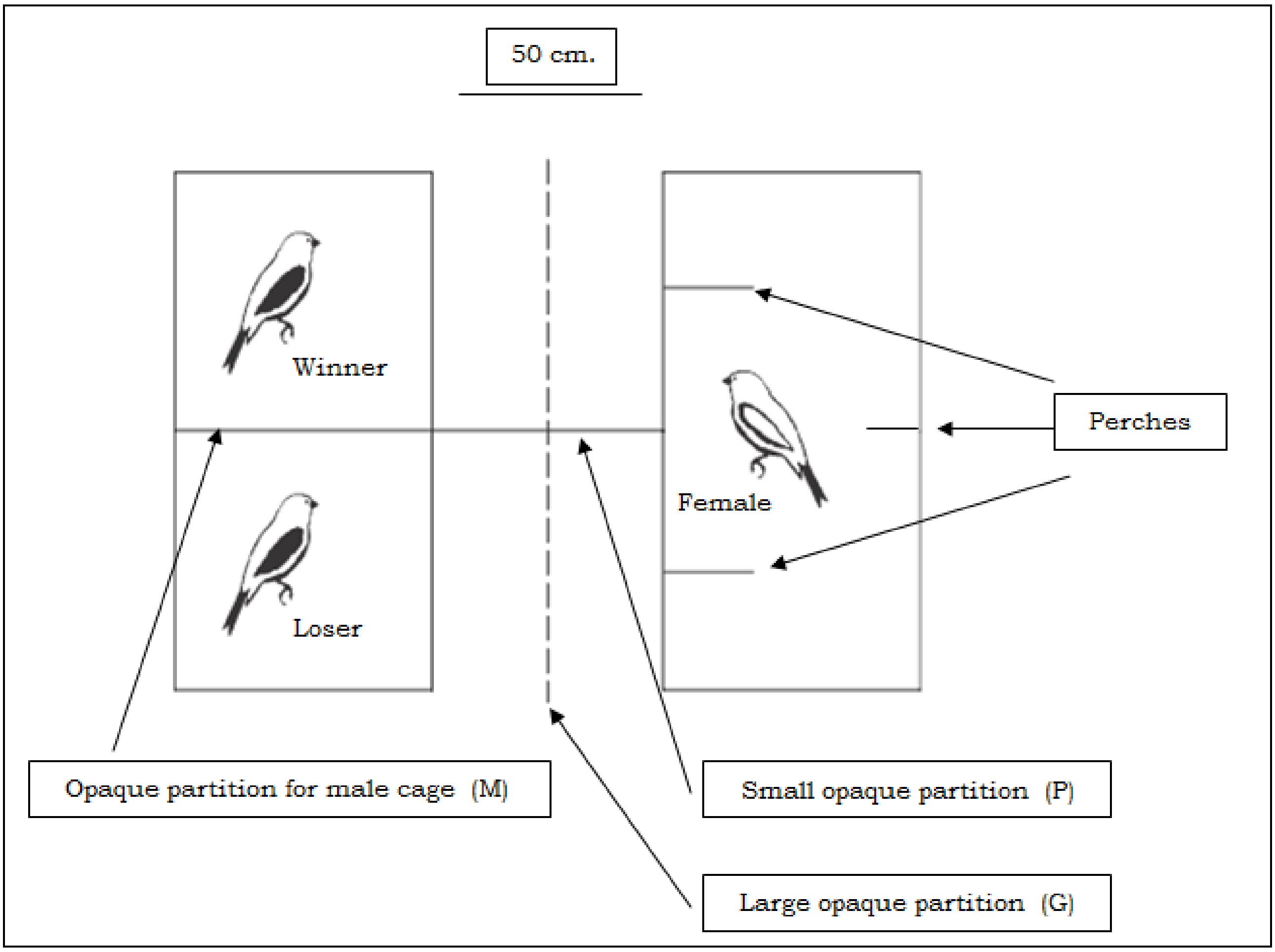


Figure 23 - Scheme of cage arrangement during the test.

Ethical note

To compensate for the 2h of food deprivation before each competition, males were provided with supplementary food after each experiment (breeding food: bakery products, egg and egg products, sugars, seeds, vegetable protein extracts, minerals, vitamins). Two previous experiments (Amy & Leboucher, 2007; Amy et al., 2008) showed that this duration was sufficient to increase motivation to interact with unfamiliar males without leading to intense levels of competition (shorter periods led to little interest in food items and no competition). To avoid needless injuries, the interactions were monitored at all times. Threat displays represent more than 90% of attacks (Amy & Leboucher, 2007). Agonistic interactions never resulted in any visible injury. Experimental authorization was delivered by the French Ministry for Agriculture and Fisheries.

Collection and separation of eggs

When females started laying, all pair was inspected daily to collect eggs, which were replaced with plastic ones. All the eggs were marked by means of a sheet, placed near them, containing an identification code (e.g,. DOREE76/N2, where DOREE76 indicates the identification ring of the female and N2 the number of the egg within the brood) and the laying date, and were photographed on a graph paper by a Nikon Coolpix 990 camera mounted on a tripod, to take standard photos (these photos allows to measure the length L and the breadth B of the eggs and to calculate the elongation index, L/B).

All eggs were immediately separated in the laboratory: weights of the whole egg, and then of shell, yolk and albumen, were taken using an electronic balance (± 0.001 g). Albumen and yolk have been collected in appropriate marked eppendorf, which have been placed in a freezer at -20°C for subsequent biochemical analyses. The first was not treated, while the second was homogenized with an equal amount (1 ml per gram of egg yolk) of a solution of NaCl 0.9% (wt/v). The thickness of the shell was measured in three different points using a precision gauge (Alpa S.p.A., Milan, Italy; precision ± 0.01 mm); the final thickness was the mean of the three measures.

Testosterone assay

The testosterone concentration in the egg yolk was estimated by radioimmunoassay at CEBC (Centre d'Études Biologiques de Chizé, CNRS-UPR1934, Villiersen-Bois, France).

In general, this protocol is based on a simple principle: the hormone to dose (hormone cold H) was in competition with the same marked hormone (hormone hot or tracker H*) for an H-specific antibody whose concentration was limiting (failing antibody dosage). After incubation at equilibrium, a bound fraction (H-Ac and H*-Ac) and a free fraction (H and H*) were obtained. Immune complexes were then separated from the free hormone and the radioactivity is counted.

For the exact protocol see the "Materials and Methods: Common Aspects" above.

Lysozyme Assay

Lysozyme activity was measured using a classical antibiogram method because the Osserman & Lawlor protocol (1966) has proved to be not sensitive enough.

Brief, the diameters of the cleared zones (inhibition zones), that the samples formed around the site of inoculation into a Petri plate containing *M. lysodeiktikus,* was an indicator of the lysozyme concentration. These diameters were proportional to the logarithm of lysozyme concentration and, compared with the standard curve, allowed to obtain values in hen egg lysozyme equivalents (HELML equivalents, expressed in µg/ml).

For the exact protocol see the "Materials and Methods: Common Aspects – Lysozyme: classical antibiogram protocol" above.

Statistical analysis

Female choice and male behaviour

First, a female have been removed from the analysis cause she has never chose a male (n = 27), so an index of female preference have been created:

$$\Delta t = t_{favourite\ male} - t_{not\ favourite\ male}$$

Legend:

Δt: female preference index;

$t_{favourite\ male}$: time spent by the female in front of the favourite male;

$t_{not\ favourite\ male}$: time spent by the female in front of the not favourite male.

Then, the same indexes have been calculated for all the variable measured during the male-male physic interaction:

$$\Delta time\ on\ food = time\ on\ food_{favourite\ male} - time\ on\ food_{not\ favourite\ male}$$

$$\Delta access\ food\ n. = access\ \ food\ n._{favourite\ male} - access\ food\ n._{not\ favourite\ male}$$

$$\Delta initiated\ interaction\ n. = initiated\ interaction\ n._{favourite\ male} - initiated\ interaction\ n._{not\ favourite\ male}$$

$$\Delta won\ interaction\ n. = won\ interaction\ n._{favourite\ male} - won\ interaction\ n._{not\ favourite\ male}$$

$$\Delta lost\ interaction\ n. = lost\ interaction\ n._{favourite\ male} - lost\ interaction\ n._{not\ favourite\ male}$$

$$\Delta number\ lost\ interactions\ = number\ lost\ interactions_{male\ prefere} - number\ lost\ interactions_{non\ prefere}$$

Data were normal and not repeated, hence a General Linear Model (GLM) have been utilized with the following variables as input:

- Dependent variable:
    - *Δt*;
- Explicative variables:
    - *Δtime on food*;
    - *Δaccess food n.*;
    - *Δinitiated interaction n.*;
    - *Δwon interaction n.*;
    - *Δlost interaction n.*

Female choice and maternal investment

First, from the analysis have been removed:

- 9 female, because they have never laid an egg (n = 19);
- Eggs with missing data (n = 68).

For the analyses the females have been separated in two groups:

- Females paired with male that they have chosen (female n = 11 and egg n = 38);
- Females paired with male that they haven't chosen (female n = 8 and egg n = 30).

For what concerns the normal variables:

- Shell weight;
- Albumen weight;
- Yolk weight;
- Testosterone concentration;
- Elongation

t-tests have been utilized.

Instead, for the normal variables:

- Egg number laid by every female;
- Egg weight;
- Shell thickness;
- Lysozyme concentration

Mann-Whitney tests have been performed.

For all the variables, results were presented like this: mean ± SE.

Statistical analyses were performed using SYSTAT 12 (SYSTAT Software Inc., San José, CA).

~

Results

~

Female choice and male behaviour

Variable explicative	**R^2 adjusted**	**$F_{(26, 26)}$**	***P***
Time on food	-0.17	-0.91	0.37
Number of access to food	20.4	1.43	0.17
Interactions started	17.3	2.33	0.028
Interactions won	5.22	0.51	0.95
Interactions lost	29.4	0.29	0.76

Table 4 - Correlation between male behaviour and female preference.

Table 4 contains the behaviour of males (when they had a competition for the food with another male) correlated with the preference of the female.

The analyses have shown that the preference of the female depended on the interactions started: more a male started interactions respect to a competitor and more time the female spent facing this male (see Table 4).

Female choice and maternal investment

	U	P
Preference		
Eggs number	82	0,89
Egg mass	843	**0,018**
Eggshell thickness	917	0.14
Lysozyme concentration	822	**0.008**

Table 5 - Mann-Whitney tests of preference related to egg number, egg mass, eggshell thickness, and lysozyme concentration and elongation index.

	t	P
Preference		
Albumen mass	0,66	0,50
Yolk mass	1,49	0,14
Eggshell mass	2,45	**0.017**
Testosterone concentration	-0.84	0.40
Elongation	3.33	**0.001**

Table 6 - t-test analysis of preference related to albumen, yolk and eggshell mass, testosterone concentration and elongation index.

Table 5 and Table 6 summarize the results of Mann-Whitney tests and t-tests performed on the variables.

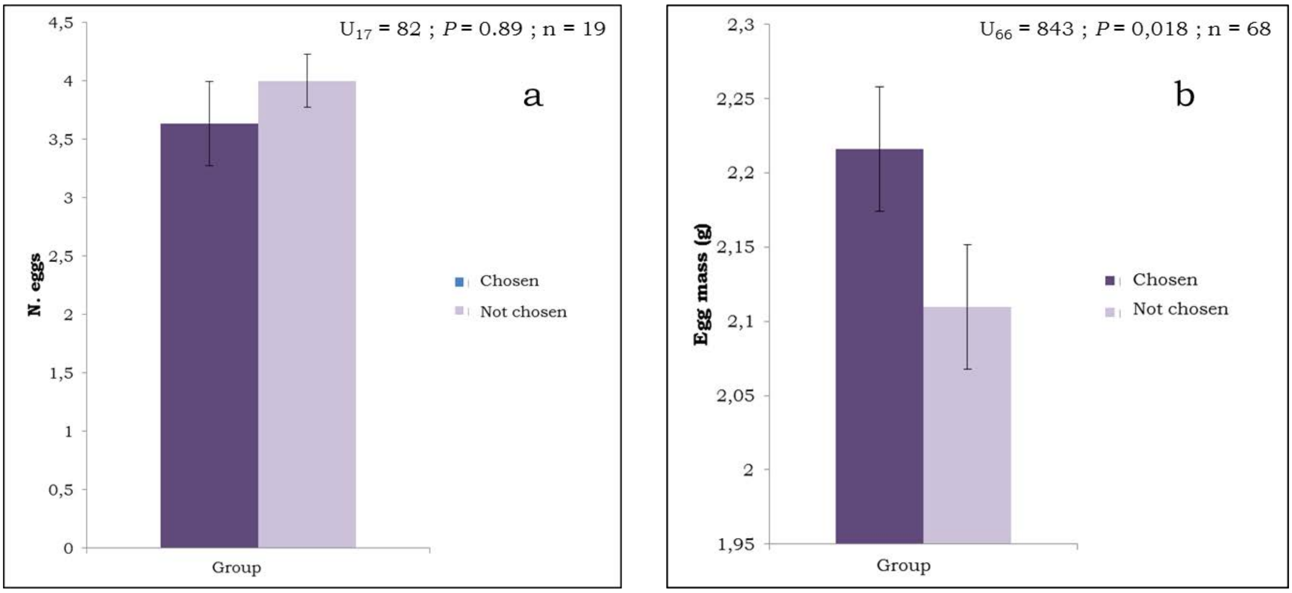


Figure 24 - Histogram a: number of eggs relating to two groups: females paired with the chosen male and female coupled with the not chosen male. Histogram b: egg mass relating to two groups. In all graphs the results of the statistics are inserted.

In two histograms of Figure 24 it's shown that there was no statistically significant difference between the two female groups (females that chose the male and females that haven't chosen him) in number of eggs laid, but a difference in egg mass was found. In particular:

- Number of eggs (Figure 24a and Table 5): chosen 3.7 ± 0.3 eggs, not chosen 4.0 ± 0.2 eggs; p-value = 0.89;
- Egg mass (Figure 24b and Table 5): chosen 2.22 ± 0.05 g, not chosen 2.12 ± 0.04 g; p-value = 0.018.

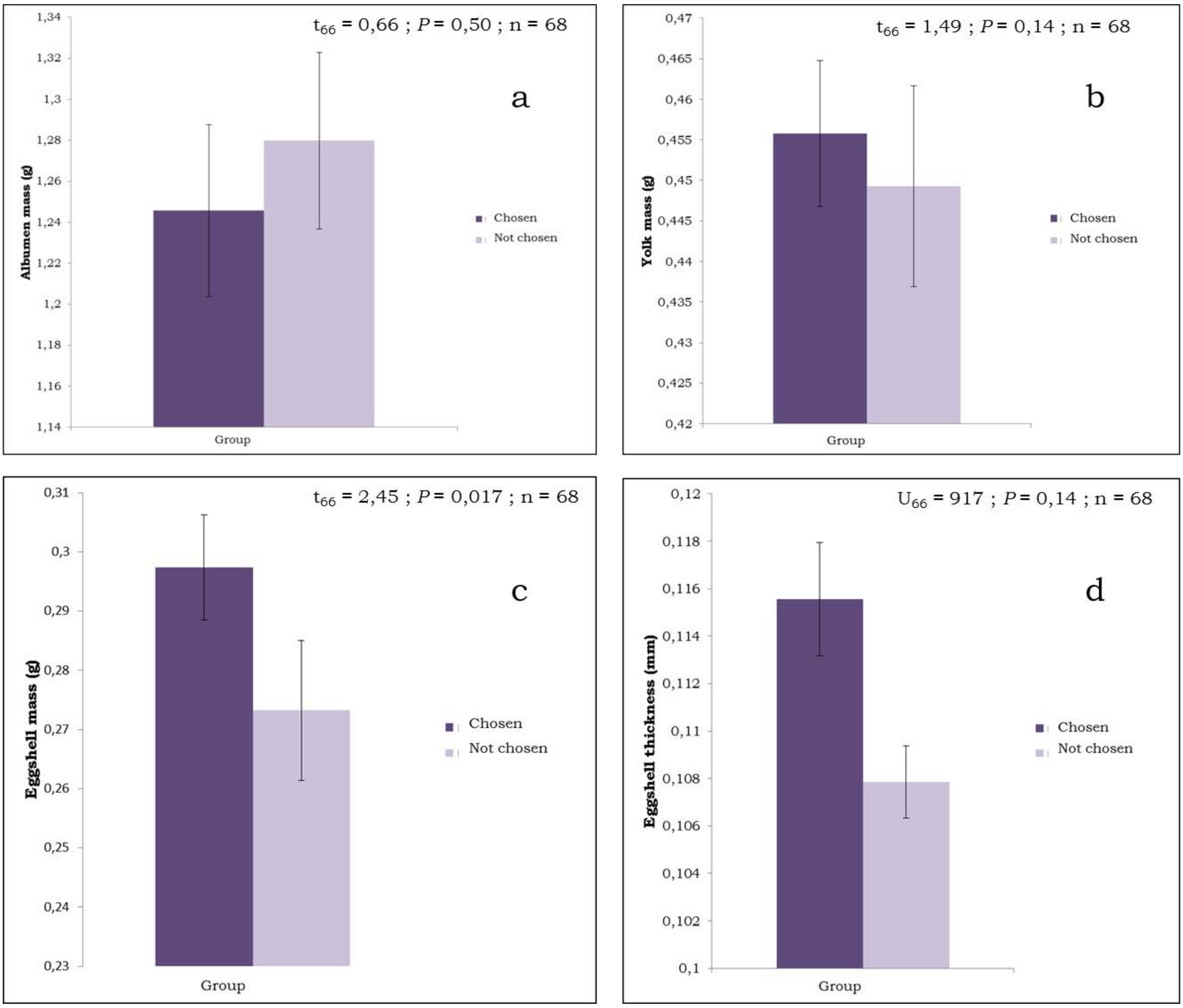


Figure 25 - Histogram a: albumen mass in two groups. Histogram b: yolk mass in two groups. Histogram c: eggshell mass in two groups. Histogram d: eggshell thickness in two groups. In all graphs the results of the statistics are inserted.

Figure 25 summarizes the trend of the macro-elements that compose the eggs in relation to the two groups examined. A statistically significant difference was found only in the eggshell mass: females that have chosen the partner laid eggs with more eggshell than the others.

In detail:

- Albumen mass (Figure 25a and Table 6): chosen 1.25 ± 0.04 g, not chosen 1.28 ± 0.05 g; p-value = 0.50;

- Yolk mass (Figure 25b and Table 6): chosen 0.455 ± 0.015 g, not chosen 0.448 ± 0.015 g; p-value = 0.14;
- Eggshell mass (Figure 25c and Table 6): chosen 0.297 ± 0.009 g, not chosen 0.274 ± 0.012 g; p-value = 0.017;
- Eggshell thickness (Figure 25d and Table 5): chosen 0.115 ± 0.003 mm, not chosen 0.108 ± 0.002 mm; p-value = 0.14.

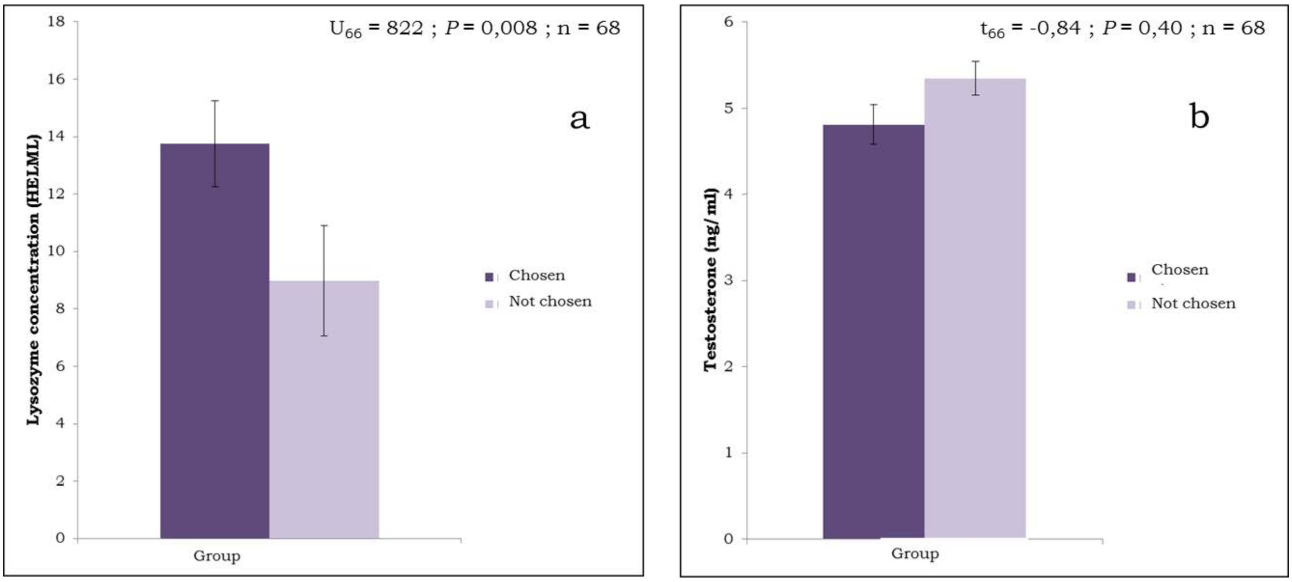


Figure 26 - Histogram a: lysozyme concentration in two groups. Histogram b: testosterone concentration in two groups. In all graphs the results of the statistics are inserted.

For what concerns the micro-components of the eggs, in particular the lysozyme (in the albumen) and the testosterone (in the yolk), a statistically significant difference was found in the lysozyme content but not in testosterone. In fact:

- Lysozyme concentration (Figure 26a and Table 5): chosen 13.9 ± 1.5 HELML, not chosen 9.0 ± 2.0 HELML; p-value = 0.008;
- Testosterone concentration (Figure 26b and Table 6): chosen 4.8 ± 0.3 ng/ml, not chosen 5.3 ± 0.2 ng/ml; p-value = 0.40.

So, a female that have chosen the partner invested more in lysozyme than a female that haven't chosen, but the same thing didn't happen for the testosterone.

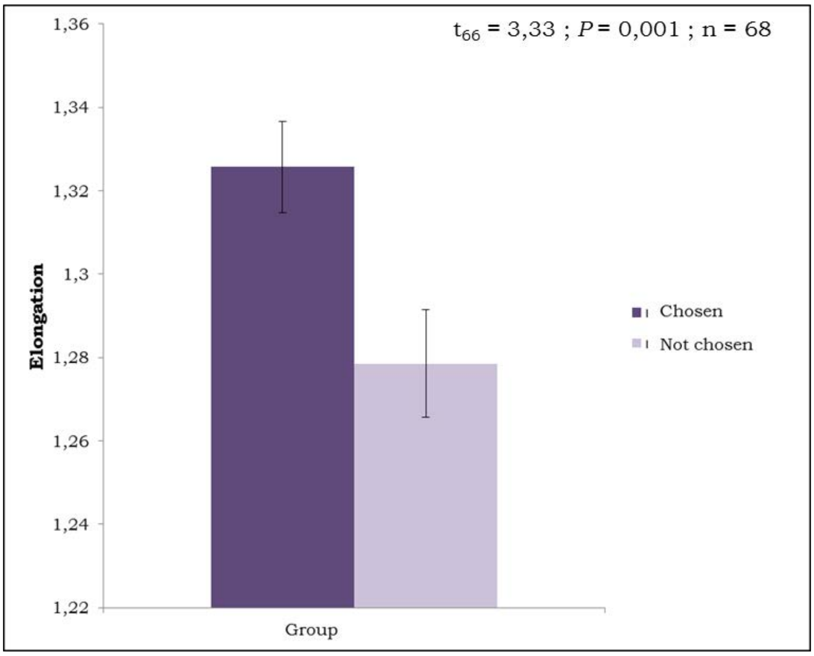


Figure 27 - Elongation index (length/breadth) in two groups. In the histogram the results of the statistics are inserted.

Figure 27 reports the differences between the two groups in regard to the elongation index.

A significant difference was found between the females of two groups: the females that chose the partner laid eggs more elongated than females that didn't choose (Figure 27a: chosen 1.33 ± 0.01, not chosen 1.28 ± 0.01; p-value = 0.001).

In conclusion, related with females paired with a male not preferred, females coupled with the male chosen laid eggs:

- Heavier;
- With a heavier eggshell;
- With more lysozyme in the albumen;
- With a higher elongation index.

~

Discussion

~

This experiment has been designed: 1) to evaluate if canary female choices or not a male that results dominant after a male-male physic competition and, consequently, 2) to assess maternal investment and testosterone and lysozyme deposition in eggs of females paired with a dominant (and chosen) male. So, interactions between two male (one dominant and one dominated) have been shown to females; after this, the female choice has been noted and finally the maternal investment has been valued.

In this experience a clear index of dominance for males hasn't been found, but several behaviours that males take in a competition for food has been characterized. So, these behaviours have been related with the preference of the female and a preference for male that starts interaction for more times was discovered.

This work shows that females paired with males that start interaction for more times and that she chose laid eggs heavier, with a shell heavier, more elongated and with more lysozyme in their albumen eggs, while number of the eggs, yolk and albumen mass, thickness shell, and the testosterone concentration didn't differ significantly in relation to partner's choice.

So, in this species a particular aggressive behaviour is related to female preference: the preferred males were those that started more aggressive interaction.

In several species female preference is related to dominance: the preferred males were dominant males. The preference for dominant male has been found in different animal taxa (Qvarnström & Forsgren, 1998): mammals (e.g., Cox & Le Boeuf, 1977; Horne & Ylönen, 1996), insects (e.g., Breed et al., 1980), fishes (e.g., Bisazza & Marin, 1991) and birds (e.g., Alatalo et al., 1991). Instead, other studies proved the opposite (Ophir & Galef, 2003; 2004; Amy et al., 2008). Zahavi (1975) suggested that males should use their ornaments both to attract females and to intimidate their opponents. Borgia (1979) proposed the "war propaganda model": in lek and territorial species females should prefer the display of an owner male, an individual that honestly communicate dominance over competitor males. Indeed in many species females are known to choose the winner in contests (Trail, 1985 in cock of the rock, *R. rupicola*; Graves et al., 1985 in domestic fowl, *G. gallus*). In the domestic cock the female even incite such competitive contests (Thornhill, 1988). Pizzarri & Birkhead (2000; 2002) demonstrated also a post-copulatory selection: female of domestic fowl favor sperm of dominant males then the sperm of subordinate ones.

In the domestic canary, a complementary effect of female preference and aggressive male behaviour on egg characteristics has been found. First, the active choice of females affected subsequent maternal investment in terms of egg mass.

This result is in contrast with the theory that the egg mass doesn't change due to the high repeatability of egg mass as a reproductive trait (Christians, 2002). The result of the present study is congruent with studies on the mallard, *A. platyrhynchos*, the chinese quail, *C. chinensis*, the peafowl, *P. cristatus*, and the zebra finch, *T. guttata*, which laid larger eggs when mated with more attractive males (Cunningham & Russell 2000; Uller & Andersson, 2005; Gilbert et al., 2006; Loyau et al., 2007), but contrasts with data obtained on the collared flycatcher, *F. hypoleuca*, the domesticated canary, the house sparrow, *P. domesticus*, and the house wren, *Troglodytes aedon* (Mazuc et al., 2003b; Tanvez et al., 2004; Michl et al., 2005, Grana et al., 2012).

Egg size can have important long-term consequences for the survival of offspring (Blomqvist et al., 1997; Starck & Ricklefs, 1998; Forbes & Wiebe, 2010) because larger eggs tend to have higher hatching success (Perrins, 1996), to lead to larger hatchlings (Magrath, 1992; Styrsky et al., 1999; Christians, 2002) and to influence fledging probability as in the lesser black-backed gull, *Larus fuscus* (Silva et al., 2008).

In this work, female preference for a more aggressive male is significantly related to lysozyme concentration in the eggs. Lysozyme is considered an important agent of maternal antibacterial immunity for the egg (Sato & Watanabe, 1976). This result disagrees with data on the grey partridge (Garcia-Fernandez, 2009; chapter I of this thesis) but it agrees with findings in the blue tit, in which females produced eggs with a higher concentration of lysozyme when mated with more attractive males (D'Alba et al., 2010). In the grey partridge, lysozyme activity has been shown to be higher in eggs laid by females fed with a high supply of β-carotene (Cucco et al., 2007); another study suggests that egg lysozyme might have positive consequences for early post-hatching survival (Prusinowska et al., 2000). Hence, lysozyme in the eggs of this species can depend on female feeding but appears to be independent to a female's mate preference. Up to now, data on the relationship between female preference and maternally derived immunity are scarce; an important exception is a study reporting a positive effect of male attractiveness on egg anti-NDV antibodies (Saino et al., 2002b). Therefore, the findings of present work improve the knowledge in this field of study of the animal behaviour and, particularly, in the relationship between the maternal investment in immunity and the preference that female expresses. In addition, the male aggressiveness probably influences positively maternal effects (specifically the allocation of components related to the innate immunity). An explication for this finding could be that the mother increase this kind of components to counteract the negative effects due to a high concentration of testosterone. In the study, a correlation between testosterone in the eggs and the female preference hasn't been found, but several works show that aggressive males have a higher concentration of testosterone compared to non-aggressive males (e.g., Hegner & Wingfield, 1987). So, a supposition could be that the progeny of an aggressive male could inherit from the father aggressiveness (and consequently have more testosterone). This supposition can't be verified because all the eggs were utilized for the analyses, but a future experiment will be interesting to do to confirm it.

For what concerns the testosterone concentration, significantly differences between the two experimental groups have not been found: females paired with the preferred male didn't invest more in testosterone compared with females coupled with the male not preferred.

This result is agree with the study on female preference for male song by Garcia-Fernandez et al. (2010b) in the canary, but it doesn't match those obtained in other species showing that females paired with attractive males deposit more testosterone in their eggs (reviewed in Kingma et al., 2009; chapter I of this thesis). Female barn swallows increased yolk androgen concentration when mated to males with experimentally elongated tails (Gil et al., 2006) or to more colourful males (Safran et al., 2008); female canaries deposited significantly more testosterone when exposed to song with attractive repertoires than when exposed to unattractive ones or maintained in acoustic isolation (Gil et al., 2004b; Tanvez et al., 2004); blue tit, *C. caeruleus*, females laid eggs with higher testosterone concentration when mated with control males than with males with an artificial reduction of the crown UV coloration (Kingma et al., 2006); zebra finches, peafowl and grey partridge increased yolk testosterone when paired with attractive males (Gil et al., 1999; Loyau et al., 2007; Garcia-Fernandez et al., 2010a). Finally, the result of the present study is agree with those of a recent meta-analysis that shows no consistent direction of adjustment of yolk androgens, immune-stimulants and anti-oxidants with respect to male attractiveness across birds, which probably reflect that they are not necessarily costly to females and that their benefits (and costs) are highly context-dependent (Horváthová et al., 2012). This finding could do a justification to this result.

Finally, in the present study has been shown that the eggs laid by females paired with the more aggressive male selected had an elongation index higher than the eggs of the female coupled with the unselected male. A recent study of Cucco et al. (2012) found that egg hatchability was higher for intermediate egg elongation values. Nothing can be said on correlation between this index and the hatchability of the canary eggs analysed, but could be interesting to provide a study in this direction.

In conclusion, this study shows that canary females preferred aggressive male and they expressed this preference producing egg qualitatively of better quality.

Further research is needed to clarify if this preference influence also the hatching of the eggs and the offspring survival. Moreover, it would be interesting to see if the offspring of aggressive males is aggressive in turn.

Chapter III: Does the city noise affect egg characteristics?

Chapter III – Abstract

In the last years the man impact is increasing more and more on the environment that surrounds him. This influence can take many forms (pollution, habitat destruction, etc.) and it must be studied in order to understand what could cause on the natural environment and on the species that inhabit it.

This chapter is focused on the influence of anthropogenic noise on sexual selection and reproductive success in a oscyne species, where the song is a fundamental means of communication: the canary.

Previous studies have shown different effects on sexual choices in different species: 1) lowering in pairs of birds living in disturbed areas in relation with other birds living in areas with less noise (Habib et al., 2007); 2) difficulty of females to recognize their reproductive partner (Swaddle & Page, 2007); 3) decrease in Copulation Solicitation Displays (Huet des Aunay, 2011).

Instead, few works are focused on reproductive success (Halfwerk et al., 2011b).

For this reason an experiment has been set up in which several females canary were subject to two conditions: one in which the song of the male was masked by the noise and another in which it was being played in a condition of silence. One group was subjected in the first cycle of reproduction to the condition known as "noise" and in the second to the one called "Silence", while the second group was treated in the reverse order. All experiments were done within soundproof "cages" and preventing females to be seen by the others, to avoid the communication between them and possible emulative behaviors.

The main results of this work for sexual selection are on CSD and on the delay between the first CSD and the begin of the presentation of the stimulus. Instead, for the reproductive success has been reported an influence of the disturbance on the number of eggs laid.

These results are substantially in line with those reported in the literature (Habib et al., 2007; Halfwerk et al., 2011b; Huet des Aunay, 2011; Schroeder et al., 2012) and are a small step to understand the effects of human impact on species that occupy noisy environments.

More still remains to be investigated in this field, such as the adaptation of oscyne species to this new ecological niche or the influence of noise on the mother-child communication during the first days of life.

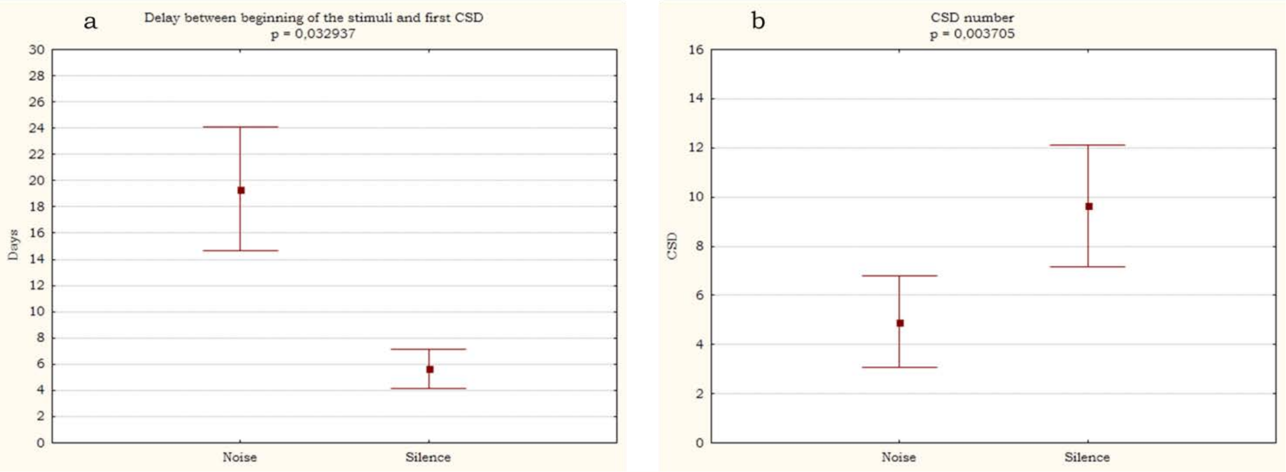


Figure 28 - a) Delay between first presentation of the stimulus and the first posture in the two groups, b) number of postures in the two groups.

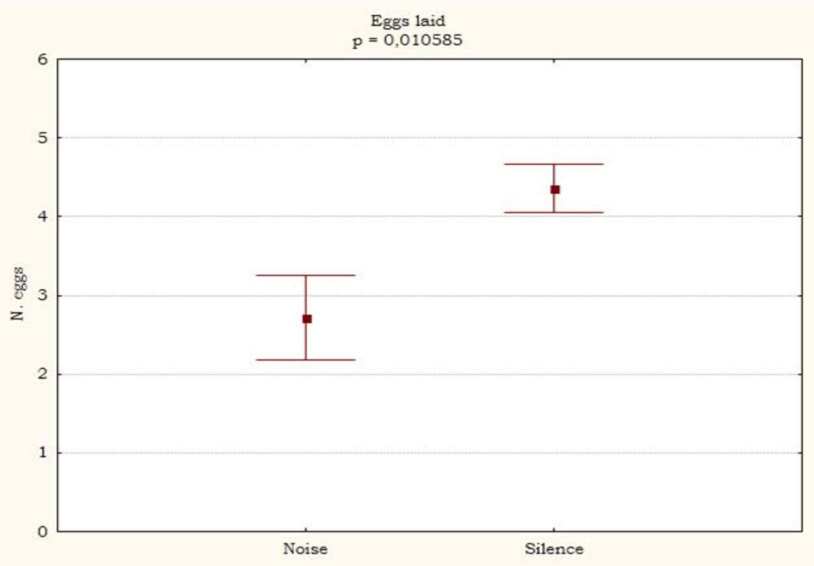


Figure 29 - Number of eggs laid by two groups.

Key-words: Anthropogenic noise, Copulation Solicitation Display, male song, maternal effects, sexual selection.

Capitolo III – Riassunto

Negli ultimi anni sta aumentando sempre di più l'impatto dell'uomo sull'ambiente che lo circonda. Questo può assumere diverse forme (inquinamento, distruzione di habitat, ecc.) e deve essere studiato per comprendere cosa può causare sull'ambiente naturale e sulle specie che lo abitano.

Questo capitolo si occupa dell'influenza del rumore antropico sulla scelta sessuale ed il successo riproduttivo in una specie oscina, dove il canto è un mezzo fondamentale di comunicazione: il canarino.

Studi precedenti hanno dimostrato diversi effetti sulla scelta sessuale di varie specie: 1) abbassamento negli accoppiamenti di uccelli che vivevano in aree disturbate rispetto ad altre zone che lo erano meno (Habib et al., 2007); 2) difficoltà delle femmine a riconoscere il proprio partner riproduttivo (Swaddle & Page, 2007); 3) diminuzione delle posture di accoppiamento (Huet des Aunay, 2011).

Pochi si sono invece concentrati sul successo riproduttivo (Halfwerk et al., 2011b).

Per questo motivo è stato predisposto un esperimento in cui diverse femmine di canarino venivano sottoposte a due condizioni: una in cui il canto del maschio veniva mascherato dal rumore ed un'altra in cui esso era riprodotto in condizione di silenzio. Un gruppo è stato sottoposto nel primo ciclo di riproduzione alla condizione denominata "Rumore" e nel secondo a quella detta "Silenzio", mentre il secondo è stato trattato nella maniera inversa. Tutti gli esperimenti sono stati fatti all'interno di "gabbie" insonorizzate ed impedendo alle femmine di vedersi, in modo da evitare la comunicazione fra esse e possibili comportamenti emulativi.

I risultati principali di questo lavoro si hanno sulle posture di sollecitazione all'accoppiamento e sul ritardo delle prime posture rispetto all'inizio della presentazione dello stimolo per quanto riguarda la scelta sessuale. Concentrandosi invece sul successo riproduttivo, è stata riscontrata un'influenza del disturbo sul numero di uova deposte.

Questi risultati sono sostanzialmente in linea con quelli presenti in letteratura (Habib et al., 2007; Halfwerk et al., 2011b; Huet des Aunay, 2011; Schroeder et al., 2012) e sono un piccolo passo per quanto riguarda la comprensione degli effetti dell'impatto antropico sulle specie che si trovano ad occupare spazi disturbati.

Molto ancora resta da indagare in questo campo, come ad esempio l'adattamento delle specie canore a questa nuova nicchia ecologica o l'influenza del rumore sulla comunicazione madre-figlio durante i primi giorni di vita.

