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**Patterns of adult behavior during the
incubation phase of parental care in the
Northern Lapwing (*Vanellus vanellus*)**

Dissertation

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Summary

Incubation of eggs is an essential assumption of successful reproduction in vast majority of bird species. However, the ways how to reach the successful end of incubation differs a lot among species in many aspects, which makes from avian reproduction one of most diverse phenomena in animal behavior at all. Specifically, in species when both parents are sharing the incubation duties, there is a huge diversity in lengths and timing of incubation bouts, as well as in the frequency and timing of parental exchanges on the nest (so-called “incubation rhythms”). Moreover, there is a huge space for communication and negotiation between parents about the particular form of these incubation rhythms. At the same time, parents need to satisfy their own, self-maintaining needs, such as feeding, sleeping, or body maintenance, which is often conflicting with the needs of developing embryo. Apart from incubation, parents can help themselves with building a nest of better thermo-insulative properties. However, during whole incubation process, parents are under the risk of nest depredation, or even of depredation of themselves.

In this thesis, different aspects of parental care during incubation are addressed in the model of the Northern Lapwing (*Vanellus vanellus*), a biparental shorebird with highly variable contribution of male to incubation. The core of thesis is a group of articles (and one manuscript), based on analysis of unique dataset of continuous video-recordings of incubating Northern Lapwings. First, we thoroughly described the within species diversity in incubation rhythms of model species (**Chapter 1**) and showed how variable male contribution to incubation shapes this incubation system. Then, we incorporated part of our dataset into the big comparative work, which enabled us to interpret the incubation rhythms of our model species from phylogenetic perspective and show how antipredator strategy of the Northern Lapwing relates to its incubation routines (**Chapter 2**).

Then we analyzed behavioral patterns preceding departures of birds from the nest and described behavioral signals serving for negotiation over parental care and helping to synchronization of parental exchanges (**Chapter 3**). We also showed that self-maintaining activities (such as sleeping and body-maintenance) in incubating females has diverse daily rhythms, which leads to maximizing of female vigilance during the high peaks of predation pressure and are shifted by the male contribution to incubation (**Chapter 4**). Finally, we diverted from the videos and based on analysis of nest-lining magnitudes showed, that Lapwings has bigger nest linings on thermally unfavorable places, but that they don't avoid having conspicuous nests in places with high risk of predation (**Chapter 5**).

To summarize, our works broadened the knowledge about possible costs connected with female choice for poorly incubating males. We also enhanced the insight into the relative importance of diverse drivers shaping the parental care.

General introduction

Variation in avian parental care during incubation

In most vertebrate animals (including humans), raising the offspring is a process that demands a great deal of time and energy. Parental care in vertebrates involves a wide range of activities, often involving territory monopolization, nest building, pregnancy or egg incubation, feeding, and defending the growing offspring (Balshine 2012, Royle et al. 2012). However, parental care is provided in extremely diverse ways. Live bearing within the mother's uterus, and egg laying into some form of nest, are widely observed. However, there are also many less usual ways of caring for the offspring including many obscure forms, such as carrying the eggs (Oppenheimer 1970, Marquez and Verrell 1991) or even postnatal development of the offspring (Oppenheimer 1970, Low 1978) in various parts of the body of a parent. At the same time, there are taxa that leave the whole process, including embryonic development, to the natural conditions (Frith 1956, Hirth 1980).

In birds, parental care generally involves building a nest, in which the female lays the eggs. The eggs are then incubated by the body heat of the parents, and the hatched offspring are either fed (in altricial birds) or at least guarded and heated until they are fledged (typically precocial birds) (Kendeigh 1952). Even here we can find exceptions. Some penguins incubate the eggs between the feet and the body of the standing parent (Maho 1977). Megapodes (Frith 1956) and to some extent the Crab Plover (*Dromas ardeola*) (De Marchi et al. 2008) leave incubation to the natural conditions, as do crocodiles, the closest living relatives of birds (Pooley 1977). Interestingly, megapodes also leave chick growth unsupervised (Göth and Vogel 2002). Generally, however, avian parental care follows the pattern described above, including egg incubation and care for the young.

However, if we focus on parental behavior while the eggs are being incubated (a crucial part of avian parental care), we find a surprisingly variable and complex phenomenon. During incubation of the eggs, all work can be done by one of the partners, but in most cases both parents are involved (Deeming 2002). Both parents may share the incubation duties (**Chapter 1&2**, Bulla et al. 2014a), or one of them (usually the male) can support the partner by feeding (Galván and Sanz 2011, Matysioková and Remeš 2014) or at least by defending the territory (or the brood) (Jenni and Collier 1972, Buitron and Nuechterlein 1989). In some species, even helpers (usually relatives) can be involved in these activities (Lennartz and Harlow 1979, Langen and Vehrencamp 1999).

Among species with clearly uniparental care during incubation, we can observe that the gyneparental system is much more common (Deeming 2002, Cockburn 2006). However, also species with sexual role reversion and androparental care can be observed (Jenni and Collier 1972, Schamel et al. 2004). This sex-role reversion can go so far that females can defend a harem of several males incubating nests, and they can even destroy the nests of other males when they take over a neighboring harem (Emlen et al. 1989). In some species, more than one type of parental care can occur at the same time (Davies and Lundberg 1984, Kålås 1986, Byrkjedal et al. 1997). In a very special case, a small number of species regularly exhibit a mating system called double-clutching, which results in simultaneous incubation by both parents, each on a different nest (Hildén 1975, Pienkowski and Greenwood 1979, Green 1984).

Naturally, this variation in avian parental systems is related to the variation of the conditions in which the embryonic development takes place. Species (and even nests within species) differ not only in terms of the incubation temperature (i.e. the temperature of the eggs when the parent is sitting on the nest) (Burger and Williams 1979, Ward 1990, Tieleman et al. 2004), but maybe more importantly in terms of the incubation attendance of the parents (i.e. the proportion of time for which the nest is being incubated) (**Chapter 1**, Skutch 1962, Chalfoun and Martin 2007, Ricklefs and Brawn 2013, Bulla et al. 2017). In some species, the parents incubate almost constantly (Jónsson et al. 2007, Bulla et al. 2014a), while in other species the incubation attendance drops to less than 50 percent (Chalfoun and Martin 2007, De Marchi et al. 2008).

Several drivers of variation in incubation attendance have been identified. Higher incubation attendance is usually typical for biparentally incubating birds (Matysioková and Remeš 2014, Bulla et al. 2017), although for example hornbill females, walled up in nest cavities, reach 100% nest attendance with only feeding support from her partner (Kinnaird and O'Brien 1999). Incubation attendance also often varies within the time of day, usually with lower values during the warmer parts of day (if the temperatures do not exceed the upper safe limit for the eggs) (**Chapter 1**, Vincze et al. 2013, Clauser and McRae 2016, Bulla et al. 2017). This pattern is typical especially for uniparentally incubating species, while biparentally incubating birds are often able to maintain incubation attendance on a constant level throughout the day (Bulla et al. 2017). There is also a latitudinal gradient in some groups, with higher incubation attendance in northern latitudes than in the tropics and in temperate zones in the southern hemisphere (Chalfoun and Martin 2007). Contradictory patterns in incubation attendance are produced by the risk of depredation. The risk of egg depredation increases the pressure for high incubation attendance (Fontaine and Martin 2006), while nest attendance decreases when there is a high risk of parent depredation (Martin 2002, Cervencí et al. 2011).

Yet another aspect of avian incubation routine where we can find huge variation among species (and also within species), is the time scheduling of incubation care between parents. On the one hand, seabirds such as albatrosses (Weimerskirch et al. 1986), petrels (Johnstone and Davis 1990, Chaurand and Weimerskirch 1994) and penguins (Maho 1977, Gauthier-Clerc et al. 2001) are known to incubate with bouts lasting many days (up to several weeks), while some small passerines exchange on the nest after periods lasting no longer than a few minutes (Bartlett et al. 2005).

Naturally, given this substantial variation in all aspects of avian parental care during incubation, an important question arises. What are the key factors that are responsible for the evolution and maintenance of such diversity?

Key factors explaining diversity in incubation care

Embryonic development

Probably the most obvious factors are the physiological requirements of the embryo. It is undeniable that if the embryo dies, all the parental care has been useless. Thus, if parents pursue successful reproduction, they absolutely have to maintain the eggs in conditions which are not lethal and which enable physiologically optimal embryo development and successful hatching of the offspring (Carey 1980, Williams 1996). The main goal in this direction is to maximize the time for which the egg is exposed to temperatures optimal for embryonic development. Although these temperatures are known for poultry, they are not known precisely for most bird species (King'ori 2011). Usual incubation temperatures among wild species vary from 30°C (Burger and Williams 1979, Haftorn 1988) to 40°C (Marder and Gavrieli-Levin 1986, Williams 1996). However, the temperatures that are truly optimal for embryonic development probably have a much narrower, species-specific range (Deeming and Jarrett 2015). Nonetheless, in many species even big deviations from this “ideal state” are widespread in the course of the incubation period (Zerba and Morton 1983, **Chapter 1**, Reneerkens et al. 2011, Cervencik et al. 2011, Bulla et al. 2017). The risk of lethal consequences arises especially when temperatures inside the egg rise above approximately 41-43°C (Bennet and Dawson 1979, Webb 1987). By contrast, decreasing temperatures tend to cause a slowdown of embryonic development until they reach the “physiological zero temperature” at around 25-27°C (Carey 1980, Haftorn 1988, Williams 1996). Below this level, the development is stopped, or rather interrupted. It can be repeatedly resumed after the temperature returns into the developmental optimum range (Bennet and Dawson 1979, Carey 1980). Temperature fluctuations of this kind often have no substantial negative impact on the offspring (Bennet et al. 1981, Aldrich and Raveling 1983, Williams 1996). In some cases, the consequences of such fluctuations include a higher risk of pre-hatching mortality (Arnold 1993, Stoleson and Beissinger 1999), or reduced weight

and poor condition of the hatchlings (Hepp et al. 2006, Carter et al. 2014, Bueno-Enciso et al. 2017a). To be complete, there are also other important but little-studied embryonic requirements, such as the relative air humidity around the egg (Grant 1982) and regular egg turning (Deeming and Jarrett 2015).

The requirements of embryonic development shape parental behavior in two important and related ways. First, the parents should minimize the time for which the nest is unattended (Martin et al. 2007, Carter et al. 2014, Bueno-Enciso et al. 2017a). Second, the parents can employ strategies that use environmental conditions to reduce the burden on themselves. They can time the incubation gaps to fall preferably within periods when the ambient temperature is closer to the temperature required by the embryos (Alrashidi et al. 2010, AlRashidi et al. 2011, Bulla et al. 2017). Thus, in temperate and Arctic species, the most convenient time for a break is around midday, when the ambient temperatures are high (**Chapter 1**, Clouser and McRae 2016, Bulla et al. 2017). In desert species, however, the breaks are during periods with a lower risk of overheating, typically during the morning and late afternoon (Wahlsberg and Voss-roberst 1983, AlRashidi et al. 2011, own unpublished data). Also, a daily pattern in the division of incubation care between the parents can play a role, since the parents may differ in the extent to which they heat up the eggs (Hawkins 1986, Kleindorfer et al. 1995, Voss et al. 2008).

Parents can influence the temperature inside the egg not only by the extent of incubation and by the timing of incubation gaps. Other ways are by nest structure (Kern 1984, Heenan 2013), by nest placement (Orr 1970, Tulp et al. 2012, Sidis et al. 2013, Kubelka et al. 2014), construction material (Kern 1984, Reid et al. 2002) or by the size of the nest lining (**Chapter 5**, Reid et al. 1999, Tulp et al. 2001). For example, Piping Plovers (*Charadrius melodus*) choose lighter-colored pebbles to enhance the reflectance of the nest for solar radiation, and thus to lower the risk of overheating if the parent is absent during the hottest part of day (Mayer et al. 2009). Crab Plovers rather move their eggs within the corridors of their burrows to optimize solar incubation and avoid the need to incubate the eggs (De Marchi et al. 2008).

To summarize, although the optimal temperature range for embryonic development is rather narrow, parents can use a wide range of options for improving their chances of reproducing successfully.

Predation pressure

A crucial factor in egg survival is the risk of nest depredation. Predation is undoubtedly the most important cause of nest failures in most bird populations (Ricklefs 1969), often leading to failure of the vast majority of breeding attempts (Sládeček et al. 2015, Kubelka et al. 2018). It is therefore obvious that parents have

to make great efforts to prevent it. Predation risk is also likely to affect parental care and behavior during incubation (**Chapter 2**, Amat 2004, Cervencí et al. 2011, Smith et al. 2012). The main task for parents in this respect is to be as inconspicuous as possible. Parents should minimize their movements and also their vocal activity at the nest (Smith et al. 2012), at least during the peak activity period of their main predators (Cervencí et al. 2011). A specific adjustment of the division of incubation duties between the sexes can also be helpful. When one sex is more conspicuous for predators during incubation, it can be expected that the more conspicuous parent will incubate during the night. This has been explicitly tested in the Red-capped Plover (*Charadrius ruficapillus*) (Ekanayake et al. 2015), but night male incubation can be found in many species of plovers with sexual plumage dimorphism (Thibault and McNeil 1995, Blanken and Nol 1998, St Clair et al. 2010).

Generally, optimizing the conditions for embryonic development helps to shorten the incubation period (Fontaine and Martin 2006, Carter et al. 2014, Bueno-Enciso et al. 2017a), and thus also the period for which eggs are exposed to predators (Mayfield 1961). In some situations, however, adaptations that are helpful from the antipredator perspective may conflict with those that are helpful for embryonic development. For example, parents may be forced to avoid incubation in the period of high predation risk, especially when there is a high risk of parent depredation together with depredation of the eggs. As an example, Redshanks (*Tringa totanus*) were shown to leave their nests unattended for many hours during the night when predation pressure was high (Cervencí et al. 2011). Similarly, parents may have to make a decision about the magnitude of the nest lining. While a massive nest lining can be very helpful for maintaining the physiological optimum in the nest (**Chapter 5**, Kern 1984, Tulp et al. 2012), it can be very conspicuous, and can easily attract visually-oriented predators (Mayer et al. 2009).

Sexual conflict

A somewhat different view of the matter appears to us if we realize that, as a consequence of complex trade-offs system, parents may not always give absolute priority to the survival of their current offspring. According to the ultimate “fitness” perspective of a bird’s life, situations can arise which will favor a lower level of parental care, even if it involves lowering the chance for a successful outcome of the current breeding attempt (McNamara et al. 1999, Lessells and McNamara 2012, Iserbyt et al. 2015). One example can be the trade-off between current reproduction and future reproduction (Barta et al. 2008, Harrison et al. 2009). For example, if the parent would be depredated as a consequence of high parental effort, it should rather reduce its current parental effort in order to increase the probability of future reproduction. The above-mentioned long night gaps in the Redshank (Cervencí et al. 2011) provide an example of this. Another example is

when a high rate of parental care lowers the parent's condition, which can increase the risk of parent depredation during migration or wintering (Hepp et al. 1990).

A particular case is when both parents are involved in parental care, and the interests of the two parents naturally come into conflict. This so-called "sexual conflict" arises from the fact that the benefits accruing from successful reproduction (i.e. increased fitness) are fully shared between the parents, while the costs (i.e. the consequences of the parental care effort) are paid by each of the parents separately (Székely et al. 2007, Harrison et al. 2009). Thus, the logical interest of each parent is to leave as many duties as possible to its partner. Theoretical models have predicted (McNamara et al. 1999, Houston et al. 2005, Lessells and McNamara 2012), and empirical data have confirmed (Schwagmeyer et al. 2002, Iserbyt et al. 2015), that parents usually respond to a decline in care provided by the partner by reducing their own effort. This can be considered an evolutionary stable strategy, except in the case of breeding in harsh conditions, such as in the Arctic or in a desert, where a reduction in parental care necessarily causes the failure of a breeding attempt (Jones et al. 2002).

To summarize, incubation behavior should always be seen in the context of trade-offs between embryo needs, parents' own needs and predation risks. An unavoidable consequence of this process is that eggs usually receive less parental care than the parents are physiologically capable of giving, and also less care than would be optimal for embryonic development.

A summary of what is currently known, and of gaps in knowledge

The sections above provide only a very brief summary of the diversity of avian parental care for eggs, and the main drivers of diversity in parental care. They indicate, however, that the phenomenon is very variable. Parental care has therefore not surprisingly been a prominent topic of interest for ornithologists since the early times of modern ornithology (Bailey 1943, Kendeigh 1952, Skutch 1957, 1962, Pitelka et al. 1974). A great deal of work has been done, and many general patterns have been under long-term investigation, e.g. the division of parental duties between parents, and also other basic parameters of incubation care (Deeming 2002, Chalfoun and Martin 2007, Deeming and Reynolds 2015, Bulla 2016). However, there are still substantial knowledge gaps and challenges for future research.

When we are searching for gaps, we can easily find one seemingly very simple. The fact is that a thorough description of the within-species variation in incubation rhythms, using a sufficiently large sample, has been made only for a very small number of species. This can be demonstrated on recently published descriptive papers on common and notoriously well-studied species such as the Blue Tit

(*Cyanistes caeruleus*) (Bueno-Enciso et al. 2017b, Bambini et al. 2018) and the Great Tit (*Parus major*) (Bueno-Enciso et al. 2017b).

To briefly introduce the term incubation rhythm, I use it to refer to the sequence of incubation bouts (i.e. when a parent is on the nest) and incubation gaps (i.e. when the nest is unattended). For biparentally incubating species, the frequency of partners exchanging on the nest is also an important component of the incubation rhythms. Other important data that can be derived are on nest attendance, i.e. the proportion of time for which the nest is incubated (**Chapter 1&2**, Afton 1980, Bulla et al. 2014a).

When discussing the terms “a description of incubation rhythms” or “a sufficiently large sample”, it is necessary to have in mind that incubation rhythms can widely vary among nests (Haftorn 1988, Fontaine and Martin 2006, Vincze et al. 2013, Bulla et al. 2014a, 2016a, 2017, Sládeček and Bulla 2018), among habitats (Cervenci et al. 2011, AlRashidi et al. 2011, Vincze et al. 2013), according to the time of day (**Chapter 1**, Zerba and Morton 1983, Ward 1990, Bulla et al. 2017), during the incubation period (Bulla et al. 2014a, Pedler et al. 2015, Zhang et al. 2017a) and during the breeding season (Bulla et al. 2014a). A thorough description of this variation is a necessary first step in order to raise more general issues, such as the causes of between-species variation (**Chapter 2**, Conway and Martin 2000, Martin et al. 2007, Chalfoun and Martin 2007) and reasons for variation among individuals within populations (Fontaine and Martin 2006, Cervenci et al. 2011, Bulla et al. 2014a, 2017). However, in order to describe most of these types of variation, it is necessary to obtain and to analyze hundreds of days of continuous incubation monitoring, since studies based on small samples or conducted in specific conditions can easily lead to erroneous generalizing conclusions about variability and causation (Bulla et al. 2014b).