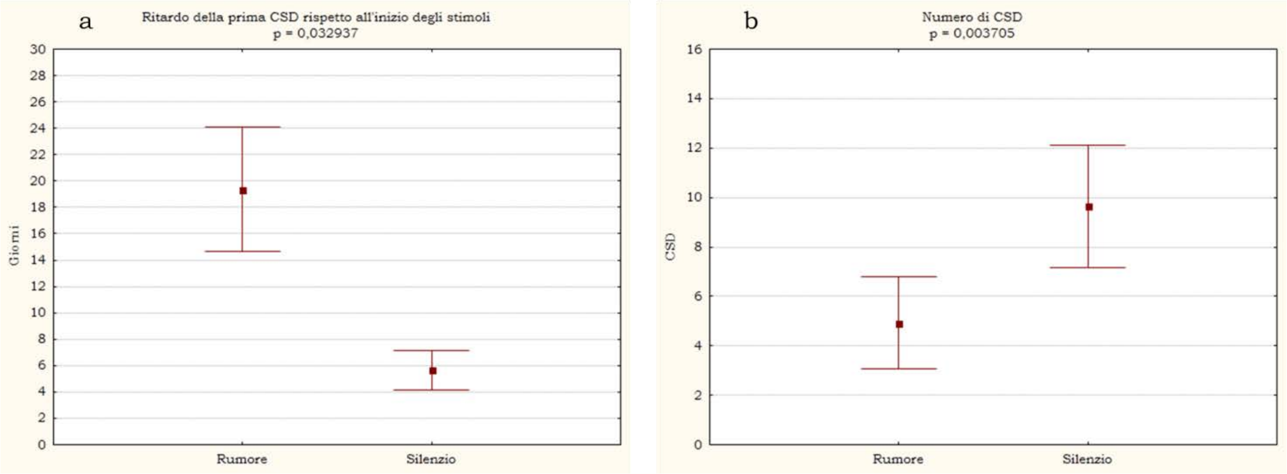


Figure 30 - a) Ritardo fra prima presentazione dello stimolo e prima postura nei due gruppi; b) numero di posture nei due gruppi.

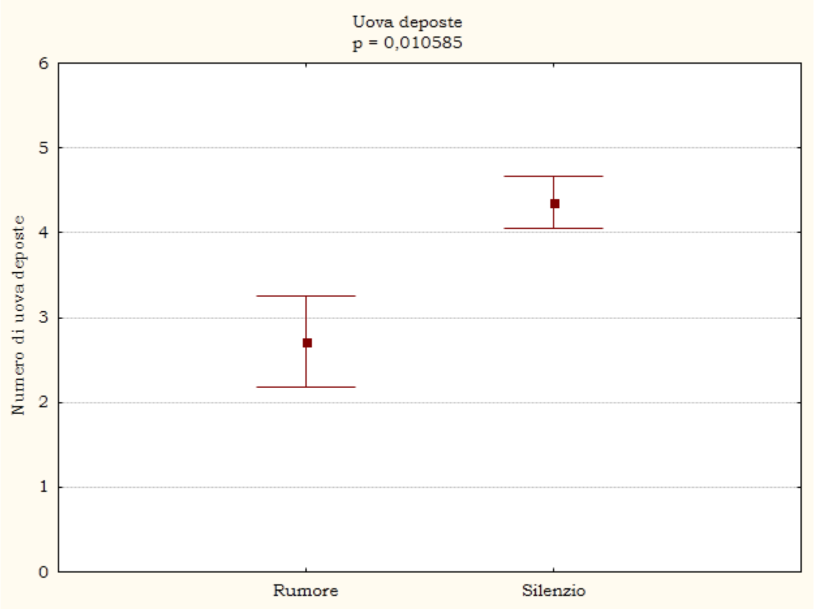


Figure 31 - Numero di uova deposte dai due gruppi.

Parole-chiave: Rumore antropico, Postura di sollecitazione all'accoppiamento, canto del maschio, effetti materni, selezione sessuale.

Chapitre III – Résumé

Au cours des dernières années l'impact de l'homme sur l'environnement est en train d'augmenter. Cela peut prendre de nombreuses formes (pollution, destruction de l'habitat, etc.) et il faut l'étudier afin de comprendre ce qui peut causer sur l'environnement naturel et les espèces.

Ce chapitre met l'accent sur l'influence du bruit d'origine anthropique sur la sélection sexuelle et le succès de reproduction dans une espèces oscine, où le chant est un moyen fondamental de communication: le canari.

Des études antérieures ont montré des effets différents sur les choix sexuels des diverses espèces: 1) l'abaissement des accouplements d'oiseaux qui vivent dans les zones perturbées par rapport à autres domaines moins déranger (Habib et al., 2007), 2) la difficulté des femmes à reconnaître leur partenaire reproductive (Swaddle & Page, 2007), 3) la diminution des postures de stimulation à l'accouplement (Huet des Aunay, 2011).

Peu d'études se sont concentré sur le succès reproducteur (Halfwerk et al., 2011b).

Pour cette raison, il a été mis en place une expérience dans laquelle plusieurs femelles de canari ont été soumis à deux conditions: l'une dans laquelle le chant du mâle a été masqués par le bruit et un autre où il a été reproduit dans un état de silence. Un groupe a été soumis dans le premier cycle de reproduction à la condition appelé «Bruit» et dans le second à celle qui est appelé "Silence", tandis que le second groupe a été traité dans l'ordre inverse. Toutes les expériences ont été réalisées dans des «cages» insonorisées pour éviter les contacts visuelles et sonores entre les femelles, de manière à éviter la communication entre eux et les possibles comportements d'émulation.

Les principaux résultats de ce travail sont sur les postures de sollicitation à l'accouplement et sur le délai entre la première présentation du stimulus et la première posture pour la sélection sexuelle. Pour ce qui concerne le succès de reproduction, une influence du dérangement sur le nombre d'œufs pondus a été signalée.

Ces résultats sont essentiellement conformes à ceux rapportés dans la littérature (Habib et al., 2007; Halfwerk et al., 2011b; Huet des Aunay, 2011; Schroeder et al., 2012) et sont un petit pas en ce qui concerne la compréhension de l'impact des activités humaines sur les espèces que occupent les espaces affectés.

Il reste encore beaucoup à étudier dans ce domaine, telles que l'adaptation des espèces oscines dans cette nouvelle niche écologique ou l'influence du bruit sur la communication mère-fils pendant les premiers jours de vie.

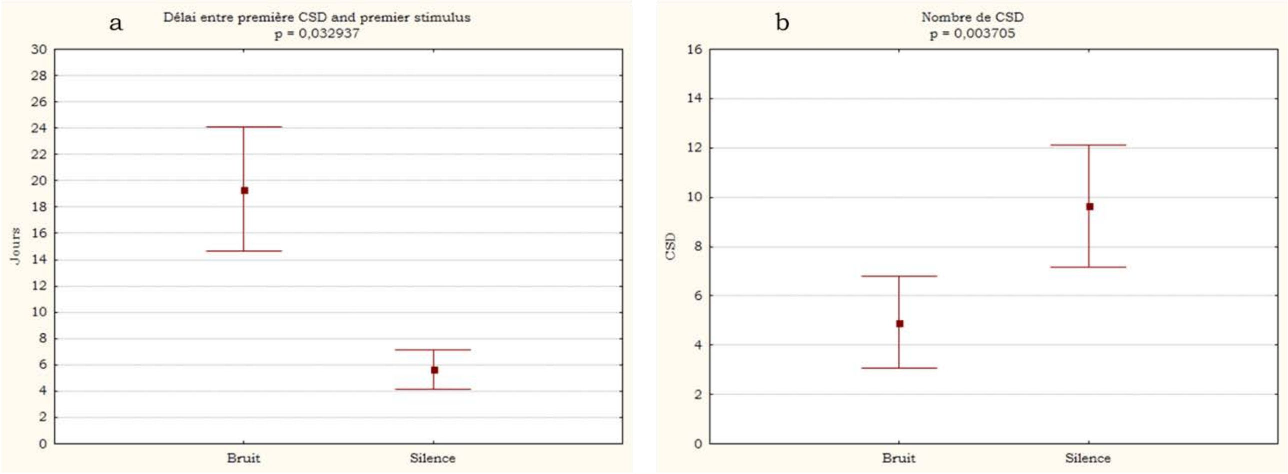


Figure 32 - a) Délai entre le jours de la première présentation du stimulus et la première posture dans les deux groupes expérimentales; b) nombre de posture dans les deux groupes experimentales.

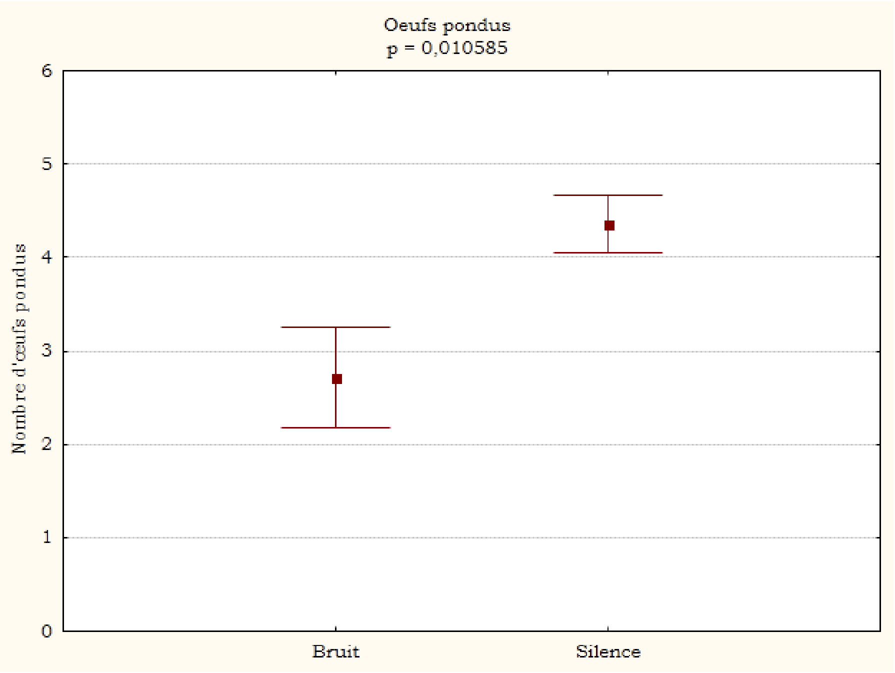


Figure 33 - Nombre d' œufs pondus par les deux groupes.

Mots clés : Bruit anthropique, posture de sollicitation à l'accouplement, chant du mâle, effets maternels, sélection sexuelle.

~

Introduction

~

In the animal world, different types of communication have been evolved: voice, chemical, visual, tactile, vibrational, electrical (Tanzarella, 2005; Breed & Moore, 2011). Voice communication is among the most widespread. There are examples in many groups of animals, both invertebrates (e.g., insects - Gerhardt & Huber, 2002) and vertebrates such as amphibians, reptiles, birds and mammals (Whitlow et al., 2000; Narins et al., 2006; Catchpole & Slater, 2008; Vergne et al., 2009). They use this type of communication to send and receive information from their environment (Laiolo, 2010).

Communication, the way by which organisms convey information to each other, is the gel that holds animal societies together: it facilitates reproduction, provides information on individual identity, status, mood and intentions (Bradbury & Vehrencamp, 1998). As it includes a substantial proportion of the behavioural repertoire of animal species, communication behaviour can become an important driver of several aspects of species biology, affecting the evolution of life histories and genes (Laiolo, 2010). Along with several other animals, humans share the use of sounds as the principal means of exchanging information. Many vertebrates (bony fishes, amphibians, reptiles, birds, mammals) and invertebrates (insects, spiders, crustaceans, nematodes) make sounds (or vibrations) for a variety of reasons, mostly for courtship and agonistic behaviours, but also for more complex social communication (Hauser, 1997; Owings et al., 1998).

For what concerns the birds, and in particular the oscines, i.e. the songbirds, the production of more or less complex songs happens thanks to the use of syringe (Tanzarella, 2005; Catchpole & Slater, 2008). The birds emit multiple signals of acoustic type that serve different functions: alarm, stress, contact, demand for food, gathering (Brichetti & Gariboldi, 2002), territorial defense (Patricelli & Blickley, 2006; Catchpole & Slater, 2008), attraction of the opposite sex during the breeding season (is generally the male that sings - Patricelli & Blickley, 2006, Warren et al., 2006; Catchpole & Slater, 2008).

Under natural conditions, the songs have adapted themselves to the environment in which they were produced; this could happen through evolution, which selected those species that are better able to adapt to the pressures to which they were getting into and that has also led to speciation (Slabbekoorn & Smith, 2002). The environmental and ecological characteristics are very important for the transmission of sound signals; standard ecological conditions often change dramatically as a function of the ecosystem and sometimes can also determine the adaptive modifications of sound communication systems. Air humidity, for example, can attenuate sounds as a function of frequencies at which they are produced. The noise caused by rivers can modify signals, changing the ratio between frequencies (increasing or attenuating some). The wind can cause variations, either directly or by acting on environmental characteristics such as vegetation. It is also known that, in environments highly disturbed by plants, these parasitize certain frequencies by changing the original signal (Catchpole & Slater, 2008).

Nowadays, with industrialization and population rise, we are faced with the formation of a new ecological niche, the urban niche, characterized by the presence of new disorders caused by man. The study of those species, that are best suited to them and then can occupy this niche, may be useful for evaluating strategies of adaptation to new environmental changes. When we talk about an urban area, we mean an area with a high density of population and human constructions (Warren et al., 2006). Therefore, in this definition are included commercial, industrial, residential areas and even those boundaries areas that, day after day, because of the population, continue to move the boundary between natural and heavily man-made world (Warren et al., 2006).

The urban environment presents both natural kind pressures as well as other specific of this area: night lighting and anthropogenic noise (Warren et al., 2006; Slabekoorn et al., 2007), that is coming from human activity. What makes this disorder so annoying for communication between birds (Slabbekoorn & Peet, 2003; Patricelli & Blickley, 2006; Wood & Yezerinac, 2006) is that it is spread over low frequencies, below 1400 Hz for most (Lohr et al., 2003). Despite the extreme heterogeneity of urban landscape, man-made changes in this environment have some common characteristics that affect communication, such as high noise levels, reflective surfaces and altered sound channels (Warren et al., 2006). Another face, that must be taken into account about anthropogenic noise disturbance, is timing: often peaks become exactly at times when the birds sing more (Brumm & Slabbekoorn, 2005; Fuller et al., 2007).

Which aspects of this problem can have an impact on bird species?

First of all, it can have a negative impact on biodiversity by reducing the survival of the species (Laiolo, 2010), increasing the risk of predation (Quinn et al., 2006) or by disturbing the transmission of the communication signal (Slabbekoorn & Peet, 2003): the noise in fact decreases the distance at which it can be correctly detected. Ultimately the transfer of information from the emitter to the receiver depends on the signal/noise ratio received by the latter (Warren et al., 2006; Pohl et al., 2009).

The answers to these problems can be multiple (Brumm & Slabbekoorn, 2005).

An example would be that of the nightingale (*Luscinia megarhyncos*), in which an adaptation of song sound intensity to the noise have been seen [Lombard effect (Brumm, 2004)]: when the intensity of noise increases too much, the nightingale sings with an higher intensity. A second usable strategy, especially in cases where the noise is concentrated at certain times or in any case is not always present, is to avoid temporal overlapping singing outside of the periods in which there is noise (Brumm & Slabbekoorn, 2005); also in european robin (*Erithacus rubecula*) it is noted that it songs before during the day, when it is in disturbed environment (Fuller et al., 2007). A third type of adaptation can be that observed in males of blackbird (*Turdus merula*), great tit (*P. major*) and singer sparrow (*Melospiza melodia*), which emit songs with higher frequencies when they are in a noisy environment compared to undisturbed one (Slabbekoorn & den Boer-Visser, 2006; Wood & Yezerinac, 2006; Ripmesteer et al., 2010b).

A study of Halfwerk et al. (2012) suggests an active role of female birds in steering male song behaviour under noisy conditions. They studied the role of social feedback on male great tit song adjustment by exclusively exposing females to artificial traffic noise inside their nest box. So males did not receive direct noise exposure during intra-pair communication, but adjusted their behaviour in the predicted direction (Halfwerk et al., 2012).

A recent study found that song components differed in their plasticity to background noise and that plasticity integration between components may further restrict the elaboration of song. Thus, the altered expression of song components under noise exposure leads to increased phenotypic integration, which is linked with reduced song complexity (Montague et al., 2013).

All the above studies have been designed to investigate the male response to the presence of noise. With regard to the success of mating, the female choice and the impact of this noise on the reproductive success, very few studies have been made until today (Habib et al., 2007; Swaddle & Page, 2007; Halfwerk et al., 2011a; 2011b, Huet des Aunay 2011; Schroeder et al., 2012). In the work of Habib et al. (2007) it's clearly proved how in populations of golden crown ovenbird (*Seiurus aurocapilla*) living in areas subject to noise the pairings of males decrease compared to other places less disturbed; this can be due to a reduction of propagation distance of the song and/or to a distorted perception of the signal by the female, that for this judges a male of low quality.

Instead, Swaddle & Page (2007) have carried out laboratory tests on zebra finches (*T. guttata*) to verify if females inserted in a context with presence of noise were able to select the male with which had already been coupled. They have been able to verify how the above situation has reduced the ability of females to recognize their own partner and has increased the selection of an unknown individual. Halfwerk et al. (2011a) performed a work on an urban bird species, the great tit (*P. major*), that experimentally shows that urban noise conditions impair male–female communication and that signal efficiency depends on song frequency in the presence of noise. They reveal a response advantage for high-frequency songs during sexual signaling in noisy conditions, whereas low-frequency songs are likely to be preferred. These data are critical for understanding the impact of anthropogenic noise on wild-ranging birds, because they provide evidence for low-frequency songs being linked to reproductive success and to be affected by noise-dependent signal efficiency. The work of Huet des Aunay (2011) has instead highlighted how females of common canary (*S. canaria*) would decrease their Copulation Solicitation Display (CSD), parameter which is evaluated in order to understand the choice and the predisposition to mate with a particular male (King & West 1977; Searcy & Marler 1981; Searcy 1992), when they came in contact with songs masked by city noise.

The work of Halfwerk et al. (2011b) has also revealed a reduction of reproductive success in the great tit: females reproduced in environments with a greater amount of noise, produced smaller clutches. This can probably be explained by the overlap of the traffic on the song of male on low frequencies.

Also Schroeder et al. (2012) investigated the underlying mechanisms in the association between fitness and anthropogenic noise in passerines birds and they found that an important aspect could be the acoustically masking parent–offspring communication.

The model used for this experiment, as previously mentioned, is the canary, altricial and oscines species. It was chosen because song is the main feature used by the male to compete with others to be chosen by females. In addition, through the CSD, it's possible to have a reliable indicator of sexual preference of the female (King & West, 1977): indeed, she responds to a particular part of the song called phrase A or "sexy phrase" (Vallet & Kreutzer, 1995). Last but not least, the domesticated canary is a very well-studied species in which females can lay eggs in the presence and absence of males. The ability of unpaired female canaries to lay eggs in the absence of mate stimulation allows to design an experiment in which male influence can be discarded (Tanvez et al., 2008).

Phrase A is a repetition of syllables containing two distinct notes that are repeated at a rate of at least 16 syllables per second and that cover a frequency band of at least 4 kHz. This frequency band and this time are very close to the limit of the singing abilities of the species (Draganoiu et al., 2002): to produce them, in fact, a male must be in good health, as it requires a good neural-sense motor coordination. Therefore, this signal indicates physical conditions (Lusianti-Fitri, 2001) and dominance status of males (Parisot, 2004). It has also been seen that in this species the females have a preference for phrases A lowered than 1.5 kHz (Pasteau et al., 2009). An analysis carried out by Huet des Aunay (2011) shows that the minimum frequency of the phrase A extends from 1 kHz to 3.6 kHz; if you apply a city noise mask, low-frequency phrases (which are preferred in natural conditions) are partially covered by noise (especially below 2 kHz), while the acute phrases (less things) are not masked.

In conclusion, the urban noise, occupying low frequencies, disturbs acoustic communication. Since this is widely used by male birds that, through their songs, defend their territory and attract and stimulate the female (Catchpole & Slater, 2008), if the noise decreases the receptiveness of the opposite sex, it's very likely that it also has an effect on the success of coupling (Habib et al., 2007) that is expressed finally in a lowering of reproduction success.

Since it's been seen in several studies that the female invests differently in their eggs depending on the attractiveness of heard song (Gil et al., 2004b; Tanvez et al., 2004; Garcia-Fernandez et al., 2013), it can be assumed that, in a disturbed environment, the females invest less in their eggs compared to other in an environment with less noise. This may explain the lowering of the reproductive success observed in nature (Halfwerk et al., 2011b). Therefore, the purpose of this study is to investigate, in controlled laboratory situation, if the anthropic noise causes repercussions on maternal investment of female canaries, at level of total weight of the egg, of macro-components (egg white, egg yolk, and shell mass) and finally physiological (different allocation of lysozyme and ovotransferrin in albumen).

In order to do this, experiments were set up in which phrases A have been diffused to a first group of females in noisy conditions during the first reproductive cycle, and then in conditions of silence during the second, while it was carried out in the reverse order for the second group.

~

Materials and Methods

~

Experimental condition

The experiment was performed under proper legislation by the French law.

All the females used for these experiments came from breeding of the Laboratoire d'Éthologie et Cognition Comparées (LECC). From the moment when tests started, females were housed individually in small cages (40 cm length x 30 cm width x 24 cm height), in turn placed into individual soundproof "boxes" (68 x 51 x 51 cm) – Figure 8 page 64, Materials and Methods: Common Aspects). Food (classical mix of seed, Beyers®, containing all necessary nutrients for breeding females, an egg-based mix, Cédé® and apple) and water were provided *ad libitum*. Each cage was provided with a piece of cuttlebone for the calcium, a nest bowl and nesting material (cotton string).

The arrangement of boxes was such to prevent all visual and sound communication among females, in order to eliminate any visual contact between individuals; for this purpose, a separation was also positioned at the middle of the room. This was done to prevent that the employed females could obtain information from the others during tests. Outside of testing periods, the boxes have been left open, for allowing female voice communication and so avoiding problems of "social deprivation".

At the time when the females were entered inside the boxes, the photoperiod (in which they were for four months at least) was also changed, passing hence from winter photoperiod, called of "Short Day" (SD: 8 h day/16h night), to summer photoperiod, called of "Long Day" (LD: 16h day/8h night). This change stimulates the reproductive cycle activation, causing the expression of sexual behaviour, avoiding the administration of estradiol (Leboucher et al., 1994).

Method, sexual preference and behavioural responses

To evaluate the female sexual preferences the Copulation Solicitation Display were used (CSD - King & West, 1977 - Figure 7, page 54). A complete CSD appears when the female crouches, lowers wings and makes them vibrating, and arches her back throwing her head back and lifting her tail. When a posture includes all four of these elements, it's assigned a score of 1; if one of these is missing, it's assigned a score of 0.5 (Kreutzer & Vallet, 1991).

Since females were positioned into soundproof boxes, the diffusion of tracks of two hours, containing 3 minutes of noise and 3 minutes of silence, started and repeated twice a day (10:00 – 12:00 and 14:00 – 16:00); for the first experimental group the phrase A was diffused into the period of noise and was repeated 3 times during the 3 minutes. Every day the first CSD was checked; the test (consisting in recording the female postures during the first presentation of the series of three phrases in the morning) started the day after the first posture.

When the egg deposition began, the eggs were removed and replaced with plastic ones. The separation will be described in the next section.

At the end of reproduction cycle, eggs, nest and cotton were removed, and the signal was stopped. Later, in the second cycle of reproduction, the stimulation was changed. The tracks was of 2 hours, containing 3 minutes of noise and 3 minutes of silence, and 3 phrases A for every period of silence. Test and collection have been carried out as described previously.

The second group of females was treated in the same way of the first, with the difference that, in the first deposition cycle, phrases A were positioned in the silence periods while in the second in those of noise.

Stimuli and noise

All the phrases A used came from the archive of song recordings of the laboratory. To avoid a possible effect of pseudoreplication (Kroodsma, 1982; 1989; Kroodsma et al., 2001) 16 different tracks have been used for the 16 females. One of these was then associated in an aleatory way to the noise condition, while another to the silence condition, for preventing the construction of a learning model of a stimulus. The females of the same session of the experiment were divided in pairs; to the first female phrase A1 was spread during the noise and phrase A2 during the silence, while the reverse was done for the second (see Table 7).

Individual	**In noise conditions**	**In silence conditions**
Female 1	Stimulus 1	Stimulus 2
Female 2	Stimulus 2	Stimulus 1
Female 3	Stimulus 3	Stimulus 4
Female 4	Stimulus 4	Stimulus 3
Female 5	Stimulus 5	Stimulus 6
Female 6	Stimulus 6	Stimulus 5
Female 7	Stimulus 7	Stimulus 8
Female 8	Stimulus 8	Stimulus 7
Female 9	Stimulus 9	Stimulus 10
Female 10	Stimulus 10	Stimulus 9
Female 11	Stimulus 11	Stimulus 12
Female 12	Stimulus 12	Stimulus 11
Female 13	Stimulus 13	Stimulus 14
Female 14	Stimulus 14	Stimulus 13
Female 15	Stimulus 15	Stimulus 16
Female 16	Stimulus 16	Stimulus 15

Table 7: Synthetic explanation of the presentation of the stimuli to females.

For the noise condition it was used a signal with an amplitude of 77 dB, which, in previous experiments carried out in the LECC laboratory, had been seen to be the most disturbing for the perception of phrases (Huet des Aunay, 2011).

As mentioned earlier, in separate sessions stimuli have been spread both in the presence of noise (condition called "77 dB") and in the absence (a condition called "SR") with a sound level of 77.1 dB ± 1.2 in the boxes (n = 8 boxes).

Collection and separation of eggs

When females started laying, all pair was inspected daily to collect eggs, which was replaced with plastic ones. All eggs were marked by means of a sheet, placed near them, containing an identification code (e.g., BLUE21/N2, where BLUE21 indicates the identification ring of the female and N2 the number of the egg within the brood) and the laying date, and were photographed on a graph paper with a Nikon Coolpix 990 camera mounted on a tripod, to take standard photos (these photos allow to measure the length L and the breadth B of the eggs and calculate the elongation index, L/B).

All eggs were immediately separated in the laboratory: weights of the whole egg, and then of shell, yolk and albumen, were taken using an electronic balance (± 0.001 g). Albumen and yolk have been collected in appropriate marked eppendorf, which have been placed in a freezer at -20°C for subsequent biochemical analyses. The first was not treated, while the second was homogenized with an equal amount (1 ml per gram of egg yolk) of a solution of NaCl 0.9% (wt/v). The thickness of the shell was measured at three different points using a precision gauge (Alpa S.p.A., Milan, Italy; precision ± 0.01 mm); the final thickness was the mean of the three measures.

Lysozyme Assay

Lysozyme activity was measured using a classical antibiogram method because the Osserman & Lawlor protocol (1966) has proved to be not sensitive enough.

Brief, the diameters of the cleared zones (inhibition zones), that the samples formed around the site of inoculation into a Petri plate containing *M. lysodeiktikus*, is an indicator of the lysozyme concentration. These diameters are proportional to the logarithm of the lysozyme concentration and, compared with the standard curve, allow to obtain values in hen egg lysozyme equivalents (HELML equivalents, expressed in µg/ml).

For the exact protocol see the "Materials and Methods Common Aspects – Lysozyme: classical antibiogram protocol" above.

Ovotransferrin Assay

A modified version of the assay described and verified by Yamanishi et al. (2002) was used. This assay measures total iron-binding capacity – the maximum amount of iron necessary to saturate all the ovotransferrin in a sample. It correlates very well with ovotransferrin concentration, as determined by comparison with immunological measurement of serum transferrin (Gambino et al., 1997; Yamanishi et al., 2002). A similar version of the assay has been used previously to measure concentrations of ovotransferrin in egg albumen (Shawkey et al., 2008; D'Alba et al., 2010). The assay consists of three reaction steps. First, ovotransferrin in the sample is saturated with ferric iron (Fe^{3+}) under alkaline conditions. Then, the unbound excess iron is reduced to Fe^{2+} by addition of ascorbic acid, and this Fe^{2+} becomes inactivated by forming coloured complexes with the chromogen FerroZine. Finally, the ovotransferrin-bound Fe^{3+} is dissociated under acidic conditions. This newly released Fe^{3+} allows further formation of the coloured Fe^{2+}–FerroZine complex. The associated increase in absorbance of the reaction mixture owing to this additional formation of the coloured complex is monitored over time (Horrocks et al., 2011).

For the exact protocol see the "Materials and Methods: Common Aspects" above.

Statistical analysis

Statistical analysis is performed by statistical softwares Systat 12® (SYSTAT Software Inc., San José, California), and Statistica 10® (StatSoft Inc., Tulsa, Oklahoma).

Paired t-tests were performed to analyse eventual differences in female responses to the stimulus and the characteristics of the eggs between the two experimental groups, composed by females that heard the male song on noise and females that heard it on silence.

After this, paired t-tests was utilized to find eventual differences in female responses and egg characteristics between the two cycle of reproduction.

Finally, Linear Mixed Models were performed to compare behaviour and egg characteristics of two experimental groups during the first cycle of reproduction and the second cycle. All the results are presented following this layout: mean ± S.E.

~

Results

~

The test included 19 females in the first cycle of reproduction (11 with song on noise and 8 with song on silence) and 19 in the second (11 with song on silence and 8 with song on noise); so, a total of 19 females with the noise and 19 with the silence (taking into account the two cycles) were tested. To clarify, the females that heard the song of male on noise in the first cycle were the same that heard the song on silence in the second, and those that heard the song on silence in the first cycle were the same that heard the song on noise in the second.

Tests lasted 29 days on average; the first CSD begun about 9 days after the first presentation of the stimulus, and the first egg was laid about 7 days later the first CSD. Finally, the females did an average of 11 CSD in mean during the test.

Difference between "Noise" group and "Silence" group

Copulation Solicitation Display

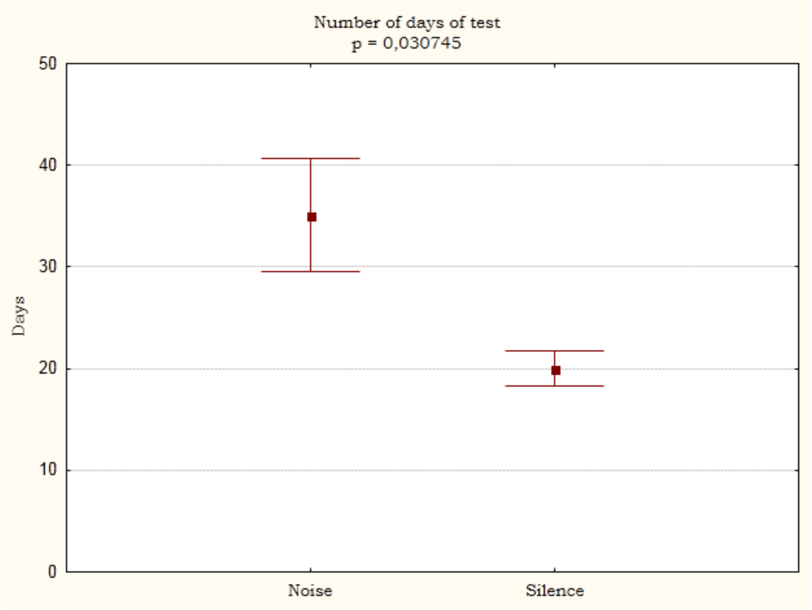


Figure 34 - Number of days of test for two groups: females that heard the male song on noise (Noise) and females that heard the song on silence (Silence).

A statistically significant difference between the group "Noise" and the group "Silence" was found (Figure 34). The test period lasted more days in the first than in the second group (Noise: 35 ± 5.5 days, Silence: 20 ± 1.7 days; p-value = 0.031).

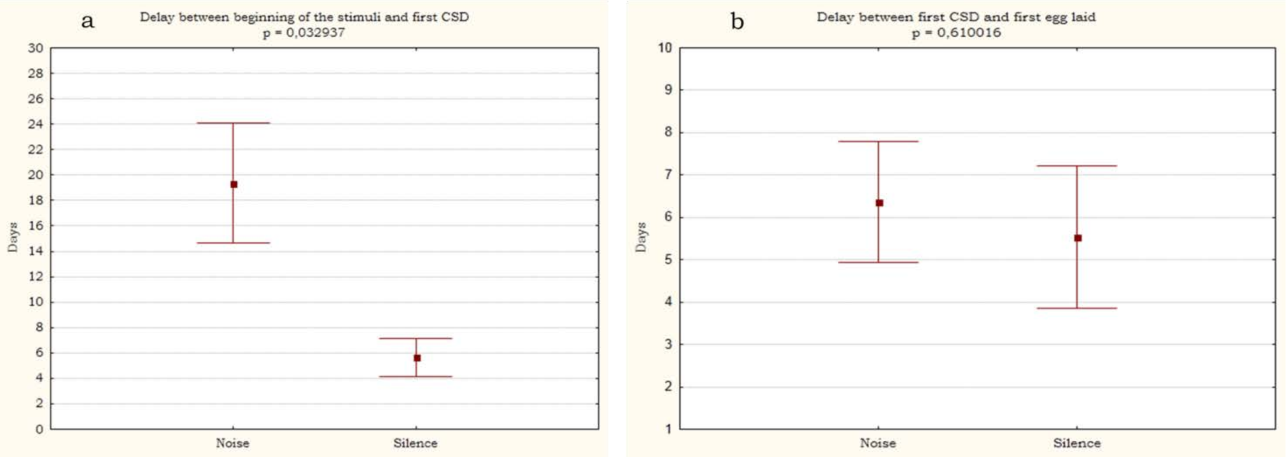


Figure 35 - Graphics with comparison between two experimental groups for what concerns the delay between the first presentation of the stimulus and the first CSD (a) and the delay between the first CSD and the first egg laid (B).

In Figure 35 there is difference between the two experimental groups for what concerns the delay between the passage to long day period (LD) and the first Copulation Solicitation Display (CSD): the females of group "Noise" began to do CSD significantly later than the group "Silence" (Noise: 19.4 ± 4.7 days, Silence: 5.6 ± 1.5 days; p-value = 0.033).

Not the same things happens on the delay between the first CSD and the first egg laid: (Noise: 6.4 ± 1.4 days, Silence: 5.6 ± 1.7 days; p-value = 0.61).

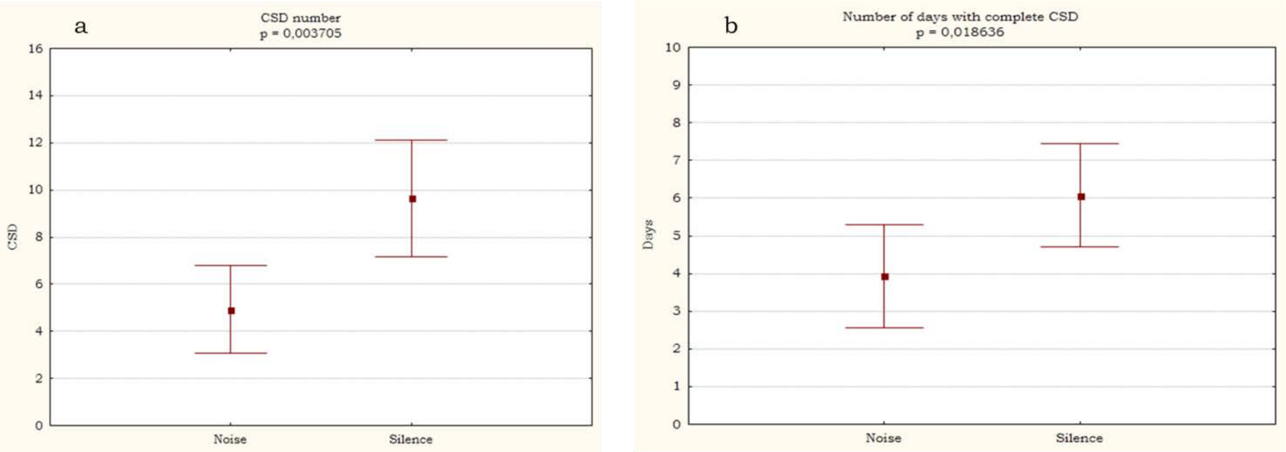


Figure 36 - Difference between two experimental groups in CSD (a) and number of days with CSD (b).

Figure 36a points out the difference between the two experimental groups in number of CSD did (Noise: 4.9 ± 1.9 CSD, Silence: 9.6 ± 2.5 CSD; p-value = 0.004): the females of groups "Silence" did more CSD than females of second group.

Figure 36b shows number of days when at least a complete CSD was observed; also here a statistical difference between the groups (Noise: 3.9 ± 1.4 days, Silence: 6.1 ± 1.4 days; p-value = 0.019) was found.

Eggs

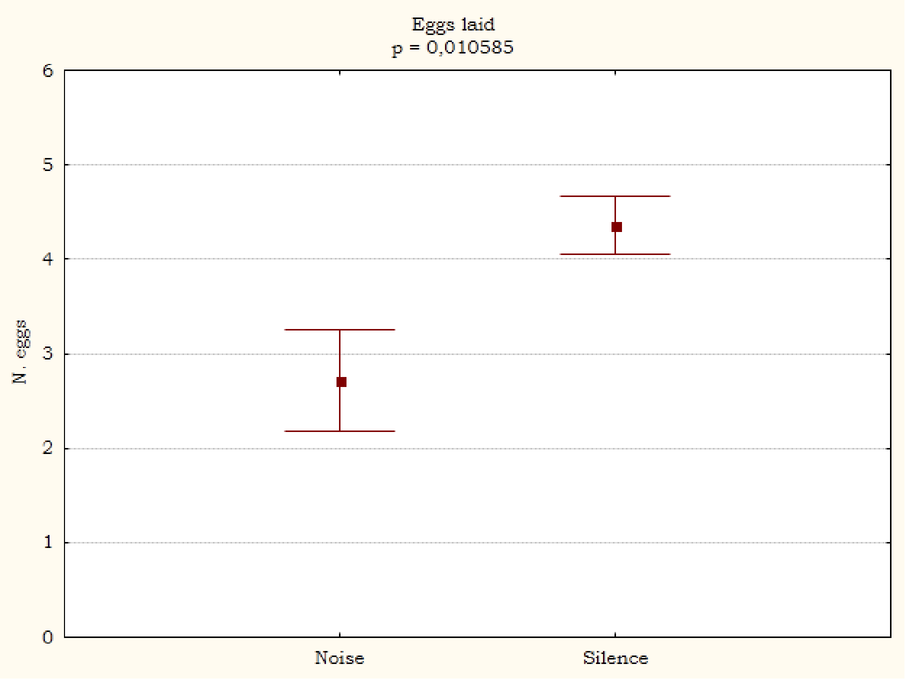


Figure 37 - Number of eggs laid by two experimental groups.

Figure 37 illustrates the number of the eggs laid by two experimental groups. A statistically significant difference (Noise: 2.7 ± 0.5 eggs, Silence: 4.3 ± 0.3 eggs; p-value = 0.011) was found: the females of group "Silence" laid significant more eggs than the females of the group "Noise".

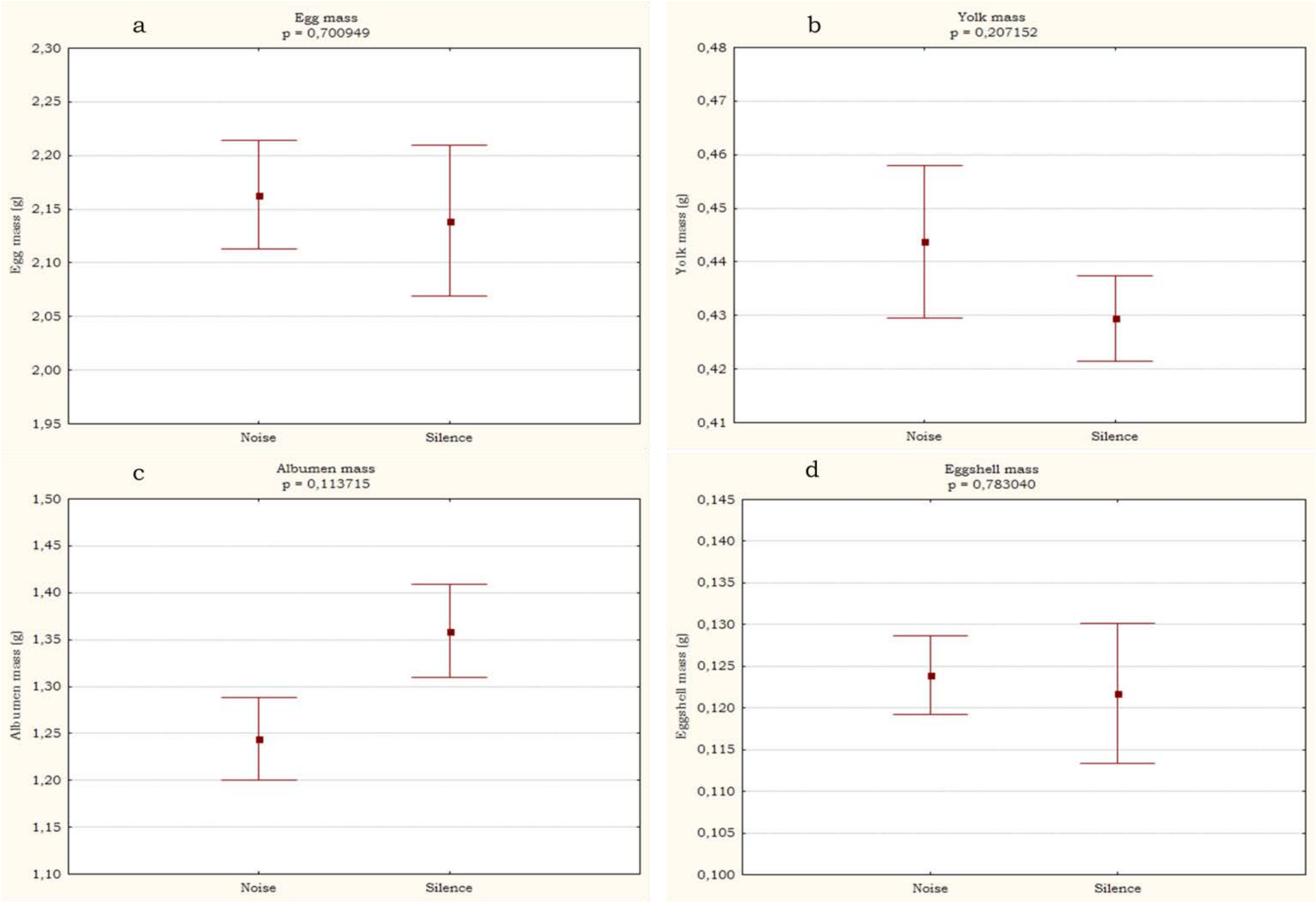


Figure 38 - Difference between two groups in egg (a), yolk (b), albumen (c) and eggshell (d) mass.

There was no difference between the two groups for what concerns egg mass and macro-components mass (Figure 38):

- Egg mass: (Noise: 2.16 ± 0.05 g, Silence: 2.14 ± 0.07 g; p-value = 0.70);
- Yolk mass: (Noise: 0.44 ± 0.02 g, Silence: 0.43 ± 0.01 g; p-value = 0.21);
- Albumen mass: (Noise: 1.24 ± 0.05 g, Silence: 1.36 ± 0.05 g; p-value = 0.11);
- Eggshell mass: (Noise: 0.12 ± 0.01 g, Silence: 0.12 ± 0.01 g; p-value = 0.78).

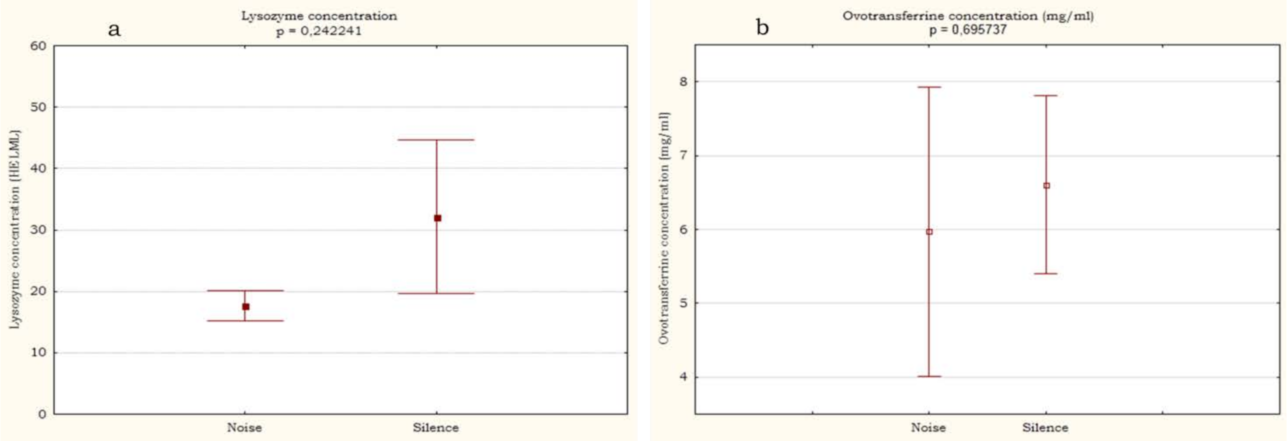


Figure 39 - Difference between two groups in lysozyme (a) and ovotransferrine (b) concentration.

Furthermore, there were no differences in lysozyme (Noise: 17.65 ± 2.38 HELML, Silence: 32.14 ± 12.55 HELML; p-value = 0.24) and ovotransferrin concentration (Noise: 5.97 ± 1.00 mg/ml, Silence: 6.60 ± 0.62 mg/ml; p-value = 0.70) between the two groups.

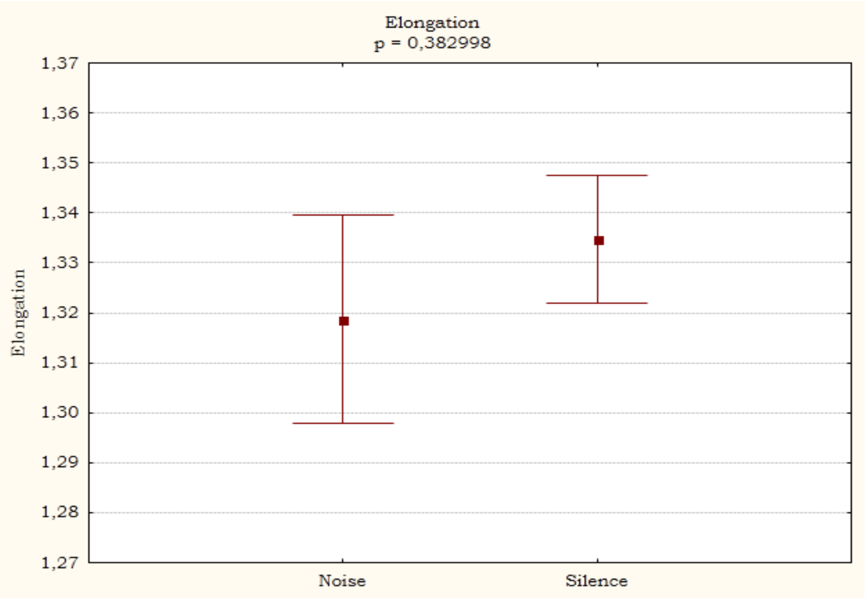


Figure 40 - Difference in elongation between two groups.

Finally, egg elongation did not differ between females of group "Noise" and females of groups "Silence" (Noise: 1.32 ± 0.02, Silence: 1.33 ± 0.02; p-value = 0.38).

Difference between first and second cycle

CSD

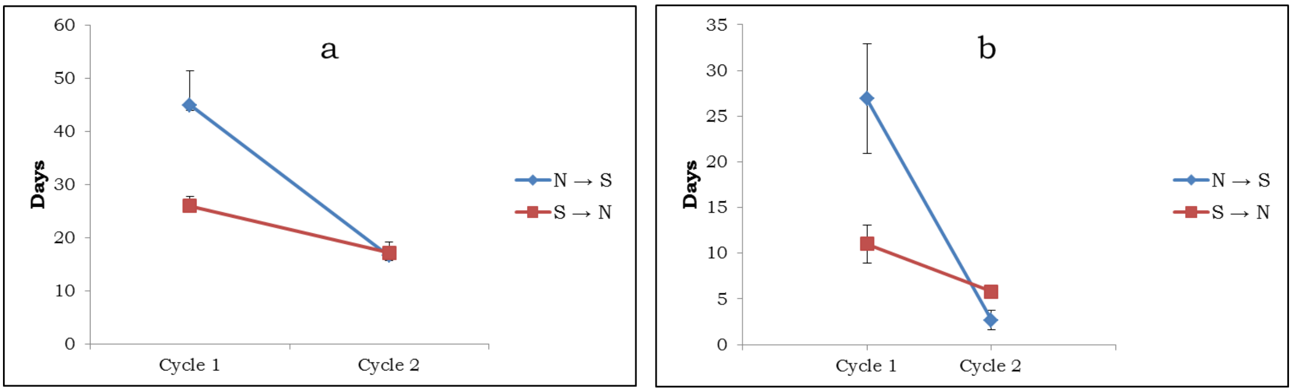


Figure 41 - In line chart a are represented the days of test for the two experimental groups into the two cycles of reproduction; in line chart b delays between the first presentation of the stimulus and the first CSD are represented. N → S is the group where the females heard in the first cycle the male song on the noise and in the second on the silence; S → N is the group where the females heard in the first cycle the male song on the silence and in the second on the noise (legend also applies to the following charts).

Figure 41 shows a significant difference between the days of test in cycle 1 and in cycle 2 for both two experimental groups, and the delay between passage to LD and first CSD in cycle 1 and in cycle 2 for both two experimental groups:

- Days of test (Figure 41a):
    - N → S: Cycle 1: 45 ± 6.5 days, Cycle 2: 16.7 ± 1.6 days; p-value = 0.001;
    - S → N: Cycle 1: 26 ± 1.8 days, Cycle 2: 17.2 ± 2.0 days; p-value = 0.022;
- Delay between the first presentation of the stimulus and the first CSD (Figure 41b):
    - N → S: Cycle 1: 27 ± 6.0 days, Cycle 2: 2.7 ± 1.1 days; p-value = 0.006;
    - S → N: Cycle 1: 11 ± 2.1 days, Cycle 2: 5.8 ± 0.7 days; p-value = 0.043.

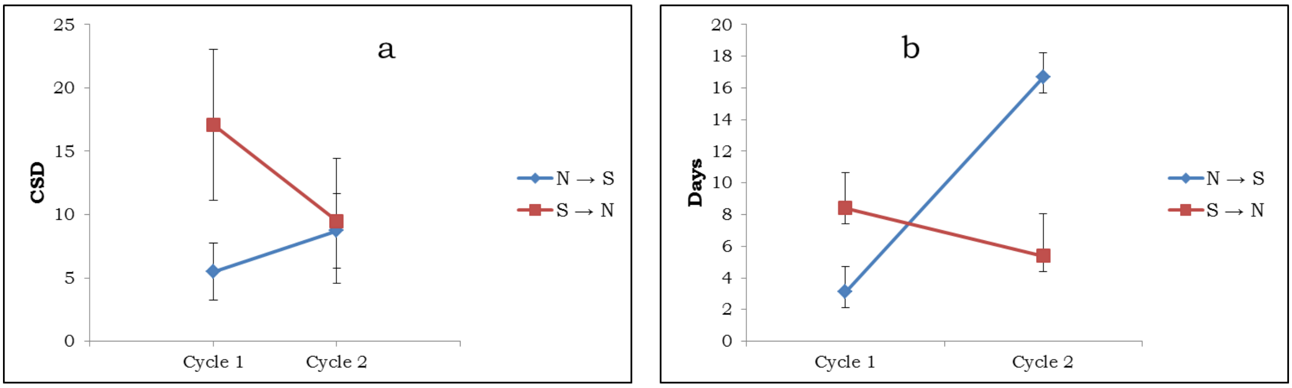


Figure 42 - Line chart a: CSD number of the two experimental groups into the two cycles of reproduction. Line chart b: days when there were CSD.

The comparisons of the number of CSD did by two groups in cycle 1 and in cycle 2 and the number of the days when a CSD show the following results:

- CSD number (Figure 42a):
  - N → S: Cycle 1: 5.5 ± 2.3 CSD, Cycle 2: 8.7 ± 3.0 CSD; p-value = 0.11;
  - S → N: Cycle 1: 17.1 ± 5.9 CSD, Cycle 2: 9.5 ± 5.0 CSD; p-value = 0.022;
- Number of days with CSD (Figure 42b):
  - N → S: Cycle 1: 3.1 ± 1.6 days, Cycle 2: 16.7 ± 1.6 days; p-value < 0.001;
  - S → N: Cycle 1: 8.4 ± 2.2 days, Cycle 2: 5.4 ± 2.7 days; p-value < 0.001.

Eggs

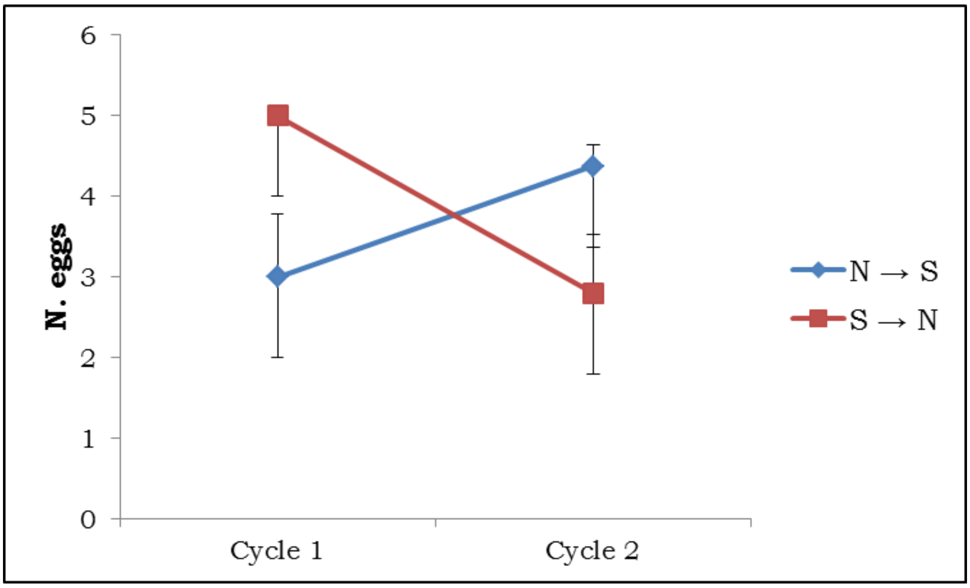


Figure 43 - Line chart of the number of eggs laid by two experimental groups into the two cycles of reproduction.

Figure 43 points out the number of eggs laid by the females of two experimental groups into the two cycles of reproduction. In detail:

- N → S: Cycle 1: 3.0 ± 0.8 eggs, Cycle 2: 4.4 ± 0.3 eggs; p-value = 0.66;
- S → N: Cycle 1: 5.0 ± 0.1 eggs, Cycle 2: 2.8 ± 0.7 eggs; p-value = 0.040.

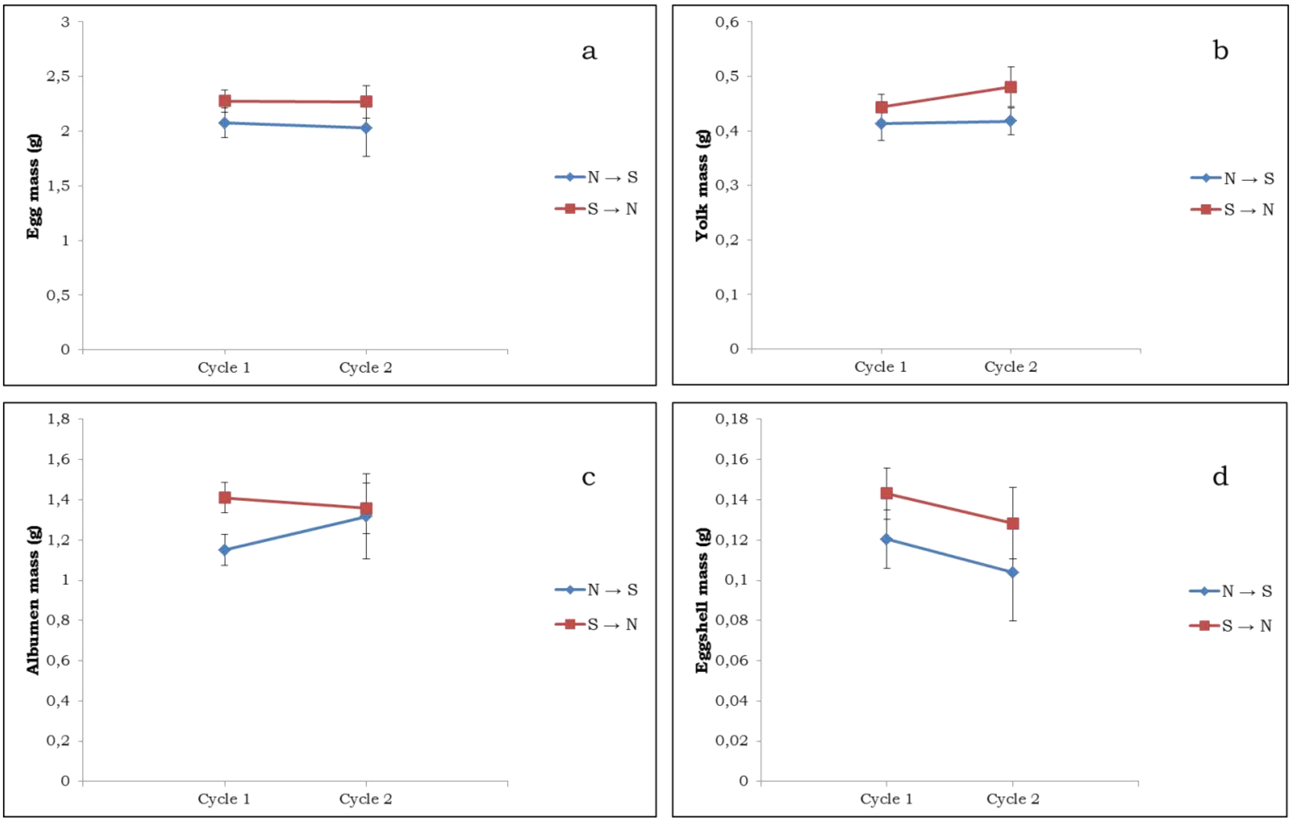


Figure 44 - Line chart a: egg mass of the eggs laid by the females of two experimental groups into the two cycles of reproduction. Line chart b: yolk mass of the eggs laid by the females of two experimental groups into the two cycles of reproduction. Line chart c: albumen mass of the eggs laid by the females of two experimental groups into the two cycles of reproduction. Line chart d: eggshell mass of the eggs laid by the females of two experimental groups into the two cycles of reproduction.

The comparisons of the egg mass and the macro-components mass of the eggs laid by two groups in cycle 1 and in cycle 2 show the following results:

- Egg mass (Figure 44a):
    - N → S: Cycle 1: 2.08 ± 0.13 g, Cycle 2: 2.03 ± 0.26 g; p-value = 0.59;
    - S → N: Cycle 1: 2.27 ± 0.10 g, Cycle 2: 2.27 ± 0.15 g; p-value = 0.28;
- Yolk mass (Figure 44b):
    - N → S: Cycle 1: 0.41 ± 0.03 g, Cycle 2: 0.42 ± 0.02 g; p-value = 0.53;
    - S → N: Cycle 1: 0.44 ± 0.02 g, Cycle 2: 0.48 ± 0.04 g; p-value = 0.023;
- Albumen mass (Figure 44c):
    - N → S: Cycle 1: 1.15 ± 0.08 g, Cycle 2: 1.32 ± 0.21 g; p-value = 0.16;
    - S → N: Cycle 1: 1.41 ± 0.08 g, Cycle 2: 1.36 ± 0.13 g; p-value = 0.58;
- Eggshell mass (Figure 44d):
    - N → S: Cycle 1: 0.12 ± 0.02 g, Cycle 2: 0.10 ± 0.02 g; p-value = 0.019;
    - S → N: Cycle 1: 0.14 ± 0.01 g, Cycle 2: 0.13 ± 0.02 g; p-value = 0.31.

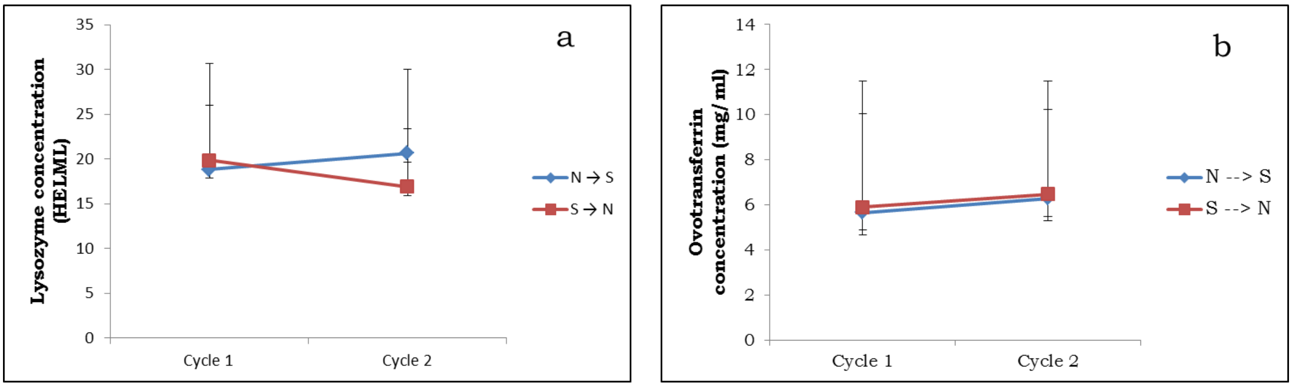


Figure 45 - Line chart a: lysozyme concentration in albumen of the eggs laid by the females of two experimental groups into the two cycles of reproduction. Line chart b: ovotransferrin concentration in albumen of the eggs laid by the females of two experimental groups into the two cycles of reproduction.

Finally, also the concentration of two major components of the albumen, the lysozyme and the ovotransferrin have been investigated.
In detail:

- Lysozyme concentration (Figure 45a):
  - N → S: Cycle 1: 18.84 ± 7.15 HELML, Cycle 2: 20.67 ± 9.39 HELML; p-value = 0.23);
  - S → N: Cycle 1: 19.83 ± 10.79 HELML, Cycle 2: 16.94 ± 6.47 HELML; p-value = 0.52;
- Ovotransferrin concentration (Figure 45b):
  - N → S: Cycle 1: 5.66 ± 4.15 mg/ml, Cycle 2: 6.29 ± 5.85 mg/ml; p-value = 0.47;
  - S → N: Cycle 1: 5.91 ± 3.76 mg/ml, Cycle 2: 6.48 ± 5.21 mg/ml; p-value = 0.74.

Differences between "Silence" and "Noise" groups

	Coefficient (SE)	F	P
First cycle			
Egg mass (n = 47)			
Condition (Noise)	-0.188 (0.039)	23.161	**<0.001**
Laying order	0.001 (0.014)	0.001	0.98
Yolk mass (n = 47)			
Condition (Noise)	-0.022 (0.013)	3.059	0.09
Laying order	-0.012 (0.004)	7.659	**0.008**
Albumen mass (n = 46)			
Condition (Noise)	-0.259 (0.050)	26.990	**<0.001**
Laying order	0.030 (0.018)	2.812	0.10
Eggshell mass (n = 46)			
Condition (Noise)	-0.022 (0.005)	15.693	**<0.001**
Laying order	-0.003 (0.002)	2.067	0.16
Lysozyme concentration (n = 34)			
Condition (Noise)	-3.983 (5.642)	0.498	0.49
Laying order	-0.315 (1.608)	0.038	0.85
Ovotransferrin concentration (n = 34)			
Condition (Noise)	-2.007 (2.161)	0.862	0.36
Laying order	1.317 (0.494)	0.314	**0.012**

Second cycle			
Egg mass (n = 47)			
Condition (Noise)	0.325 (0.097)	11.200	**0.002**
Laying order	0.033 (0.034)	0.961	0.33
Yolk mass (n = 47)			
Condition (Noise)	0.053 (0.021)	6.605	**0.014**
Laying order	-0.006 (0.007)	0.645	0.43
Albumen mass (n = 43)			
Condition (Noise)	0.147 (0.070)	4.450	**0.041**
Laying order	0.055 (0.024)	5.066	**0.030**
Eggshell mass (n = 45)			
Condition (Noise)	0.025 (0.011)	12.443	**0.001**
Laying order	-0.001 (0.002)	0.221	0.64
Lysozyme concentration (n = 33)			
Condition (Noise)	-5.434 (9.026)	0.362	0.55
Laying order	-1.133 (2.647)	0.183	0.67
Ovotransferrin concentration (n = 33)			
Condition (Noise)	-0.561 (1.664)	0.114	0.74
Laying order	-1.174 (0.580)	4.3092	0.052

Table 8 - Multivariate mixed-model analysis of egg mass and egg macro-components in two cycle of reproduction, with condition (noise-silence) as factor, and position in the laying order of the focal egg as covariates. In the models, the female identity was inserted as a random factor.

First cycle: difference in CSD

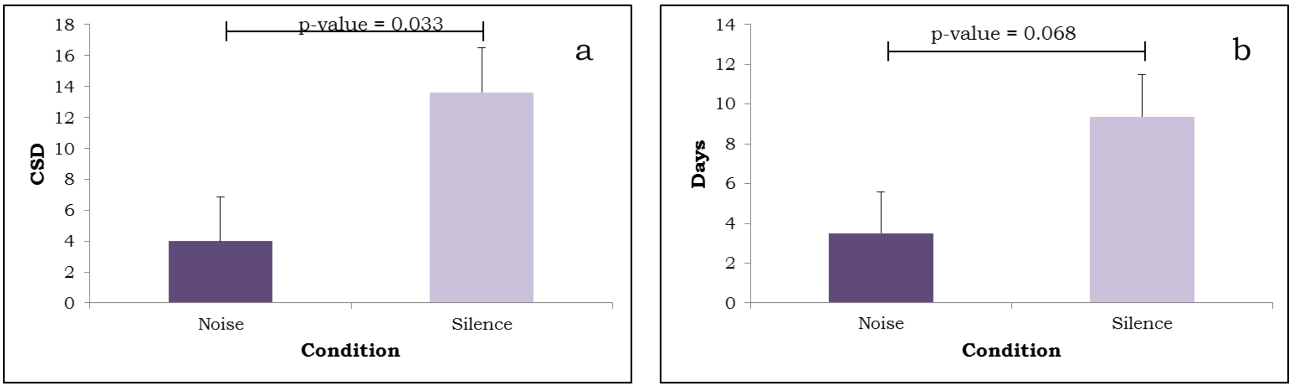


Figure 46 - Histogram a: difference in CSD number in first cycle between females that heard the male song on noise (Noise) and females that heard it on silence (Silence). Histogram b: difference in days with CSD in first cycle between females that heard the male song on noise (Noise) and females that heard it on silence (Silence).

Figure 46a (and Table 8) illustrates a statistical difference between females of group "Noise" and females of group "Silence" in number of CSD: the first did less CSD than the second (Noise: 4.0 ± 2.9 CSD, Silence: 13.6 ± 2.9 CSD; p-value = 0.033).

Instead, in Figure 46b (and Table 8) there is no difference between the two groups for what concerns the day when a CSD was detected (Noise: 3.5 ± 2.1 days, Silence: 9.38 ± 2.1 days; p-value = 0.033).

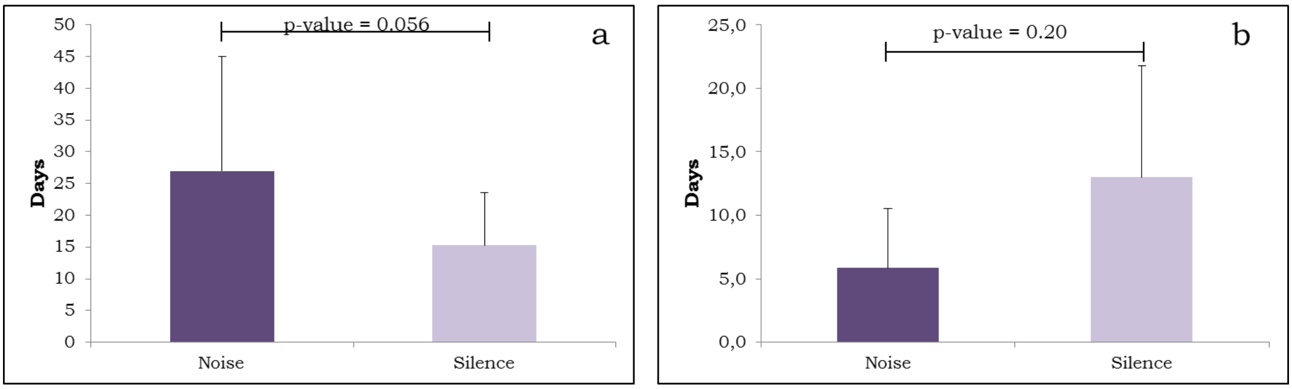


Figure 47 - Histogram a: difference in delay between the first presentation of the stimulus and the first CSD in first cycle between two groups. Histogram b: difference in delay between first CSD and first egg laid in first cycle between two groups.

Both for delay between first stimulus and first CSD and delay between first CSD and first egg, there wasn't any significant difference between groups:

- Delay between the first presentation of the stimulus and the first CSD (Figure 47a and Table 8): Noise: 27.0 ± 18.0 days, Silence: 15.3 ± 8.3 days; p-value = 0.056);
- Delay CSD-first egg (Figure 47b and Table 8): Noise: 5.8 ± 4.7 days, Silence: 13.0 ± 8.8 days; p-value = 0.20).

Second cycle: difference in CSD

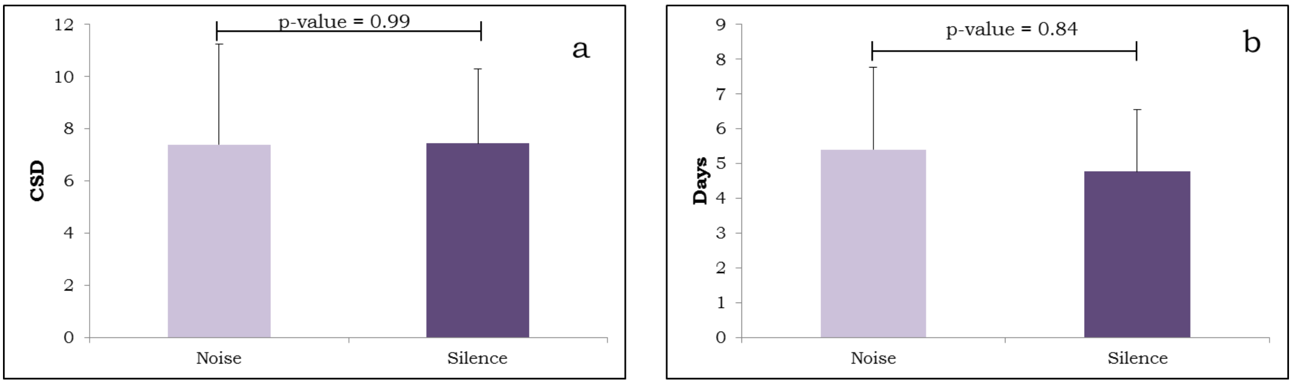


Figure 48 - Histogram a: difference in CSD number in second cycle between two groups. Histogram b: difference in days with CSD in second cycle between two groups.

Figure 48 (and Table 8) shows no difference between females of group "Noise" and females of group "Silence" in number of CSD (Noise: 7.4 ± 3.8 CSD, Silence: 7.4 ± 2.9 CSD; p-value = 0.99) and day when a CSD was observed (Noise: 5.4 ± 2.4 days, Silence: 4.8 ± 1.8 days; p-value = 0.84).

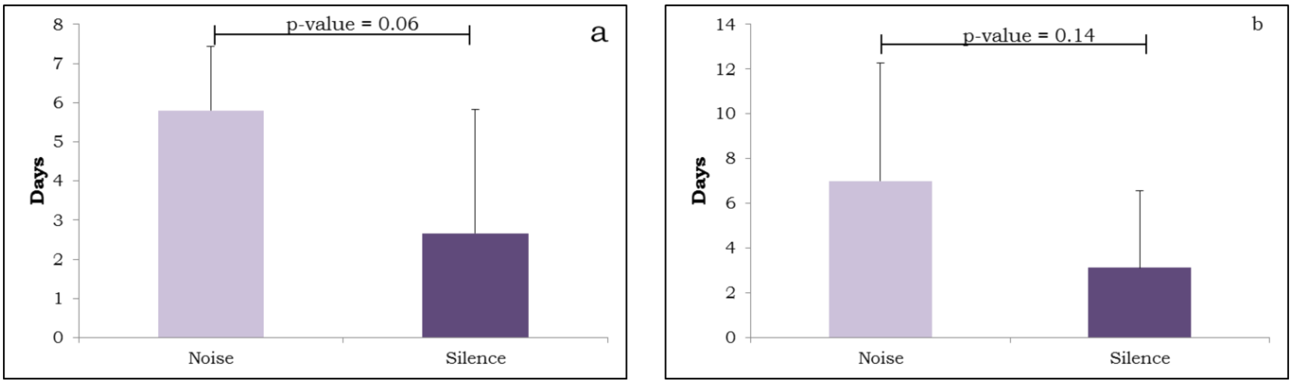


Figure 49 - Histogram a: difference in delay between the first presentation of the stimulus and the first CSD in second cycle between two groups. Histogram b: difference in delay between first CSD and first egg in second cycle between two groups.

Both for delay between first stimulus presentation and first CSD and delay between first CSD and first egg, there were no significant differences:

- Delay between the first presentation of the stimulus and the first CSD (Figure 49a and Table 8): Noise: 5.8 ± 1.6 days, Silence: 2.6 ± 3.2 days; p-value = 0.06);
- Delay CSD-first egg (Figure 49b and Table 8): Noise: 7.0 ± 5.3 days, Silence: 3.1 ± 3.4 days; p-value = 0.14).

First cycle: differences in egg characteristics

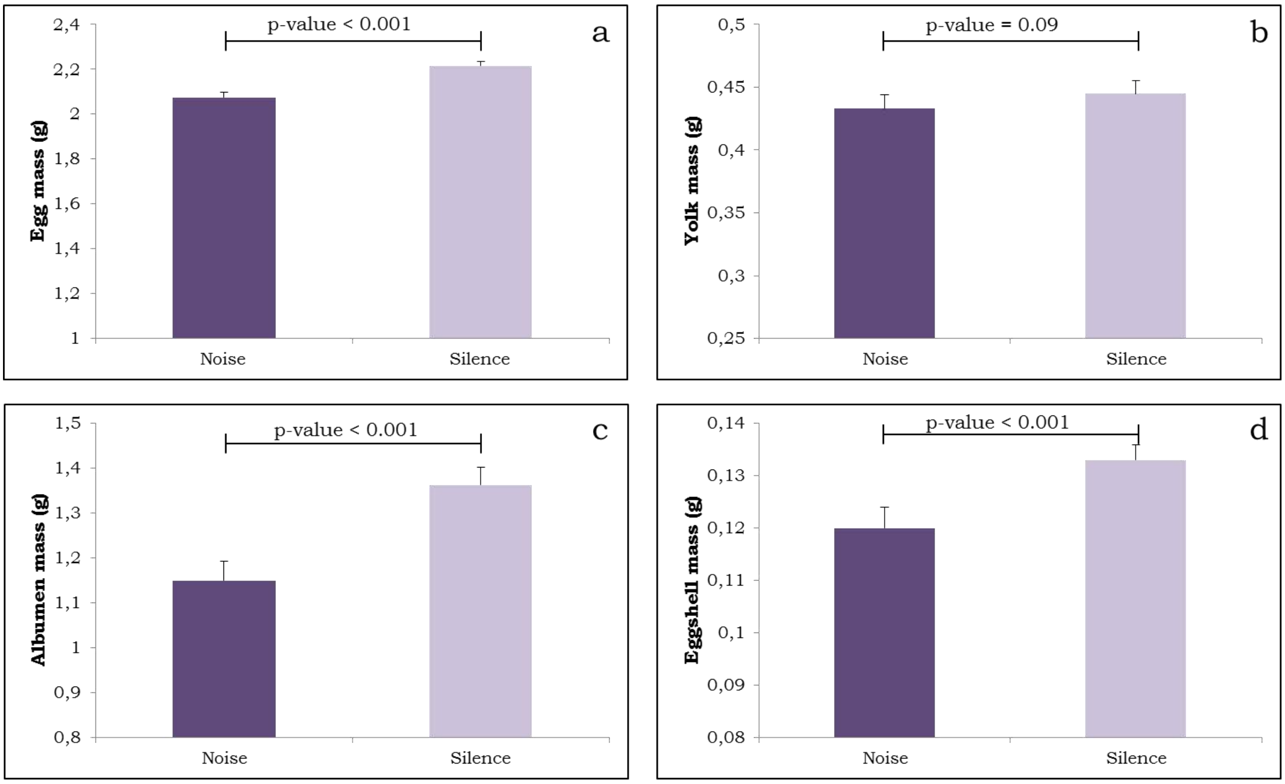


Figure 50 - Histogram a: difference in egg mass in first cycle between two groups. Histogram b: difference in yolk mass in first cycle between two groups. Histogram c: difference in albumen mass in first cycle between two groups. Histogram d: difference in eggshell mass in first cycle between two groups.

Figure 50 points out statistically significant differences between females of group "Noise" and females of group "Silence" in egg, albumen, and eggshell mass:

- Egg mass (Figure 50a and Table 8): Noise: 2.08 ± 0.02 g, Silence: 2.22 ± 0.02 g; p-value < 0.001);
- Albumen mass (Figure 50c and Table 8): Noise: 1.15 ± 0.04 g, Silence: 1.36 ± 0.03 g; p-value < 0.001);
- Eggshell mass (Figure 50d and Table 8): Noise: 0.12 ± 0.004 g, Silence: 0.13 ± 0.003 g; p-value < 0.001).

Instead, there was no significant difference in yolk mass (Figure 50d and Table 8 - Noise: 0.43 ± 0.01 g, Silence: 0.45 ± 0.01 g; p-value = 0.09).

In short, females of group "Silence" laid eggs heavier with more albumen and eggshell than those of group "Noise".

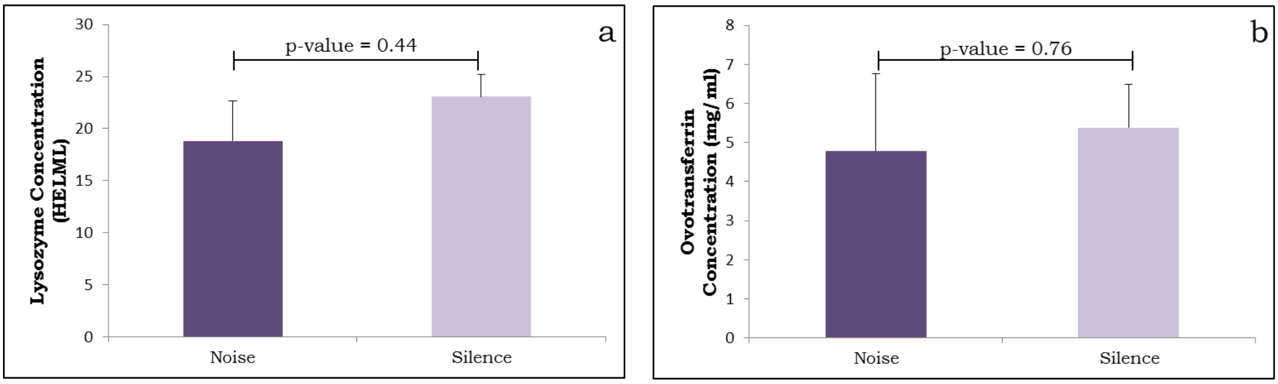


Figure 51 - Histogram a: difference in lysozyme concentration in first cycle between two groups. Histogram b: difference in ovotransferrin concentration in first cycle between two groups.