Analyses of continuous incubation monitoring lasting hundreds of days was virtually impossible before the expansion of modern technologies such as video recording (**Chapter 1-4**, Jongbloed et al. 2006, Smith et al. 2012, Zhang et al. 2017b) and temperature data-logging (Ward 1990, Reneerkens et al. 2011, Bulla et al. 2017, Moreau et al. 2018). Studies based on direct observations by a researcher usually worked with very small and scattered datasets with severely limited potential for describing the patterns (Liker and Székely 1999a, Bulla et al. 2012). Moreover, observations could usually be made only during daylight, which in most species caused an unavoidable bias. The only exception were birds with extremely long incubation bouts, such as procellariiform birds (Johnstone and Davis 1990, Chaurand and Weimerskirch 1994) and penguins (Maho 1977, Davis 1988), where one visit to the breeding colony per day is enough to capture most of the between-nest variation in incubation rhythms. The study by Cantar and Montgomerie (1985)

is in my opinion very remarkable, and it illustrates the problems faced by investigators less than 40 years ago. Cantar and Montgomerie used a celluloid-film camera that exposed one frame each minute. To obtain approximately 120 days of records from no more than eight nests of the White-rumped Sandpiper (*Calidris fuscicollis*), the authors performed the amazing task of analyzing more than 172 000 pictures manually.

With modern equipment and technology, it is very much easier to collect and extract incubation data automatically. Video recorders are able to take many days of continuous and relatively easily workable recordings. Many studies have used temperature data-logging (Ward 1990, Bulla et al. 2017, Moreau et al. 2018), which however, does not allow individual birds to be recognized. This approach is therefore convenient for uniparental species only. Radio Frequency Identification Devices (RFID) have been used successfully for individual recognition of the incubating parent by checking the presence of birds equipped with individual RFID tags within the nest scrape (**Chapter 2**, Bulla et al. 2014a, 2017). Light-level geolocators (**Chapter 2**) and other specific techniques (Coulson and Wooller 1984) have also been used.

The prevalent method used for extracting Northern Lapwing (*Vanellus vanellus*) incubation behavior throughout this dissertation was continuous monitoring by small cameras placed in the vicinity of nests (**Chapter 1-4**). It is a very time-consuming process to analyze video recordings, but the use of cameras provided an opportunity, unlike all the other methods mentioned above, to go far beyond simply asking who, when and how much each of the parents incubates, and how this varies among the nests. While analyzing the video records of incubation, one can not only consider the simple presence or absence of incubating birds (**Chapter 1&2**) but also cover many other detailed aspects of parental behavior. Thus, we were able to describe behavioral patterns preceding the exchange of parents on the nest (**Chapter 3**) and to make a detailed classification of the behavior of the female during incubation (**Chapter 4**).

These aspects of behavior during incubation provide an insight into several almost unstudied issues of incubating parents. The first topic is how the parents communicate and negotiate about the division of incubation duties and about the timing of exchange on the nest. The answer to this question seems to be relatively simple in species with large territories, where the parents are unable to communicate continuously during incubation. The only way for incubating penguins or albatrosses to exchange with the partner is to wait until the partner returns from several hundred kilometer long trip (Seddon 1989, Weimerskirch 1995). However, species that spend the whole incubation period within a few acres of land with little vegetation (as in the case of our focal species) have much better

opportunities for continuous communication and also for negotiating about parental exchanges on the nest. We therefore hypothesize that some mechanisms have evolved for handling the exchange on nests through continuous communication between the partners moving within the territory. Although such negotiation processes very probably occur in many biparental species, and behavioral compatibility has recently been recognized as a factor that potentially has a great influence on the reproductive output in biparental species (Ihle et al. 2015), studies investigating this phenomenon are still scarce (Ball and Silver 1983, Niebuhr and McFarland 1983, Boucaud et al. 2016, 2017). Moreover, existing studies are often based on captive birds in laboratory conditions (Ball and Silver 1983, Boucaud et al. 2016).

The second phenomenon, which has been studied even less, is the timing of self-maintaining activities during incubation (Javůrková et al. 2011). Incubating parents usually need to resolve the trade-offs between benefits and costs due to the time spent on caring for themselves and on caring for the clutch (Chalfoun and Martin 2007, Lothery et al. 2014). Pursuing some activities (e.g. foraging) while sitting on the nest can be impossible or may lead to an increased risk of depredation. For example, preening during incubation can inform predators about the nest position (Smith et al. 2012). Sleeping can reduce the vigilance level of a sitting bird, and can increase the risk of a predator arriving unobserved (Lima et al. 2005). However, the demands of incubation constrain the birds to pursue these activities when they are not on the nest. How birds are able to resolve these trade-offs, and how they are related to the division of incubation duties between the partners, is an almost totally unstudied topic.

Shorebirds as a model group for a study of diversity in incubation care

Shorebirds are a very variable group with great diversity of mating systems and incubation patterns (Thomas et al. 2006, 2007, Székely et al. 2014), although in many other aspects of breeding biology they are rather uniform. Most shorebird species lay four maculated eggs, the clutches are laid into a shallow scrape on the ground and, after hatching, precocial and cryptic chicks are brooded (Cramp and Simmons 1983). Thus, compared with other aspects of their life, the diversity of incubation patterns among waders is truly surprising. We can find species that incubate biparentally (Vincze et al. 2013, **Chapter 2**, Bulla et al. 2014a), gyneparentally (Cantar and Montgomerie 1985, Løfaldli 1985), and androparentally (Jenni and Collier 1972, Ridley 1980). In addition, species exhibiting double clutching are known (Hildén 1975). Even more interestingly, a big portion of this diversity can often be found within a single species (**Chapter 2**, Kålås 1986). Given these facts, shorebirds can be considered as a convenient “non-

conventional” model group for studying mating systems (Székely et al. 2006, Székely 2019) and incubation rhythms (Bulla 2016).

Specifically, the widely-distributed Eurasian and predominantly temperate Northern Lapwing, the focal species of this dissertation, is known to be usually biparentally incubating, although a polygynous species (with 20 to 60 % of polygynous males) (Šálek 2005). The Northern Lapwing breeds solitarily, or in loose colonies on grasslands and arable land (Šálek 1993, Byrkjedal et al. 1997, Liker and Székely 1999b). Lapwings are relatively well-visible while the nests, often with a big and conspicuous nest lining, are being incubated (**Chapter 5**). However, they are very aggressive in attacking intruding predators, which is a task especially assigned to the male (Elliot 1985, Kis et al. 2000). Although they deter daily predators very efficiently, lapwings suffer a high predation rate, especially from night-operating mammals, such as Red Fox (*Vulpes vulpes*) and Rock Marten (*Martes foina*) (**Chapter 4, Unpublished data**). Although Lapwing males are very active in defending territory against both predators and conspecifics, their contribution to incubation has repeatedly been shown to be very variable on a between-nest scale (**Chapter 1**, Liker and Székely 1999a, Lislevand et al. 2004, Jongbloed et al. 2006). The Northern Lapwing therefore seems to be a very good model species for a study of parental care and its within-species flexibility.

Outline of the thesis

The aim of this thesis was to describe various aspects of parental care in the Northern Lapwing during incubation. We used video-recordings of breeding Northern Lapwings for an analysis of the variation in their incubation rhythms (Bulla et al. 2016a, b, Sládeček and Bulla 2018, Sládeček et al. 2019a) and for a comparison of these rhythms with related shorebird species (**Chapter 2**). Then we focused in greater detail on the behavior of video-taped parents in order to describe the behavioral signaling between the partners while negotiating about incubation exchange (**Chapter 3**, Sládeček et al. 2019b), and to test whether male nest attendance is related to female self-maintaining behavior during incubation (**Chapter 4**). Finally (**Chapter 5**, Kubelka et al. 2019), we described patterns of nest lining magnitude, and tested whether this is related to the thermoregulation requirements or to the need for crypsis, as an anti-predator strategy.

Incubation rhythm of the Northern Lapwing

In **Chapter 1**, we used continuous video recordings of 113 nests to provide a description of the diversity in incubation rhythms of the focal species. We placed a set of small cameras with a digital video recorder close to the nests to obtain around 3 days of continuous video recordings (median, range 1-22 days). Specifically, we investigated the between-nest variation in overall nest attendance

and other metrics of the incubation rhythm, and also how this rhythm changes within the day, throughout the incubation period and the breeding season. We also investigated how variation in male nest attendance relates to this variation in overall nest attendance.

Then, we included a part of this dataset in a big comparative study across 91 populations of 32 shorebird species (**Chapter 2**). This study described a huge among-species and within-species variation in incubation rhythms related to phylogeny, anti-predator strategy, energetics and environmental conditions of the breeding environment as potential drivers of this diversity. Within this thesis, participation in this work enabled us to place the results on Northern Lapwing in the broader context of the Shorebird group.