For what concerns two of the major components of the albumen, no statistically significant differences were found between two experimental groups:

- Lysozyme concentration (Figure 51a and Table 8): Noise: 18.84 ± 3.79 HELML, Silence: 23.07 ± 2.13 HELML; p-value = 0.44);
- Ovotransferrin concentration (Figure 51b and Table 8): Noise: 4.79 ± 1.96 mg/ml, Silence: 5.38 ± 1.10 mg/ml; p-value = 0.76).

Second cycle: differences in egg characteristics

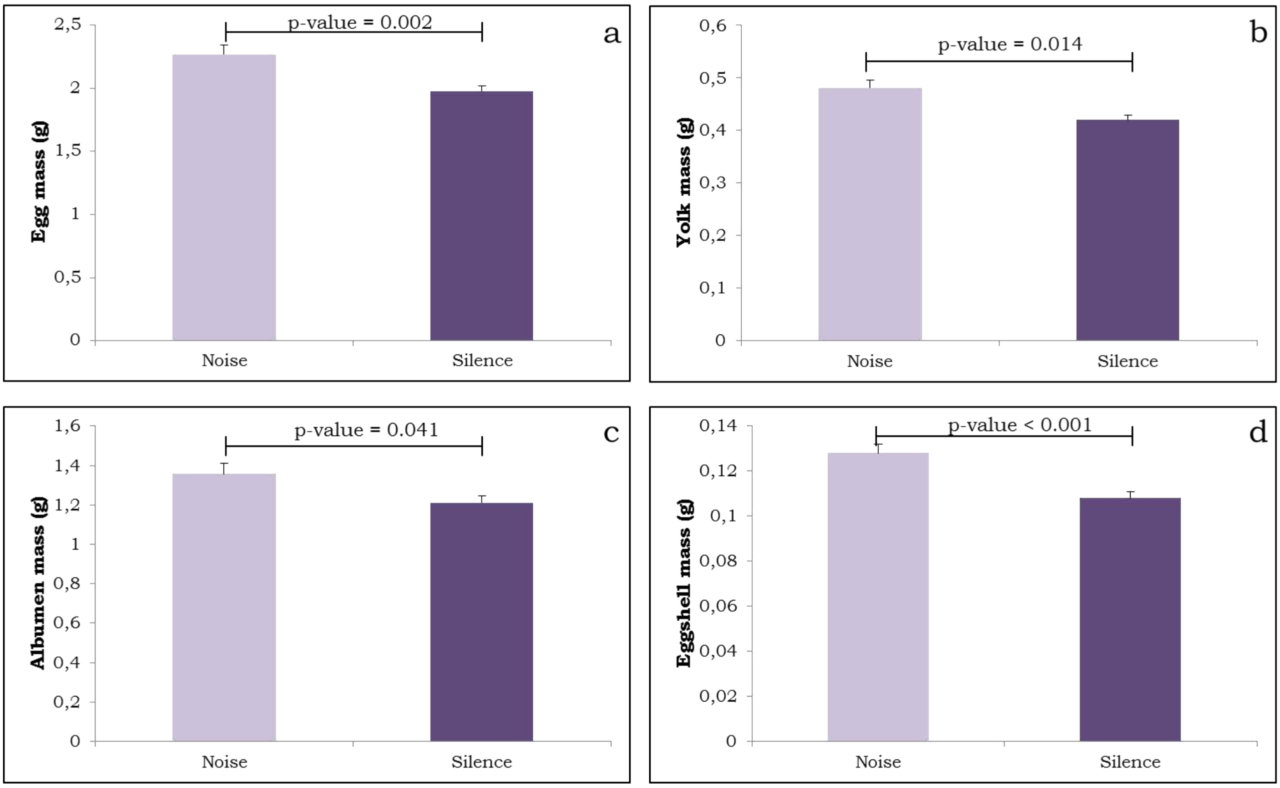


Figure 52 - Histogram a: difference in egg mass in second cycle between two groups. Histogram b: difference in yolk mass in second cycle between two groups. Histogram c: difference in albumen mass in second cycle between two groups. Histogram d: difference in eggshell mass in second cycle between two groups.

Figure 52 illustrates statistically significant differences between females of "Noise" group and females of "Silence" group in egg, yolk, albumen, and eggshell mass:

- Egg mass (Figure 52a and Table 8): Noise: 2.27 ± 0.08 g, Silence: 1.98 ± 0.05 g; p-value = 0.002);
- Yolk mass (Figure 52b and Table 8): Noise: 0.02 ± 4.7 g, Silence: 0.42 ± 0.01 g; p-value = 0.014);
- Albumen mass (Figure 52c and Table 8): Noise: 1.36 ± 0.05 g, Silence: 1.21 ± 0.03 g; p-value = 0.041);
- Eggshell mass (Figure 52d and Table 8): Noise: 0.13 ± 0.004 g, Silence: 0.11 ± 0.003 g; p-value < 0.001).

Females of "Noise" group laid heavier eggs, with more yolk, albumen and eggshell than females of "Silence" group.

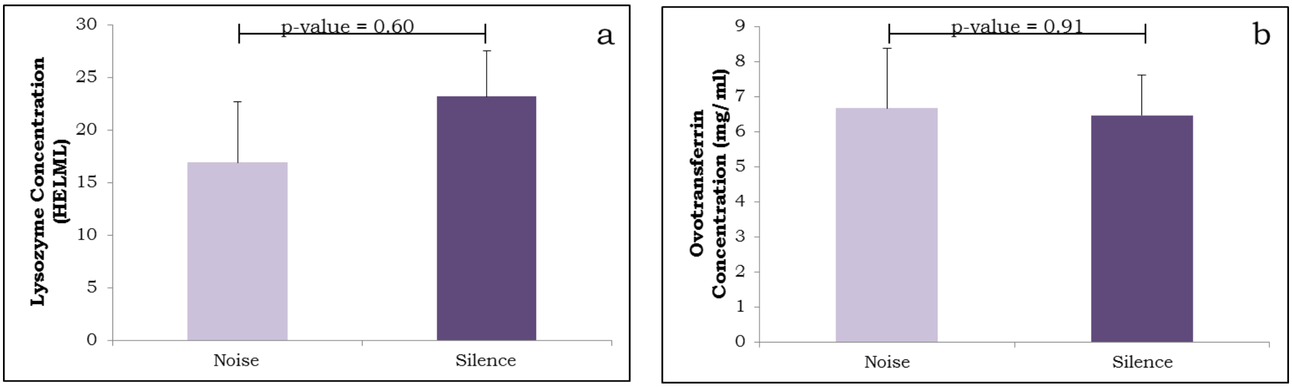


Figure 53 - Histogram a: difference in lysozyme concentration in second cycle between two groups. Histogram b: difference in ovotransferrin concentration in second cycle between two groups.

Both lysozyme and ovotransferrin concentration did not differ significantly:

- Lysozyme concentration (Figure 53a and Table 8): Noise: 16.94 ± 5.77 HELML, Silence: 23.21 ± 4.36 HELML; p-value = 0.60);
- Ovotransferrin concentration (Figure 53b and Table 8): Noise: 6.68 ± 1.73 mg/ml, Silence: 6.47 ± 1.15 mg/ml; p-value = 0.91).

~

Discussion

~

In the field of noise effects on the life-history traits of the birds, very few studies have been performed until today with regard to the success of mating, the female choice and the impact of this noise on the reproductive success (Habib et al., 2007; Swaddle & Page, 2007; Halfwerk et al., 2011a; 2011b; Huet des Aunay, 2011; Schroeder et al., 2012).

To experimentally test for such trade-offs, in this laboratory study females canary heard the male song on two different condition: with noise and with silence. The experience took two cycle of reproduction, reversing the stimuli between the first and the second cycle. Birds were all of the same age to avoid possible age effects (Bonneaud et al., 2003; Cabezas-Diaz et al., 2005), and food was provided *ad libitum* to reduce environment-related variations. The behavioural responses of the female to the song and the following maternal investment have been evaluated to understand if and how noisy condition influences them.

To estimate behavioural responses, the Copulation Solicitation Display, days between the first presentation of the stimulus and the first CSD, days when CSD have been observed, and days between first CSD and first egg laid have been evaluated.

Results (joining the condition of noise and silence in two reproduction cycles) show that females in noise condition were tested for more days (because they delayed the responses), did first CSD later, did less CSD and for less days than females in silence condition. These results are concordant with works of Habib et al. (2007) and Huet des Aunay (2011). In the first, it's clearly proved how in populations of golden crown ovenbird living in areas subject to noise the pairings of males decrease compared to other places less disturbed; for the authors, this can be due to a reduction of propagation distance of the song and/or to a distorted perception of the signal by the female, that for this judges a male of low quality. Also The work of Huet des Aunay (2011) has highlighted how females of common canary decreased their CSD (King & West 1977; Searcy & Marler 1981; Searcy 1992), when they came in contact with songs masked by city noise. Therefore, the results obtained in this present experiment are probably due to a mask of the male song that could reduce perception of the female or that could induce the female to think of being faced to a male of low quality.

Considering the same females in two cycle of reproduction, the results show that females that have heard song in noise condition in first cycle and in silence in the second had a decrease in test days and days between the first presentation of the stimulus and the first CSD, and an increase in days when CSD have been observed. Females that experimented song in silence in the first cycle and in noise in the second had a decrease in test days, days between the first presentation of the stimulus and the first CSD, CSD number and days when CSD have been observed. Regarding to the reducing of the test days and days between the first presentation of the stimulus and the first CSD in both the situations, a first consideration is that the second cycle is faster than the first anyhow. In natural conditions has been seen that the laying of the eggs in second cycle starts earlier than in first cycle. For what concerns the number of CSD and the day when CSD have been observed, these results confirm that in noisy condition females have problems to hear the song of the male (Huet des Aunay, 2011).

Finally for the behavioural responses, comparing the two groups of females in first cycle and then in the second, in first a higher number of CSD in group silence has been observed, while in the second no statistically significant differences have been found. Also this result confirms what seen in the other analyses.

Concluding the discussion on a possible influence of the noise in the sexual selection, these results are in line with the idea that noise disturbs the inter-sexual communication during the reproduction season (Habib et al., 2007; Huet des Aunay, 2011).

The second aim of present work is to understand if these problems in sexual selection affected also the subsequent period of reproduction. To investigate this, all the eggs laid by the canary females were collected, all the components were weighted, and lysozyme and ovotransferrin concentration [two important molecules related to the maternal effects (e.g., D'Alba et al., 2010)] have been analyzed.

Comparing "noise" and "silence" groups, a higher dimension of the clutches has been observed in the second one. No differences have been found in mass of the macro-components of the eggs and in lysozyme and ovotransferrin concentrations. This results is confirmed also by the analyses on the females that heard the song on silence in the first cycle of reproduction and noise in the second; they reported a statistically significant diminution in the eggs number. To actual knowledge, only two previous studies have investigated correlation between noise and reproductive success (Halfwerk et al., 2011b; Schroeder et al., 2012). In particular, Halfwerk et al. (2011b) found an impact of traffic noise on avian reproductive success manifested by smaller clutches and fewer fledged chicks in noisier areas. They explicated four possible mechanisms, all related to signal masking to some degree, which could explain how anthropogenic noise has a negative impact on avian reproductive success. The most important for present study is the first that is related to interference with acoustic assessment of mate quality. Female birds are known to rely on song in assessment of male quality and subsequent investment decisions (Holveck & Riebel 2009). High noise levels could reduce perceived song quality and cause females to breed later, allocate less energy to the eggs or provide less maternal care to the chicks. The data of Halfwerk's work show that spectral overlap between noise and great tit song best predicts patterns in clutch size, suggesting that noise may indeed interfere with song-based assessment of male quality and subsequently lower female investment (Halfwerk et al., 2011b). This explanation could fit with the results of the present study on dimension of clutches, even if it is not reflected in investment in the macro-components of the eggs (yolk, albumen, etc.) and in lysozyme and ovotransferrin. Probably the mass of the macro-components it's more linked with females condition, genetics or particular environmental situation (e.g., temperature – Cucco et al., 2009), and the noise doesn't affect these traits. This idea finds partial confirmation in the results obtained correlating the two groups in the two cycle of reproduction: the females that laid heavier eggs with more albumen and eggshell during the experience with song on silence (in rapport to the other groups in the same cycle but with song on noise) continued to laid "better" eggs also in the second cycle, when they are submitted to song on noise. These results could find explanation or in the genetic quality of the females or in a long effect of the noise/silence: females that heard song on noise in the first cycle continue to laid poor eggs and females that heard song on silence continue to laid good eggs. In conclusion, this study shows a negative influence of the noise both in sexual selection (longer time for choice) and in reproductive success (smaller clutches).

Present study is a first approach to this new field of the behavioural ecology and gives some indications for future works. Analyses on other components of the maternal effects (e.g., testosterone) should be performed to understand if noise has influence on hormonal or other components.

Finally, it could be interesting to study the characteristics of the offspring born in disturbed environments and also to examine communication between them and the parents to confirm in controlled condition what has been found in the wild situation by Schroeder et al. (2012).

Chapter IV: Effects of immune challenge and supernormal clutch production on egg quality in the red-legged partridge.

Chapter IV – Abstract

Life-history theory provides an evolutionary framework that analyse how organisms allocate their resources in relation to different priorities related to survival and reproduction (Stearns, 1992). An important chapter is on the trade-offs between immune function, health conditions and resources to invest in reproduction (Sheldon & Verhulst, 1996).

There are studies showing that the investment in immunity against parasites reduces parental investment in terms of clutch size, mass of eggs laid or allocated components (Råberg et al., 2000; Burnham et al., 2003; Uller et al., 2006; Marzal et al., 2007; Viscione et al., 2008; Cucco et al., 2010).

Another factor that can decrease the quality of the eggs is a supernormal clutch production caused, for example, by the disappearance of eggs already laid.

From these assumptions the study presented in this chapter is born; here, two experimental groups (composed by females immunized by NDV virus vaccination and females not immunized) were compared. In addition to immunization, a supernormal clutch production has been stimulated by the disappearance of eggs just laid.

No results in terms of health of females or egg quality was found in relation to vaccination, while changes were observed in the last eggs laid. In particular, there has been a decrease in terms of egg weight, width, yolk and albumen weight, and concentration of lysozyme. The first parameters shown an inverse U-shaped pattern, in which the eggs of best quality are those in the middle of the order of deposition, while with regard to the lysozyme a linear decrease was found.

The lower quality of the last eggs laid can be explained by a progressive energy loss by mother.

In conclusion, this study shows that females subjected to two different types of stress do not seem to have problems with an increased immune activity, while they suffer a progressive energy loss due to an over-production of eggs.

Key-words: Egg quality, laying order, maternal investment, NDV vaccine challenge, supernormal clutch.

Capitolo IV – Riassunto

La teoria della *life-history* fornisce un quadro evoluzionistico che analizza come gli organismi allochino le loro risorse tenendo conto delle diverse priorità inerenti la sopravvivenza e la riproduzione (Stearns, 1992). Un capitolo importante è quello sui *trade-offs* fra la funzione immunologica, le condizioni di salute e le risorse da investire nella riproduzione (Sheldon & Verhulst, 1996).

Vi sono studi che dimostrano come l'investimento nell'immunità dai parassiti vada a ridurre quello parentale in termini di dimensioni della covata, massa delle uova deposte o componenti allocate (Råberg et al., 2000; Burnham et al., 2003; Uller et al., 2006; Marzal et al., 2007; Viscione et al., 2008; Cucco et al., 2010).

Un altro fattore che può diminuire la qualità delle uova è la produzione di un sovrannumero di queste provocato, per esempio, dalla scomparsa di quelle già deposte.

Da questi presupposti nasce lo studio presentato in questo capitolo, dove vengono comparati due gruppi sperimentali, uno composto da femmine di pernice rossa sottoposte ad una vaccinazione per il virus del NDV e l'altro da femmine non vaccinate. Oltre all'immunizzazione è stata stimolata la produzione di un sovrannumero di uova tramite il prelevamento delle stesse appena venivano deposte.

Nessun risultato in termini di salute delle femmine o di qualità delle uova è stato trovato in relazione alla vaccinazione, mentre variazioni sono state apprezzate nelle ultime uova deposte. In particolare si è assistito ad una diminuzione in termini di peso, larghezza, peso di tuorlo e albume e concentrazione di lisozima. I primi parametri mostrano un andamento ad U rovesciata, dove le uova di miglior qualità si trovano a metà dell'ordine di deposizione, mentre per quanto riguarda il lisozima si nota una diminuzione lineare.

La minor qualità delle ultime uova deposte può essere spiegata con una progressiva perdita di energie da parte della madre.

In conclusione, questo studio mostra come femmine sottoposte a due tipi di stress diverso non sembrano avere problemi legati ad una aumentata attività immunitaria mentre subiscono una perdita progressiva di energia dovuta ad una sovra-produzione di uova.

Parole-chiave: Qualità dell'uovo, ordine di deposizione, investimento materno, vaccino NDV, sovra-produzione di uova.

Chapitre IV – Résumé

La théorie de l'histoire de vie fournit un cadre évolutif dans lequel on analyse comment les organismes allouent leurs ressources en tenant compte des priorités différentes inhérent la survie et la reproduction (Stearns, 1992). Un chapitre important est sur le compromis entre la fonction immunitaire, la santé et les ressources nécessaires pour la reproduction (Sheldon & Verhulst, 1996).

Il y a des études qui montrent que l'investissement dans l'immunité contre les parasites va à réduire l'investissement parental en termes de taille de la couvée, masse d'œufs pondus ou composants alloués (Råberg et al., 2000 ; Burnham et al., 2003 ; Uller et al., 2006 ; Marzal et al., 2007 ; Viscione et al., 2008 ; Cucco et al., 2010).

Un autre facteur qui peut diminuer la qualité des œufs est la production d'une nombre excédentaire des œufs causée, par exemple, par la disparition de celles qui sont déjà déposées.

A partir de ces principes l'étude présentée dans ce chapitre est né, où on a comparé deux groupes expérimentaux, l'un composé par femelles de perdrix rouge soumis à une vaccination contre le virus de NDV et l'autre par femelles pas vaccinées. En plus, aussi la production d'un surplus d'œufs a été stimulé enlevant les mêmes juste après la ponte.

Aucun résultat en termes de santé des femmes ou de qualité d'œufs a été trouvé lié à la vaccination, alors que des changements ont été appréciés dans les derniers œufs pondus. En particulier, il y avait une diminution en termes de poids, largeur, poids du jaune et de l'albumen et de la concentration de lysozyme. Les premiers paramètres suivent une tendance à U inversé, où les œufs de meilleure qualité sont situés au milieu de l'ordre de dépôt ; pour ce qui concerne le lysozyme une diminution linéaire a été trouvée.

La baisse de la qualité des derniers œufs peut être explique par une perte progressive de l'énergie de la mère.

En conclusion, cette étude montre que les femelles soumises à deux types de contraintes ne semblent pas avoir des problèmes avec une augmentation de l'activité immunitaire, mais elles subissent une perte progressive de l'énergie due à une surproduction d'œufs.

Mots clés : Qualité de l'œuf, ordre de dépôt, investissement maternel, NDV vaccin, surproduction d'œufs.

~

Introduction

~

Life-history theory provides a theoretical evolutionary framework in which to analyse how organisms allocate their resources in relation to different priorities for survival and reproduction (Stearns, 1992). All animals have evolved optimal allocation strategies to counteract the aggression of parasites, organisms able to reduce the fitness of their hosts (Price, 1980). Adaptive defence against parasites is mainly down to the immune apparatus (Boughton et al., 2011), a complex and heterogeneous system whose maintenance can be energetically costly and resource-limited (Loye & Zuk, 1991). In this light, life-history theory predicts the existence of trade-offs among immune function, body condition, and reproductive allocation (Sheldon & Verhulst, 1996).

There is experimental evidence showing that an investment in immunity may impair reproduction. In oviparous species, immune-challenged females may lower the frequency of relaying (Ilmonen et al., 2000), reduce brood size (Råberg et al., 2000; Marzal et al., 2007) or decrease reproductive investment in terms of egg mass (Uller et al., 2006; Cucco et al., 2010). In the house martin, *Delichon urbica*, reduced egg androgen deposition has been observed in response to an immune system challenge (Gil et al., 2006), while an immune challenge altered the yolk fatty acid composition in the chicken, *G. gallus* (Burnham et al., 2003; Viscione et al., 2008). In some cases, immune-challenged females showed a worsening of their health condition (Costantini & Møller, 2009), accompanied by a decrease in egg quality (Cucco et al., 2010). Rutkowska et al. (2012) examined the relationship between the strength of maternal immune response and offspring viability and immune response in captive zebra finches *T. guttata*. In three independent experiments, the females were challenged with sheep red blood cells and they found offspring survival until adulthood to be negatively related to maternal antibody titres. That effect was consistent among all experiments. This is the first study showing the relationship between the strength of the immune response and between-generational fitness costs in birds (Rutkowska et al., 2012).

So, the reproductive costs associated with the upregulation of immunity have been well-documented and constitute a fundamental trade-off between reproduction and self-maintenance. However, recent experimental work suggests that parents may increase their reproductive effort following immunostimulation as a form of terminal parental investment as prospects for future reproduction decline. Bowers et al. (2012) tested the trade-off and terminal investment hypotheses in a wild population of house wrens (*T. aedon*) by challenging the immune system of breeding females with lipopolysaccharide, a non-lethal antigen. Immunized females showed no evidence of reproductive costs; instead, they produced offspring of higher phenotypic quality, but in a sex-specific manner. Relative to control offspring, sons of immunized females had increased body mass and their sisters exhibited higher cutaneous immune responsiveness to phytohaemagglutinin injection, constituting an adaptive strategy of sex-biased allocation by immune-challenged females to enhance the reproductive value of their offspring. Thus, their results are consistent with the terminal investment hypothesis, and suggest that maternal immunization can induce pronounced transgenerational effects on offspring phenotypes (Bowers et al., 2012).

Some studies have focused on the effects of immune challenge of the mothers in the pregnancy period on the behaviour of the progeny (so, they investigated the long-term effects of this immunization Hava et al., 2006; Taylor et al., 2012). For example, it was seen that mice whose mothers were treated with LPS during pregnancy show less aggression and more social grooming behaviour in adulthood (Hava et al., 2006). Instead, Taylor et al. (2012) hypothesized that prenatal immune activation alters juvenile social play behaviour just as it alters adult behaviour, and their results suggest a way in which prenatal immune activation may differentially affect the development of social behaviour in males and females.

For oviparous animals, clutch production represents a huge investment of energy and reserves in a limited period (Nager, 2006). Females can adaptively modify egg composition through the differential deposition of substances that influence embryo growth and health [maternal effects (Mousseau & Fox, 1998)]. Since the allocation of substances in a clutch is costly (Gil et al., 1999; Pilz et al., 2003), a differential within-clutch allocation can be hypothesized and can be related to the expected fitness of the young (Stearns, 1992). Although, variation of avian eggs is largely attributable to differences among species, eggs can also vary considerably within species (Ricklefs, 1974). Williams et al. (1993) suggested that females allocate nutrients to eggs within a clutch according to the fitness that each egg has by virtue of its position in the laying sequence. Indeed, detailed investigations of intra-clutch variation of egg components in different species have shown a variety of relationships between egg size or components and laying sequence (Kennamer et al., 1997; Lessells et al., 2002). Egg characteristics generally vary with laying order according to species-specific patterns, such as (1) increasing, (2) decreasing or (3) increasing up to the middle eggs and decreasing thereafter (Aparicio, 1999). Furthermore, many bird species ('indeterminate layers') lay additional eggs in response to egg removal (Kennedy, 1991). However, the quality (composition) of the eggs decreases with increasing egg number (Heaney et al., 1998; Nager et al., 2000).

Nidifugous birds are of special interest for the study of female investment in eggs, since they are specialized in a vast nutritional investment in each egg (because they do not feed the chicks after hatching). It has been observed that the eggs of nidifugous birds contain a high amount of yolk (Sotherland & Rahn, 1987; Carey, 1996). Nidifugous birds generally lay large clutches (Ricklefs, 1974), in which the high quality of the egg characteristics must be maintained so as not to decrease the fitness expectancies of propagules (Kennedy, 1991). In this study, the relationship between female health condition and egg quality (total mass, shell, yolk, albumen, and concentration of lysozyme, a substance involved in innate antibacterial immunity) was analysed in the red-legged partridge, *A. rufa*. Birds were bred in captivity to reduce environment-related variations [a discussion of the advantages and disadvantages of experiments in captive vs. wild conditions is given in Boughton et al. (2011)]. Breeding females were subjected to egg removal (inducing a protracted reproductive effort) and to an immune challenge (Newcastle disease virus, NDV). The red-legged partridge is an indeterminate layer that has a naturally high investment in eggs (Cramp & Simmons, 1980; Alonso et al., 2008). Moreover, it is highly susceptible to immune challenge with the NDV vaccine (Geral et al., 1976).

The aim of the present study was to experimentally test the hypothesis of a physiological trade-off among various functions (French et al., 2009), including immune response, self-maintenance, and reproductive output. This work wished to determine whether stimulation of the immune system would impair female condition or egg quality, and if females would be able to maintain egg characteristics despite a supernormal clutch production.

~

Materials and Methods

~

45 pairs (22 in 2008, 23 in 2009) selected from a breeding farm stock in Alessandria, northwest Italy were studied (Cucco et al., 2011a). All birds were one year old and in their first reproductive season. Throughout the year, the birds were maintained in natural light and temperature conditions. The rearing food was a powdered mixture commonly used by aviculturists to provide proper nutrition during egg laying. Food and water were provided *ad libitum*. In the pre-breeding season, partridges were housed in small groups in outdoor aviaries (20 m long × 3 m wide × 2 m high). Groups were exclusively composed of individuals of the same sex, and females and males had only visual or acoustic contacts during this period.

In 2009, two weeks before laying the first egg, breeding partridges were randomly assigned to one of two groups: a vaccinated group (Vaccine09) and a control group (Control09). Females were immunized orally with NDV live vaccine (Bio-Vac NDV 1000 doses made by Fatro in Ozzano Emilia, Italy) in accordance with the procedure of Kiss et al. (2003). Boosters were given three times at two-week intervals. Newcastle disease is highly contagious, prevalent worldwide, and causes severe economic loss to the poultry industry (Alexander, 1997). NDV was chosen because partridges are highly susceptible to experimental infection with NDV (Geral et al., 1976). Moreover, NDV has been shown to induce both humoral and cell-mediated immune responses, and the cell-mediated response is considered important for conferring resistance to velogenic NDV (Marino & Hanson, 1987). In 2008, no vaccination occurred (Control08 group).

The number of days elapsed between vaccination and laying of the first egg was 24.1 ± 3.2 days (mean ± S.E.). Vaccinated and control females did not differ in laying date ($t_{19} = 0.79$, $P = 0.27$).

Measurements on breeding females

Four body, blood, and immune response variables (body mass, erythrosedimentation rate, haematocrit, and immune response to phytohaemagglutinin injection) were measured before the vaccination and before the breeding period began (March). Body mass was measured with an electronic balance (±0.01 g accuracy). Blood was drawn from the brachial vein into 75 ml heparinized capillary tubes to measure the erythrosedimentation rate and haematocrit value. The erythrosedimentation rate is diagnostic of many acute and chronic diseases, including infections and rheumatic and inflammatory diseases (Merilä & Svensson, 1995). The erythrosedimentation rate was measured as the ratio between the length of the capillary tube not occupied by blood cells and the total length after the capillaries had stood vertically for 4h in a refrigerator at 4°C. Haematocrit is a serological variable diagnostic of acute and chronic diseases, bacterial infections, anaemia, and dehydration, or may reflect nutritional deficiencies of some minerals (Rupley, 1997). Blood samples were centrifuged in a portable apparatus for 4 min at 4000 rpm, and the haematocrit was expressed as the volume of that part of the capillary occupied by blood cells/blood volume in the capillary. The phytohaemagglutinin test was used to estimate the cell-mediated immune response. Subcutaneous injection with phytohaemagglutinin produces a local inflammation proportional to the intensity of T-lymphocyte cell-mediated immunocompetence (Smits et al., 1999), and its relative thickness (wing-web index) is directly related to the immune condition (Tella et al., 2008). The thickness of the wing-web area of the breeding individuals was measured with a spessimeter (Alpa spa, Milan, Italy, accuracy ±0.01 mm); the birds were then injected with 0.25 mg of phytohaemoagglutinin (L-8754; Sigma®) diluted in 0.05 mL phosphate-buffered saline solution. After 24h, the web thickness at the injection site was re-measured.

Egg collection and analyses

The aviaries were inspected and eggs collected (N = 554 in 2008, 566 in 2009) daily during the laying period. This egg removal schedule caused a supernormal deposition of eggs. Instead of a typical clutch [maximum of 10–12 eggs (Cramp & Simmons, 1980)], females laid a mean of 24.9 eggs. All eggs were weighed with an electronic balance (±0.1 g accuracy), and their length and width were measured with a caliper (±0.05 mm accuracy). Some of these eggs were taken for shell, yolk, and albumen weight measurements and for lysozyme analysis (laboratory eggs, N = 132 in 2008, 125 in 2009). The second, third, fifth, eighth, eleventh, and fourteenth eggs of all females was token, with the exception of five females that stopped laying before 14 eggs were laid. After separating the yolk from the albumen, the components were frozen at −20°C.

Lysozyme activity was measured using the lyso-plate method (Osserman & Lawlor, 1966). Brief, the diameters of the cleared zones (inhibition zones) that the samples formed around the site of inoculation into a Petri plate containing *Mycrococcus lysodeiktikus* was an indicator of the lysozyme concentration. These diameters were proportional to the logarithm of the lysozyme concentration and, compared with the standard curve, allowed to obtain values in hen egg lysozyme equivalents (HELML equivalents, expressed in µg/ml).

For the exact protocol see the "Materials and Methods: Common Aspects – Lysozyme: protocol Osserman & Lawlor, (1966)" above.

Statistical analysis

The effects of vaccine treatment on female mass, erythrosedimentation rate, haematocrit, and immune response were tested by repeated-measures analysis of variance (ANOVA), with vaccine treatment as an independent categorical variable.

Egg characteristics were analysed using multivariate mixed models with egg mass and egg characteristics as dependent variables. The vaccine treatment was inserted as a fixed effect, and clutch was included as a random effect to control for among-female variation. The possible effect of female condition was tested by inserting mass, haematocrit, blood erythrosedimentation rate, and immune response in the models as covariates. The effect of egg mass and position in the laying order was tested by inserting these variables as covariates. Position in the laying order was inserted both as a linear and a quadratic term to allow a U-shaped pattern of variation, with intermediate eggs being different from first and last eggs laid.

Initially, all variables were simultaneously entered in the mixed models. Then, a stepwise backward procedure of selection of independent variables by eliminating, at each step, the variable that had the least predictive power was used. The stepwise backward procedure led to a final model containing only the variables reaching the 0.05 level of significance.

Statistical analyses were performed using SYSTAT 12 (SYSTAT Software Inc., San José, CA).

~

Results

~

Effect of vaccination and prolonged laying on adults

At the beginning of the breeding period, birds of the two groups (Vaccine09 and Control09) did not differ significantly in mass, haematocrit, erythrosedimentation rate or immune response (**Errore. L'origine riferimento non è stata trovata.**). Similarly, at the end of the breeding period, there were no significant differences between vaccinated and control females.

Parameter	GROUP				ANOVA	
	Vaccine		Control		$F_{1,21}$	*P*
Beginning of the experimental period*						
Mass (g)	446.6	± 9.99	436.2	± 10.54	0.510	*0.49*
Haematocrit	0.567	± 0.014	0.556	± 0.016	0.279	*0.60*
Erythrosedimentation rate	0.765	± 0.033	0.743	± 0.038	0.205	*0.66*
Immune response (mm)	0.566	± 0.068	0.387	± 0.078	3.008	*0.10*
End of the experimental period*						
Mass (g)	463.7	± 11.07	469.5	± 12.38	0.123	*0.73*
Haematocrit	0.662	± 0.030	0.614	± 0.035	1.108	*0.31*
Erythrosedimentation rate	0.735	± 0.028	0.739	± 0.040	0.009	*0.93*
Immune response (mm)	0.427	± 0.073	0.476	± 0.085	0.177	*0.68*

Table 9: Comparison of mean values of mass, haematological parameters, and immune response to PHA in groups of breeding red-legged partridges tested with different vaccine treatment (N = 23 females, mean ± s.e. are reported). * *Repeated measures ANOVA.*

In all groups, the values recorded at the end of the supernormal laying effort did not differ significantly from those measured at the beginning (Table 9).

Effect of vaccination on egg characteristics

In 2009, vaccinated females (Vaccine09 group females) and control females (Control09 group females) did not differ significantly in the number of eggs laid (mean ± S.D.: 25.92 ± 8.28 eggs for the Vaccine09 group, 24.20 ± 7.80 eggs for the Control09 group; t_{21} = 0.496; P = 0.62). In 2008, the females (Control08 group) laid a similar number of eggs (25.78 ± 6.95 eggs).

	Complete model			Stepwise model	
Variable	t	*P*	Coefficient (SE)	t	*P*
Egg mass (N = 1104)					
Vaccine 2009	0.165	0.87	0.067 (0.404)	-	-
Control 2008	-1.301	0.19	-0.711 (0.547)	-	-
Position laying order	6.955	**0.001**	0.116 (0.017)	6.618	**0.001**
Position * position	-5.566	**0.001**	-0.003 (0.001)	-5.135	**0.001**
Female mass	0.758	0.45	0.003 (0.004)	-	-
Haematocrit	1.346	0.18	4.988 (3.706)	-	-
Erythrosedimentation	-0.343	0.73	-0.005 (0.015)	-	-
Immune reaction	-0.252	0.80	-0.093 (0.370)	-	-
Length (N = 1081)					
Vaccine 2009	0.974	0.33	0.246 (0.253)	-	-
Control 2008	-0.817	0.41	-0.281 (0.343)	-	-
Position laying order	-5.700	**0.001**	-0.082 (0.014)	-5.650	**0.001**
Position * position	3.098	**0.002**	0.001 (0.001)	3.020	**0.003**
Female mass	0.610	0.54	0.002 (0.003)	-	-
Haematocrit	1.391	0.16	3.218 (2.313)	-	-
Erythrosedimentation	0.472	0.64	0.005 (0.010)	-	-
Immune reaction	-1.798	0.07	-0.418 (0.233)	-2.044	**0.041**
Egg mass	20.777	**0.001**	0.54 (0.026)	21.715	**0.001**
Breadth (N = 1082)					
Vaccine 2009	-0314	0.75	-0.033 (0.104)	-	-
Control 2008	-3.830	**0.001**	-0.540 (0.141)	-5.086	**0.001**
Position laying order	6.071	**0.001**	0.046 (0.008)	5.783	**0.001**
Position * position	-5.160	**0.001**	-0.001 (0.001)	-4.899	**0.001**
Female mass	-1.191	0.23	-0.001 (0.001)	-	-
Haematocrit	0.514	0.608	0.487 (0.947)	-	-
Erythrosedimentation	-0.252	0.80	-0.001 (0.004)	-	-
Immune reaction	2.148	**0.032**	0.207 (0.096)	2.086	**0.037**
Egg mass	29.972	**0.001**	0.401 (0.013)	31.603	**0.001**
Shell mass (N = 252)					
Vaccine 2009	1.270	0.21	0.124 (0.097)	-	-
Control 2008	-1.736	0.08	-0.231 (0.133)	-3.592	**0.001**
Position laying order	-2.207	**0.028**	-0.057 (0.026)	-4.385	**0.001**
Position * position	1.147	0.25	0.002 (0.002)	-	-
Female mass	0.196	0.85	0.001 (0.001)	-	-
Haematocrit	0.662	0.51	0.591 (0.892)	-	-
Erythrosedimentation	0.845	0.39	0.003 (0.004)	-	-
Immune reaction	-1.110	0.27	-0.096 (0.087)	-	-
Egg mass	4.390	**0.001**	0.094 (0.021)	4.584	**0.001**
Yolk mass (N =252)					
Vaccine 2009	-0.668	0.51	-0.119 (0.178)	-	-
Control 2008	-2.343	**0.020**	-0.562 (0.240)	-	-
Position laying order	2.833	**0.005**	0.101 (0.036)	3.107	**0.002**
Position * position	-2.603	**0.010**	-0.006 (0.002)	-2.985	**0.003**

Female mass	1.235	0.22	0.002 (0.002)	-	-
Haematocrit	-0.11	0.91	-0.178 (1.620)	-	-
Erythrosedimentation	-1.100	0.27	-0.007 (0.007)	-	-
Immune reaction	1.550	0.12	0.248 (0.160)	-	-
Egg mass	7.848	**0.001**	0.247 (0.032)	8.663	**0.001**
Albumen mass (N =250)					
Vaccine 2009	0.376	0.71	0.087 (0.232)	-	-
Control 2008	-1.200	0.232	-0.376 (0.313)	-	-
Position laying order	3.822	**0.001**	0.171 (0.045)	3.750	**0.001**
Position * position	-2.717	0.007	-0.008 (0.003)	-2.637	**0.009**
Female mass	0.042	0.97	0.001 (0.02)	-	-
Haematocrit	0.762	0.45	1.613 (2.117)	-	-
Erythrosedimentation	-0.086	0.93	-0.001 (0.009)	-	-
Immune reaction	-0.358	0.72	-0.075 (0.209)	-	-
Egg mass	7.330	**0.001**	0.293 (0.040)	8.272	**0.001**
Lysozyme (N = 148)					
Vaccine 2009	1.473	0.144	184.7 (125.3)	-	-
Control 2008	3.791	**0.001**	671.7 (177.2)	4.372	**0.001**
Position laying order	-1.001	0.32	-60.3 (60.2)	-2.401	**0.018**
Position * position	0.498	0.62	2.25 (4.51)	-	-
Female mass	-1.109	0.27	-1.53 (1.38)	-	-
Haematocrit	-0.047	0.96	-56.7 (1202.6)	-	-
Erythrosedimentation	1.188	0.24	5.74 (4.83)	-	-
Immune reaction	-1.587	0.11	-123.1 (318.4)	-	-
Egg mass	1.868	0.06	58.3 (31.3)	-	-

Table 10: Multivariate mixed model analysis of egg mass and egg characteristics, with vaccine as factor, and female mass, haematocrit, erythrosedimentation rate, immune reaction, and position in the laying order of the focal egg as covariates. In the models female identity was inserted as a random factor.

Vaccination had no significant effect on egg characteristics. In 2009, eggs laid by vaccinated females did not differ from eggs laid by control females (**Errore. L'origine riferimento non è stata trovata.**). However, there was a significant difference between eggs laid by control females in 2008 and 2009: the eggs laid in 2008 had a smaller breadth, shell and yolk mass, and had more lysozyme in their albumen (Table 10).

Egg characteristics were significantly related to immunocompetence, but not to the other indices of female health condition (mass, haematocrit, and erythrosedimentation rate). Immune reaction was negatively related to egg length and positively related to egg breadth; hence females in better condition laid more spherical eggs.

Effect of supernormal laying on egg characteristics

Position in the laying order was positively related to egg mass, breadth, yolk mass, and albumen mass (Figure 54), while there was a decrease in egg length, shell weight, and lysozyme concentration along the laying order (**Errore. L'origine riferimento non è stata trovata.**). There were significant negative quadratic coefficients for egg mass, breadth, yolk mass, and albumen mass (Table

9). This determined an inverse U-shaped pattern of variation, with higher values for intermediate egg positions, while the extreme eggs (first- and last-laid eggs) showed lower values (Figure 54). The only positive quadratic coefficient was found for egg length; in this case, the eggs at extreme positions in the laying order were longer.

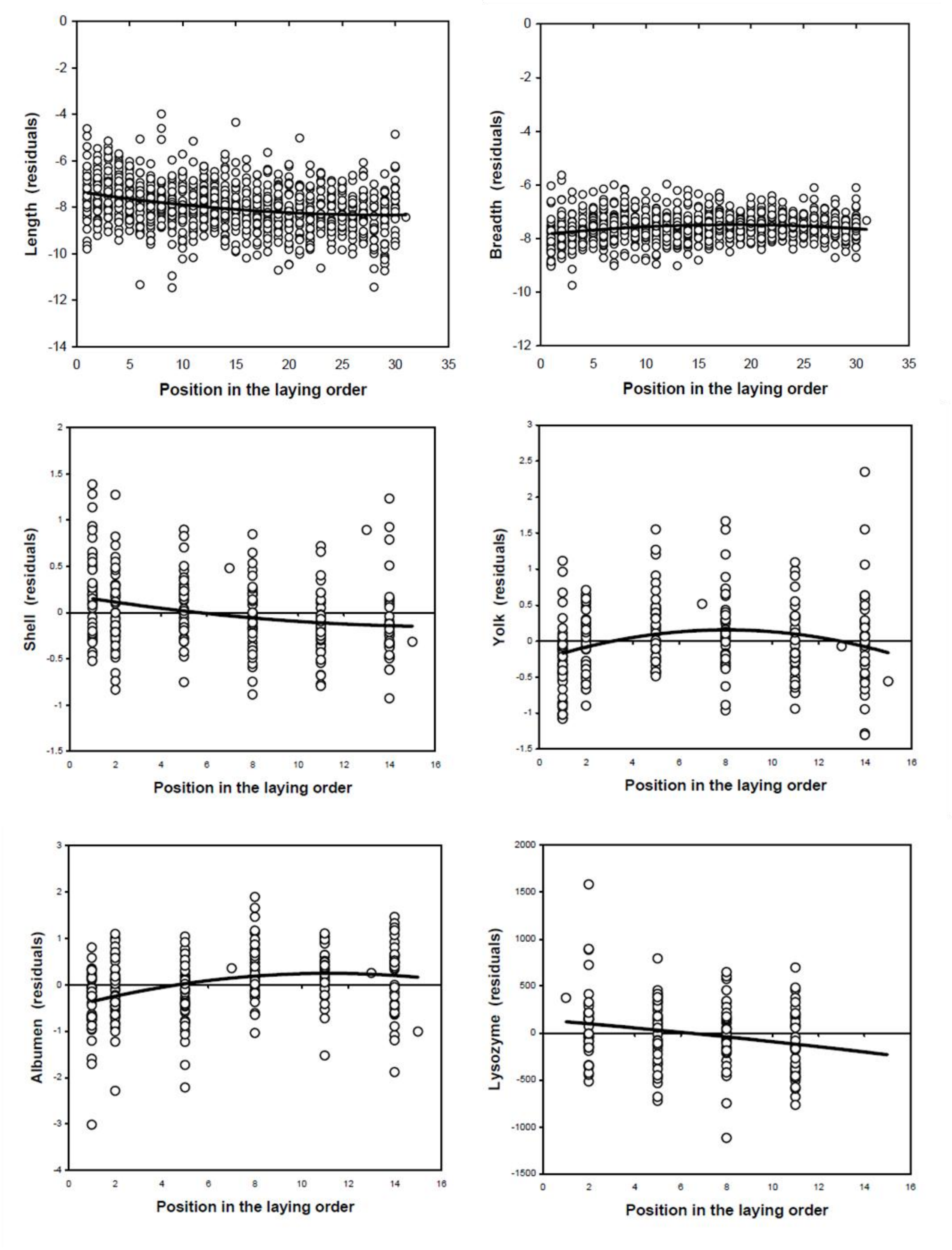


Figure 54 - Relationship between egg length, breadth, and components (residuals: deviations from within-clutch means) and laying sequence for clutches of red-legged partridges. Second-order polynomial lines are shown for each measured variable.

~

Discussion

~

Evolutionary theory predicts that the immune response should be traded against other vital functions, such as growth, self-maintenance, and/or reproductive output (Norris & Evans, 2000; Hanssen et al., 2004). In this study, to experimentally test for such trade-offs, female red-legged partridges were challenged with the NDV vaccine and induced them, via egg removal, to produce supernormal clutches. Birds were all of the same age (first year) to avoid possible age effects (Bonneaud et al., 2003; Cabezas-Diaz et al., 2005), and food was provided *ad libitum* to reduce environment-related variations.

Female body condition variables (mass, haematocrit, erythrosedimentation rate, and immune response to phytohaemagglutinin injection) are not significantly affected by the vaccine challenge and none of the health parameters differed significantly between the beginning and the end of the protracted egg deposition. These results indicate that conditions are not affected by the two experimental challenges. The vaccine treatment also had no effect on egg characteristics, whereas the increased effort due to laying supernumerary eggs led to a significant decrease in egg quality.

Previous experiments under *ad libitum* food conditions have shown both negative (Bertrand et al., 2006b) and nil effects of immune challenge on female body mass (Martin et al., 2003; Pap et al., 2008; Cucco et al., 2010). On the other hand, studies in the wild have generally shown a negative effect of immune challenge on body mass (Ots et al., 2001; Hanssen, 2006). Some studies have shown that mass loss can be accompanied by an increase in basal metabolic rate, probably resulting in weight loss (Ots et al., 2001; Eraud et al., 2005), and in the pigeon *Columba livia* an immune challenge induced a decrease in mass and also caused higher oxygen consumption and an increase of cloacal temperature (van de Crommenacker et al., 2010). The results of this work for body mass are in line with the idea that the energetic cost of activating an immune response can be low (Svensson et al., 1998; Lee et al., 2005). However, an alternative explanation is that it is difficult to detect high energetic costs in terms of mass in this experimental set-up: as the females did not have food limitations, they could easily recover the energy allocated in mounting the immune response.

In the present study, there is no significant effect of vaccine challenge on immunocompetence or blood parameters. In particular, there is no effect on haematocrit, indicating that the NDV immune challenge did not cause anaemia in the breeding females (Williams et al., 2004; Wagner et al., 2008). This result is similar to that reported in the grey partridge *P. perdix* (Cucco et al., 2010). In the present study, the erythrosedimentation rate and the cellular immune response to phytohaemagglutinin injection are not markedly influenced by the NDV immune challenge. In a similar experimental set-up with the grey partridge, challenged females showed an impaired erythrosedimentation rate, a blood parameter diagnostic of many diseases and infections (Heylen & Matthysen,

2008) and a good indicator of individual condition (Masello & Quillfeldt, 2004). The difference between grey and red-legged partridges could be related to a different susceptibility to NDV of the two species (Geral et al., 1976; Aldous & Alexander, 2008). The finding of this study on cellular immune response is similar to observations in the great tit *P. major* (Nilsson et al., 2007). In general, data on the red-legged partridge suggest that the immune challenge does not impair female health. However, it is difficult to obtain a complete picture of state of health, as its different aspects (blood parameters, humoral and cell-mediated immunity) can be differentially affected. Further studies are needed to determine whether other components are impaired (Hõrak et al., 2006; Sarv & Hõrak, 2009).

Immune-challenged partridges laid eggs similar in mass to the control eggs in the present study. This indicates that red-legged partridges are able to invest in egg production even with stimulation of the immune system. There are few data on possible detrimental effects of immune stimulation on egg mass. In birds, a slight decrease in egg mass after an immune challenge was found in the grey partridge (Cucco et al. 2010). A decrease in egg mass was observed in two reptiles (Uller et al., 2006; French et al., 2007) and a cricket (Shoemaker & Adamo, 2007), but in two of these cases it was found only in extreme circumstances – a food scarcity regimen (French et al., 2007) or a very high dose of immune-stimulating lipopolysaccharides (Shoemaker & Adamo, 2007). It should be noted that the role of egg mass in influencing future prospects of survival is still a matter of debate because the relationship is difficult to infer from correlational studies (Krist, 2011) and a general pattern has not been agreed on due to conflicting results (Williams, 1994).

In this study, albumen, yolk, egg shell, and the concentration of the antibacterial substance lysozyme did not change significantly after vaccination of the mothers. This indicates that the preservation of egg mass is accompanied by maintenance of egg quality. In avian studies, the few specific data on egg quality after a maternal immune challenge concern a decrease of yolk testosterone in the house martin *D. urbica* (Gil et al., 2006) and a change of yolk fatty acid profile in laying hens (Burnham et al., 2003; Viscione et al., 2008). The result of the present study is similar to that reported in the grey partridge, in which an immune challenge did not alter the concentration of two antibacterial substances (lysozyme and avidin) in the albumen (Cucco et al., 2010).

Female condition was measured at the beginning and at the end of a prolonged period of laying, during which there was supernormal egg production. The female mass and blood parameters at the end of egg deposition were not statistically different from those at the beginning. This suggests that, after prolonged egg production, the females did not experience deterioration in health or energetic conditions. This findings are in line with those of Willie et al. (2010) on the health of the zebra finch *T. guttata* and of Christians (2000) on mass stores in the starling *S. vulgaris*. Willie et al. (2010) investigated the effect of repeated cycles of egg production on haematological traits in female zebra finches. Females maintained haematocrit and haemoglobin concentration at some minimal functional level independently of reproductive effort, supporting the hypothesis that egg laying females have functionally reduced haematocrit and haemoglobin concentration. Regarding energy stores, starling females experimentally induced to lay one extra egg did not have reduced protein and lipid stores (Christians, 2000). In contrast, Monaghan et al. (1998) found that producing one extra egg reduced the lean dry mass of the lesser black-backed gull *L. fuscus*.

It is conceivable that laying supernormal clutches could have a detrimental effect on egg quality. Indeed, a decrease in egg mass, breadth, yolk mass, albumen mass, and lysozyme content are observed in the last-laid eggs. The relationship between laying order and egg quality is inverse U-shaped, with smaller values at the beginning and at the end of the laying sequence. The low quality of the first eggs is not unexpected: it is known that in this and other partridge species the first-laid eggs are usually of low quality and are laid scattered in an area different from the definitive nest (Cramp & Simmons, 1980).

To date, few studies have reported changes in egg quality in response to supernormal egg production. In each case, the specific egg component was different: a decrease in shell calcium was found in the common tern *Sterna hirundo* (Heaney et al., 1998), a decrease of yolk proteins in the zebra finch (Williams & Miller, 2003), a lower yolk-to-albumen ratio (Verboven et al., 2010) and an increased water content with a decrease of yolk lipids in the lesser black-backed gull (Nager et al., 2000). Female zebra finches, lesser black-backed gulls, and common terns laying extended clutches were able to maintain egg mass in the extra eggs (Heaney et al., 1998; Williams and Miller, 2003), while in the red-legged partridge (this study) and in the great tit the extra eggs were smaller than control eggs (Mänd et al., 2007).

In conclusion, this study examined the possible trade-offs among immune response, selfmaintenance, and prolonged reproductive output in breeding red-legged partridges. Results show that of the two challenges, immune and supernormal egg deposition, the former did not cause evident costs, while the latter did not impair female condition but affected the quality of last-laid eggs. Future studies might clarify whether egg phenotype will translate into offspring growth, survival, and recruitment.

Overall Discussion

The three species chosen for the experiences have proved to be extremely good models. Their ecological and behavioural characteristics and the simplicity of breeding play a fundamental role for the comprehension of the maternal effects and the differential allocation.

The allocation of resources into the eggs (yolk, albumen, antimicrobial molecules, hormones) is subjected to multiple factors, such as male characteristics, environmental aspects or female condition. Experimentation in controlled environment allows to realise simple situations from which reliable results can be extrapolated. In particular, the experiences described in this thesis were performed in laboratory for the canary, where also the photoperiod could be controlled, and in semi-natural condition for the grey and red-legged partridges.

Canary and partridges are monogamous species that keep the partner along the whole reproduction season, although the couple could change between years. These species have been well studied for what concerns sexual selection and characteristics implicated in female choice, song characteristics (in the canary), and investment in eggs in several condition, so there is a strong theoretical basis. Male dominance and subsequent selection of grey partridge females is less studied (Garcia-Fernandez, 2009) and some questions are still open. For this reason the first chapter is focused on this topic. Also the characteristics selected by the canary females when faced to a physical male competition are little known (chapter II). Another interesting topic is the influence of the environment on the sexual selection and maternal effects. In particular, in literature is gaining increasing interest the impact of anthropic noise on the animal biology. Chapter three attempts to investigate the influence of this environmental characteristic. Finally, chapter four is focused on the influence of immune challenge and supra-normal laying on the maternal investment.

The species utilized for the experiences has been selected also for another fact: the partridges are nidifugous species, while canary is nidicolous. In simples terms, the maternal investment in the partridges is substantially prenatal, whereas in canary is both pre and postnatal. Selecting these species it's possible to have a complete panoramic on the maternal effects.

Summary of the main results

This thesis bases on the allocation of the resources into the eggs as means of rapid offspring phenotype adaptation to the environmental heterogeneity made by mothers (Mousseau & Fox, 1998). The environmental heterogeneity can take different forms: in these works it is simulated by male dominance or male agonistic characteristics, anthropic noise and immune-challenge. To investigate the effects of these components on the investment, all the eggs have been weighted and measured, some of them (the totality in the experiences with canary) were collected, separated into their principal components (yolk, albumen, and eggshell) and the concentration in lysozyme (in all experiences) ovotransferrin (except in the immune-challenge and dominance in canary studies), and testosterone (only in works on the dominance in the grey partridge and in canary) have been evaluated.

In chapter I, the influence of the hierarchic status of the males on the prenatal investment is observed. After a pre-test in which the male ranking was established, and the formation of ten groups of two males where was easy to appreciate the different rank, it was allowed to females to see a physic competition between two males, and after the female preference was investigated. After this, some females were paired with the dominant (and chosen) male, while others were coupled with the subordinate (and not chosen) one. The large number of eggs laid by grey partridge females allowed to collect some eggs for the laboratory analyses and to incubate the remaining to evaluate the hatching rate. The main findings are that females paired with dominant males (and compared with females coupled with dominated ones) laid eggs with more testosterone (Figure 14 page 90) and that hatch more (Figure 19 page 95). This fact could find a justification correlating the two things: a high concentration of testosterone enhance development of hatching muscle (Lipar & Ketterson, 2000), implicated in the breaking of the eggshell during the hatching. In addition, a slight increase of albumen mass, and slight decrease of yolk mass and shell mass and an increase of testosterone concentration with laying order have been found. The progressive increase of albumen mass with laying sequence is similar to that reported in the red-legged partridge (Cucco et al., 2011b) and it confirms a previous study on the grey partridge (Cucco et al., 2007). The possible adaptive value of this patterns is still an open question, which deserves more studies and experimental designs. Probably, this variation also in the same species in different experiments can be related to external environmental factors and individual differences. The negative relationship between position in the laying order and yolk mass is in line with the theory of a natural depletion of the mother resources with the progress of deposition: females have limited resources and the decline could simply reflect exhaustion of maternal resources. The increase of testosterone concentration is concordant to Garcia-Fernandez work (2009) that explicated this tendency as a beneficial for the inter nest competition. Finally, also a correlation between hatchability of the eggs and the elongation index has been found: eggs with higher or lower elongation hatched at a lower rate. The result is in accord with several studies (Baspinar et al., 1997; Erisir & Ozbey, 2005; Cucco et al., 2012).

The aim of the work described in second chapter is evaluate if canary female chooses or not a male that results dominant after a male-male physic competition and understand if this choice influences maternal investment (albumen and yolk mass, testosterone and lysozyme deposition in eggs). To do this, interactions between two males (one dominant and one subordinate) have been shown to females, female choice has been noted and finally the maternal investment has been valued. This work shows that females paired with the chosen male (that start interaction for more times for first) laid eggs heavier (Figure 24b page 123), with a shell heavier (Figure 25c page 124), more elongated (Figure 27 page 126), and with more lysozyme in their albumen eggs (Figure 26a page 125). Literature shows that larger eggs tend: 1) to have higher hatching success (Perrins, 1996), 2) to influence the dimensions of hatchlings (Magrath, 1992; Styrsky et al., 1999; Christians, 2002), and 3) to influence fledging probability (Silva et al., 2008). Switching to lysozyme, an explication could be that the mother increase it to counteract possible negative effects due to a high concentration of testosterone. In this study, a correlation between testosterone in the eggs and the female preference has not been found, but it has been shown that the progeny of an aggressive male could inherit from the father aggressiveness (and consequently have more testosterone). Finally, the result on the elongation index is potentially accordant with the results of chapter one, but in this work the hatchability has not been investigate. A future study could be focused on this topic.

In chapter III are presented the results obtained in an experience where females heard the male attractive song masked by noise or on silence; this protocol has been performed to understand if the anthropic noise influences sexual selection and maternal investment in the canary, an oscine bird where the song is more important for social behaviour. Summarizing the main findings, the work shows an influence of noise on the Copulation Solicitation Display and on the delay between the start of song presentation and the first CSD. For what concerns maternal investment, females in silence condition laid more eggs than females in noise condition. This work is concordant with the few studies present in literature (Habib et al., 2007; Halfwerk et al., 2011b; Huet des Aunay, 2011; Schroeder et al., 2012).

Forth chapter is focused on effects of immune challenge and supernormal clutch production on egg quality in the red-legged partridge. In the experiment, female red-legged partridges have been challenged with the NDV vaccine and induced, via egg removal, to produce supernormal clutches. No effects due to immune challenge have been found both on female health and maternal investment, probably because red-legged partridges are little susceptible to NDV vaccine and also they are able to invest in egg production even with stimulation of the immune system. Conversely, a decrease in egg components in the last-laid eggs were observed, that could be linked to a progressive loss of energy by the females. The few studies before performed have reported changes in egg quality, but in each case the specific egg component was different (e.g., Heaney et al., 1998; Williams & Miller, 2003; Verboven et al., 2010). In conclusion, results show that of the two challenges, immune and supernormal egg deposition, the former did not cause evident costs, while the latter did not impair female condition but affected the quality of last-laid eggs. Future studies might clarify whether egg phenotype will translate into offspring growth, survival, and recruitment.

Finally, if the different experiments are analysed together, we see how several types of environmental stressors affect the reproductive success both in terms of sexual selection and parental investment.

Comparison between nidifugous and nidicolous species

The partridges are monogamous, and the young are precocial. Chicks born with down and are able to move. However, they are not completely independent up to the age of 15-20 days.

The canary is also monogamous and altricial species. The young born blind and without thermoregulation. They are totally dependent on their parents for ten days after hatching during which they are unable to leave the nest. As soon as their feathers are grown, they begin to leave the nest. For 15 days after they return to nest to obtain food.

Postnatal investment is more important in canaries where parents spend a lot of time and energy to feed the nestlings than in partridge where parents monitor young, but don't much care of them.

Instead, the partridges invest more in eggs: in fact, females canary produce about 4 or 5 eggs for cycle of reproduction, and do 3 cycle in a season; in the partridges, eggs are 15-20 and they are laid only one time in the reproduction season.

In grey partridge male characteristics influenced the concentration of testosterone; the same thing wasn't present in canary.

Leaving the nest a few hours after hatching, in small groups called companies, under the control of their parents, young partridge can potentially meet again very quickly. The influence of testosterone on aggressiveness in young may allow a brood to access to food before near broods.

In the canary, in which young remain in the nest for a long time, the clutch does not meet another early. A general increase in the concentration of testosterone in the eggs of the brood could be deleterious, because would increase the aggressiveness.

Perspectives

This thesis has tried to find answers to some questions of behavioural ecology, but at the same time it has opened others.

The study of Ophir & Galef (2004) showed that the sexual experience influence the female choice faced to an aggressive behaviour of a dominant male: females at first reproduction chose the dominant male, while females experienced chose the dominated. The same fact could happened also in the grey partridge?

In addition to this, the work on dominance in the grey partridge didn't analyse the survival and the behaviour of broods after the hatching. It would be interesting to investigate whether the son of a dominant male is dominant in turn and also his testosterone concentration and health parameters.

In the experience on physical competition in the canary all the eggs has been utilized for the laboratory analyses; an evolution of this work could be to clarify if the female preference influence also the hatching of the eggs and the offspring survival. Moreover, it would be interesting to see also in this species if the offspring of aggressive males is aggressive in turn, and hormonal concentration.

For what concerns the influence of anthropic noise in the sexual behaviour and reproductive success a long way yet to be travelled. It would be very interesting investigate also the mother-son communication in a disturbed environment, to understand if noise influenced also this part of the birds life. In addition, the continuous increase of anthropic areas can lead to carry out studies on animal adaptation to a new ecological niche.

Also in the field of immunity a lot of things must to be investigated. The trade-off between reproduction success and health is one of the most important, and new studies could allow to better understand the physiological mechanisms implicated in this trade-offs (e.g., Reactive Oxygen Species formation).

Finally, other components are present into the eggs, both antimicrobial and hormonal or linked to the immune competence (e.g. immunoglobulines). Future studies may allow to understand if these components vary in particular environmental condition, or according to male characteristics.

Bibliography

- Abbott D.H., Keverne E.B, Bercovitch F.B., Shively C.A., Mendoza S.P., Saltzman W., Snowdon C.T., Ziegler T.E., Banjevic M., Garland T.Jr & Sapolskyj R.M. 2003. Are subordinates always stressed? A comparative analysis of rank differences in cortisol levels among primates. Hormones and Behavior 43: 67–82.
- Adolph S.C. & Porter W.P. 1993. Temperature, activity, and lizard life histories. The American Naturalist 142: 273–295.
- Ahmed A.M., Baggott S.L., Maingon R. & Hurd H. 2002. The costs of mounting an immune response are reflected in the reproductive fitness of the mosquito *Anopheles gambiae*. Oikos 97: 371–377.
- Alatalo R.V., Höglund J. & Lundberg A. 1991. Lekking in the black grouse - a test of male viability. Nature 352: 155–156.
- Aldous E.W. & Alexander D.J. 2008. Newcastle disease in pheasants (*Phasianus colchicus*): a review. The Veterinary Journal 175: 181–185.
- Alexander D.J. 1997. Newcastle disease. In: Diseases of Poultry. Calnek B.W., Barnes H.J., Reid W.M. & Yoder H.W. (Editors). Pp. 541–570. Iowa State University Press. Ames, Iowa. USA.
- Alonso-Alvarez C., Bernars S., Devevey G., Prost J., Faivre B. & Sorci G. 2004. Increased susceptibility to oxidative stress and proximate cost of reproduction. Ecology Letters 7: 363–368.
- Alonso-Alvarez C., Bernars S., Devevey G., Prost J., Faivre B., Chastel O. & Sorci G. 2006. An experimental manipulation of life-history trajectories and resistance to oxidative stress. Evolution 60: 1913–1924.
- Alonso M.E., Prieto R., Gaudioso V.R., Pérez J.A. Bartolomé D. & Díez C. 2008. Influence of the pairing system on the behaviour of farmed red-legged partridge couples (*Alectoris rufa*). Applied Animal Behaviour Science 115: 55–66.
- Amy M. & Leboucher G. 2007. Male canaries can visually eavesdrop on conspecific food interactions. Animal Behaviour 74: 57–62.
- Amy M., Monbureau M., Durand C., Gomez D., Thery M. & Leboucher G. 2008. Female canary mate preferences: differential use of information from two types of male-male interaction. Animal Behaviour 76: 971–982.
- Anderson W., Kim Y.-K. & Gowaty P. 2007. Experimental constraints on female and male mate preferences in *Drosophila pseudoobscura* decrease offspring viability and reproductive success of breeding pairs. Proceedings of the National Academy of Sciences of the USA 104: 4484–4488.
- Andersson M. 1994. Sexual selection. Princeton University Press. Princeton, New Jersey. USA.
- Andersson S., Uller T., Lõhmus & Sundström F. 2004. Effects of egg yolk testosterone on growth and immunity in a precocial bird. Journal of Evolutionary Biology 17: 501–505.

- Andrès J.A., Maroja L.S., Bogdanowicz S.M., Swanson W.J. & Harrison R.G. 2006. Molecular evolution of seminal proteins in field crickets. Molecular Biology and Evolution 23: 1574–1584.
- Ankney C.D. & Alisauskas R.T. 1991. Nutrient-reserve dynamics and diet of breeding female gadwalls. Condor 93: 799–810.
- Anton M., Nau F. & Nys Y. 2005. Bioactive egg components and their potential uses. World's Poultry Science Journal 62: 429–438.
- Aparicio J.M. 1999. Intra-clutch egg size variation in the Eurasian Kestrel: advantages and disadvantages of hatching from large eggs. The Auk 116: 825–830.
- Archawaranon M., Dove L. & Wiley R.H. 1991. Social inertia and hormonal control of aggression and dominance in white-throated sparrows. Behaviour 118: 42–65.
- Ardia D.R., Matthew F., Wasson M.F. & Winkler D.W. 2006. Individual quality and food availability determine yolk and egg mass and egg composition in tree swallows *Tachycineta bicolor*. Journal of Avian Biology 37: 252–259.
- Ariew A. & Lewontin R.C. 2004. The confusion of fitness. The British Journal for the Philosophy of Science 55: 347–363.
- Arnold S.J. 1994. Bateman's principles and the measurement of sexual selection in plants and animals. The American Naturalist 144: S126–S149.
- Arnqvist G. 1998. Comparative evidence for the evolution of genitalia by sexual selection. Nature 393: 784–786.
- Arnqvist G. & Rowe L. 2002. Correlated evolution of male and female morphologies in water striders. Evolution 56: 936–947.
- Askenmo C. 1979. Reproductive effort and return rate of male pied flycatchers. The American Naturalist 114: 748–752.
- Babisch W. 2003. Stress hormones in the research on cardiovascular effects of noise. Noise Health 5: 1–11.
- Baptista L.F., DeWolfe B. & Avery-Beausoleil L. 1987. Testosterone, aggression, and dominance in Gambel's white-crowned sparrows. Wilson Bulletin 99: 86–91.
- Barber J.R., Crooks K.R. & Fristrup K.M. 2009. The costs of chronic noise exposure for terrestrial organisms. Trends in Ecology and Evolution 25: 180–189.
- Barnett S.A. 1958. Physiological effects of social stress in wild rats. I. The adrenal cortex. Journal of Psychosomatic Research 3: 1–11.
- Barnett S.A., Eaton J.C. & McCallum C.H. 1960. Physiological effects of "social stress" in wild rats—II. Liver glycogen and blood glucose. Journal of Psychosomatic Research 4: 251–260.
- Baspinar E., Yildiz M.A., Ozkan M.M. & Kavuncu O. 1997. The effect of egg weight and shape index on hatchability in Japanese quail eggs. Turkish Journal of Veterinary and Animal Sciences 21: 53–56.

- Bateman A.J. 1948. Intra-sexual selection in *Drosophila*. Heredity 2: 349–368.
- Bayly K.L., Evans C.S. & Taylor A. 2006. Measuring social structure: a comparison of eight dominance indices. Behavioural Processes 73: 1–12.
- Beani L. & Dessì F. 1995. Mate choice in the grey partridge, *Perdix perdix*: role of physical and behavioural male traits. Animal Behaviour 49: 347–356.
- Beaugrand J.P. 1983. Modèles de dominance et théorie de l'évolution. In: Darwin après Darwin. Lévy J. & Cohen H.E. (Editors). Pp. 110–137. Presses de l'Université du Quėbec. Quėbec, Quėbec. Canada.
- Bell G. 1980. The costs of reproduction and their consequences. The American Naturalist 116: 45–76.
- Bell G. & Koufopanou V. 1986. The cost of reproduction. In: Oxford Surveys in Evolutionary Biology. Dawkins R. & Ridley M. (editors). Oxford University Press. Oxford, UK.
- Bentley G.E., Wingfield J.C., Morton M.L. & Ball G.F. 2000. Stimulatory effects on the reproductive axis in female songbirds by conspecific and eterospecific male song. Hormones and Behavior 37: 179–189.
- Berglund A., Bisazza A. & Pilastro A. 1996. Armaments and ornaments: an evolutionary explanation of traits of dual utility. Biological Journal of the Linnean Society 58: 385–399.
- Bernstein I.S. 1981. Dominance: the baby and the bathwater. Journal of Behavioral and Brain Science 4: 419–457.
- Bertrand S., Alonso-Alvarez C., Devevey G., Faivre B., Prost J. & Sorci G. 2006a. Carotenoids modulate the trade-off between egg production and resistance to oxidative stress in zebra finches. Oecologia 147: 576–584.
- Bertrand S., Criscuolo F., Faivre B. & Sorci G. 2006b. Immune activation increases susceptibility to oxidative tissue damage in Zebra Finches. Functional Ecology 20: 1022–1027.
- Birdlife International. 2013a. Species factsheet: *Serinus canaria*. Downloaded from: http://www.birdlife.org on 06/01/2013.
- BirdLife International. 2013b. Species factsheet: *Alectoris rufa*. Downloaded from http://www.birdlife.org on 06/01/2013.
- Birkhead T.R. & Möller A.P. 1998. Sperm competition and sexual selection. Academic Press. London, UK.
- Birkhead T.R. & Pizzari T. 2002. Postcopulatory sexual selection. Nature Reviews Genetics 3: 262–273.
- Bisazza A., Marconato A. & Marin G. 1989. Male competition and female choice in *Padogobius martensi* (Pisces, Gobiidae). Animal Behaviour 38: 406–413.
- Bisazza A. & Marin G. 1991. Male size and female mate choice in the eastern mosquitofish (*Gambusia holbrooki*: Poeciliidae). Copeia 3: 730–735.

- Blanchard D.C., Spencer R.L., Weiss S.M., Blanchard R.J., McEwen B. & Sakai R.R. 1995. Visible burrow system as a model of chronic social stress: behavioral and neuroendocrine correlates. Psychoneuroendocrinology 20: 117–134.
- Blanchard R.J. & Blanchard D.C. 1989. Antipredator defensive behaviors in a visible burrow system. Journal of Comparative Psychology 103: 70–82.
- Blomqvist D., Johansson O.C. & Götmark F. 1997. Parental quality and egg size affect chick survival in a precocial bird, the lapwing *Vanellus vanellus*. Oecologia 110: 18–24.
- Blount J.D., Houston D.C. & Møller A.P. 2000. Why egg yolk is yellow. Trends in Ecology and Evolution 15: 47–49.
- Blount J.D., Surai P.F., Nager R.G., Houston D.C., Møller A.P., Trewby M.L. & Kennedy M.W. 2002. Carotenoids and egg quality in the lesser black-backed gull *Larus fuscus*: a supplemental feeding study of maternal effects. Proceedings of the Royal Society B: Biological Sciences 269: 29–36.
- Bluhm C.K. & Gowaty P.A. 2004. Reproductive compensation for offspring viability deficits by female mallards, *Anas platyrhynchos*. Animal Behaviour 68: 985–992.
- Board R.G. 1982. Properties of avian eggshells and their adaptive value. Biological reviews of the Cambridge Philosophical Society 57: 1–28.
- Bolton M. 1991. Determinants of chick survival in the lesser black-backed gull: relative contributions of egg size and parental quality. Journal of Avian Ecology 60: 949–960.
- Bolund E., Schielzeth H. & Forstmeier W. 2009. Compensatory investment in zebra finches: females lay larger eggs when paired to sexually unattractive males. Proceedings of the Royal Society B: Biological Sciences 276: 707–715.
- Boncoraglio G., Rubolini D., Romano M., Martinelli R. & Saino N. 2006. Effects of elevated yolk androgen on perinatal begging behaviour in yellow-legged gulls (*Larus michahellis*) chicks. Hormones and Behavior 50: 442–447.
- Bonisoli-Alquati A., Rubolini D., Romano M., Cucco M., Fasola M., Caprioli M. & Saino N. 2010. Egg antimicrobials, embryo sex and chick phenotype in the yellow-legged gull. Behavioral Ecology and Sociobiology 64: 845–855.
- Bonneaud C., Mazuc J., Gonzalez G., Haussy C., Chastel O., Faivre B. & Sorci G. 2003. Assessing the cost of mounting an immune response. The American Naturalist 161: 367–369.
- Boonekamp J.J., Ros A.H.F. & Verhulst S. 2008. Immune activation suppresses plasma testosterone level: a meta-analysis. Biology Letters 4: 741–744.
- Borgia G. 1979. Sexual selection and the evolution of mating systems. In: Sexual selection and reproductive competition in insects. Blum M.S. & Blum N.A. (Editors). Pp. 19–80. Academic Press. New York, New York. USA.

- Bottoni L., Massa R., Lea R.W. & Sharp P.J. 1993. Mate choice and reproductive success in the red-legged partridge (*Alectoris rufa*). Hormones and Behavior 27: 308-317.
- Bouchard F. & Rosenberg A. 2004. Fitness, probability and the principles of natural selection. The British Journal for the Philosophy of Science 55: 693–712.
- Boughton R.K., Joop G. & Armitage S.A.O. 2011. Outdoor immunology: methodological considerations for ecologists. Functional Ecology 25: 81–100.
- Bowden R.M., Ewert M.A. & Nelson C.E. 2000. Environmental sex determination in a reptile varies seasonally and with yolk hormones. Proceedings of the Royal Society B: Biological Sciences 267: 1745–1749.
- Bowers E.K., Smith R.A., Hodges C.J., Zimmerman L.M., Thompson C.F. & Sakaluk S.K. 2012. Sex-biased terminal investment in offspring induced by maternal immune challenge in the house wren (*Troglodytes aedon*). Proceedings of the Royal Society B: Biological Sciences 279: 2891–2898.
- Bradbury J.W. & Vehrencamp S.L. 1998. Principles of Acoustic Communication. Sinauer Associates. Sunderland, Massachusetts. USA.
- Breed M.D. & Moore J. 2011. Animal behavior. Academic press. Waltham, Massachusetts. USA.
- Breed M.D., Smith S.K. & Gall B.G. 1980. Systems of mate selection in a cockroach species with male dominance hierarchies. Animal Behaviour 28: 130–134.
- Brett J.R. 1958. Implications and assessments of environmental stress. In: The investigation of fish-power problems. H. R. MacMillan lectures in fisheries. Larkin P. (Editor). Pp. 69–83. University of British Columbia. Vancouver, British Columbia. Canada.
- Brian D.N. & Pitcher T.E. 2005. Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. Molecular Ecology 14: 19–38.
- Brichetti P. & Fracasso G. 2004. Ornitologia Italiana Volume II. Alberto Perdisa Editore. Bologna, Italy.
- Brichetti P. & Gariboldi P.B. 2002. Manuale di ornitologia volume 3. Edagricole. Bologna. Italia.
- Briskie J.V., Montgomerie E. & Birkhead T.R. 1997. The evolution of sperm size in birds. Evolution 51: 937–945.
- Brommer J.E. 2000. The evolution of fitness in life-history theory. Biological Reviews 75: 377–404.
- Brommer J.E. 2004. Immunocompetence and its costs during development: an experimental study in blue tit nestlings. Proceedings of the Royal Society B: Biological Sciences Supplement 271: S110–S113.
- Brown G.R. & Silk J.B. 2002. Reconsidering the null hypothesis: is maternal rank associated with birth sex ratios in primate groups?

Proceedings of the National Academy of Sciences of the USA 99: 11252–11255.

- Brumm H. 2004. The impact of environmental noise on song amplitude in a territorial bird. Journal of Animal Ecology 73: 434–440.
- Brumm H. & Slabbekoorn H. 2005. Acoustic communication in noise. Advances in the Study of Behavior 35: 151–209.
- Brumm H. & Todt D. 2002. Noise-dependent song amplitude regulation in a territorial songbird. Animal Behaviour 63: 891–897.
- Bryant D.M. 1979. Reproductive costs in the house martin. Journal of Animal Ecology 48: 655–675.
- Bulmer M.G. & Parker G.A. 2002. The evolution of anisogamy: a game-theoretic approach. Proceedings of the Royal Society B: Biological Sciences 269: 2381–2388.
- Burley N.T. 1986. Sexual selection for aesthetic traits in species with biparental care. The American Naturalist 127: 415–445.
- Burley N.T. 1988. The differential-allocation hypothesis: an experimental test. The American Naturalist 132: 611–628.
- Burley R.W. & Vadehra D.V. 1989. The avian egg chemistry and biology. John Wiley and Sons Inc. New York, New York.USA.
- Burnham M.R., Peebles E.D., Branton S.L. & Whitmarsh S.K. 2003. Effect of F-strain *Mycoplasma gallisepticum* inoculation at twelve weeks of age on the egg yolk composition in commercial egg laying egg. Poultry Science 82: 577–584.
- Byers J.A. & Waits L. 2006. Good genes sexual selection in nature. Proceedings of the National Academy of Sciences of the USA 103: 16343–16345.
- Cabezas-Diaz S., Virgos E. & Villafuerte R. 2005. Reproductive performance changes with age and laying experience in the red-legged partridge *Alectoris rufa*. Ibis 147: 316–323.
- Caligaris R. 1997. Influenza della crenoterapia solfurea sul lisozima nel siero e nel secreto nasale. Medicina Clinica e Termale 38: 53–61.
- Calow P. 1973. The relationship between fecundity, phenology and longevity: a systems approach. The American Naturalist 107: 559–574.
- Calow P. 1979. The cost of reproduction – a physiological approach. Biological review 54: 23–40.
- Campbell B. & Lack E. 1985. A dictionary of birds. British Ornithologists Union. Buteo Books. Vermillion, Sout Dakota. USA.
- Carere C. & Balthazart J. 2007. Sexual versus individual differentiation: the controversial role of avian maternal hormones. Trend in Endocrinology & Metabolism 18: 73–80.
- Catchpole C.K. & Slater P.J.B. 2008. Bird song: Biological themes and variations. Cambridge University Press. Cambridge, UK.

- Carey C. 1996. Avian Energetics and Nutritional Ecology. Chapman & Hall. New York, New York. USA.
- Carey C., Rahn H. & Parisi P. 1980. Calories water, lipid, and yolk in avian eggs. The Condor 82: 335–343.
- Charnov E.L. & Krebs J.R. 1973. On clutch size and fitness. Ibis 116: 217–219.
- Cheverud J.M. & Moore A.J. 1994. Quantitative genetics and the role of the environment provided by relatives in the evolution of behavior. In: Quantitative genetic studies of behavioral evolution. Boake C.R.B. (editor). Pp. 67–100. University of Chicago Press. Chicago, Illinois. USA.
- Chippindale A.K., Chu T.J. & Rose M.R. 1996. Complex trade-offs and the evolution of starvation resistance in *Drosophila melanogaster*. Evolution 50: 753–766.
- Christians J.K. 2000. Producing extra eggs does not deplete macronutrient reserves in European Starlings *Sturnus vulgaris*. Journal of Avian Biology 31: 312–318.
- Christians J.K. 2002. Avian egg size: variation within species and inflexibility within individuals. Biological Reviews of the Cambridge Philosophical Society 77: 1–26.
- Clark C.M. & Galef B.G.Jr. 1995. Prenatal influences in reproductive life history strategies. Trends in Ecology and Evolution 10: 151–153.
- Clutton-Brock T.H. 1990. Reproductive success. University of Chicago Press. Chicago, Illinois. USA.
- Clutton-Brock T.H. 1991. The evolution of parental care. Princeton University Press. Princeton, New Jersey. USA.
- Clutton-Brock T.H. 2009. Structure and function in mammalian societies. Philosophical Transactions of the Royal Society B: Biological Sciences 364: 3229–3242.
- Clutton-Brock T.H., Guinness F.E. & Albon S. 1982. Red deer: behavior and the ecology of two sexes. University of Chicago Press. Chicago, Illinois. USA.
- Clutton-Brock T.H. & Parker G.A. 1995. Sexual coercion in animal societies. Animal Behaviour 49: 1345–1365.
- Costantini D. & Møller A.P. 2009. Does immune response cause oxidative stress in birds? A meta-analysis. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 145: 137–142.
- Cox C.R. & Le Bœuf B.J. 1977. Female incitation of male competition: a mechanism in sexual selection. The American Naturalist 111: 317–335.
- Cramp S. & Simmons K.E.L. 1980. The birds of western Paleartic. Oxford University Press. Oxford, UK.
- Creel S. 2001. Social dominance and stress hormones. Trends in Ecology & Evolution 16: 491–497.