Sex-specific signaling during the parental incubation exchange

By making a detailed analysis of parental behavior during their incubation exchanges (i.e. shortly before their departures from the nest), we investigated whether the parents communicate by using behavioral signals (**Chapter 3**). We also investigated how these signals help to synchronize parental care and how the intensity and the efficiency of these signals relates to the variation in overall nest attendance.

Female self-maintenance behavior during incubation

Based on a time-budget analysis of the 24-hour period of video recordings from 55 nests, we analyzed patterns of female sleeping and preening (as self-maintenance activities) on the nest during incubation (**Chapter 4**). First, we described the daily rhythm of these two activities. Since there is considerably higher predation pressure on lapwings during the night than during daylight, we hypothesized that both of these activities are suppressed during the hours of darkness, in order to maximize the inconspicuousness of the incubating female. Second, we hypothesized that females who were paired with more nest-attentive males would spend less time on both of the activities during incubation. That is, we hypothesized that help from their male mates allows the females to deal with self-maintenance within their time out of the nest, and thus achieve higher vigilance during incubation.

Variability of nest lining magnitude

In **Chapter 5**, we analyzed the patterns of nest lining magnitude from more than 600 nests. Based on previously published works, we have formulated and tested two main hypotheses. First, on the basis of the “thermoregulation hypothesis”, we predicted that there would be a bigger nest lining on nests with a cooler microclimate, and this would therefore be associated with an earlier start to

incubation, cooler habitats or greater moisture around the nest. At the same time, we suggested that a big nest lining would be conspicuous for predators and would thus be associated with a higher predation rate. Moreover, based on the “antipredator hypothesis”, we predicted smaller nest lining in places with a higher predation risk, e.g. on the edges of breeding associations (Šálek and Šmilauer 2002) or in the vicinity of potential perches for avian predators.

Availability of supporting information

I present supporting information at the end of each chapter. Moreover, in order to increase the reproducibility of the scientific work, we have provided all data and also all R-scripts to reproduce all the analyses and visualizations presented in this thesis (Bulla et al. 2016a, Sládeček and Bulla 2018, Sládeček et al. 2018, 2019c, Sládeček and Kubelka 2019). Moreover, for some chapters (**Chapters 1,2&4**) we have also visualized the raw data; these plots are also freely available online (Bulla et al. 2016a, Sládeček and Bulla 2018, Sládeček et al. 2019c).

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Chapter 1

Diversity of incubation rhythms in a facultatively uniparental shorebird – the Northern Lapwing

Martin Sládeček, Eva Vozabulová, Miroslav E. Šálek & Martin Bulla

In birds, incubation by both parents is a common form of care for eggs. Although the involvement of the two parents may vary dramatically between and within pairs, as well as over the course of the day and breeding season, detailed descriptions of this variation are rare, especially in species with variable male contributions to care. Here, we continuously video-monitored 113 nests of Northern Lapwings *Vanellus vanellus* to reveal the diversity of incubation rhythms and parental involvement, as well as their daily and seasonal variation. We found great between-nest variation in the overall nest attendance (68–94%; median = 87%) and in how much males attended their nests (0–37%; median = 13%). Notably, the less the males attended their nests, the lower was the overall nest attendance, even though females partially compensated for the males' decrease. Also, despite seasonal environmental trends (e.g. increasing temperature), incubation rhythms changed little over the season and 27-day incubation period. However, as nights shortened with the progressing breeding season, the longest night incubation bout of females shortened too. Importantly, within the 24h-day, nest attendance was highest, incubation bouts longest, exchange gaps shortest and male involvement lowest during the night. Moreover, just after sunrise and before sunset males attended the nest the most. to conclude, we confirm substantial between nest differences in Lapwing male nest attendance, reveal how such differences relates to variation in incubation rhythms, and describe strong circadian incubation rhythms modulated by sunrise and sunset.

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Diversity of incubation rhythms in a facultatively uniparental shorebird – the Northern Lapwing

Martin Sládeček¹, Eva Vozabulová¹, Miroslav E. Šálek¹ & Martin Bulla ^{1,2,3}

In birds, incubation by both parents is a common form of care for eggs. Although the involvement of the two parents may vary dramatically between and within pairs, as well as over the course of the day and breeding season, detailed descriptions of this variation are rare, especially in species with variable male contributions to care. Here, we continuously video-monitored 113 nests of Northern Lapwings *Vanellus vanellus* to reveal the diversity of incubation rhythms and parental involvement, as well as their daily and seasonal variation. We found great between-nest variation in the overall nest attendance (68–94%; median = 87%) and in how much males attended their nests (0–37%; median = 13%). Notably, the less the males attended their nests, the lower was the overall nest attendance, even though females partially compensated for the males' decrease. Also, despite seasonal environmental trends (e.g. increasing temperature), incubation rhythms changed little over the season and 27-day incubation period. However, as nights shortened with the progressing breeding season, the longest night incubation bout of females shortened too. Importantly, within the 24h-day, nest attendance was highest, incubation bouts longest, exchange gaps shortest and male involvement lowest during the night. Moreover, just after sunrise and before sunset males attended the nest the most. To conclude, we confirm substantial between nest differences in Lapwing male nest attendance, reveal how such differences relates to variation in incubation rhythms, and describe strong circadian incubation rhythms modulated by sunrise and sunset.

A parent incubating eggs is a rare site across the animal kingdom^{1,2}, but not so in birds³. In the vast majority of avian species, incubating parents actively maintain egg temperatures in a range that is optimal for embryonic development (e.g. by siting, shading or wetting the eggs). Moreover, in almost 50% of bird families incubation by both parents is the most common form of care for eggs³. Yet, species vary greatly in how parents divide and time their incubation, that is species vary in their incubation rhythms^{4–6}.

In some species, such as seabirds, one parent sits on the nest continuously for several days (e.g. refs^{7–9}). In others, one parent sits continuously on the nest for a few hours^{10–13} or even only for few minutes¹⁴. In some species, both sexes share incubation duties nearly equally^{6,13,15}; in others, one sex incubates far more than the other^{16–19}, be it in terms of nest attendance^{16–19} and/or incubation efficiency (i.e. incubation temperatures)^{16,20,21}. Thus, although the general between-species differences in how parents divide and time their incubation are somewhat known, detailed descriptions over the day and season (i.e. as ambient temperatures and predation pressure change) are uncommon^{6,15,22–26} and often limited to species with incubation bouts lasting several days^{7–9}. Moreover, although between- and within-pair differences in incubation rhythms might be considerable^{6,13}, and in extreme cases one parent may even desert its incubating partner²⁴, detailed analysis of such between- and within-pair differences is often also lacking.

Here, we used continuous video-monitoring of 113 nests to describe the incubation rhythms of the Northern Lapwing *Vanellus vanellus*, a common Palearctic shorebird with variable male contribution to incubation^{27–31}. Current knowledge about incubation of Northern Lapwings is mostly based on brief sampling periods of a few hours^{27,29–31} (but see ref.²⁸). Our continuous data allowed for a detailed description of daily and seasonal variation in how sexes divide their incubation duties between and within pairs. In shorebirds, including our

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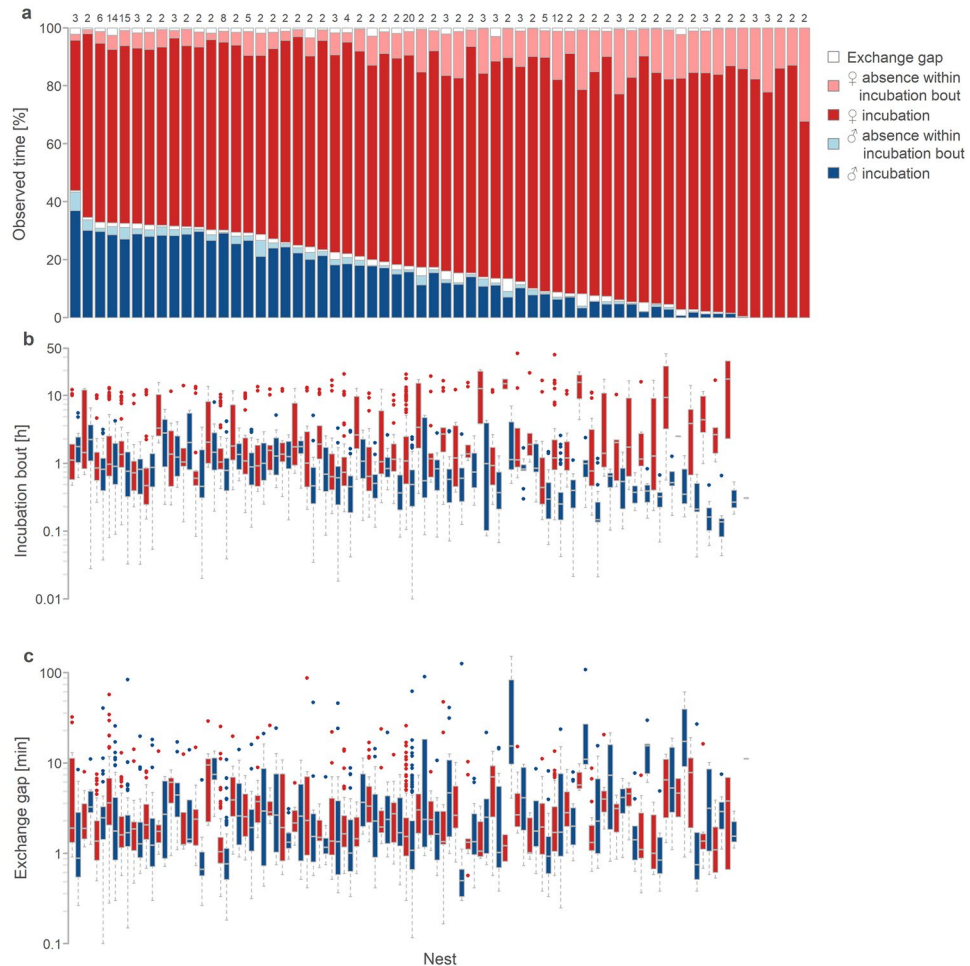