- Cronin H. 1991. The ant and the peackock: altruism and sexual selection from Darwin to today. Cambridge University Press. Cambridge, UK.
- Cucco M., Grenna M. & Malacarne G. 2012. Female condition, egg shape and hatchability: a study on the grey partridge. Journal of Zoology 287: 186–194.
- Cucco M., Grenna M., Pellegrino I. & Malacarne G. 2011a. Egg-sequence rather than mating preference influences female egg investment in the red-legged partridge. Ethology Ecology & Evolution 23: 343-357.
- Cucco M., Grenna M., Pellegrino I. & Malacarne G. 2011b. Effects of immune challenge and supernormal clutch production on egg quality in the red-legged partridge. Evolutionary Ecology Research 13: 269–282.
- Cucco M., Guasco B., Malacarne G., Ottonelli R. & Tanvez A. 2008. Yolk testosterone levels and dietary carotenoids influence growth and immunity of grey partridge chicks. General and Comparative Endocrinology 156: 418–425.
- Cucco M., Guasco B., Malacarne G. & Ottonelli R. 2007. Effects of β-carotene on adult immune condition and antibacterial activity in the eggs of the grey partridge, *Perdix perdix*. Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology 147: 1038–1046.
- Cucco M., Guasco B., Ottonelli R, Balbo V. & Malacarne G. 2009. The influence of temperature on egg composition in the grey partridge *Perdix perdix*. Ethology Ecology & Evolution 21: 63–77.
- Cucco M., Pellegrino I. & Malacarne G. 2010. Immune challenge affects female condition and egg size in the Grey Partridge. Journal of Experimental Zoology 313: 597–604.
- Cunningham E.J.A. & Russell A.F. 2000. Egg investment is influenced by male attractiveness in the mallard. Nature 404: 74–77.
- D'Alba L., Shawkey M., Korsten P., Vedder O., Kingma S., Komdeur J. & Beissinger S. 2010. Differential deposition of antimicrobial proteins in Blue Tit (*Cyanistes caeruleus*) clutches by laying order and male attractiveness. Behavioral Ecology and Sociobiology 64: 1037–1045.
- Darwin C. 1859. On the origin of Species by means of natural selection, or the preservation of favoured races in the struggle for life. John Murray. London, UK.
- Darwin C. 1871. The descent of man, and selection in relation to sex. John Murray. London, UK.
- De Goeij D.C., Dijkstra H. & Tilders F.J. 1992. Chronic psychosocial stress enhances vasopressin, but not corticotropin-releasing factor, in the external zone of the median eminence of male rats: relationship to subordinate status. Endocrinology 131: 847–853.
- Dijkstra C., Bult A., Bijlsma S., Daan S., Meijer T. & Zijlstra M. Brood Size Manipulations in the Kestrel (*Falco tinnunculus*): Effects on Offspring and Parent Survival. Journal of Animal Ecology 59: 269–285.

- Dijkstra H., Tilders F.J., Hiehle M.A. & Smelik P.G. 1992. Hormonal reactions to fighting in rat colonies: prolactin rises during defence, not during offence, Physiology & Behavior 51: 961–968.
- Dobzhansky T. 1968a. Adaptedness and fitness. In: Population biology and evolution. Lewontin R.C. (editor). Pp. 109–121. Syracuse University Press. Syracuse, New York. USA.
- Dobzhansky T. 1968b. On some fundamental concepts of Darwinian biology. In: Evolutionary biology. Dobzhansky T., Hecht M.K. & Steere W.C. (editors). Pp. 1–24. Appleton Century-Crofts. New York, New York. USA.
- Dooling R.J. & Popper A.N. 2007. The effects of highway noise on birds. Report to California Department of Transportation, contract 43A0139. (http://www.dot.ca.gov/hq/env/bio/avian_bioacoustics.htm).
- Dorus S., Evans P.D., Wyckoff G.J., Choi S.S. & Lahn B.T. 2004. Rate of molecular evolution of the seminal protein gene SEMG2 correlates with levels of female promiscuity. Nature Genetics 36: 1326–1329.
- Doughty P. & Shine R. 1997. Detecting life history trade-offs: measuring energy stores in "capital" breeders reveals costs of reproduction. Oecologia 110: 508–513.
- Doughty P. & Shine R. 1998. Reproductive energy allocation and long-term energy stores in a viviparous lizard (*Eulamprus tympanum*). Ecology 79: 1073–1083.
- Douglas H.D. & Conner W.E. 1999. Is there a sound reception window in coastal environments? Evidence from shorebird communication systems. Naturwissenschaften 86: 228–230.
- Doutrelant C., McGregor P.K. & Oliveira R.F. 2001. The effect of an audience on intrasexual communication in male Siamese fighting fish, *Betta splendens*. Behavioral Ecology 12: 283–286.
- Draganoiu, T.I., Nagle L. & Kreutzer M. 2002. Directional female preference for an exaggerated male trait in canary (*Serinus canaria*) song. Proceedings of the Royal Society of London. Series B: Biological Sciences 269: 2525-2531.
- Drent R.H. & Daan S. 1980. The prudent parent: energetic adjustments in avian breeding. Ardea 68: 225–252.
- Drews C. 1993. The concept and definition of dominance in animal behaviour. Behaviour 125: 283–313.
- Drickamer L.C., Gowaty P.A. & Holmes C.M. 2000. Free female mate choice in house mice affects reproductive success and offspring viability and performance. Animal Behaviour 59: 371–378.
- Drobney R.D. 1980. Reproductive bioenergetics of wood ducks. The Auk 97: 480–490.
- Dunham A.E., Grant B.W. & Overall K.L. 1989. Interfaces between biophysical and physiological ecology and the population ecology of terrestrial vertebrate ectotherms. Physiological Zoology 62: 335–355.

- Dusenbery D.B. 2006. Selection for high gamete encounter rates explains the evolution of anisogamy using plausible assumptions about size relationships of swimming speed and duration. Journal of Theoretical Biology 241: 33–38.
- Eberhard W.G. 1996. Female control: sexual selection by cryptic female choice. Princeton University Press. Princeton, New Jersey. USA.
- Ehrlich P.R., Dobkin D.S. & Wheye D. 1988. The birder's handbook: a field guide to the natural history of North American birds: including all the species that regularly breed north of Mexico. Simon and Schuster. New York, New York. USA.
- Eising C.M., Eikenaar C., Schwabl H. & Groothuis T.G. 2001. Maternal androgens in black headed gull (*Larus ridibundus*) eggs: consequences for chick development. Proceedings of the Royal Society B: Biological Sciences 268: 839–846.
- Eising C.M. & Groothuis T.G.G. 2003. Yolk androgens and begging behaviour in black-headed gull chicks: an experimental field study. Animal Behaviour 66: 1027–1034.
- Eising C.M., Müller W. & Groothuis T.G.G. 2006. Avian mothers create different phenotypes by hormone deposition in their eggs. Biology Letters 22: 20–22.
- Ellis L. 1995. Dominance and reproductive success among nonhuman animals. Ethology and Sociobiology 16: 257–333.
- Ely D.L. & Henry J.P. 1978. Neuroendocrine response patterns in dominant
- and subordinate mice, Hormones and Behavior 10: 156–169.
- Emlen S.T. & Oring L.W. 1977. Ecology, sexual selection, and the evolution of mating systems. Science 197: 215–223.
- Endler J.A. 1986. Natural selection in the wild. Princeton University Press. Princeton, New Jersey. USA.
- Eraud C., Duriez O., Chastel O. & Faivre B. 2005. The energetic cost of humoral immunity in the Collared Dove *Streptotelia decaocto*: is the magnitude sufficient to force energy-based trade-offs? Functional Ecology 19: 110–118.
- Eraud C., Jacquet A. & Faivre B. 2009. Survival cost of an early immune soliciting in nature. Evolution 63: 1036–1043.
- Erisir Z. & Ozbey O. 2005. The effects of egg weight and shape index on hatching characteristics in bronze turkeys. The Indian Veterinary Journal 82: 967–968.
- Fair J.M., Hansen E.S. & Ricklefs R.E. 1999. Growth, developmental stability and immune response in juvenile Japanese quails (*Coturnix coturnix japonica*). Proceedings of the Royal Society B: Biological Sciences 266: 1735–1742.
- Faivre B., Grégoire A., Pérault M., Cézilly F. & Sorci G. 2003. Immune activation rapidly mirrored in a secondary sexual trait. Science 300: 103.

- Fay R.R. 1988. Hearing in Vertebrates: A Psychophysics Databook. Hill-Fay Associates. Dallas, Texas. USA.
- Fay R.R. & Popper A.N. 2000. Evolution of hearing in vertebrates: inner ears and processing. Hearing Research 149: 1–10.
- Finkler M.S., van Orman J.B. & Sotherland P.R. 1998. Experimental manipulation of egg quality in chickens: influence of albumen and yolk on the size and body composition of near-term embryos in a precocial bird. Journal of Comparative Physiology B 168: 17–24.
- Fisher R.A. 1930. The genetical theory of natural selection. Clarendon Press. Oxford, UK.
- Fiske P., Rintamäki P.T. & Karvonen E. 1998. Mating success in lekking males: a meta-analysis. Behavioral Ecology 9: 328–338.
- Fitze P.S., Tschirren B., Gasparini J. & Richner H. 2007. Carotenoid-based plumage colors and immune function: is there a trade-off for rare carotenoids? The American Naturalist 169: S137–S144.
- Fokkema D.S., Koolhaas J.M. & van der Gugten J. 1995. Individual characteristics of behavior, blood pressure, and adrenal hormones in colony rats. Physiology & Behavior 57: 857–862.
- Follett B.K., Hinde R.A., Steel E. & Nicholls T.J. 1973. The influence of photoperiod on nest building, ovarian development and luteinizing hormone secretion in canaries (*Serinus canarius*). Journal of Endocrinology 59: 151–162.
- Folstad I. & Karter A.J. 1992. Parasites, bright males, and the immunocompetence handicap. The American Naturalist 139: 603–622.
- Fong D.W., Kane T.C. & Culver D.C. 1995. Vestigialization and loss of nonfunctional characters. Annual Review of Ecology, Evolution, and Systematics 26: 249–268.
- Fontenot M.B., Kaplan J.R., Manuck S.B., Arango V. & Mann J.J. 1995. Long-term effects of chronic social stress on serotonergic indices in the prefrontal cortex of adult male cynomolgus macaques. Brain Research 705: 105–108.
- Forbes S. & Wiebe M. 2010. Egg size and asymmetric sibling rivalry in red-winged blackbirds. Oecologia 163: 361–372.
- Forman R.T.T. 2000. Estimate of the area affected ecologically by the road systemin the United States. Conservation Biology 14: 31–35.
- Forsgren E. 1997. Female sand gobies prefer good fathers over dominant males. Proceedings of the Royal Society B: Biological Sciences 264: 1283–1286.
- Forsgren E., Amudsen T., Borg Å.A. & Bjelvenmark J. 2004. Unusually dynamic sex roles in a fish. Nature 429: 551–554.
- Forstmeier W., Coltman D.W. & Birkhead T.R. 2004. Maternal effects influence the sexual behavior of sons and daughters in the zebra finch. Evolution 58: 2574–2583.

- Freedberg S., Bowden R.M., Ewert M.A., Sengelaub D.R. & Nelson C.E. 2006. Long-term sex reversal by oestradiol in amniotes with heteromorphic
- sex chromosomes. Biology letters 2: 378–381.
- Freeland W.J. 1981. Parasitism and behavioural dominance among male mice. Science 213: 461–462.
- French S.S., Johnston G.I.H. & Moore M.C. 2007. Immune activity suppresses reproduction in food-limited female tree lizards *Urosaurus ornatus*. Functional Ecology 21: 1115–1122.
- French S.S., Moore M.C. & Demas G.E. 2009. Ecological immunology: the organism in context. Integrative and Comparative Biology 49: 246–253.
- Freitak D., Ots I., Vanatoa A. & Horak P. 2003. Immune response is energetically costly in white cabbage butterfly pupae. Proceedings of the Royal Society B: Biological Sciences 270: S220–222.
- French S.S., Moore M.C. & Demas G.E. 2009. Ecological immunology: the organism in context. Integrative and Comparative Biology 49: 246–253.
- Fugle G.N., Rothstein S.I., Osenberg C.W. & McGinley M.A. 1984. Signals of status in wintering white-crowned sparrows, *Zonotrichia leucophrys gambelii*. Animal Behaviour 32: 86–93.
- Fuller R.A., Warren P.H. & Gaston K.J. 2007. Daytime noise predicts nocturnal singing in urban robins. Biology Letters 34: 368–370.
- Gadgil M. & Bossert W. 1970. Life history consequences of natural selection. The American Naturalist. 104: 1–24.
- Gambino R., Desvarieux E., Orth M., Matan H., Ackattupathil T., Lijoi E. Wimmer, C., Bower J. & Gunter E. 1997. The relation between chemically measured total iron-binding capacity concentrations and immunologically measured transferrin concentrations in human serum. Clinical Chemistry 43: 2408–2412.
- Garamszegi L.Z., Merino S., Török J., Eens M. & Martínez J. 2006. Indicators of physiological stress and the elaboration of sexual traits in the collared flycatcher. Behavioral Ecology 17: 399–404.
- Garcia-Fernandez V. 2009. Qualité du partenaire et qualité de l'œuf chez les oiseaux. PhD thesis. Université Paris Ouest Nanterre – Università degli Studi del Piemonte Orientale.
- Garcia-Fernandez V., Guasco B., Tanvez A., Cucco M., Leboucher G. & Malacarne G. 2010a. Influence of mating preferences on yolk testosterone in the grey partridge. Animal Behaviour 80: 45–49.
- Garcia-Fernandez V., Amy M., Lacroix A., Leboucher G. & Malacarne G. 2010b. Eavesdropping on male singing interactions leads to differential allocation of eggs. Ethology 116: 662-670.
- Garcia-Fernandez V., Draganoiu T.I., Ung D., Lacroix A., Malacarne G & Leboucher G. 2013. Female canaries invest more in response to an exaggerated male trait. *In press*.

- Garratt M., Vasilaki A., Stockley P., McArdle F., Jackson M. & Hurst J.L. 2011. Is oxidative stress a physiological cost of reproduction? An experimental test in house mice. Proceedings of the Royal Society B: Biological Sciences 278: 1098–1106.
- Gasparini J., Bize P., Piault R., Wakamatsu K., Blount J.D., Ducrest A.-L. & Roulin A. 2009. Strength and cost of an induced immune response are associated with heritable melanin-based colour trait in female tawny owls. Journal of Animal Ecology 78: 608 –616.
- Geral M.F., Lautie R. & Bodin G. 1976. Study of the experimental infection of game birds (pheasant, red legged partridge and common partridge) with Newcastle disease virus. Veterinary Medical Review 127: 1537–1574.
- Gerhardt H.C. & Huber F. 2002. Acoustic Communication in Insects and Anurans: Common Problems and Diverse Solutions. The University of Chicago Press. Chicago, Illinois. USA.
- Giansanti F., Leboffe L., Pitari G., Ippoliti R. & Antonini G. 2012. Physiological roles of ovotransferrin. Biochimica et Biophysica Acta 1820: 218–225.
- Giansanti F., Rossi P., Massucci M.T., Botti D., Antonini G., Valenti P. & Seganti L. 2002. Antiviral activity of ovotransferrin discloses an evolutionary strategy for the defensive activities of lactoferrin. Biochemistry and Cell Biology 80: 125–130.
- Gil D. 2008. Hormones in avian eggs: physiology, ecology and behavior. Advances in the Study of Animal Behaviour 38: 337–398.
- Gil D., Graves J., Hazon N. & Wells A. 1999. Male attractiveness and differential testosterone investment in zebra finch eggs. Science 286: 126–128.
- Gil D., Leboucher G., Lacroix A., Cue R. & Kreutzer M.L. 2004a. Sexually attractive phrases increase yolk androgens deposition in Canaries (*Serinus canaria*). Hormones and Behavior 45: 64–70.
- Gil D., Leboucher G., Lacroix A., Cue R. & Kreutzer M.L. 2004b. Female canaries produce eggs with greater amounts of testosterone when exposed to attractive male song. Hormones and Behavior 45: 64–70.
- Gil D., Marzal A., de Lope F., Puerta M. & Møller A.P. 2006. Female house martins (*Delichon urbica*) reduce egg androgen deposition in response to a challenge of their immune system. Behavioral Ecology and Sociobiology 60: 96–100.
- Gilbert L., Williamson K.A., Hazon N. & Graves J.A. 2006. Maternal effects due to male attractiveness affect offspring development in the zebra finch. Proceedings of the Royal Society B: Biological Sciences 273: 1765–1771.
- Gill F.B. 1996. Ornitology. 2th edition. Freeman W.H. & Company. Pp. 355–357; 361–363; 368–371. New York, New York. USA.
- Gill, F.B. 2007. Ornithology. 3rd edition. Freeman W.H. & Company. Pp. 405–409; 418–421; 423–425. New York, New York. USA.

- Goncalves I.B., Mobley K.B., Ahnesjö I., Sagebakken G., Jones A.G. & Kvarnemo C. 2010. Reproductive compensation in broad-nosed pipefish females. Proceedings of the Royal Society B: Biological Sciences 277: 1581–1587.
- Gosler A.G., Greenwood J.D. & Perrins C. 1995. Predation risk and the cost of being fat. Nature 377: 621–623.
- Gowaty P.A. 2008. Reproductive compensation. Journal of Evolutionary Biology 21: 1189–1200.
- Gowaty P.A., Anderson W.W., Bluhm C.K., Drickamer L.C., Kim Y.K. & Moore A.J. 2007. The hypothesis of reproductive compensation and its assumptions about mate preferences and offspring viability. Proceedings of the National Academy of Sciences of the USA 104: 15023–15027.
- Gowaty P.A., Drickamer L.C. & Schmid-Holmes S. 2003. Male house mice produce fewer offspring with lower viability and poorer performance when mated with females they do not prefer. Animal Behaviour 65: 95–103.
- Grafen A. 1990. Biological signals as handicaps. Journal of Theoretical Biology 144: 517–546.
- Graham I. & Williams J. 1975. A comparison of glycopeptides from the transferrins of several species. Biochemical Journal 145: 263–279.
- Grana S.C., Sakaluk S.K., Bowden R.M., Doellman M.A., Vogel L.A. & Thompson C.F. 2012. Reproductive allocation in female house wrens is not influenced by experimentally altered male attractiveness. Behavioral Ecology and Sociobiology 66: 1247–1258.
- Graves H.B., Hable C.P. & Jenkins T.H. 1985. Sexual selection in *Gallus*: effects of morphology and dominance on female spatial behavior. Behavioural Processes 11: 189–197.
- Grindstaff J.L. 2008. Maternal antibodies reduce costs of an immune response during development. Journal of Experimental Biology 211: 654–660.
- Groothuis T.G.G., Müller W., von Engelhardt N., Carere C. & Eising C. 2005. Maternal hormones as a tool to adjust offspring phenotype in avian species. Neuroscences and Biobehavioral Reviews 29: 329–352.
- Groothuis T.G.G. & Schwabl H. 2002. Determinants of within- and among-clutch variation in levels of maternal hormones in blackheaded gull eggs. Functional Ecology 16: 281–289.
- Guasco B. 2006. Effetti materni e qualità dell'uovo nella starna (*Perdix perdix*). PhD thesis. Università degli Studi del Piemonte Orientale.
- Gustafsson L., Qvarnström A. & Sheldon B.C. 1995. Trade-offs between life-history traits and a secondary sexual character in male collared flycatchers. Nature 375: 311–313.
- Gwinner H. & Schwabl H. 2005. Evidence for sexy son hypothesis in European starlings (*Sturnus vulgaris*) Behavioral Ecology and Sociobiology 58: 375–382.

- Habib L., Bayne E.M. & Boutin S. 2007. Chronic industrial noise affects pairing success and age structure of ovenbirds *Seiurus aurocapilla*. Journal of Applied Ecology 44: 176–184.
- Halfwerk W., Bot S., Buikx J., van der Veldeb M., Komdeurb J. & ten Catea C. 2011a. Low-frequency songs lose their potency in noisy urban conditions. Proceedings of the National Academy of Sciences of the USA 108: 14549–14554.
- Halfwerk W., Holleman L.J.M., Lessells C.M. & Slabbekoorn H. 2011b. Negative impact of traffic noise on avian reproductive success. Journal of Applied Ecology 48: 210–219.
- Halfwerk W., Bot S. & Slabbekoorn H. 2012. Male great tit song perch selection in response to noise-dependent female feedback. Functional Ecology 26: 1339–1347.
- Hanssen S.A. 2006. Cost of immune challenge and terminal investment in a long-lived bird. Ecology 87: 2440–2446.
- Hanssen S.A., Hasselquist D., Folstad I. & Erikstad K. E. 2004. Cost of immunity: immune responsiveness reduces survival in a vertebrate. Proceedings of the Royal Society B: Biological Sciences 271: 925–930.
- Hargitai R., Arnold K.E., Herényi M., Prechl J. & Török J. 2009. Egg composition in relation to social environment and maternal physiological condition in the collared flycatcher. Behavioral Ecology and Sociobiology 63: 869–882.
- Harris W.E. & Uller T. 2009. Reproductive investment when mate quality varies: differential allocation versus reproductive compensation. Philosophical Transactions of the Royal Society B: Biological Sciences 364: 1039–1048.
- Harvell C.D. 1990. The ecology and evolution of inducible defenses. The Quarterly Review of Biology 65: 323–340.
- Hasselquist D. & Nilsson J.-Å. 2012. Physiological mechanisms mediating costs of immune responses: what can we learn from studies of birds? Animal Behaviour 83: 1303–1312.
- Hauser M.D. 1997. The Evolution of Communication. MIT Press. Cambridge, Massachusetts. USA.
- Hava G., Vered L., Yael M., Mordechai H. & Mahoud H. 2006. Alterations in behavior in adult offspring mice following maternal inflammation during pregnancy. Developmental Psychobiology 48:162–168.
- Hayakawa S., Kondo H., Nakamura R. & Sato Y. 1983. Effect of β-ovomucin on the solubility of β-ovomucin and further inspection of the structure of ovomucin complex in thick egg white. Agricultural and Biological Chemistry 47: 815–820.
- Heaney V., Nager R.G. & Monaghan P. 1998. Effect of increased egg production on egg composition in the common tern *Sterna hirundo*. Ibis 140: 693–696.

- Hegner R.E. & Wingfield J.C. 1987. Social status and circulating levels of hormones in Flocks of House Sparrows, *Passer domesticus*. Ethology 76: 1–14.
- Heylen D.J.A. & Matthysen E. 2008. Effect of tick parasitism on the health status of a passerine bird. Functional Ecology 22: 1099–1107.
- Hinde R.A. 1958. The nest-building behaviour of domesticated canaries. Proceedings of the Royal Society B: Biological Sciences 131: 1–48.
- Hinde R.A. & Steel E. 1975. The dual role of daylength in controlling canary reproduction. Symposia of the Zoological Society of London 35: 245–259.
- Hoeschele M., Moscicki M.K., Otter K.A., van Oort H., Fort K.T., Farrell T.M., Lee H., Robson S.W.J. & Sturdy C.B. 2010. Dominance signalled in an acoustic ornament. Animal Behaviour 79: 657–664.
- Höglund J. & Alatalo R.V. 1995. Leks. Princeton University Press. Princeton, New Jersey. USA.
- Holveck M.J. & Riebel K. 2009. Low-quality females prefer low-quality males when choosing a mate. Proceedings of the Royal Society B: Biological Sciences 277: 153–160.
- Hõrak P., Zilmer M., Saks L., Ots I., Karu U. & Zilmer K. 2006. Antioxidant protection, carotenoids and the costs of immune challenge in greenfinches. Journal of Experimental Biology 209: 4329–4338.
- Horne T.J. & Ylönen H. 1996. Female bank voles (*Clethrionomys glareolus*) prefer dominant males; but what if there is no choice? Behavioral Ecology and Sociobiology 38: 401–405.
- Horrocks N.P.C., Tieleman B.I. & Matson K.D. 2011. A simple assay for measurement of ovotransferrin – a marker of inflammation and infection in birds. Methods in Ecology and Evolution 2: 518–526.
- Horváthová T., Nakagawa S. & Uller T. 2012. Strategic female reproductive investment in response to male attractiveness in birds. Proceedings of the Royal Society B: Biological Sciences 279: 163–170.
- Houston A.I. & McNamara J.M. 1999. Models of adaptive behaviour: an approach based on state. Cambridge University Press. Cambridge, UK.
- Houston A.I., Welton N.J. & McNamara J.M. 1997. Acquisition and maintenance costs in the long-term regulation of avian fat reserves. Oikos 78: 331–340.
- Huet des Aunay G. 2011. Communication dans le bruit : Comment les femelles canaris domestiques, *Serinus canaria* rêagissent-elles aux chants des mâles ? Master 2 thesis. Université Paris Ouest Nanterre.
- Hughey V.L. & Johnson E.A. 1987. Antimicrobial Activity of lysozyme against bacteria involved in food spoilage and food-borne Disease. Applied and Environmental Microbiology 53: 2165–2170.
- Hunt J. & Hodgson D. 2010. What is fitness, and how do we measure it? In: Evolutionary behavioral ecology. Westneat D.F. & Fox C.W. (editors). Pp. 46–70. Oxford University Press. Oxford, UK.

- Hunter F.M., Harcourt R., Wright M. & Davis L. 2000. Strategic allocation of ejaculates by male Adélie penguins. Proceedings of the Royal Society B: Biological Sciences 267: 1541–1545.
- Huopalahti R., López-Fandiño R., Anton M. & Schade R. 2007. Bioactive egg compounds. Springer. Berlin, Germany.
- Hurd P.L. & Enquist M. 2001. Threat display in birds. Canadian Journal of Zoology 79: 931–942.
- Hussel D.T.J. 1972. Factors affecting clutch size in arctic passerines. Ecological Monographs 49: 317–364.
- Ilmonen P., Taarna T. & Hasselquist D. 2000. Experimentally activated immune defence in female pied flycatcher results in reduced breeding success. Proceedings of the Royal Society B: Biological Sciences 267: 665–670.
- Ilmonen P., Taarna T. & Hasselquist D. 2002. Are incubation costs in female pied flycatchers expressed in humoral immune responsiveness or breeding success? Oecologia 130: 199–204.
- Janzen F.J., Wilson M.E., Tucker J.K. & Ford S. P. 1998. Endogenous yolk steroid hormones in turtles with different sex-determining mechanisms. General and Comparative Endocrinology 111: 306–317.
- Jarup L., Babisch W., Houthuijs D., Pershagen G., Katsouyanni K., Cadum E., Dudley M.-L., Savigny P., Seiffert I., Swart W., Breugelmans O., Bluhm G., Selander J., Haralabidis A., Dimakopoulou K., Sourtzi P., Velonakis M. & Vigna-Taglianti F. 2008. Hypertension and exposure to noise near airports: the HYENA study. Environmental Health Perspectives 116: 329–333.
- Jennions M.D. & Kokko H. 2010. Sexual selection. In: Evolutionary behavioral ecology. Westneat D.F. & Fox C.W. (editors). Pp. 343–364. Oxford University Press. Oxford, UK.
- Jennions M.D., Møller A.P. & Petrie M. 2001. Sexually selected traits and adult survival: a meta-analyses. Quartely Review of Biology 76: 3–36.
- Jivoff P. & Hines A.H. 1998. Female behaviour, sexual competition and mate guarding in the blue crab, *Callinectes sapidus*. Animal Behaviour 55: 589–603.
- Johnsen T.S., Zuk M. & Fessler E.A. 2001. Social dominance, male behavior and mating in a mixed-sex flocks of red jungle fowl. Behaviour 138: 1–18.
- Johnson A.L. 2000. Reproduction in the females. In: Sturkie's Avian Physiology. 5th edition. Whittow G.C. (Editor). Pp. 569–596. Academic Press. Waltham, Massachusetts. USA.
- Jones A.J. & Ratterman N.L. 2009. Mate choice and sexual selection: what have we lerned since Darwin? Proceedings of the National Academy of Sciences of the United States of America 106: 10001–10008.

- Jones A.G., Rosenqvist G., Berglund A. & Avise J.C. 2005. The measurement of sexual selection using Bateman's principles: an experimental tests in the sex-role-reversed pipefish *Syngnathus typhle*. Integrative and Comparative Biology 45: 874–884.
- Jones I.H., Stoddart D.M. & Mallick J.J. 1995. Towards a sociobiological model of depression. A marsupial model (*Petaurus breviceps*). The British Journal of Psychiatry 166: 475–479.
- Kaplan J.R., Adams M.R., Clarkson T.B., Manuck S.B., Shively C.A. & Williams J.K. 1996. Psychosocial factors, sex differences, and atherosclerosis: lessons from animal models. Psychosomatic Medicine 58: 598–611.
- Kaplan J.R., Chen H. & Manuck S.B. 2009. The relationship between social status and atherosclerosis in male and female monkeys as revealed by meta-analysis. American Journal of Primatology 71: 732–741.
- Kaplan J.R., Manuck S.B., Clarkson T.B., Lusso F.M. & Taub D.M. 1982. Social status, environment, and atherosclerosis in *Cynomolgus monkeys.* Arteriosclerosis 2: 359–368.<
- Kaplan J.R., Manuck S.B., Fontenot M.B. & Mann J.J. 2002. Central nervous system monoamine correlates of social dominance in cynomolgus monkeys (*Macaca fascicularis*). Neuropsychopharmacology 26: 431–443.
- Karasov W.H. & Anderson R.A. 1998. Correlates of average daily metabolism of field-active zebra-tailed lizards (*Collisaurus draconoides*). Physiological Zoology 71: 93–105.
- Keiper R.R. & Receveur H. 1992. Social interactions of free-ranging Przewalski horses in semi-reserves in the Netherlands. Applied Animal Behaviour Science 33: 303–318.
- Kennamer R.A., Alsum S.A. & Colwell S.V. 1997. Composition of wood duck eggs in relation to egg size, laying sequence, and skipped days of laying. The Auk 114: 479–487.
- Kennedy E.D. 1991. Determinate and indeterminate egg-laying patterns: a review. The Cooper Ornithological Society 93: 106–124.
- Ketterson E.D. & Nolan V.Jr. 1992. Hormones and life histories: An integrative approach. The American Naturalist 140: S33–S62.
- Kilner R. & Johnston R.A. 1997. Begging the question: are offspring solicitation behaviours signals of need? Trends in Ecology and Evolution 12: 11–15.
- King J.R. 1973. Energetics of reproduction in birds. In: Breeding biology in birds. Farner D.S. (editor). Pp. 78–107. National Academy Sciences. Washington, Washington DC. USA.
- King A. & West M. 1977. Species identification in the North American cowbird: appropriate responses to abnormal song. Science 195: 1002–1004.
- Kingma S.A., Komdeur J., Vedder O., von Engelhardt N., Korsten P. & Groothuis T.G.G. 2006. Rapid changes in maternal yolk hormone

deposition in response to manipulated male attractiveness. In: Avian Sex Allocation and Ornamental Coloration. A Study on Blue Tits. Korsten P. (Editor). Pp. 73–86. PhD thesis. University of Groningen. http://dissertations.ub.rug.nl/FILES/faculties/science/2006/p.korsten/PartII/c5.pdf.

- Kingma S.A., Komdeur J., Vedder O., von Engelhardt N., Korsten P. & Groothuis T.G.G. 2009. Manipulation of male attractiveness induces rapid changes in avian maternal yolk androgen deposition. Behavioral Ecology 20: 172-179.
- Kirkpatrick M. & Barton N.H. 1997. The strength of indirect selection on female mating preferences. Proceedings of the National Academy of Sciences of the USA. 94: 1282–1286.
- Kirkpatrick M. & Lande R. 1989. The evolution of maternal characters. Evolution 43: 485–503.
- Kirkpatrick M. & Ryan M.J. 1991. The evolution of mating preferences and the paradox of the lek. Nature 350: 33–38.
- Kirschel A.N.G., Blumstein D.T., Cohen R.E., Buermann W., Smith T.B. & Slabbekoorn H. 2009. Birdsong tuned to the environment: green hylia song varies with elevation, tree cover, and noise. Behavioral Ecology 20: 1089–1095.
- Kiss Z., Bardos L., Szabo C., Lengyel L. & Szabo W. 2003. Effect of beta-carotene supplementation on plasma and yolk IgY levels induced by NDV vaccination in Japanese quail. International Journal for Vitamin and Nutrition Research 73: 285–289.
- Klump G.M. 1996. Bird communication in a noisy world. In: Ecology and Evolution of Acoustic Communication in Birds. Kroodsma D.E. & Miller E.H. (Editors). Pp. 321–328. Cornell University Press. Ithaca, New York. USA.
- Kokko H. & Jennions M.D. 2008. Parental investment, sexual selection and sex ractios. Journal of Evolutional Biology 21: 919–948.
- Kokko H., Brooks R., Jennions M.D., & Morley J. 2003. The evolution of mate choice and mating biases. Proceedings of the Royal Society B: Biological Sciences 270: 653–664.
- Kolm N. 2001. Females produce larger eggs for large males in a paternal mouthbrooding fish. Proceedings of the Royal Society B: Biological Sciences 268: 2229–2234.
- Kolmer K. & Heinze J. 2000. Rank orders and division of labour among unrelated cofounding ant queens. Proceedings of the Royal Society B: Biological Sciences 267: 1729–1734.
- Kotiaho J.S., Simmons L.W., Hunt J. & Tomkins J.L. 2003. Males influence maternal effects that promote sexual selection: a quantitative genetic experiment with dung beetles *Onthophagus taurus*. The American Naturalist 161: 852–859.

- Kreutzer M. L. & Vallet E. M. 1991. Differences in the Responses of Captive Female Canaries to Variation in Conspecific and Heterospecific Songs. Behaviour 117: 106–116.
- Krimbas C.B. 2004. On fitness. Biology and Philosophy 19: 185–203.
- Krist M. 2011. Egg size and offspring quality: a meta-analysis in birds. Biological Reviews 86: 692–716.
- Kroodsma D.E. 1976. Reproductive development in a female songbird: differential stimulation by quality of male song. Science 192: 574–575.
- Kroodsma D.E. 1982. Learning and the ontogeny of sound signals in birds. In: Acoustic communication in birds (v.2). Kroodsma D.E., Miller E.H., Ouellet H. (Editors). Pp. 1–23. Academic Press. Waltham, Massachusetts. USA.
- Kroodsma D. E. 1989. Inappropriate experimental designs impede progress in bioacoustic reasearch: a reply. Animal behaviour 38: 717–719.
- Kroodsma, D.E., Byers B.E., Goodale E., Johnson S. & Liu W.-C. 2001. Pseudoreplication in playback experiments, revisited a decade later. Animal Behaviour 61: 1029–1033.
- Kuijper B., Pen I. & Weissing F.J. 2012. A guide to Sexual Selection Theory. The Annual Review of Ecology, Evolution, and Systematics 43: 287–311.
- Kurvers R.H.J.M., Eijkelenkamp B., van Oers K., van Lith B., van Wieren S.E., Ydenberg R.C. & Prins H.H.T. 2009. Personality differences explain leadership in barnacle geese. Animal Behaviour 78: 447–453.
- Lack, D. 1968. Ecological adaptations for breeding in birds. Methuen & Co. London, UK.
- Laiolo P. 2010. The emerging significance of bioacoustics in animal species conservation. Biological Conservation 143: 1635–1645.
- LaMunyon C.W. & Ward S. 1999. Evolution of sperm size in nematodes: sperm competition favours larger sperm. Proceedings of the Royal Society B: Biological Sciences 266: 263–267.
- Landwer A.J. 1994. Manipulation of egg production reveals costs of reproduction in the tree lizard (*Urosaurus ornatus*). Oecologia 100: 243–249.
- Le Boeuf B.J. & Mesnick S. 1990. Sexual behaviour of male northern elephant seals: I. Lethal injures to adult females. Behaviour 116: 143–162.
- Leboucher G., Kreutzer M.L. & Dittami J. 1994. Copulation-solicitation displays in female canaries (*Serinus canaria*): Are oestradiol implants necessary? Ethology 97: 190–197.
- Leboucher G. & Pallot K. 2004. Is he all he says he is? An investigation into intersexual eavesdropping in the domestic canary (*Serinus canaria*). Animal Behaviour 68: 957–963.
- Leboucher G., Vallet E., Nagle L., Béguin N., Bovet D., Hallé F., Draganoiu T.I., Amy M. & Kreutzer M.L. 2012. Studying female reproductive activities in relation to male song: domestic canary as a model. In: Advances in the

study of behavior Volume 44. Brockmann H.J., Roper T.J., Naguib M., Mitani J.C. & Simmons L.W. (editors). Pp. 183–223. Academic Press. Waltham, Massachusetts. USA.

- Lee K.A., Martin L.B. II & Wikelski M.C. 2005. Responding to inflammatory challenges is less costly for a successful avian invader, the house sparrow (*Passer domesticus*), than its less-invasive congener. Oecologia 145: 244–251.
- Leporati L. 1983. La starna. Edagricole. Bologna, Italy.
- Lessells C.M., Dingemanse N.J. & Both C. 2002. Egg weights, egg component weights and laying gaps in great tits (*Parus major*) in relation to ambient temperature. The Auk 119: 1091–1103.
- Li-Chan E.C., Powrie W.D. & Nakai S. 1995. The chemistry of eggs and egg products. In: Egg science and technology. Stadelman W.J. & Cotterill O.J. (Editors). Pp. 105–175. The Haworth Press. Binghamton, New York. USA.
- Lima S.L. 1986. Predation risk and unpredictable feeding conditions: determinants of body mass in birds. Ecology 67: 366–376.
- Linden M. & Möller A.P. 1989. Cost of reproduction and covariation of life history traits in birds. Trends in Ecology & Evolution 6: 183–185.
- Lindström J. 1999. Early development and fitness in birds and mammals. Trends in Ecology & Evolution 9: 343–348.
- Lipar J.L., Ketterson E.D., Nolan V. & Casto J.M. 1999. Egg yolk layers vary in the concentration of steroid hormones in two avian species. General and Comparative Endocrinology 115: 220–227.
- Lipar J.L. & Ketterson E.D. 2000. Maternally derived yolk testosterone enhances the development of the hatching muscle in the red-winged blackbird *Agelaius phoeniceus*. Proceedings of the Royal Society B: Biological Sciences 267: 2005–2010.
- Locatello L. & Neat F.C. 2005. Reproductive allocation in *Aidablennius sphynx* (Teleostei, Blenniidae): females lay more eggs faster when paired with larger males. Journal of Experimental Zoology Part A: Comparative Experimental Biology 303A: 922–926.
- Lochmiller R.L. 1996. Immunocompetence and animal population regulation. Oikos 76: 594–602.
- Lochmiller R.L. & Deerenberg C. 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity? Oikos 88: 87–98.
- Lodé T. 2001. Les stratégies de reproduction des animaux. Editions Dunod Sciences. Paris, France.
- Lohr B., Wright T.F. & Dooling R.J. 2003. Detection and discrimination of natural calls in masking noise by birds: estimating the active space of a signal. Animal Behaviour 65: 763–777.
- Lovern M.B. & Wade J. 2001 Maternal plasma and egg yolk testosterone concentrations during embryonic development in green anoles (*Anolis carolinensis*). General and Comparative Endocrinology124: 226–235.

- Lovern M.B. & Wade J. 2003. Yolk testosterone varies with sex in eggs of the lizard, *Anolis carolinensis*. Journal of Experimental Zoology 295: 206–210.
- Loyau A., Saint Jalme M., Mauget R. & Sorci G. 2007. Male sexual attractiveness affects the investment of maternal resources into the eggs in peafowl (*Pavo cristatus*). Behavioral Ecology and Sociobiology 61: 1043–1052.
- Loye J.E. & Zuk M. 1991. Bird–Parasite Interactions: Ecology, Evolution, Behaviour. Oxford University Press. Oxford, UK.
- Luckinbill L.S., Arking R., Clare M.J., Cirocco W.C. & Buck S.A. 1984. Selection for delayed senescence in *Drosophila melanogaster*. Evolution 38: 996–1004.
- Lusianti-Fitri L. 2001. Les chants des canaris domestiques: leurs relations avec des caractéristiques physiologiques et le statut social des mâles émetteurs. PhD thesis. Université Paris Ouest Nanterre.
- Luther D.A. & Derryberry E.P. 2012. Birdsongs keep pace with city life: changes in song over time in an urban songbird affects communication. Animal Behaviour 83: 1059–1066.
- Lyman C.P. & Chatfield P.O. 1955. Physiology of hibernation in mammals. Physiological Reviews 35: 403–425.
- Magrath R.D. 1990. Hatching asynchrony in altricial birds. Biological Reviews 65: 587–622.
- Magrath R.D. 1992. The effect of egg mass on the growth and survival of blackbirds: A field experiment. Journal of Zoology 227: 639–653.
- Majolo B., Aureli F. & Schino G. 2012. Meta-analysis and animal social behaviour. Evolutionary Ecology 26: 1197-1211.
- Mallick J., Stoddart D.M., Jones I. & Bradley A.J. 1994. Behavioral and endocrinological correlates of social status in the male sugar glider (*Petaurus breviceps* Marsupialia: Petauridae). Physiology & Behavior 55: 1131–1134.
- Mänd R., Tilgar V., Kilgas P. & Mägi M. 2007. Manipulation of laying effort reveals habitat-specific variation in egg production constraints in Great Tits (*Parus major*). Journal of Ornithology 148: 91–97.
- Mansour N., Lahnsteiner F. & Patzner R.A. 2007. Distribution of lipid droplets is an indicator for egg quality in brown trout, *Salmo trutta fario*. Aquaculture 273: 744–747.
- Marino O.C. & Hanson R.P. 1987. Cellular and humoral response of in ovo-bursectomized chickens to experimental challenge with velogenic Newcastle disease virus. Avian Diseases 31: 293–301.
- Martin L.B., Scheuerlein A. & Wikelski M. 2003. Immune activity elevates energy expenditure of house sparrows: a link between direct and indirect costs? Proceedings of the Royal Society B: Biological Sciences 270: 153–8.

- Marzal A., Revireigo M., de Lope F. & Møller A.P. 2007. Fitness costs of an immune response in the house martin (*Delichon urbica*). Behavioral Ecology and Sociobiology 61: 1573–1580.
- Masello J.F. & Quillfeldt P. 2004. Are haematological parameters related to body condition, ornamentation and breeding success in wild burrowing parrots *Cyanoliseus patagonus*? Journal of Avian Biology 35: 445–454.
- Mazuc J., Bonneaud C., Chastel O. & Sorci G. 2003a. Social environment affects female and egg testosterone levels in the house sparrow (*Passer domesticus*). Ecology Letters 6: 1084–1090.
- Mazuc J., Chastel O. & Sorci G. 2003b. No evidence for differential maternal allocation to offspring in the house sparrow (*Passer domesticus*). Behavioral Ecology 14: 340–346.
- McCormick M.I. 1999. Experimental test of the effect of maternal hormones on larval quality of a coral reef fish. Oecologia 118: 412–422.
- McGregor P. (editor). 2005. Animal Communication Networks. Cambridge University Press. Cambridge, UK.
- McGregor P.K. & Dabelsteen T. 1996. Communication networks. In: Ecology and Evolution of Acoustic Communication in Birds. Kroodsma D.E. & Miller E.H. (Editors). Pp. 409–425. Comstock Publishing Associates. Ithaca, New York. USA.
- McGregor P.K. & Peake T. 2000. Communication networks: social environments for receiving and signaling behaviour. Acta Ethologica 2: 71–81.
- Mead L.S. & Arnold S.J. 2004. Quantitative genetic models of sexual selection. Trends in Ecology and Evolution. 19: 264–271.
- Mennill D.J., Ratcliffe L.M. & Boag P.T. 2002. Female eavesdropping on male song contests in songbirds. Science 296: 873.
- Merilä J. & Svensson E. 1995. Fat reserves and health state in migrant Goldcrest *Regulus regulus*. Functional Ecology 9: 842–848.
- Michl G., Torok J., Peczely P., Garamszegi L.Z. & Schwabl H. 2005. Female collared flycatchers adjust yolk testosterone to male age, but not to attractiveness. Behavioral Ecology 16: 383–388.
- Minvielle F. & Oguz Y. 2002. Effects of genetics and breeding on egg quality of Japanese quail. World's Poultry Science Journal 58: 291–295.
- Mizutani K., Toyoda M. & Mikami B. 2012. X-ray structures of transferrins and related proteins. Biochimica et Biophysica Acta - General Subjects 1820: 203–211.
- Moburg G.P. 1991. How behavioral stress disrupts the endocrine control of reproduction in domestic animals. Journal of Dairy Science 74: 304–311.
- Mockford E.J. & Marshall R.C. 2009. Effects of urban noise on song and response behaviour in great tits. Proceedings of the Royal Society B: Biological Sciences 276: 2979–2985.

- Møller A.P., Biard C., Blount J.D., Houston D.C., Ninni P., Saino N. & Surai P.F. 2000. Carotenoid-dependent signals: indicators of foraging efficency, immunocompetence, or detoxification ability? Avian and Poultry Biology Reviews 11: 137–159.
- Møller A.P. & Jennions M.D. 2001. How important are direct fitness benefits of sexual selection? Naturwissenschaften 88: 401–415.
- Møller A.P. & Alatalo R.V. 1999. Good-genes effects in sexual selection. Proceedings of the Royal Society B: Biological Sciences 266: 85–91.
- Monaghan P., Metcalfe, N.B. & Torres R. 2009. Oxidative stress as mediator of life history trade-offs: mechanisms, measurements and interpretations. Ecology Letters 12: 75–92.
- Monaghan P., Nager R.G. & Houston D.C. 1998. The price of eggs: increased investment in egg production reduces the offspring rearing capacity of parents. Proceedings of the Royal Society B: Biological Sciences 265: 1731–1735.
- Montague M.J., Danek-Gontard M. & Kunc H.P. 2013. Phenotypic plasticity affects the response of a sexually selected trait to anthropogenic noise. Behavioral Ecology 24: 343–348.
- Moore A.J., Brodie E.D. & Wolf J.B. 1997. Interacting phenotypes and the evolutionary process. 1. Direct and indirect genetic effects of social interactions. Evolution 51: 1352–1362.
- Moore I.T. & Jessop T.S. 2003. Stress, reproduction, and adrenocortical modulation in amphibians and reptiles. Hormones and Behavior 43: 39–47.
- Mousseau T.A. & Fox C.W. 1998. The adaptive significance of maternal effects. TREE 13: 403–407.
- Müeller L.D., Guo P. & Ayala F.J. 1991. Density dependent natural selection and trade-offs in life history traits. Science 253: 433-435.
- Müller W., Eising C.M., Dijkstra C. & Groothuis T.G.G. 2002. Sex differences in yolk hormones depend on maternal social status in leghorn chickens (*Gallus gallus domesticus*). Proceedings of the Royal Society B: Biological Sciences 269: 2249–2255.
- Müller W., Vergauwen J. & Eens M. 2008. Yolk testosterone, postnatal growth and song in male canaries. Hormones and Behavior 54: 125–133.
- Murphy M.T., Sextona K., Dolona A.C. & Redmonda L.J. 2008. Dawn song of the eastern kingbird: an honest signal of male quality? Animal Behaviour 75: 1075–1084.
- Mussa P.P. & Boano G. 1990. Piemonte. Gestione Faunistica e Legislazione. Eda Torino. Torino, Italy.
- Nager R.G. 2006. The challenge of making eggs. Ardea 94: 323–346.
- Nager R.G., Monaghan P. & Houston D.C. 2000. Within-clutch trade-offs between the number and quality of eggs: experimental manipulations in gulls. Ecology 81: 1339–1350.

- Nagle L., Kreutzer M.L. & Vallet E.M. 1993. Obtaining copulation solicitation displays in female canaries without estradiol implants. Experientia 49: 1022–1023.
- Naguib M. & Todt D. 1997. Effects of dyadic interactions on other conspecific receivers in nightingales. Animal Behaviour 54: 1535–1543.
- Narins P., Feng A., Fay R. & Popper A. 2006. Hearing and Sound Communication in Amphibians. Springer. Berlin, Germany.
- Navara K.J., Hill G.E. & Mendoça M.T. 2005. Variable effects of yolk androgens on growth, survival, and immunity in eastern bluebird nestlings. Physiological and Biochemical Zoology 78: 570–578.
- Navara K.J., Badyaev A.V., Mendonca M.T. & Hill G.E. 2006a. Yolk antioxidants vary with male attractiveness and female condition in the house finch (*Carpodacus mexicanus*). Physiological and Biochemical Zoology 79: 1098–1105.
- Navara K.J., Hill G.E. & Mendonca M.T. 2006b. Yolk androgen deposition as a compensatory strategy. Behavioral Ecology and Sociobiology 60: 392–398.
- Navara K.J., Badyaev A.V., Mendonca M.T. & Hill G.E. 2006c. Yolk antioxidants vary with male attractiveness and female condition in the house finch (*Carpodacus mexicanus*). Physiological and Biochemical Zoology 79: 1098–1105.
- Navara K.J., Siefferman L.M., Hill G.E. & Mendonça M.T. 2006d. Yolk androgens vary inversely to maternal androgens in eastern bluebirds: an experimental study. Functional Ecology 20: 449–456.
- Neff B.D. & Pitcher T.E. 2005. Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. Molecular Ecology 14: 19–38.
- Neumann C., Duboscq J., Dubuc C., Ginting A., Irwan A.M., Agil M., Widding A. & Engelhardt A. 2011. Assessing dominance hierarchies: validation and advantages of progressive evaluation with Elo-rating. Animal Behaviour 82: 911–921.
- Nicholls E.H., Burke T. & Birkhead T.R. 2001. Ejaculate allocation by male sand martins *Riparia riparia*. Proceedings of the Royal Society B: Biological Sciences 268: 1265–1270.
- Nicholls T.J. & Storey C.R. 1977. The effect of duration of the daily photoperiod on recovery of photosensitivity in photorefractory canaries (*Serinus canarius*). General and Comparative Endocrinology 31: 72–74.
- Nilsson J.-Å., Granbom M. & Råberg L. 2007. Does the strength of an immune response reflect its energetic cost? Journal of Avian Biology 38: 488–494.
- Nisbet I.C.T. 1978. Dependence of fledging success on egg-size, parental performance and egg-composition among Common and Roseate terns, *Sterna hirundo* and *S. dougallii*. Ibis 120: 207–215.

- Norris K. & Evans M.R. 2000. Ecological immunology: life history trade-offs and immune defense in birds. Behavioral Ecology 11: 19–26.
- Nur N. 1984a. The consequences of brood size for breeding blue tits. I. Adult survival, weight change and the cost of reproduction. Journal of Animal Ecology 53: 479–496.
- Nur N. 1984b. The consequences of brood size for breeding blue tits. II. Nestling weight, offspring survival and optimal brood size. Journal of Animal Ecology 53: 497–517.
- Nussey D.H., Pemberton J.M., Pilkington J.G. & Blount J.D. 2009. Life history correlates of oxidative damage in a free-living mammal population. Functional Ecology 23: 809–817.
- Ojanen M., Orell M. & Vaisanen R.A. 1979. Role of heredity in egg size variation in the great tit *Parus major* and the pied flycatcher *Ficedula hypoleuca*. Ornis Scandinavica 10: 22–28.
- Ołdakowski Ł., Piotrowska Ż., Chrząścik K.M., Sadowska E.T., Koteja P. & Taylor J.R.E. 2012. Is reproduction costly? No increase of oxidative damage in breeding bank voles. The Journal of Experimental Biology 215: 1799–1805.
- Ophir A.G. & Galef B.G. 2003. Female japanese quail that 'eavesdrop' on fighting males prefer losers to winners. Animal Behaviour 66: 399–407.
- Ophir A.G. & Galef B.G. 2004. Sexual experience can affect use of public information in mate choice. Animal Behaviour 68: 1221–1227.
- Ophir A.G., Persaud K.N. & Galef B.G.J. 2005. Avoidance of relatively aggressive male Japanese quail (*Coturnix japonica*) by sexually experienced conspecific females. Journal of Comparative Psychology 119: 3–7.
- Osserman E.F. & Lawlor D.P. 1966. Serum and Urinary Lysozym (Muramidase) in Monocyctic and Monomyelocytic Leukaemia. The Journal of Experimental Medicine 124: 921–922.
- Ots I. & Hörak P. 1996. Great tits *Parus major* trade health for reproduction. Biological Sciences 263: 1443–1447.
- Ots I., Kerimov A.B., Ivankina E.V., Ilyina T.A. & Horak P. 2001. Immune challenge affects basal metabolic activity in wintering great tits. Proceedings of the Royal Society B: Biological Sciences 268: 1175–1181.
- Otter K., McGregor PK., Terry A.M.R., Burford F.R.L., Peake T.M. & Dabelsteen T. 1999. Do female great tits (*Parus major*) assess males by eavesdropping? A field study using interactive song playback. Proceedings of the Royal Society B: Biological Sciences 266: 1305–1309.
- Otter K.A. & Ratcliffe L.M. 2005. Enlightened decisions: female assessment and communication networks. In: Animal Communication Networks. McGregor P.K. (Editor). Pp. 133–151. Cambridge University Press Cambridge, UK.
- Owings H., Beecher M.D. & Thompson N.S. 1998. Perspectives in Ethology: Communication. Plenum Press. New York, New York. USA.

- Pap P.L., Vagasi C.I., Czirjak G.A. & Barta Z. 2008. Diet quality affects postnuptial molting and feather quality of the house sparrow (*Passer domesticus*): interaction with humoral immune function? Canadian Journal of Zoology 86: 834–842.
- Parisot M. 2004. Le statut social et le choix du partenaire chez le canari domestique commun (*Serinus canaria*): indices comportementaux, hormonaux et vocaux. PhD thesis. Université Paris XIII.
- Parisot M., Tanvez A., Lacroix A., Vallet E. & Leboucher G. 2005. Social competition and plasma T profile of domesticated canaries: an experimental test of the challenge hypothesis. Hormones and Behavior 48: 225–232.
- Parker G.A. 1970. Sperm competition and its evolutionary consequences in the insects. Biological Reviews 45: 527–567.
- Parker G.A. 1982. Why are there so many tiny sperm? Sperm competition and the maintenance of two sexes. Journal of Theoretical Biology 96: 281–294.
- Parker G.A. 1984. Sperm competition and the evolution of animal mating systems. In: Sperm competition and the evolution of animal mating systems. Smith R.L. (editor). Pp. 1–60. Academic Press. Orlando, Florida. USA.
- Parker G.A. 1998. Sperm competition and the evolution of ejaculates: Towards a theory base. In: Sperm competition and sexual selection. Birkhead T.R. & Møller A.P. (editors). Pp. 3–54. Academic Press. London, UK.
- Parker G.A., Baker R.R. & Smith V.G.F. 1972. The origin and evolution of gamete dimorphism and the male-female phenomenon. Journal of Theoretical Biology 36: 529–553.
- Parker T.H., Knapp R. & Rosenfield J.A. 2002. Social mediation of sexually selected ornamentation and steroid hormone levels in male jungle fowl. Animal Behaviour 64: 291–298.
- Parris K.M., Velik-Lord M. & North J.M.A. 2009. Frogs call at a higher pitch in traffic noise. Ecology and Society: 14: 25.
- Partecke J. & Schwabl H. 2008. Organizational effects of maternal testosterone on reproductive behavior of adult house sparrows. Developmental Neurobiology 68: 1538-1548.
- Partridge L., Prowse N. & Pignatelli P. 1999. Another set of responses and correlated responses to selection on age at reproduction in *Drosophila melanogaster*. Proceedings of the Royal Society B: Biological Sciences 266: 255–261.
- Pasteau M., Nagle L. & Kreutzer M. 2009. Preferences and predispositions of female canaries (*Serinus canaria*) for loud intensity of male sexy phrases. Biological Journal of the Linnean Society 96: 808–814.
- Patricelli G.L. & Blickley J.L. 2006. Avian communication in urban noise: causes and consequences of vocal adjustement. The Auk 123: 639–649.

- Perrins C.M. 1996. Eggs, egg formation and the timing of breeding. Ibis 138: 2–15.
- Peters A.P., Delhey K., Denk A.G. & Kempenaers B. 2004. Trade-offs between immune investment and sexual signalling in male mallards. The American Naturalist 164: 51–59.
- Petes L.E., Menge B.A. & Harris A.L. 2008. Intertidal mussels exhibit energetic trade-offs between reproduction and stress resistance. Ecological Monographs 78: 387–402.
- Petrie M. & Williams A. 1993. Peahens lay more eggs for peacocks with larger trains. Proceedings of the Royal Society B: Biological Sciences 251: 127–131.
- Phelps C.F. & Antonini E. 1975. A study of the kinetics of iron and copper binding to hen ovotransferrin. Biochemical Journal 147: 385–391.
- Pike T.W. & Petrie M. 2003. Potential mechanisms of avian sex manipulation. Biological Reviews 78: 553–574.
- Pike T.W. & Petrie M. 2005. Maternal body condition and plasma hormones affect offspring sex ratio in peafowl. Animal Behaviour 70: 745–751.
- Pilz K.M., Quiroga M., Schwabl H. & Adkins-Regan E. 2004. European starling chicks benefit from high yolk testosterone levels during a drought year. Hormones and Behavior 46: 179–192.
- Pilz K.M. & Smith H.G. 2004. Egg yolk androgen levels increase with breeding density in the European starling (*Sturnus vulgaris*). Functional Ecology 18:58–66.
- Pilz K.M., Smith H.G., Sandell M.I. & Schwabl H. 2003. Interfemale variation in egg yolk androgen allocation in the European stearling: do high-quality females invest more? Animal Behaviour 65: 841–850.
- Piper W.H. 1997. Social dominance in birds: early findings and new horizons. In: Current Ornithology. Vol. 14. Nolan V., Ketterson E.D. & Thompson C.F. (Editors). Pp. 125–187. Plenum Press. New York, New York. USA.
- Pitnick S. & Markow T.A. 1994. Male gametic strategies: sperm size, testis size, and the allocation of ejaculate among successive mates by the sperm-limited fly Drosophila pachea and its relatives. The American Naturalist 143: 785–819.
- Pizzari T. & Birkhead T.R. 2000. Female feral fowl eject sperm of subdominant males. Nature 405: 787–789.
- Pizzari T. & Birkhead T.R. 2002. The sexually-selected sperm hypothesis: sex-biased inheritance and sexual antagonism. Biological Reviews of the Cambridge Philosophical Society 77: 183–209.
- Poesel A., Dabelsteen T. & Pedersen S.B. 2007. Implications of conspecific background noise for features of blue tit, *Cyanistes caeruleus*, communication networks at dawn. Journal of Ornithology 148: 123–128.

- Pohl H. 1994. Entrainment properties of the circadian system chanching with reproductive state and molt in the canary. Physiology & Behavior 55: 803–810.
- Pohl N.U., Leadbeater E., Slabbekoorn H., Klump G.M. & Langermann U. 2012. Great tits in urban noise benefit from high frequencies in song detection and discrimination. Animal Behaviour 83: 711–721.
- Pohl N.U., Slabbekoorn H., Klump G.M. & Langemann U. 2009. Effects of signal features and environmental noise on signal detection in the great tit, *Parus major*. Animal Behaviour 78: 1293–1300.
- Poisbleau M., Fritz H., Guillemain M. & Lacroix A. 2005. Testosterone and social dominance status in captive male dabbling ducks in winter. Ethology 111: 493–509.
- Pomarède M. 1992. Le canari. Précis de canariculture, 2ème edition. Edition du point vétérinaire. Maison-Alfort, France.
- Potts G.R. 1986. The Partridge. Pesticides, predation and conservation. Collins London. London, UK.
- Pradhan G.R. & Van Schaik C.P. 2009. Why do females find ornaments attractive? The coercion-avoidance hypothesis. Biological Journal of the Linnean Society 96: 372–382.
- Price P.W. 1980. Evolutionary biology of parasites. Princeton University Press. Princeton, New Jersey. USA.
- Prusinowska I., Jankowski J., Sowinski G. & Wawro K. 2000. An evaluation of lysozyme usability in Turkey improvement. Czech Journal of Animal Science 45: 225–228.
- Qvarnström A. & Forsgren E. 1998. Should females prefer dominant males? TREE 13: 498–501.
- Qvarnström A. & Price T. 2001. Maternal effects, paternal effects, and sexual selection. Trends in Ecology & Evolution 16: 95–100.
- Queller D.C. 1997. Why do females care more than males? Proceedings of the Royal Society B: Biological Sciences 264: 1555–1557.
- Quinn J.L., Whittingham M.J., Butler S.J. & Cresswell W. 2006. Noise, predation risk compensation and vigilance in the chaffinch *Fringilla coelebs*. Journal of Avian Biology 37: 601–608.
- Råberg L., Nilsson J.-Å., Ilmonen P., Stjernman M. & Hasselquist D. 2000. The cost of an immune response: vaccination reduces parental effort. Ecological Letters 3: 382–386.
- Rabat A. 2007. Extra-auditory effects of noise in laboratory animals: the relationship between noise and sleep. Journal of the American Association for Laboratory Animal Science 46: 35–41.
- Ramm S.A., Oliver P.L., Ponting C.P., Stockley P. & Emes R.D. 2008. Sexual selection and the adaptative evolution of mammalian ejaculate proteins. Molecular Biology and Evolution 25: 207–219.

- Räsänen K. & Kruuk L.E.B. 2007. Maternal effects and evolution at ecological time scales. Functional Ecology 21: 408–421.
- Rasmussen E. 1999. Isolation and analysis of lysozyme from egg whites. Journal of Agricultural and Food Chemistry 47: 1420–1429.
- Ratikainen I.I. & Kokko H. 2010. Differential allocation and compensation: who deserves the silver spoon? Behavioral Ecology 21: 195–200.
- Rawas A., Moreton K., Muirhead H. & Williams J. 1989, Preliminary crystallographic studies on duck ovotransferrin. Journal of Molecular Biology 208: 213–214.
- Reed W.L. & Vleck C.M. 2001. Functional significance of variation in egg-yolk androgens in the American coot. Oecologia 128:164–171.
- Reznick D.N. 1985. Costs of reproduction: an evaluation of the empirical evidence. Oikos 44: 257–267.
- Reznick D.N. 1992. Measuring the costs of reproduction. Trends in Ecology & Evolution 7: 42–45.
- Richner H. 1989. Phenotypic correlates of dominance in carrion crows and their effects on access to food. Animal Behaviour 38: 606–612.
- Ricklefs R.E. 1974. Energetics of reproduction in birds. In: Avian energetics. Paynter R.A. (editor). Pp. 152-297. Nuttall Ornithological Club. Cambridge, UK.
- Ricklefs R.E. 1977. Composition of eggs of several bird species. The Auk 94: 350–356.
- Ríos-Chelén A.A. 2009. Bird song: the interplay between urban noise and sexual selection. Oecologia Brasiliensis 13: 153–164.
- Ripmeester E.A.P., Mulder M. & Slabbekoorn H. 2010a. Habitat-dependent acoustic divergence affects playback response in urban and forest populations of the European blackbird. Behavioral Ecology 21: 876–883.
- Ripmeester E.A.P., Kok J.S., van Rijssel J.C. & Slabbekoorn H. 2010b. Habitat-related birdsong divergence: a multi-level study on the influence of territory density and ambient noise in European blackbirds. Behavioral Ecology and Sociobiology 64: 409–418.
- Ritchison G. 1983. The function of singing in female black-headed grosbeaks (*Pheucticus melanocephalus*): family-group maintenance. The Auk 100: 105–116.
- Robinson D.S. 1987. The chemical basis of albumen quality. In: Egg Quality. Current Problems and Recent Advances. Wells R.G. & Belyavin C.G. (Editors). Pp. 171–191. Butterworths. London, UK.
- Rodriguez-Llanes J.M., Verbeke G. & Finlayson C. 2009. Reproductive benefits of high social status in male macaques (Macaca). Animal Behaviour 78: 643–649.
- Roff D.A. 1992a. The evolution of life histories. Chapman & Hall. New York, DC. USA.

- Roff D.A. 1992b. Evolution of life histories: theory and analysis. Chapman and Hall. New York, New York. USA.
- Rogers H.J. & Perkins H.R. 1968. Cell walls and membranes. Spon. London, UK.
- Rogers C.M. & Smith J.N.M. 1993. Life-history theory in the nonbreeding period: trade-offs in avian fat reserves. Ecology: 74: 419–426.
- Romanoff A.L. & Romanoff A. 1949. The avian egg. John Wiley e Sons. New York, New York. USA.
- Rose M. & Charlesworth B. 1980. A Test of Evolutionary Theories of Senescence. Nature 287: 141–142.
- Rose M.R. 1984. Laboratory evolution of postponed senescence in *Drosophila melanogaster*. Evolution 38: 1004–1010.
- Royle N.J., Surai P.F. & Hartley I.R. 2003. The effect of variation in dietary intake on maternal deposition of antioxidants in zebra finch eggs. Functional Ecology 17: 472–481.
- Rubolini D., Romano M., Boncoraglio G., Ferrari R.P., Martinelli R., Galeotti P., Fasola M. & Saino N. 2005. Effects of elevated egg corticosterone levels on behavior, growth, and immunity of yellow-legged gull (*Larus michahellis*) chicks. Hormones and Behavior 47: 592–605.
- Rubolini D., Romano M., Martinelli R., Leoni B. & Saino N. 2006. Effects of prenatal yolk androgens on armaments and ornaments of the ringnecked pheasant. Behavioural Ecology and Sociobiology 59: 549–560.
- Rupley A.E. 1997. Manual of Avian Practice. W.B. Saunders. Philadelphia, Pennsylvania. USA.
- Rutkowska J., Martyka R., Arct A & Cichoń M. 2012. Offspring survival is negatively related to maternal response to sheep red blood cells in zebra finches. Oecologia 168:355–359.
- Rutstein A.N., Gilbert L., Slater P.J.B. & Graves J.A. 2004. Mate attractiveness and primary resource allocation in the zebra finch. Animal Behaviour 68: 1087–1094.
- Ruuskanen S., Doligez B., Pitala N. Gustaffson L. & Laaksonen T. 2012. Long-term fitness consequences of high yolk androgen levels: sons pay the costs. Functional Ecology 26: 884–894.
- Ruuskanen S. & Laaksonen T. 2010. Yolk hormones have sex-specific long-term effects on behavior in the pied flycatcher (*Ficedula hypoleuca*). Hormones and Behavior 57: 119–127.
- Safran R.J., Pilz K.M., McGraw K.J., Correa S.M. & Schwabl H. 2008. Are yolk androgens and carotenoids in barn swallow eggs related to parental quality? Behavioral Ecology and Sociobiology 62: 427–438.
- Saino N., Bertacche V., Ferrari R.P., Martinelli R., Møller A.P. & Stradi R. 2002a. Carotenoid concentration in barn swallow eggs is influenced by laying order, maternal infection and paternal ornamentation. Proceedings of the Royal Society B: Biological Sciences 269: 1729–1733.

- Saino N., Ferrari R.P., Martinelli R., Romano M., Rubolini D. & Moller A. 2002b. Early maternal effects mediated by immunity depend on sexual ornamentation of the male partner. Proceedings of the Royal Society B: Biological Sciences 269: 1729–1733.
- Saino N., Dall'Ara P., Martinelli R. & Møller A.P. 2002c. Early maternal effects and antibacterial immune factors in the eggs, nestlings and adults of the barn swallow. Journal of Evolutionary Biology 15: 735–743.
- Saino N., Martinelli R., Biard C., Gil D., Spottiswoode C.N., Rubolini D., Surai P.F. & Møller A.P. 2007. Maternal immune factors and the evolution of secondary sexual characters. Behavioral Ecology 18: 513–520.
- Saino N., Romano M., Ferrari R.P., Martinelli R. & Moller A.P. 2003. Maternal antibodies but not carotenoids in barn swallow eggs covary with embryo sex. Journal of Evolutionary Biology 16: 516–522.
- Sapolsky R.M. & Bennett A.E. 1990. Award paper, Adrenocortical function, social rank, and personality among wild baboons. Biological Psychiatry 28: 862–878.
- Sarv T. & Hõrak P. 2009. Phytohaemagglutinin injection has a long-lasting effect on immune cells. Journal of Avian Biology 40: 569–571.
- Sato Y. & Watanabe K. 1976. Lysozyme in hen blood serum. Poultry Science 55: 1749–1756.
- Schade A.L. & Caroline L. 1944. Raw hen egg white and the role of iron in growth inhibition of *Shigella dysenteriae*, *Staphylococcus aureus*, *Escherichia coli* and *Saccharomyces cerevisiae*. Science 100: 14–15.
- Schaffer W.M. 1972. Evolution of optimal reproductive strategies. PhD thesis. Princeton University.
- Schaffer W.M. 1974a. Selection for optimal life histories: the effects of age structure. Ecology 55: 291–303.
- Schaffer W.M. 1974b. Optimal reproductive effort in fluctuating environments. The American Naturalist 108: 783–790.
- Schjelderup-Ebbe T. 1935. Social behaviour of birds. In: A Handbook of Social Psycology. Murchison C. (Editor). Pp. 947–972. Russel & Russel. New York, New York. USA.
- Schino G. 2004. Birth sex ratio and social rank: consistency and variability within and between primate groups. Behavioral Ecology 15: 850–856.
- Schomer P. & Beck G. 2010. Wind-induced pseudo-noise and leaf-rustle noise. Noise Control Engineering Journal 58: 121–131.
- Schreck C.B. Contreras-Sanchez W. & Fitzpatrick M.S. 2001. Effects of stress on fish reproduction, gamete quality, and progeny. Aquaculture 197: 3–24.
- Schroeder J., Nakagawa S., Cleasby I.R. & Burke T. 2012. Passerine birds breeding under chronic noise experience reduced fitness. PLoS ONE 7: e39200.

- Schwabl H. 1993. Yolk is a source of maternal testosterone for developing birds. Proceedings of the National Academy of Sciences of the USA 90: 11446–11450.
- Schwabl H. 1996a. Environment modifies the testosterone levels of a female bird and its eggs. Journal of Experimental Zoology 276: 157–163.
- Schwabl H. 1996b. Maternal testosterone in the avian egg enhances postnatal growth. Comparative Biochemistry and Physiology A 114: 271–276.
- Schwabl H., Mock D.W. & Gieg J.A. 1997. A hormonal mechanism for parental favouritism. Nature 386: 231.
- Schwarzkopf L. 1993. Costs of reproduction in water skins. Ecology 74: 1970–1981.
- Schwarzkopf L. 1994. Measuring trade-offs: a review of studies of costs of reproduction in lizards. In: Lizard ecology. Historical and experimental prespectives. Vitt L.J. & Pianka E.R. (editors). Pp. 7–29. Princeton University Press. Princeton, New Jersey. USA.
- Searcy W.A. 1992. Measuring responses of female birds to male song. In: Playback and studies of animal communication. McGregor P.K. (Editor). Pp. 175–189. Plenum Press. New York, New York. USA.
- Searcy W.A. & Marler P. 1981. A test for responsiveness to song structure and programming in female sparrows. Science 213: 926–928.
- Selye H. 1963. Stress and the adaptation syndrome. In: Cyclopedia of Medicine, Surgery, and Specialties, Vol. XIII. Pp. 365-366F. F.A. Davis Company. New York, New York. USA.
- Servedio M.R. & Lande R. 2006. Population genetic models of male and mutual mate choice. Evolution 60: 674–685.
- Seviour E.M. & Board R.G. 1972. Bacterial growth in albumin taken from the eggs of domestic hens and waterfowl. British Poultry Science 13: 557–575.
- Shawkey M.D., Kosciuch K.L., Liu M., Rohwer F.C., Loos E.R., Wang J.M. & Beissinger S.R. 2008. Do birds differentially distribute antimicrobial proteins within clutches of eggs? Behavioral Ecology 19: 920–927.
- Sheldon B.C. 1993. Sexually transmitted disease in birds: occurrence and evolutionary significance, Philosophical Transactions of the Royal Society B: Biological Sciences 339: 491–497.
- Sheldon B.C. 2000. Differential allocation: tests, mechanisms and implications. Trends in Ecology & Evolution 15: 397–401.
- Sheldon B.C. & Verhulst S. 1996. Ecological immunology: costly parasite defenses and trade-offs in evolutionary ecology. Trends in Ecology & Evolution 11: 317–321.
- Sheldon B.C. & West S.A. 2004. Maternal dominance, maternal condition, and offspring sex ratio in ungulate mammals. The American Nataturalist 163: 40–54.

- Shively C.A. 1998. Social subordination stress, behavior, and central monoaminergic function in female cynomolgus monkeys, Biological Psychiatry 44: 882–891.
- Shively C.A., Grant K.A., Ehrenkaufer R.L., Mach R.H. & Nader M.A. 1997a. Social stress, depression, and brain dopamine in female cynomolgus monkeys. Annals of the New York Academy of Sciences 807: 574–577.
- Shively C.A., Laber-Laird K. & Anton R.F. 1997b. Behavior and physiology of social stress and depression in female cynomolgus monkeys. Biological Psychiatry: 871–882.
- Shoemaker K.L. & Adamo S.A. 2007. Adult female crickets, *Gryllus texensis*, maintain reproductive output after repeated immune challenges. Physiological Entomology 32: 113–120.
- Sibly R.M. & Calow P. 1986. Physiological Ecology of Animals. An evolutionary approach. Blackwell Scientific Publications. Palo Alto, California. USA.
- Siegel H.S. 1980. Physiological stress in birds. Bioscience 30: 529–534.
- Silk J.B. 1983. Local resource competition and facultative adjustment of sex ratio in relation to competitive abilities. The American Naturalist 121: 56–66.
- Silk J.B. 2007. The adaptive value of sociality in mammalian groups. Philosophical Transactions of the Royal Society B: Biological Sciences 362: 539–559.
- Silk J.B., Willoughby E. & Brown G.R. 2005. Maternal rank and local resource competition do not predict birth sex ratios in wild baboons. Proceedings of the Royal Society B: Biological Sciences 272: 859–864.
- Silva M.C., Boersma P.D., MacKay S. & Strange I.J. 2008. Egg size and parental quality in thin-billed prions, *Pachyptila belcheri*: effects on offspring fitness. Animal Behaviour 74: 1408–1412.
- Skiba R. 2000. Mögliche Dialektselektion des Regenrufes beim Buchfink (*Fringilla coelebs*) durch Lärmbelastung: Prüfung einer Hypothese. Journal of Ornithology 141: 160–167.
- Slabbekoorn H. & den Boer-Visser A. 2006. Cities Change the Songs of Birds. Current Biology 16: 2326–2331.
- Slabbekoorn H. & Peet M. 2003. Birds sing at a higher pitch in urban noise. Nature 424: 267.
- Slabbekoorn H. & Ripmeester E.A. 2008. Birdsong and anthropogenic noise: implications and applications for conservation. Molecular Ecology 17: 72–83.
- Slabbekoorn H. & Smith T.B. 2002. Bird song, ecology and speciation. Philosophical Transactions of the Royal Society B 357: 493–503.

- Slabbekoorn H., Yeh P. & Hunt K. 2007. Sound transmission and song divergence: a comparison of urban and forest acoustics. The Condor 109: 67–78.
- Smits J.E., Bortolotti G.R. & Tella J.L. 1999. Simplifying the phytohaemagglutinin skin-testing technique in studies of avian immunocompetence. Functional Ecology 13: 567–572.
- Sockman K.W. 2008. Ovulation order mediates a trade-off between pre-hatching and post-hatching viability in an altricial bird. PLoS ONE 3: e1785.
- Soler J.J., de Neve L., Pérez-Contreras T., Soler M. & Sorci G. 2003. Trade-off between immunocompetence and growth in magpies: an experimental study. Proceedings of the Royal Society B: Biological Sciences 270: 241–248.
- Sotherland P.R & Rahn H. 1987. On the composition of bird eggs. The Condor 89: 48–65.
- Speakman J.R. 2008. The physiological costs of reproduction in small mammals. Philosophical Transactions of the Royal Society B: Biological Sciences 363: 375–398.
- Spencer H. 1866. The principles of biology. Appleton. London, UK.
- Staicer C.A., Spector D.A. & Horn A.G. 1996. The dawn chorus and other diel patterns in acoustic signalling. In: Ecology and Evolution of Acoustic Communication in Birds. Kroodsma D.E. & Miller E.H. (Editors). Pp. 426–453. Cornell University Press. Ithaca, New York. USA.
- Starck J. 1998. Avian Growth and Development. Oxford University Press. Oxford, UK.
- Starck J.M. & Ricklefs R.E. 1998. Avian growth and development. Evolution within the altricial-precocial spectrum. Oxford Ornithology series. Oxford University Press. Oxford, UK.
- Steams S.C. 1992. The evolution of life histories. Oxford University Press. Oxford, UK.
- Stearns S.C. 1989. Trade-offs in life history evolution. Functional Ecology 3: 259-268.
- Stearns S.C. 1992. The evolution of life histories. Oxford University Press. Oxford, UK.
- Steel E., Follett B.K. & Hinde R.A. 1975. The role of short days in the termination of photorefractoriness in female canaries (*Serinus canarius*). Journal of Endocrinology 64: 451–464.
- Stefanski V., Knopf G. & Schulz S. 2001. Long-term colony housing in Long–Evans rats: immunological, hormonal, and behavioral consequences. Journal of Neuroimmunology 114: 122–130.
- Stevens D.J., Hansell M.H., Freel J.A. & Monaghan P. 1999. Developmental trade-offs in caddis flies: increased investment in larval defense alters adult

resource allocation. Proceedings of the Royal Society B: Biological Sciences 266: 1049–1054.

- Storey C.R. & Nicholls T.J. 1976. Some effects of manipulation of daily photoperiod on the rate of onset of a photorefractory state in canaries (*Serinus canarius*). General and Comparative Endocrinology 30: 204–208.
- Strasser R. & Schwabl H. 2004. Yolk testosterone organises behavior and male plumage coloration in house sparrows (*Passer domesticus*). Behavioral Ecology and Sociobiology 56: 491–497.
- Styrsky J.D., Eckerle K.P. & Thompson C.F. 1999. Fitness-related consequences of egg mass in nestling house wrens. Proceedings of the Royal Society B: Biological Sciences 266: 1253–1258.
- Sugino H., Nitoda T. & Juneja L.R. 1997. General chemical composition of hen eggs. In: Hen eggs. Their Basic and Applied Science. Yamamoto T., Juneja L.R., Hatta H. & Kim M. (editors). CRC Press LLC. Boca Raton, Florida. USA.
- Surai P.F., Speake B.K., Wood N.A.R., Blount J.D., Bortolotti G.R. & Sparks N.H.C. 2001. Carotenoid discrimination by the avian embryo: a lesson from wild birds. Comparative Biochemistry and Physiology - Part B: Biochemistry & Molecular Biology 128: 743–750.
- Svensson E., Råberg L., Koch C. & Hasselquist D. 1998. Energetic stress, immunosuppression and the costs of an antibody response. Functional Ecology 12: 912–919.
- Swaddle J.P. & Page L.C. 2007. High levels of environmental noise erode pair preferences in zebra finches: implications for noise pollution. Animal Behaviour 74: 363–368.
- Tamashiro K.L.K., Nguyen M.M.N. & Sakay R.R. 2005. Social stress: from rodents to primates. Frontiers in Neuroendocrinology 26: 27–40.
- Tanvez A., Amy M., Chastel O. & Leboucher G. 2009. Maternal effects and β-carotene assimilation in Canary chicks. Physiology & Behavior 96: 389–393.
- Tanvez A., Béguin N., Chastel O., Lacroix A. & Leboucher G. 2004. Sexually attractive phrases increase yolk androgens deposition in canaries (*Serinus canaria*). General and Comparative Endocrinology 138: 113–120.
- Tanvez A., Parisot M., Chastel O. & Leboucher G. 2008. Does maternal social hierarchy affect yolk testosterone deposition in domesticated canaries? Animal Behaviour 75: 929–934.
- Tanzarella S. 2005. Perception et communication chez les animaux. De Boeck. Paris, France.
- Taylor G.T., Weiss J. & Rupich R. 1987. Male rat behavior, endocrinology and reproductive physiology in a mixed-sex, socially stressful colony. Physiology & Behavior 39: 429–433.
- Taylor P.V.,Veenema A.H., Paul M.J., Bredewold R., Isaacs S. & de Vries G.J. 2012. Sexually dimorphic effects of a prenatal immune challenge on

social play and vasopressin expression in juvenile rats. Biology of Sex Differences 3:15.