Figure 1. Between and within nest variation in incubation. **(a)** Between- and within-nest variation in nest attendance. Each bar represents one nest and proportion of female incubation bouts (red), male incubation bouts (blue) and exchange gaps (gaps preceding female incubation bouts are above female bars and those preceding male incubation bouts are above male bars). Dark colours indicate actual incubation (individual sitting on the nest) and light colours indicate the absence of a parent (no incubation) within its incubation bouts. Numbers above the bars indicate the number of days with incubation data. Nests (bars) are ordered from the highest to the lowest male nest-attendance ($N = 60$ nests). **(b,c)** Between- and within-nest variation in incubation bouts **(b)** and exchange gaps **(c)** according to sex (female in red, male in blue). Each pair of box plots (female and male) corresponds to the nest (bar) in **(a)** ($N = 2239$ bouts and gaps from 55 biparental nests). Box plots depict median (vertical line inside the box), 25–75th percentiles (box), 25th and 75th percentiles minus or plus $1.5 \times$ interquartile range, respectively, or the minimum and maximum value, whichever is smaller (whiskers) and outliers (circles). **(a–c)** The between nest variation is unlikely driven by when the nest started within the breeding season or within which part of the incubation period it was monitored (Tables S3, S7, S8³³).

Lapwing population, nest attendance highly correlates with actual incubation (warming or shading of eggs^{13,24,32}; Supplementary actograms³³). Also, females and males seem to heat the clutch to the similar temperatures (^{13,24,32,34}; Supplementary actograms³³).

We specifically investigated (1) between-nest variation in overall nest attendance (proportion of observed time parents were sitting on the nest or shading the eggs), (2) how male nest attendance relates to this between-nest variation in nest attendance, as a single parent cannot incubate with high nest attendance indefinitely, and (3) tested how incubation rhythm (female and male contribution) changed within a day, throughout the incubation period and season as food availability^{35,36}, temperature and predation pressure^{37,38}, as well as brood value vary over temporal time scales^{39–41}.

Results

Overall nest attendance. Northern Lapwing parents incubated their eggs 87% of time (median, range: 68–94%, $N = 60$ nests with more than 2 days of recording; Fig. 1a, dark red and blue). Actual incubation bouts, defined as the time between the arrival of a parent at the nest and its departure, followed by the incubation of its partner (i.e. the total time allocated to a single parent including incubation recesses), covered 98% of observed time (median, range: 95–100%, $N = 55$ nests incubated by both parents; Fig. 1a, red and blue). In other words,

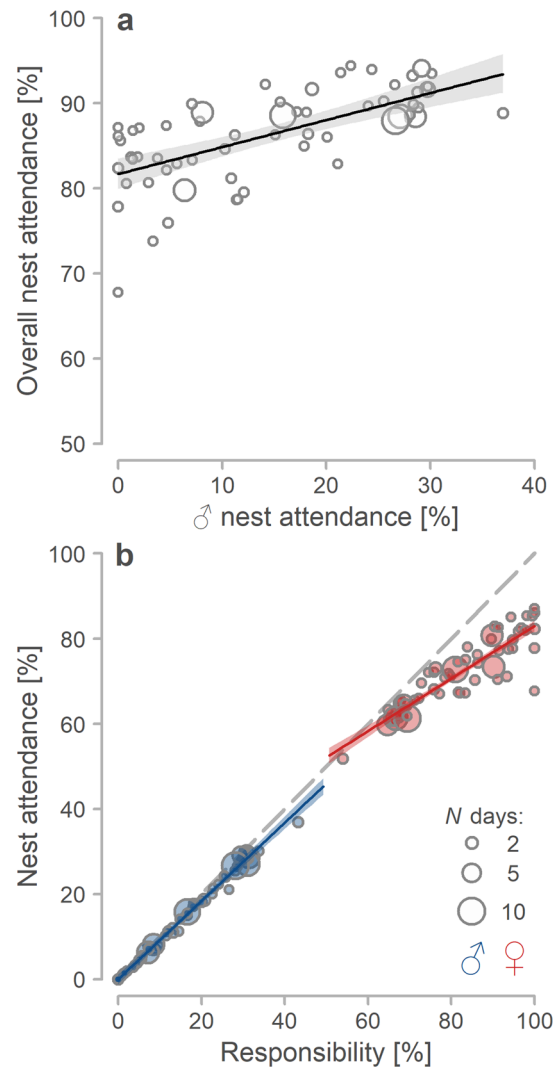


Figure 2. Contribution of females and males to overall nest attendance. **(a)** Relationship between male nest attendance and overall nest attendance ($N=60$ nests). **(b)** Relationship between responsibility (proportion of all parent's incubation bouts within observed time) and nest attendance for females (red) and males (blue; $N=120$ parents from 55 biparental nests). **(a,b)** Circles represent individual nests **(a)** or parents **(b)** and their size, the number of days with incubation data. Lines with shaded areas indicate model prediction with 95% credible intervals based on the joint posterior distribution of 5,000 simulated values based on model outputs (Tables S1 and S2³³) and generated by the 'sim' function in R⁹⁰. Included are only nests with at least two days of incubation data and days with at least 90% of recording. The dashed line in **b** indicates full compensation for the reduced care of a partner.

one parent was nearly always responsible for the nest (sum of incubation bouts). Exchange gaps, defined as the time between the departure of one parent from the nest and the return of its partner, thus accounted for only 2% of observed time (median, range: 0.3–5%, $N=55$ nests incubated by both parents).

Females incubated more than males because they attended the nest 72% of observed time (median, range: 52–87%, $N=60$ nests with more than 2 days of recording; Fig. 1a in dark red) and were responsible for the nest, that is their incubation bouts covered, 82% of observed time (median, range: 54–100%; Fig. 1a in dark and light red; note that 100% represents 5 nests incubated solely by females). Females were absent from the nest during their incubation bouts (i.e. incubation recesses covered) 10% of the time (median, range: 2–32%; Fig. 1a in light red). In contrast, males attended the nests 13% of the time (median, range: 0–37%; Fig. 1a in dark blue) and were responsible for the nest 15% of the time (median, range: 0–43%; Fig. 1a in dark and light blue). Recesses during male incubation bouts covered 7% of the time (median, range: 0–22%; $N=55$ nests with male incubation; Fig. 1a in light blue). Note that overall female nest attendance was always higher than that of males and the two were strongly negatively correlated ($r = -0.87$; Fig. S3a).

Overall nest attendance decreased by 3% (95% CI: 2.2–3.1%) as male nest attendance decreased by 10% (Figs 1a and 2a, Table S1³³). Thus, females 'partially compensated' for this decrease (i.e. incubated for 62% of male absence, 95% CI: 56–67%). As female responsibility for the nest increased, their nest attendance increased

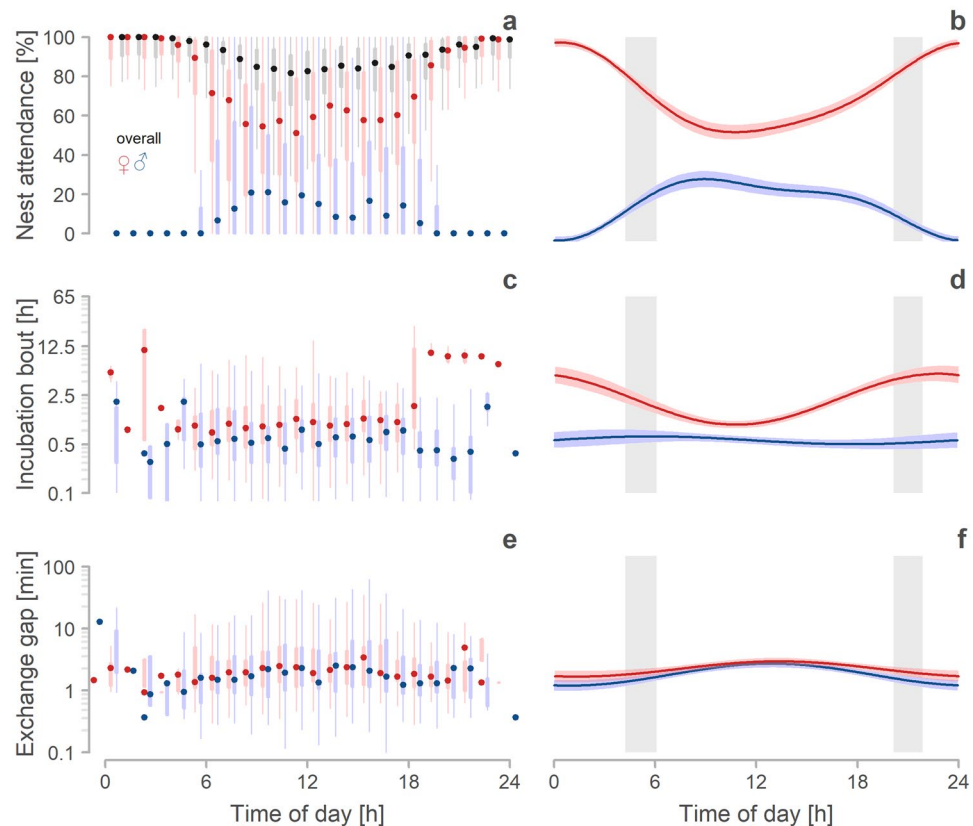


Figure 3. Daily changes in incubation behaviour. (a,c,e) Daily variation in overall (dark grey), female (red) and male (blue) nest attendance (a), bout length (c) and exchange gap (e). Points depict hourly median weighted by sample size for each nest. Thicker lines indicate 25–75th percentiles, thinner lines 25th and 75th percentiles minus or plus $1.5 \times$ interquartile range, respectively, or the minimum and maximum value, whichever is smaller. Note that outliers are not depicted. Included are only those hours with complete incubation records ($N = 7933$ hours from 113 nests; median 61 hours per nest, range: 24–482) and complete incubation bouts ($N = 3184$ bouts from 107 biparentally incubated nests; median 20 bouts and exchange gaps per nest, range: 1–297). (b,d,f) Predicted relationships between time of day and nest attendance (b), bout length (d) and exchange gap length (f) according to sex (female in red, male in blue). Lines with shaded areas indicate model prediction with 95% credible intervals based on the joint posterior distribution of 5,000 simulated values from mode outputs (Tables S4, S5 and S8³³) and generated by the ‘sim’ function in R³⁰. Grey bars indicate the period between the earliest and the latest sunrise and sunset during incubation monitoring.

as well, but less than would be expected under the ‘full compensation hypothesis’ (Figs 1a and 2b, Supplementary Table S2³³; note that in Fig. 2b hypothetical full compensation is indicated by dashed line and in case of no compensation the points would be parallel to x-axis).

Daily nest attendance. Daily nest attendance mirrored the overall nest attendance (median = 88%, range: 50–98%, $N = 191$ days from 78 nests) and also decreased by 3.6% (95% CI: 2.1–4.1%) with every 10% decrease in male nest attendance (Supplementary Fig. S1 and Table S3³³). Daily nest attendance was repeatable in females (0.54, 95% CI: 0.37–0.67), as well as in males (0.7, 95% CI: 0.58–0.8). Daily nest attendance (overall, female or male) was unrelated to the day of the incubation period or day when the nest was started within the breeding season (Table S3³³). However, overall nest attendance varied strongly within a day, being highest and nearly continuous during the night (median nest attendance between 22:00 and 4:00 was 100%) and lowest during the day (Fig. 3a – in dark grey). Females were almost always the incubating sex at night, and dominated the nest attendance also during the day (Fig. 3a,b; Table S3³³). Specifically, female nest attendance dropped after sunrise, while male nest attendance peaked after sunrise and also before sunset (Fig. 3a,b; Table S3).

Incubation bouts and exchange gaps. In biparental nests (i.e. nests where both females and males incubated), incubation bouts lasted 44 minutes (median, range: 1 second –42 hours, $N = 3184$ bouts from 107 nests) and varied greatly between and within nests (Fig. 1b, Supplementary Actograms³³) and especially over the day (Fig. 3c,d); bouts (especially of females) were longer during the night than during the day (Fig. 3c,d). Female incubation bouts lasted 60 minutes (median, range: 1 minute –42 hours; $N = 1518$) whereas male incubation bouts lasted 32 minutes (median, range: 1 second –7.9 hours; $N = 1666$). Notably, on average and regardless of time, female bouts were always longer than those of males (Fig. 3c,d; Table S5³³), although during the daytime females had shorter median incubation bouts than males at 30% of nests (Fig. S3b³³). Also note that median female and

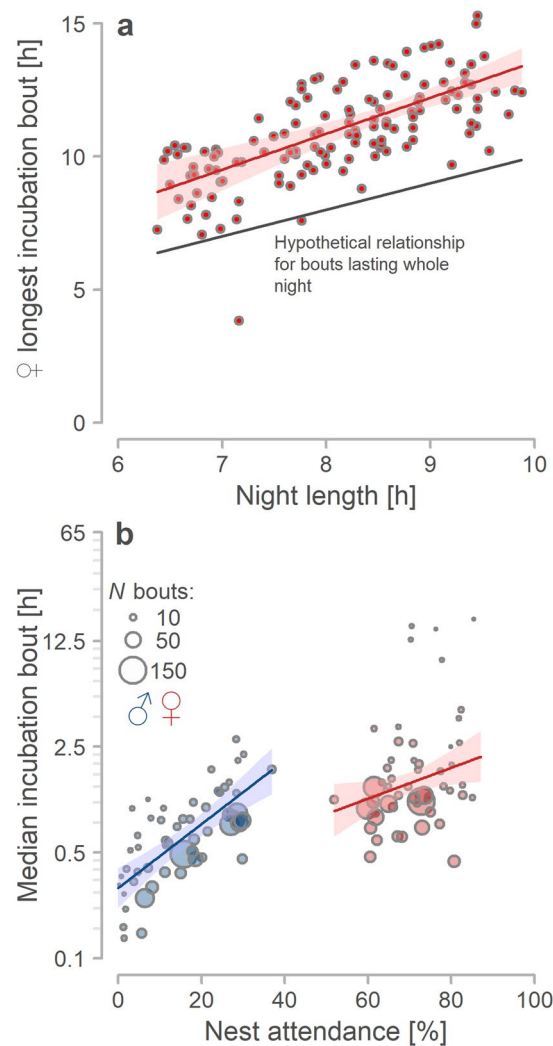


Figure 4. Bout length correlates. **(a)** Longest female incubation bout during a particular night in relation to the night length - defined as time when the Sun is $>6^\circ$ under the horizon. Points indicate bouts ($N = 133$ bouts from 55 nests; median 2 nights per nest, range 1–14 night; included are only bouts with at least 60% of their length in nights). The thick grey line indicates a situation when incubation bouts would last the whole night. **(b)** Median bout length in relation to sex (females in red, males in blue) and nest attendance. Circles represent individual parents, their size the number of days with incubation data. ($N = 110$ parents from 55 biparentally incubated nests with more than two days of continuous recording). **(a,b)** Lines with shaded areas indicate model prediction with 95% credible intervals based on the joint posterior distribution of 5,000 simulated based on model outputs (Tables S6 and S7³³) and values generated by the ‘sim’ function in R⁹⁰.

male bout lengths were uncorrelated (Fig. S3b³³). Overall, incubation bouts were similar across the incubation period and unrelated to the day when the nest started within the breeding season, but note the tendency in males for shorter incubation bouts as the incubation period progressed (Fig. S2, Table S5³³). Essentially, as the breeding season progressed and nights became shorter, longest night incubation bouts of females shortened too (Fig. 4a, Table S6³³). Also, median bout length of females and males positively correlated with their nest attendance (Figs 1 and 4b; Table S7³³).

Exchange gaps lasted 1.9 minutes (median; range 6 seconds – 2.5 hours; $N = 3184$ exchange gaps from 107 nests). Length of exchange gaps was unrelated to the day in the incubation period and the day when the nest started within the season. However, exchange gaps fluctuated over the course of the day, being longest in the middle of the day (noon median = 2.49 minutes, range: 0.13–39.5 minutes, interquartile range: 1.32–5.32), and shortest during early mornings (5:00 o'clock median = 1.16 minutes, range: 0.35–19.3 minutes, interquartile range: 0.68–2.05) and evenings (19:00 o'clock median = 1.41 minutes, range: 0.28–87.5, interquartile range: 0.61–2.63; Fig. 3e,f; note that there is only negligible number of exchange gaps during the night). Also, exchange gaps that occurred before female incubation bouts were approximately 24 seconds longer (estimate, 95% CI: 15–35 seconds) than those before male incubation bouts (Fig. 3e,f; Table S8³³).

Discussion

Using continuous video monitoring, we quantitatively described incubation rhythms in a central European population of a common Palearctic shorebird, the Northern Lapwing. Our data set allowed us to confirm and reveal three main aspects of Northern Lapwing incubation rhythms. We show that (1) nests varied substantially in overall nest attendance and overall nest attendance strongly correlated with male nest attendance. We further reveal that (2) females partially compensated for the general lack of male nest attendance and that (3) the incubation rhythms varied little over the incubation period and season, but varied strongly over the course of the day. We discuss these three aspects in detail below.

Overall incubation rhythm. Overall nest attendance was 87% (median; range: 67 to 94%; Fig. 1a), which is in line with findings from other Lapwing populations from the Netherlands and Norway^{27–29,31}. Yet, it remains unclear why Northern Lapwings do not incubate more, given that closely related species can achieve higher nest attendance even when incubating uniparentally and in more northerly regions^{42,43}. Perhaps breeding in a temperate climate does not require as continuous nest attendance as in other harsher climates (but see temperate species in ref.³⁴). Essentially, the between nest variability in nest attendance – which can vary by as much as a 6.5 hours per day – seems huge and is much larger than nest attendance fluctuations known to influence embryo development^{32–34}, offspring quality and survival^{44–49} or length of the incubation period^{23,32,33,35}.