- Teather K.L. & Weatherhead P.J. 1995. The influence of age and access to females on dominance in captive male brown-headed cowbirds (*Molothrus ater*). Canadian Journal of Zoology 73: 1012–1018.
- Tella J.L., Lemus J.A., Carrete M. & Blanco G. 2008. The PHA test reflects acquired T-cell mediated immunocompetence in birds. PLoS ONE 3: e3295.
- Thierry B. 2000. Covariation of conflict management patterns across macaque species. In: Natural conflict resolution. Aureli F. & de Waal F.B.M. (Editors). Pp. 106–128. University of California Press. Berkeley, California. USA.
- Thornhill R. 1983. Cryptic female choice and its implications in the scorpionfly *Harpobittacus nigriceps*. The American Naturalist 122: 765–788.
- Thornhill R. 1988. The jungle fowl hen's cackle incites male competition. Verhandlungen Deutschen Zoologisches Gesellshaft 81: 145–154.
- Tinkle D.F. 1969. The concept of reproductive effort and its relation to the evolution of life histories in lizards. The American Naturalist 103: 427–434.
- Tinkle D.W. & Hadley N.F. 1975. Lizard reproductive effort: caloric estimates and comments on its evolution. Ecology 56: 427–434.
- Togashi T. & Cox P.A. 2011. The Evolution of Anisogamy: A Fundamental Phenomenon Underlying Sexual Selection. Cambridge University Press. Cambridge, UK.
- Tollrian R. & Harvell C.D. 1999. The ecology and evolution of inducible defenses. Princeton University Press. Princeton, New Jersey. USA.
- Tomkins J.L., Radwan J., Kotiaho J.S. & Tregenza T. 2004. Genic capture and resolving the lek paradox. Trends in Ecology and Evolution 19: 323–328.
- Torres R. & Velando A. 2007. Male reproductive senescence: the price of immuneinduced oxidative damage on sexual attractiveness in the blue-footed booby. Journal of Animal Ecology 76: 1161–1168.
- Townsend C.R. & Calow P. 1981. Physiological ecology. An evolutionary approach to resource use. Blackwell Scientific Publications. Oxford, New York. USA.
- Trail P.W. 1985. Courtship disruption modifies mate choice in a lek-breeding bird. Science 227: 778–780.
- Tranter H.S. & Board R.G. 1982. The antimicrobial defence of avian eggs: biological perspective and chemical basis. Journal of Applied Biochemistry 4: 295–338.
- Trillmich F. 1986. Are endotherms emancipated? Some considerations on the cost of reproduction. Oceologia 69: 631–633. Trillmich F. 1986. Are endotherms emancipated? Some considerations on the cost of reproduction. Oceologia 69: 631–633.

- Trivers R.L. 1972. Parental investment and sexual selection. In: Sexual selection and the descent of man, 1871-1971. Campbell B. (Editor). Pp. 136–179. Aldine-Atherton. Chicago, Illinois. USA.
- Trivers R.L. & Willard D. 1973. Natural selection of parental ability to vary the sex ratio of offspring. Science 179: 90–92.
- Uller T., Eklof J. & Andersson S. 2005. Female egg investment in relation to male sexual traits and the potential for transgenerational effects in sexual selection. Behavioral Ecology and Sociobiology 61: 541–550.
- Uller T., Isaksson C. & Olsson A. 2006. Immune challenge reduces reproductive output and growth in a lizard. Functional Ecology 20: 873–879.
- Valenti P., De Stasio A., Mastromerino P., Seganti L., Sinibaldi L. & Orsi N. 1981a. Influence of bicarbonate and citrate on the bacteriostatic action of ovotransferrin towards staphylococci. FEMS Microbiology Letters 10: 77–79.
- Valenti P., Guarino M., Visca P., Von Hunolstein C., Antonini G., De Stasio A. & Orsi N. 1981b. Resistance of genus proteus to ovotransferrin. Bollettino dell'Istituto sieroterapico Milanese 604: 284–287.
- Valenti P., Antonini G., Von Hunolstein C., Visca P., Orsi N. & Antonini E. 1983. Studies on the antimicrobial activity of ovotransferrin. International Journal of Tissue Reactions V: 97–105.
- Valenti P., Visca P., Antonini G. & Orsi N. 1985. Antifungal activity of ovotransferrin towards genus candida. Mycopathologia 89: 169–175.
- Valenti P., Visca P., Antonini G., Orsi N. & Antonini E. 1987. The effect of saturation with Zn^{2+} and other metal ions on the antibacterial activity of ovotransferrin. Medical Microbiology and Immunology 176: 123–130.
- Valera F., Hoi H. & Krištín A. 2003. Male shrikes punish unfaithful females. Behavioral Ecology 14: 403–408.
- Vallet E. & M. Kreutzer 1995. Female canaries are sexually responsive to special song phrases. Animal Behaviour 49: 1603–1610.
- Valone T.J. 1989. Group foraging, public information, and patch estimation. Oikos 56: 357–363.
- van de Crommenacker J., Horrocks N.P.C., Versteegh M.A., Komdeu J., Tieleman B.I. & Matson K.D. 2010. Effect of immune supplementation and immune challenge on oxidative status and physiology in a model bird: implications for ecologists. Journal of Experimental Biology 213: 3527–3535.
- Van Duyse E., Pinxten R. & Eens M. 2002. Effects of testosterone on song, aggression, and nestling feeding behavior in male great tits, *Parus major.* Hormones and Behavior 41: 178–186.
- Van Noordwijk A.J., van Balen J.H. & Scharloo W. 1980. Heritability of ecologically important traits in the great tit. Ardea 68: 193–203.

- van Schaik C.P. & Hrdy S.B. 1991. Intensity of local resource competition shapes the relationship between maternal rank and sex ratios at birth in cercopithecinae primates. The American Naturalist 138: 1555–1562.
- Veiga J.P., Vinuela J., Cordelo P.J., Aparicio J.M. & Polo V. 2004. Experimentally increased testosterone affects social rank and primary sex ratio in the spotless starling. Hormones and Behavior 46: 47–53.
- Verboven N., Monaghan P., Nager R.G. & Evans N.P. 2010. The effect of maternal state on the steroid and macronutrient content of lesser black-backed gull eggs. Physiological and Biochemical Zoology 83: 1009–1022.
- Vergne A.L., Pritz M.B. & Mathevon N. 2009. Acoustic communication in crocodilians: from behaviour to brain. Biological reviews. 84: 391–441.
- Verhulst S., Riedstra B. & Wiersma P. 2005. Brood size and immunity costs in zebra finches *Taeniopygia guttata*. Journal of Avian Biology 36: 22–30.
- Verzijden M.N., Ripmeester E.A.P., Ohms V.R., Snelderwaard P. & Slabbekoorn H. 2010. Immediate spectral flexibility in singing chiffchaffs during experimental exposure to highway noise. Journal of Experimental Biology 213: 2575–2581.
- Vieira S.L. 2007. Chicken Embryo Utilization of Egg Micronutrients. Brazilian Journal of Poultry Science 9: 1–8.
- Virgin Jr. C.E. & Sapolsky R.M. 1997. Styles of male social behavior and their endocrine correlates among low-ranking baboons. American Journal of Primatology 42: 25–39.
- Viscione K.A., Branton S.L., Gerard P.D., Whitmarsh S.K. & Peebles E.D. 2008. Effects of a prelay 6/85-Strain *Mycoplasma gallisepticum* inoculation alone or in conjunction with subsequent F-strain *M. gallisepticum* inoculations during lay on the internal egg characteristics of commercial egg-laying hens. Poultry Science 87: 1120–1124.
- Voigt C. & Leitner S. 1998. Breeding biology of the island canary *Serinus canaria* (aves: Fringillidae) on the desertas island ilhéu chão. Boletim do Museu Municipal Funchal (História Natural) 50: 117–124.
- Von Hunolstein C., Ricci M.L., Valenti P. & Orefici G. 1992. Lack of activity of transferrins towards streptococcus spp. Medical Microbiology Immunology 181: 351–357.
- Wade J., Gong A. & Arnold A.P. 1997. Effects of embryonic estrogen on differentiation of the gonads and secondary sexual characteristics of male zebra finches. Journal of Experimental Zoology 278: 405–411.
- Wagner E.C., Stables C.A. & Williams T.D. 2008. Hematological changes associated with egg production: direct evidence for changes in erythropoiesis but a lack of resource dependence? Journal of Experimental Biology 211: 2960–2968.
- Wallace B.P., Sotherland P.R., Tomillo P.S., Bouchard S.S., Reina R.D., Spotila J.R. & Paladino F.V. 2006. Egg components, egg size, and hatchling

size in leatherback turtles. Comparative Biochemistry and Physiology Part A 145: 524–532.

- Ward S. 1996. Energy expenditure of female barn swallows, *Hirundo rustica*, during egg formation. Physiological Zoology 69: 930–951.
- Warren P.S., Katti M., Ermann M. & Brazel A. 2006. Urban bioacoustics: it's not just noise. Animal Behaviour 71: 491–502.
- Warren R.P. & Hinde R.A. 1961. Does the male stimulate oestrogen secretion in female canaries? Science 133: 1354–1355.
- Wedell N., Gage M.J.G. & Parker G.A. 2002. Sperm competition, male prudence and sperm-limited females. Trends in Ecology & Evolution 17: 313–320.
- Weiner J. 1992. Physiological limits to sustainable energy budgets in birds and mammals: ecological implications. Trends in Ecology & Evolution 7: 384–388.
- Wellman-Labadie O., Picman J. & Hincke M.T. 2007. Avian antimicrobial proteins: structure, distribution and activity. World's Poultry Science Journal 63: 421–438.
- West S.A. & Sheldon B.C. 2002. Constraints in the evolution of sex ratio adjustment. Science 295: 1685–1688.
- Whitlow W.L.A., Popper A.N., Fay R.R. 2000. Hearing by whales and dolphins. Springer. Berlin, Germany.
- Whittingham L. & Schwabl H. 2002. Maternal testosterone in tree swallow eggs varies with female aggression. Animal Behaviour 62: 63–67.
- Wiley R.H. & Poston J. 1996. Indirect mate choice, competition for mates, and coevolution of sexes. Evolution 50: 1371–1381.
- Williams G.C. 1966. Natural selection, the cost of reproduction and a refinement of Lack's principle. The American Naturalist 100: 687–690.
- Williams J. 1968. A comparison of glycopeptides from the ovotransferrin and serum transferrin of the hen. Biochemistry Journal 108: 57–67.
- Williams T.D. 1994. Intraspecific variation in egg size and egg composition in birds: effects on offspring fitness. Biological Reviews of the Cambridge Philosophical Society 69: 35–59.
- Williams T.D. 2005. Mechanisms underlying the costs of egg production. Bioscience 55: 39–48.
- Williams T.D., Challenger W.O., Christians J.K., Evanson M., Love O. & Vezina F. 2004. What causes the decrease in haematocrit during egg production? Functional Ecology 18: 330–336.
- Williams T.D., Christians J.K., Alken J.J. & Evanson M. 1999. Enhanced immune function does not depress reproductive output. Proceedings of the Royal Society B: Biological Sciences 266: 753–757.
- Williams T.D., Lank D.B. & Cooke F. 1993. Is intraclutch egg-size variation adaptive in the lesser snow goose. Oikos 67: 250–256.

- Williams T.D. & Miller. M. 2003. Individual and resource-dependent variation in ability to lay supranormal clutches in response to egg removal. The Auk 120: 481–489.
- Williamson K.A., Surai P.F. & Graves J.A. 2006. Yolk antioxidants and mate attractiveness in the zebra finch. Functional Ecology 20: 354–359.
- Willie J., Travers M. & Williams T.D. 2010. Female zebra finches (*Taeniopygia guttata*) are chronically but not cumulatively 'anemic' during repeated egg laying in response to experimental nest predation. Physiological and Biochemical Zoology 83: 119–126.
- Wilson A.J., Coltman D.W., Pemberton J.M., Overall A.D.J., Byrne K.A. & Kruuk L.E.B. 2005. Maternal genetic effects set the potential for evolution in a free-living vertebrate population. Journal of Evolutionary Biology 18: 405–414.
- Wilson E.O. 1975. Sociobiology. The New Synthesis. Harvard University Press. Cambridge, Massachusetts. USA.
- Wilson R.S. & Booth D.T. 1998. Effect of tail loss on reproductive output and its ecological significance in the skink *Eulamprus quoyii*. Journal of Herpetology 32: 128–131.
- Wingfield J.C. 1984. Androgens and mating systems: testosterone-induced polygyny in normally monogamus birds. The Auk 101: 665–671.
- Wingfield J.C., Hegner R.E., Dufty A.M.J. & Ball G.F. 1990. The "challenge hypothesis": theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. The American Naturalist 136: 829–846.
- Wingfield J.C & Sapolsky R.M. 2003. Reproduction and resistance to stress: when and how. Journal of Neuroendocrinology 15: 711–724.
- Wingfield J.C., Jacobs J.D., Soma K.K., Maney D.L., Hunt K.E., Wisti-Peterson D., Meddle S., Ramenofsky M. & Sullivan K. 2003. Testosterone, aggression, and communication: ecological bases of endocrine phenomena. In: The Design of Animal Communication. Hauser M.D. & Konishi M. (Editors). Pp. 255–283. MIT Press. Bradford Book. Cambridge, Massachusetts. USA.
- Witter M.S. & Cuthill I.C. 1993. The ecological costs of avian fat storage. Philosophical Transactions of the Royal Society B: Biological Sciences 340: 73–92.
- Wolf J.B., Brodie E.D. & Moore A.J. 1999. The role of maternal and paternal effects in the evolution of parental quality by sexual selection. Journal of Evolutionary Biology 12: 1157–1167.
- Wood W.E. & Yezerinac S.M. 2006. Song sparrow (*Melospiza melodia*) song varies with urban noise. The Auk 123: 650–659.
- Wu J. & Acero-Lopez A. 2012. Ovotransferrin: Structure, bioactivities, and preparation. Food Research International 46: 480–487.

- Yamanishi H., Iyama S., Yamaguchi Y., Kanakura Y. & Iwatani Y. 2002. Modification of fully automated total iron-binding capacity (TIBC) assay in serum and comparison with dimension TIBC method. Clinical Chemistry 48: 1565–1570.
- Yang J.-N. 2010. Cooperation and the evolution of anisogamy. Journal of Theoretical Biology 264: 24–36.
- Zahavi A. 1975. Mate selection – a selection for a handicap. Journal of Theoretical Biology 53: 205–214.
- Zeh J.A. & Zeh D.W. 1996. The evolution of polyandry I: intragenomic conflict and genetic incompatibility. Proceedings of the Royal Society B: Biological Sciences 263: 1711–1717.
- Zeh J.A. & Zeh D.W. 1997. The evolution of polyandry II: postcopulatory defences against genetic incompatibility. Proceedings of the Royal Society B: Biological Sciences 264: 69–75.
- Zera A.J. & Harshman L.G. 2001. The physiology of life history trade-offs in animals. Annual Review of Ecology and Systematics 32: 95–126.
- Zera A.J., Potts J. & Kobus K. 1998. The physiology of life history trade-offs: experimental analysis of a hormonally-induced life history trade-off in *Gryllus assimilis*. The American Naturalist 152: 7–23.
- Zuk M. 1996. Disease, endocrine-immune interactions, and sexual selection. Ecology 77: 1037–1042.

Attachments

Papers published:

- Cucco M., Grenna M. & Malacarne G. 2012. Female condition, egg shape and hatchability: a study on the grey partridge. Journal of Zoology 287: 186–194.
- Cucco M., Grenna M., Pellegrino I. & Malacarne G. 2011. Egg-sequence rather than mating preference influences female egg investment in the red-legged partridge. Ethology Ecology and Evolution 23: 343–357.
- Cucco M., Grenna M., Pellegrino I. & Malacarne G. 2011. Effects of immune challenge and supra-normal clutch production on egg quality in the red-legged partridge. Evolutionary Ecology Research 13: 269–282.

Papers in preparation:

- Grenna M., Cucco M., Leboucher G. & Malacarne G. Dominant males influence egg allocation of female grey partridges.
- Huet des Aunay G.*, Grenna M.*, Draganoiu T., Nagle L., Malacarne G., Leboucher G. Does the city noise affect egg characteristics?
- Ung D.*, Grenna M.*, Amy M., Malacarne G. & Leboucher, G. What determines the preference of a canary female faced to a male-male physical interaction? And how does an eventually preference influence egg allocation?

*authors contributed equally to the paper and both should be considered as 1st author.

Summary

Résumé thèse

L'étude porte sur l'allocation de ressources aux oeufs d'oiseaux selon les caractéristiques du mâle partenaire ou de l'environnement. Les données étudiées sont : taille de la ponte et caractéristiques des oeufs (masse, masse des constituants, concentration de testostérone, lysozyme, ovotransferrin). Les caractéristiques du mâle prises en compte sont s'il est choisi ou non par la femelle et son statut social. En plus, l'effet du bruit et d'un vaccination couplé avec un surproduction des oeufs sont pris en compte.

Deux expériences ont été menées sur le canari domestique (*Serinus canaria*) en laboratoire, au sein du LECC de Paris Ouest Nanterre.

Deux expériences ont eu lieu en milieu semi-naturel au DiSIT de l'Università degli Studi del Piemonte Orientale (Italie) sur la perdrix grise (*Perdix perdix*) et rouge (*Alectoris rufa*).

Chez la perdrix grise, nous observons effets positifs de la préférence et du statut social sur la testostérone et sur le taux d'éclosion. Cette hormone peut rendre les oisillons nidifuges plus compétitifs vis-à-vis des couvées voisines.

Chez le canari, on observe un effet positif de la préférence sur le poids des oeufs et sur le lysozyme, important substance antimicrobienne. En plus, on a trouvé un influence négative du bruit sur la sélection sexuelle et sur le numéro des oeufs pondues.

Chez la perdrix rouge, nous observons variations significatives des components de l'oeuf en relation à la surproduction des oeufs (épuisement des ressources).

En conclusion, la femelle n'est pas seule à déterminer les caractéristiques des oeufs. Le mâle partenaire et l'environnement ont aussi une influence sur leur composition et donc sur le développement des jeunes.

Résumé chapitres

Chapitre I – Résumé

Ce chapitre se concentre sur les effets du statut social du mâle sur l'allocation des ressources dans l'œuf.

Dans la littérature, plusieurs études existent qui indiquent que la préférence des femelles pour un mâle attractif peut accroître les investissements maternelle (Burley, 1988 ; Schwabl, 1996a ; 1996b ; Sheldon, 2000). Dans la perdrix rouge, par exemple, a été vu une augmentation du nombre d'œufs pondus quand la femelle peut se reproduire avec le partenaire choisi (Bottoni et al., 1993). En ce qui concerne les androgènes, des travaux ont montré comment les femmes peuvent faire varier la concentration d'androgènes en réponse à des stimuli visuels du mâle (Gil et al., 1999 ; Saino et al., 2002b ; Gowati et al., 2007) ou aux caractéristiques du chant (Gil et al., 2004a).

La dominance est un concept fondamental dans l'étude de l'organisation sociale. Certaines études ont été portés sur la relation entre cette caractéristique sociale du mâle et la sélection sexuelle fait par la femelle ; les résultats obtenus sont contradictoires : quelque fois le choix récompense le dominante tandis que d'autres est en faveur du dominé (Cox & Le Boeuf, 1977 ; Alatalo et al., 1991 ; Horne & Ylönen, 1996 ; Qvanström & Forsgren, 1998 ; Johnsen et al., 2001 ; Ophir & Galef, 2003 ; 2004 ; Amy et al., 2008).

Cette étude a été réalisée sur 20 mâles et 20 femelles de perdrix grise. Après un pré-test qui est servi à comprendre le rang social de ces 20 mâles, dix groupes de deux, dans lesquels la différence en termes de dominance était évident, ont été formés. Chaque groupe a été montré à deux femelles différentes, qui ont pu apprécier les caractéristiques des mâles et montrer leur préférence. A la fin de ces tests, 10 femelles ont été accouplées avec le mâle dominant (et choisi) et 10 avec le dominé. Tous les œuf ont été pesés et mesurés, certains ont été emmenés au laboratoire pour analyser les composantes principales et la concentration de lysozyme, ovotransferrine et testostérone ; les autres ont été laissés à incuber pour l'évaluation du taux d'éclosion.

Notre hypothèse était que les femelles accouplées avec des mâles dominants allouent une plus grande quantité de ressources dans leurs œufs.

Les principaux résultats de ce travail ont été que les femelles accouplées avec des mâles dominants et choisi, par rapport à d'autres qui ont reçu une mâle dominé, ont pondu œufs avec plus de testostérone et un taux plus élevé d'éclosion. Aucune corrélation a été trouvée avec le poids de l' œuf, de l'albumen, du jaune et de la coquille et la concentration de lysozyme et ovotransferrine.

Autres résultats de l'étude ont été celles concernant les quantités des composants des œufs par rapport à l'ordre de dépôt (diminution du jaune et de la coquille et augmentation du blanc et de la testostérone) et une plus grande éclosion des œufs avec un indice d'allongement intermédiaire.

En conclusion, le rang social du mâle liée à la préférence de la femelle a changé la répartition des substances hormonales dans les œufs et la pourcentage du taux d'éclosion.

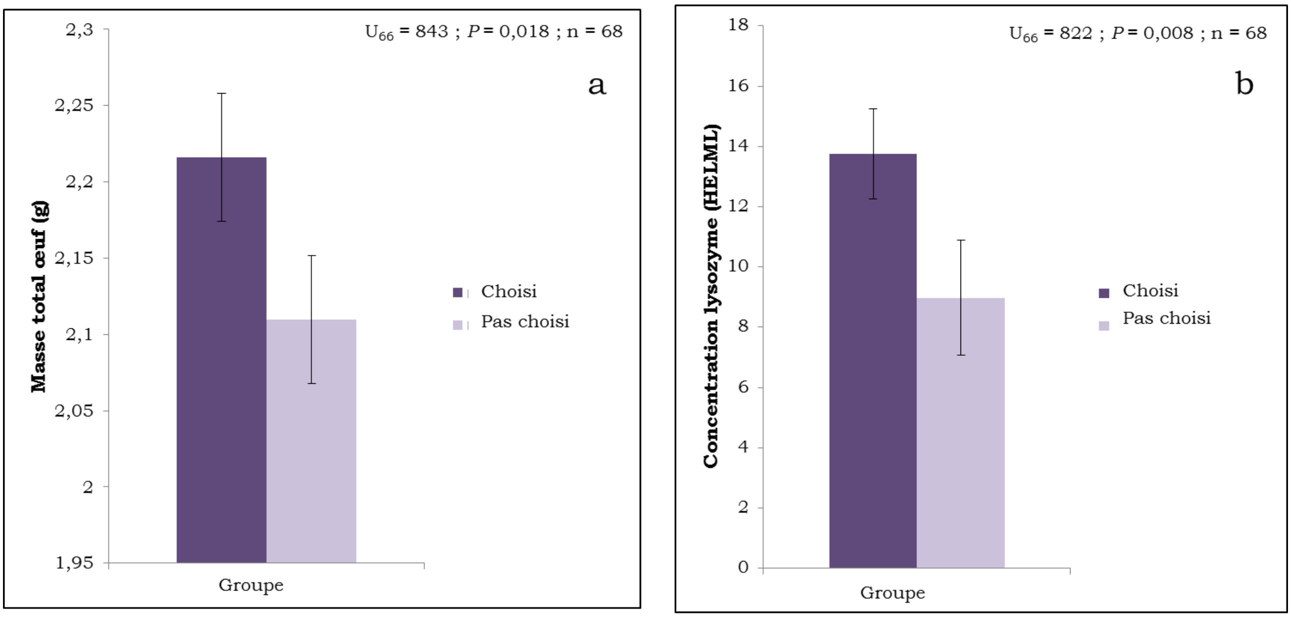


Figure Errore. Nel documento non esiste testo dello stile specificato.**-1 : a) Concentration de la testostérone par rapport au groupe expérimental ; b) taux d'éclosion par rapport au groupe expérimental.**

Mots clés : Dominance du mâle, choix de la femelle, effets maternels, concentration de testostérone, taux d’éclosion.

Chapitre II – Résumé

Le but de l'étude présentée dans ce chapitre a été de comprendre s'il y a une préférence pour un mâle dominant chez les femelles de canari et si ce facteur influence l’investissement maternel.

Dans plusieurs articles sur les réseaux de communication a été considéré comme les femelles utilisent les informations publiques (Valone, 1989), telles que l'interaction entre différents mâles, afin d'évaluer la qualité et déterminer ainsi ses choix (par exemple, Doutrelant & McGregor, 2001 ; Leboucher & Pallot, 2004) ou pour régler ses comportements de copulation à l’extérieur de la paire (par exemple, Otter et al., 1999 ; Mennill et al., 2002).

Les types d'interaction entre les mâles à partir de lesquelles on peut obtenir des informations sur leur qualité sont essentiellement trois : concurrence fondée sur les caractéristiques visuelles; concurrence avec le chant et concurrence physique. Ce travail, en particulier, a mis l'accent sur l'étude du troisième type d'interaction.

Pour faire ça, une expérience a été mis en place où un compétition entre deux mâles pour l'accès à la nourriture a été montré à une femelle. Après ces affrontements, la choix des femelles a été évaluée et les œufs ont été prélevés pour effectuer les analyses en laboratoire des principaux éléments et des certaines molécules dans l’albumen et dans le jaune.

Dans cette étude, on a découvert que la femelle effectue son choix en se basant sur un comportement particulier du mâle : les femelles utilisées ont passé plus de temps (indice de choix) devant les mâles qui avaient attaqué à plusieurs reprises l'autre pour premier.

Le couplage avec le mâle choisi a conduit à des résultats différents : les œufs produits par le groupe de femelles accouplées avec le mâle préféré ont pondu œufs plus lourds, dont la coquille était plus lourde, avec un indice d'allongement plus élevé et avec une concentration plus élevée du lysozyme.

Le poids plus grande des œufs produits par femelles couplées avec le mâle préféré est en accord avec des études précédentes (Cunningham & Russell, 2000 ; Uller et al., 2005 ; Gilbert et al., 2006 ; Loyau et al, 2007) et il est considéré comme un indice de bonne qualité des œufs (Magrath, 1992 ; Perrins, 1996 ; Blomqvist et al., 1997 ; Starck & Ricklefs, 1998 ; Styrsky et al., 1999 ; Christians, 2002 ; Silva et al., 2008 ; Forbes & Wiebe, 2010).

Le lysozyme est plutôt considéré comme un agent antimicrobien importante et sa plus grande concentration dans les œufs produits par les femelles accouplées avec des mâles les plus agressifs peut être une tentative pour contrer les effets négatifs sur la santé des concentrations élevées de testostérone.

Dans cette étude des différences statistiquement significatives n'ont pas été trouvé entre les deux groupes en ce qui concerne la concentration de testostérone ; autres études antérieures ont montré, cependant, que les fils des mâles agressifs sont agressifs eux-mêmes et ont une plus grande quantité de testostérone. La concentration plus élevée du lysozyme pourrait trouver une explication dans ce fait.

En conclusion, les femelles accouplées avec des mâles préféré après avoir visionné des interactions de type physique avec des autres pondent des œufs de meilleure qualité en termes de masse totale de l'œuf et de la concentration de lysozyme.

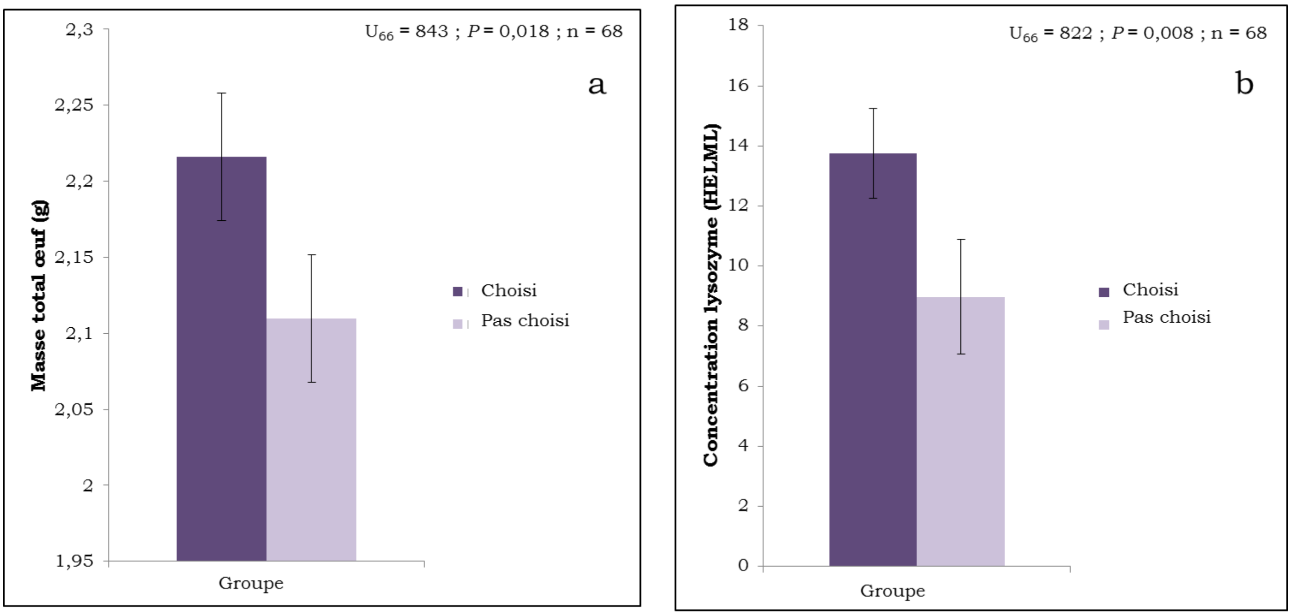


Figure Errore. Nel documento non esiste testo dello stile specificato.**-2: a) Histogramme qui corrèle la masse de l'œuf et le groupe expérimental. b) histogramme qui corrèle la concentration de lysozyme et le groupe expérimental.**

Mots clés : Interactions physiques mâle-mâle, choix de la femelle, effets maternels, concentration de lysozyme, qualité de l' œuf.

Chapitre III – Résumé

Au cours des dernières années l'impact de l'homme sur l'environnement est en train d'augmenter. Cela peut prendre de nombreuses formes (pollution, destruction de l'habitat, etc.) et il faut l'étudier afin de comprendre ce qui peut causer sur l'environnement naturel et les espèces.

Ce chapitre met l'accent sur l'influence du bruit d'origine anthropique sur la sélection sexuelle et le succès de reproduction dans une espèces oscine, où le chant est un moyen fondamental de communication: le canari.

Des études antérieures ont montré des effets différents sur les choix sexuels des diverses espèces: 1) l'abaissement des accouplements d'oiseaux qui vivent dans les zones perturbées par rapport à autres domaines moins déranger (Habib et al., 2007), 2) la difficulté des femmes à reconnaître leur partenaire reproductive (Swaddle & Page, 2007), 3) la diminution des postures de stimulation à l'accouplement (Huet des Aunay, 2011).

Peu d’études se sont concentré sur le succès reproducteur (Halfwerk et al., 2011b).

Pour cette raison, il a été mis en place une expérience dans laquelle plusieurs femelles de canari ont été soumis à deux conditions: l'une dans laquelle le chant du mâle a été masqués par le bruit et un autre où il a été reproduit dans un état de silence. Un groupe a été soumis dans le premier cycle de reproduction à la condition appelé «Bruit» et dans le second à celle qui est appelé "Silence", tandis que le second groupe a été traité dans l'ordre inverse. Toutes les expériences ont été réalisées dans des «cages» insonorisées pour éviter les contacts visuelles et sonores entre les femelles, de manière à éviter la communication entre eux et les possibles comportements d'émulation.

Les principaux résultats de ce travail sont sur les postures de sollicitation à l’accouplement et sur le délai entre la première présentation du stimulus et la première posture pour la sélection sexuelle. Pour ce qui concerne le succès de reproduction, une influence du dérangement sur le nombre d'œufs pondus a été signalée.

Ces résultats sont essentiellement conformes à ceux rapportés dans la littérature (Habib et al., 2007; Halfwerk et al., 2011b; Huet des Aunay, 2011; Schroeder et al., 2012) et sont un petit pas en ce qui concerne la compréhension de l’impact des activités humaines sur les espèces que occupent les espaces affectés.

Il reste encore beaucoup à étudier dans ce domaine, telles que l'adaptation des espèces oscines dans cette nouvelle niche écologique ou l'influence du bruit sur la communication mère-fils pendant les premiers jours de vie.

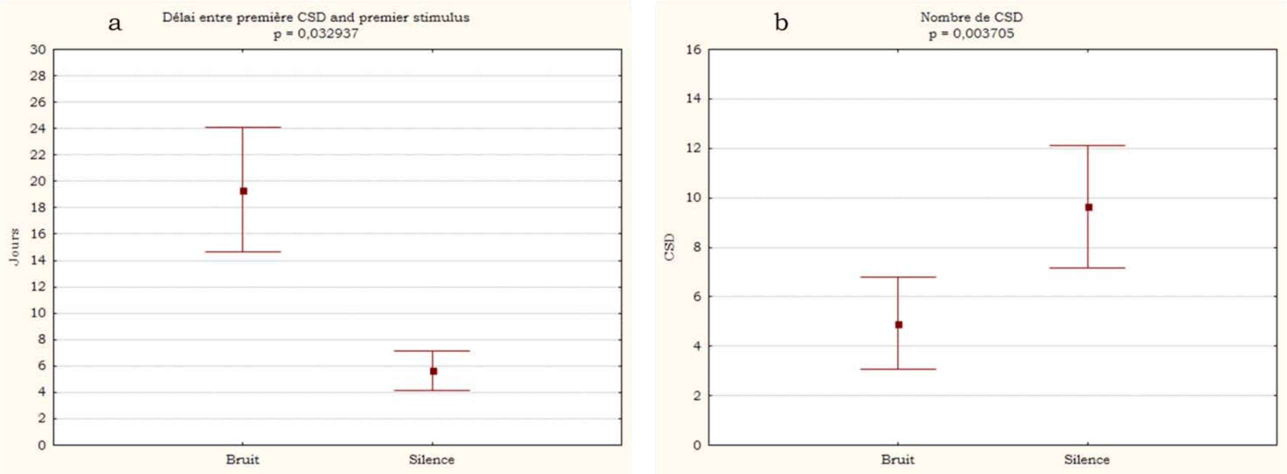


Figure Errore. Nel documento non esiste testo dello stile specificato.**-3 : a) Délai entre le jours de la première présentation du stimulus et la première posture dans les deux groupes expérimentales; b) nombre de posture dans les deux groupes experimentales.**

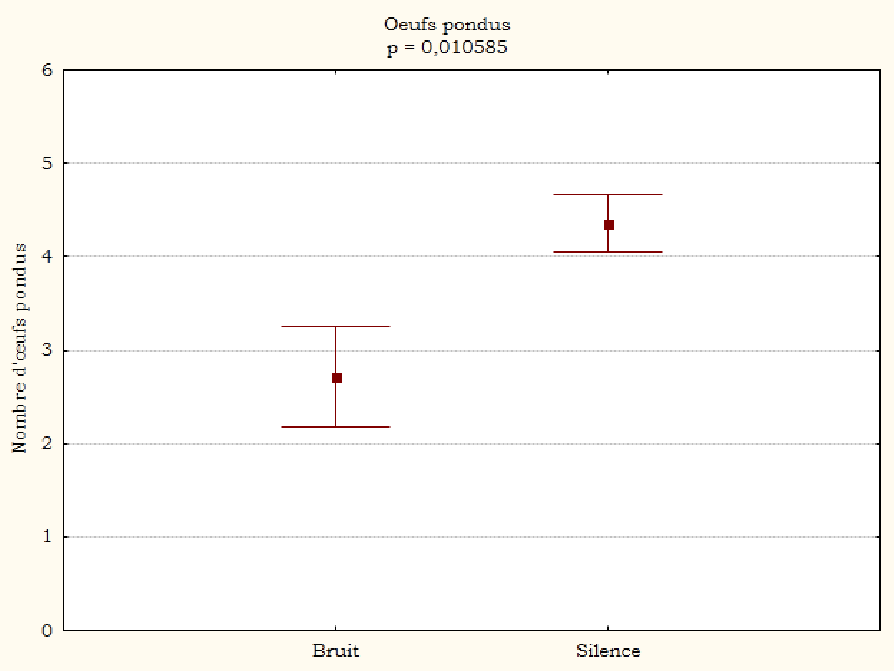


Figure Errore. Nel documento non esiste testo dello stile specificato.**-4 : Nombre d' œufs pondus par les deux groupes.**

Mots clés : Bruit anthropique, posture de sollicitation à l’accouplement, chant du mâle, effets maternels, sélection sexuelle.

Chapitre IV – Résumé

La théorie de l'histoire de vie fournit un cadre évolutif dans lequel on analyse comment les organismes allouent leurs ressources en tenant compte des priorités différentes inhérent la survie et la reproduction (Stearns, 1992). Un chapitre important est sur le compromis entre la fonction immunitaire, la santé et les ressources nécessaires pour la reproduction (Sheldon & Verhulst, 1996).

Il y a des études qui montrent que l'investissement dans l'immunité contre les parasites va à réduire l'investissement parental en termes de taille de la couvée, masse d'œufs pondus ou composants alloués (Råberg et al., 2000 ; Burnham et al., 2003 ; Uller et al., 2006 ; Marzal et al., 2007 ; Viscione et al., 2008 ; Cucco et al., 2010).

Un autre facteur qui peut diminuer la qualité des œufs est la production d'une nombre excédentaire des œufs causée, par exemple, par la disparition de celles qui sont déjà déposées.

A partir de ces principes l'étude présentée dans ce chapitre est né, où on a comparé deux groupes expérimentaux, l'un composé par femelles de perdrix rouge soumis à une vaccination contre le virus de NDV et l’autre par femelles pas vaccinées. En plus, aussi la production d'un surplus d'œufs a été stimulé enlevant les mêmes juste après la ponte.

Aucun résultat en termes de santé des femmes ou de qualité d'œufs a été trouvé lié à la vaccination, alors que des changements ont été appréciés dans les derniers œufs pondus. En particulier, il y avait une diminution en termes de poids, largeur, poids du jaune et de l’albumen et de la concentration de lysozyme. Les premiers paramètres suivent une tendance à U inversé, où les œufs de meilleure qualité sont situés au milieu de l'ordre de dépôt ; pour ce qui concerne le lysozyme une diminution linéaire a été trouvée.

La baisse de la qualité des derniers œufs peut être explique par une perte progressive de l'énergie de la mère.

En conclusion, cette étude montre que les femelles soumises à deux types de contraintes ne semblent pas avoir des problèmes avec une

augmentation de l'activité immunitaire, mais elles subissent une perte progressive de l'énergie due à une surproduction d'œufs.

Mots clés : Qualité de l’œuf, ordre de dépôt, investissement maternel, NDV vaccin, surproduction d'œufs.