Moreover, we found that nest attendance positively correlated with the length of incubation bouts (Fig. 4b) and that exchange gaps between incubation bouts were short (median = 1.9 minutes; Fig. 3c). These findings suggest that the departures of a parent from its nest (within its incubation bout) do not trigger their partner to come and incubate^{30,50}, although this partner spends most of its time inside the breeding territory and thus usually sees its nest^{29,31}. Hence, in Northern Lapwings other (to date unknown) cues drive the decision of parents to return to the nest and incubate. These findings resemble those from other species, albeit in most of those the off-nest partner is far from the nest^{7,8,13}.

We also found that male nest attendance varied from 0 to 37% of observed time, with a median of only 13% (mean = 14%; Fig. 1). Such male contributions are lower than in other Northern Lapwing populations (mean: 20% in ref.²⁸, 27% in ref.²⁹; median: 19% in ref.³¹, 22% in ref.⁵¹). This difference might be partly driven by the day-time only monitoring of incubation in most previous studies^{29,31,51}. If we include only day-light period, male nest attendance in our data rises to 19% (both median and mean). Importantly, the immense variability in male nest attendance is rare among shorebirds⁶. Yet, we know little about the drivers of this between population variability in male contribution to incubation. There is some evidence that the population differences in *Charadrius* plovers correlate with differences in local climate or operational sex ratio of the population^{22,23,52}. In non-shorebird species, the population differences in male care (in general) were linked to population differences in the food abundance^{53,54}.

The low male nest attendance across Northern Lapwing populations indicates that males either invest into parental care less than females or invest into parental care differently than by incubating, e.g. by guarding the nest (and the female) against predators^{29,55}. Importantly, the variation in male nest attendance may reflect the male's mating status (not recorded in this study), as some Northern Lapwing males tend to have more than one female (reviewed in ref.⁵⁶). Indeed, in other species, the amount of polygyny is linked to the variation in male nest attendance^{57,58}. Nevertheless, in Northern Lapwings, the available evidence for this relationship is inconsistent, perhaps because it is based on non-continuous monitoring^{27,29,30}.

Relationship between female and male nest attendance. Notably, we also found that when male nest attendance was low, female nest attendance was higher, but not enough to fully compensate for the male decrease. Hence, with decreasing male nest attendance, overall and daily nest attendance decreased as well (see Fig. 2 for overall effects, Fig. S1 for daily effects³³). These findings suggest that, unlike in other species such as geese with uniparental female incubation and close to 100% nest attendance⁵⁹, the Northern Lapwing females have a limited capacity to incubate continuously (i.e. nor our uniparental females, nor Lapwing females in other studies maintained continuous close to 100% nest attendance throughout the incubation period^{28,29}). Such limited capacity to incubate continuously is in line with nest attendance of uniparentally incubating shorebirds^{24,42}. Although our results are only correlational and thus experiments (e.g. temporal removal of a male⁶⁰) are needed to elucidate these patterns of partial compensation, the findings are in line with previous empirical and theoretical work^{61–64}, which suggests that a biparental care will be evolutionary stable only if a parent compensates partially for a reduced parental care of its partner. Such partial compensation is feasible only in environments (like in this temperate Northern Lapwing population) where a decrease in parental care does not necessarily translate into breeding failure, e.g. due to cooling or overheating of eggs^{22–24,65}.

Seasonal and daily variation in incubation rhythms. *Season.* Incubation rhythms were generally stable during the incubation period and season (Tables S3, S5, S8³³) but varied strongly within a day (Fig. 3). The general lack of variation in Northern Lapwing nest attendance across the incubation period and season contrasts with findings from other species where, for example, incubation bouts lengthen over the incubation period and then shorten just before hatching^{13,25,66}. Note that the lack of variation across the incubation period in our study may also reflect a lack of statistical power, that is 5–8 days of incubation data at the start and end of incubation period may still not be enough, in face of within- and between-nest variation. However, we also found that female night bouts shortened as nights shortened with the progressing breeding season. We propose that this seasonal pattern results from daily incubation rhythm of Lapwings where females take nearly sole responsibility for their nest and incubate continuously with one or few long incubation bouts over the whole night. As nights become shorter, the night incubation bouts also shorten – something worth investigating in other species.

Daily variation. Overall nest attendance and female nest attendance were highest during the night and lowest during the day; in contrast, males rarely incubated at night and their nest attendance peaked (Fig. 3b) after sunrise and before sunset.

Why do Northern Lapwings incubate so differently during the day and night? Anti-predation strategy does explain variation in incubation rhythms of shorebirds on a comparative scale; species that rely on camouflage when incubating (i.e. are cryptic) have longer incubation bouts than those which do not⁶. Thus, while Northern Lapwings actively attack predators and have short incubation bouts during the day^{29,55}, they may minimize number of changeovers on the nest during the night when mammalian predators are more active, leading to long incubation bouts. This may reduce olfactory cues as incubating birds may smell less than unincubated clutch⁶⁷ and will reduce visual cues. Also, poor visibility during the night may prohibit Lapwings from attacking mammalian predators. Perhaps, more importantly, attacking a mammal may not deter it from further searching for the eggs. Indeed, in our population all video-recorded egg predation events occurred during the night and by mammals (mainly by Red fox *Vulpes vulpes* and Stone Marten *Martes foina*; unpublished data).

Apart from anti-predation strategy and predatory risk, the day-night differences in lapwing nest attendance may arise from circadian variation in ambient temperatures and food availability. First, as ambient temperature falls during the night, continuous incubation might be necessary to sustain embryonic development^{45,46}. Indeed, also other species (including the uniparental ones) increase their night-time nest attendance^{24,68,69}. Second, as food availability might be lower during the night (e.g. due to lower activity of arthropods), Lapwings may prefer daytime foraging and have limited or no need to be off the nest during the night. However, this seems improbable, since Northern Lapwings forage more and with higher food intake during the night⁷⁰. Similarly, other shorebirds have either similar food intake across day and night or higher intake during the night⁷¹.

The lack of male night nest attendance (Fig. 3a) corresponds with findings from other Lapwing populations^{28,30}. We found some night incubation only in 5 out of 55 incubating males (9%) and the five males took care of less than 1% of the nocturnal incubation effort; In Norway males never incubated at night and the Netherlands 10 out of 20 males attended the nest at night (50%), but again only with 8% of night attendance. Lack of male night nest attendance (i.e. female-only night incubation) is reported also in related plover species of genus *Pluvialis*^{6,34,72}. Still, why do Lapwing males incubate so rarely during the night? One hypothesis suggests that the brighter parent should incubate at night⁷³. However, in Northern Lapwings (as well as in other species genus *Vanellus* and *Pluvialis*), sexes are rather similar. If anything, males are the more ornamented sex^{74,75} and hence should incubate during the night, which is not the case. Importantly, the sexual colour dimorphism of Northern Lapwings is similar to sexual colour dimorphism of other *Charadrius* species with predominantly male night incubation^{11,76–78}. Alternatively, Northern Lapwing (and *Pluvialis*) males might be less efficient incubators, e.g. warm the eggs to lower temperatures (as is the case in other species^{16,18,20,21}), which would favour female to attend the nest during times with lower ambient temperatures, that is during the night⁷⁹. The efficiency of male incubation in Lapwings or other closely related species with female night nest attendance is to date unexplored, but our limited descriptive evidence suggest minimal difference between Lapwing females and males in incubation temperatures (Supplementary Actograms³³).

Notably, we depicted the distribution of male nest attendance across the day with peaks after sunrise and before sunset (Fig. 3a). A similar (but role-reversed) situation seems to be present in some Kentish plover (*Charadrius alexandrinus*) populations²². We speculate that by incubating after sunrise and before sunset males may allow females to replenish their energy stores after and before long night incubation bouts. We thus propose testing whether females lacking male contribution to care will weigh less and incubate less in the morning, at the end of the day or at the end of the incubation period than females with male contributions to incubation⁸⁰.

Conclusion

To conclude, with continuous monitoring of 113 Northern Lapwing nests we demonstrate (a) how male contribution to incubation links to the substantial within population variability in incubation rhythms, and that (b) the incubation rhythms were generally stable over the days, but strongly fluctuated across 24 h-day, being modulated not only by day and night, but also by sunrise and sunset. The next step is to experimentally investigate what drives the variation in male incubation and whether the sunrise and sunset driven modulations (or even other modulations) of the circadian incubation rhythms are common also in other populations, species and environments.

Methods

Data collection. In April–May of 2015 and 2016 we monitored incubation of Northern Lapwings in České Budějovice basin, Doudlebia, Czech Republic (49.25°N, 14.08°E), on approximately 40 square kilometres of agricultural land. We searched for nests by systematically scanning fields and meadows with telescopes, or by walking through areas with high nest densities. If a nest was found during laying (i.e. with a lower clutch size than during later nest visits), we estimated its start of incubation by assuming that females laid one egg per day and started incubation when the clutch was complete (usually four, rarely three eggs). If a nest was found with a full clutch, we estimated its start of incubation based on the median height and angle at which the eggs floated in water⁸¹ and assuming an incubation period of 27 days (unpublished data).

We monitored incubation with a custom designed video recording system (Jan Petrů, Czech Republic), consisting of an external lens (Ø 2 cm, length 4 cm) mounted on a ~30 cm long twig and placed 1.5 meters from the nest in a southward direction to minimize the time the lens faced the sun, which would have overexposed the videos and made individuals hard to recognize. Infra-red light (within the lens) of 10 out of 15 systems was used to record the night time nest attendance. The digital recorder stored videos in 10–15 frames per second in 640 × 480 pixels resolution for about four days. The system was powered by a 12-V, 44-Ah battery buried together with the recorder (in a waterproof case) under the ground (Supplementary Picture S1³³). Two to three people

installed the equipment, which took about 10 minutes per nest. To minimize the number of visits to a particular site, we often equipped several nests at a time. Note that parents (regardless of the sex) returned to the nest within 46 minutes after installation (median; for females: median = 45 minutes, 2.5th quantile = 9 minutes, 97.5th quantile = 3.7 hours; for males: median = 56 minutes, 2.5th quantile = 22 minutes, 97.5th quantile = 4.3 hours). Thus, the camera could have influenced behaviour of some sensitive individuals.

In addition, at six of the video-recorded nests, we also recorded nest temperature and surface temperature next to the nest using MSR 145B5 dataloggers (0.1 °C accuracy) and small external probes placed among the eggs^{6,13}.

All field procedures were performed in accordance with the relevant guidelines and regulations, and approved by the institutional committee, based on the institutional accreditation No. 63479/2015-MZE-17214 of Ministry of Agriculture of the Czech Republic.

Extraction of incubation behaviour. We extracted incubation behaviour from video recordings in AVS Media Player (<http://www.avs4you.com/AVS-Media-Player.aspx>) by noting the date and time (to the nearest second) when a bird came to the nest (both legs in the nest) or left the nest. We thus define incubation as both sitting on the eggs (warming) or standing above them (turning them or shading them from direct sunlight). We distinguished females and males via individual and sex-specific plumage traits such as crest length, or the extent of melanin ornaments on the face and breast⁷⁴ – a technique widely used to distinguish female and male of this species^{28,56,82–85} because the overlap of crest length or breast colour between the sexes is minimal⁷⁴.

We further noted any disturbance caused by the field team, agricultural work, general public or interaction with other animals (note that only bouts with disturbance from the field team were excluded from the analyses). Bouts with technical difficulties and with low visibility, when parents were hard to recognize (e.g. during direct sunlight or heavy rains), were classified as uncertain and excluded from the analyses (<1% of recorded time; see Supplementary Actograms for details, raw incubation data and extracted incubation bouts³³).

Definition of incubation variables. We defined nest attendance as the proportion of time a nest was actually incubated by one of the parents, i.e. a parent being on the nest (including shading of eggs), which excludes incubation recesses. Specifically, ‘overall nest attendance’ indicates attendance for the whole time a nest was monitored; ‘daily nest attendance’ indicates attendance for a particular day and nest; ‘hourly nest attendance’ indicates attendance for a particular hour in a particular day and nest. Female or male nest attendance denotes proportion of incubation by a particular sex during a respective time interval (e.g. overall, day, hour or incubation bout).

Furthermore, we define incubation bouts as the total time allocated to a single parent (i.e. the time between the arrival of a parent at the nest and its departure, followed by the incubation of its partner) and exchange gaps as the time between the departure of one parent from the nest and the return of its partner. Note that incubation bouts include also incubation recesses and that an incubation bout of one parent is an off-nest bout of the off-duty parent.

Last, responsibility at each nest indicates a proportion of monitored time taken by all incubation bouts of a given parent (i.e. the sum of all incubation bouts of a given parent divided by the total observation time).

Sample sizes. We monitored 107 nests (46 in 2015 and 61 in 2016) for a median 3 days (range: 1–7 days). Because we caught and individually marked only 5% of the monitored parents, we cannot rule out the possibility that some parents were monitored during multiple breeding attempts. However, we believe this was rare because (a) in both years we observed only up to 25% of our breeding population (~200 nests) and because (b) out of 73 individuals caught during 2014–2017, we observed only 10% during subsequent years. Thus, the repeated sampling, if any, is rare and hence its consequence on our analysis minimal.

To increase the number of nests monitored for nearly the whole incubation period, we included another 6 nests from a different study (also from the Czech Republic, 49.90°N, 15.98°E) monitored for 14 days (median; range: 8–22 days^{6,34}; the Lapwing part of the study collected the incubation data between 2009 and 2011 using the same method as we do here^{6,34}).

However, not all incubation data and nests were suitable for all analyses. For the analyses of overall nest attendance, we used only nests with at least two complete days of recorded incubation ($N = 60$ nests with median of 3 days per nest; range: 2–20 days). For the analyses of daily nest attendance, we used only nests with at least one day of recorded incubation ($N = 191$ days from 78 nests with median of 2 days per nest; range: 1–20 days). For both, nest level and daily nest attendance data, we used only days monitored for more than 90% of the day. For the analyses of hourly nest attendance, we used only nests with at least 24 hours of recording and only hours with continuous incubation recording ($N = 113$ nests with a median of 61 hours, range: 24–482 hours). We used the same nests (but excluding uniparental ones) for the analyses of incubation bouts and exchange gaps ($N = 107$ nests with median of 20 incubation bouts and exchange gaps per nest, range: 1–297 bouts and exchange gaps).

Statistical analysis. All procedures were performed in R version 3.3.0⁸⁶. General linear models were fitted using the ‘lm’ function⁸⁶ and mixed-effect models using the ‘lmer’ function from the ‘lme4’ R package⁸⁷. For each model parameter we report effect size and model predictions as medians and the Bayesian 95% credible intervals (95%CI) represented by 2.5 and 97.5 percentiles from the posterior distribution of 5 000 simulated or predicted values obtained by the ‘sim’ function from the ‘arm’ R package. We estimated the repeatability of female and male daily nest attendance (i.e. between-days stability in division of incubation) using the ‘rpt’ function from the ‘rptR’ R package, restricted maximum likelihood method (REML), gaussian model, and 5 000 bootstrapped runs⁸⁸.

All continuous predictors (except for time) were z-transformed (mean centered and divided by standard deviation)⁸⁹. Whenever we tested for the rhythmicity in a response (period of 12 h or 24 h), we transformed time to radians ($2 \times \text{time} \times \pi / \text{period of interest}$) and then fitted the sinus and cosinus of radians⁶. Where appropriate,

models were weighted by the square root of monitored time (e.g. number of days or proportion of monitored time within the day; see Supplementary Tables for details).

Overall nest attendance. To explain between-nest variation in overall nest attendance we fitted overall nest attendance (%) as a response and male nest attendance as a predictor (Table S1). To explore the relationship between nest attendance and responsibility of each parent, we fitted nest attendance as a response, and sex of the parent in interaction with its responsibility for the nest (%) as predictors (Table S2). As female and male nest attendance and responsibility at a given nest may not be independent of each other, we further fitted nest identity as a random intercept.

Daily nest attendance. We investigated the correlates of variation in between- and within- day nest attendance with two mixed-effect models with gaussian response variable. In the first (Table S3), we specified daily nest attendance as a response and three predictors: the number of days from the beginning of the incubation, i.e. day in incubation period ('Day of incubation'), day when incubation started ('Start of incubation') and male daily nest attendance ('Male attendance'). Furthermore, we specified nest identity as a random intercept and male daily nest attendance as a random slope. In the second (Table S4), we specified hourly nest attendance as a response. As hourly nest attendance of female and male were inversely correlated ($r_s = -0.72$), to ensure independence of data points we randomly sampled for each hour one sex so that each hour had only one sex associated with it. We then fitted sex in interaction with time transformed (in the above described manner) with 12 hour periodicity ('12 time'), as well as in interaction with time transformed with 24 hour periodicity ('24 time'). 'Day in season' and parent identity nested in nest identity were fitted as random intercepts and time predictors were fitted as random slopes.

Incubation bouts and exchange gaps. To investigate variation in incubation bouts and exchange gaps we fitted four models. In the first model (Table S5), we fitted incubation bout (ln-transformed) as a response and sex, 'Day of incubation' and 'Start of incubation', all in interaction with time (with 24 hour rhythmicity) as predictors. To eliminate temporal autocorrelation, we included also the length of the previous incubation bout (ln-transformed). Nest identity and day in season were fitted as random intercepts. As the influence of time may differ between the nests or over the season, we further fitted time and day in incubation period as random slopes.

In the second model (Table S6), we fitted the longest female night bout (ln-transformed) as a response. Such bout had at least 60% of its length in the night (i.e. when sun was $>6^\circ$ below the horizon). We specified the length of the night as a predictor, the nest identity as a random intercept and the length of the night as a random slope.

In the third model (Table S7), median bout per parent and nest (ln-transformed) was fitted as a response variable and overall nest attendance per parent in interaction with sex as predictors. Nest identity was used as a random intercept. In the fourth model (Table S8), we fitted the length of exchange gap (ln-transformed) as a response and sex, 'Day of incubation' and 'Start of incubation', all three also in interaction with time (24 hour rhythmicity) as predictors. Nest identity was specified as a random intercept and '24 time' and 'Day of incubation' as random slopes.

Note that in some models we attempted to use more complicated random structures, but the models never converged (for further details see legends in Supplementary Tables³³).

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Author Contributions

M.S., E.V. and M.Š. collected the data; M.S. and E.V. extracted the incubation data from videos, M.S., M.B. and M.Š. conceived the paper, M.S. and M.B. analysed and visualised the data, drafted the paper and with input from M.Š. and E.V. wrote the final paper.

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SUPPLEMENTARY INFORMATION for

Diverse incubation rhythms in a facultatively uniparental shorebird – the northern lapwing

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Picture S1. Example of video-recording equipment at the nest. The camera lens monitors the nest, its cable is hidden under the ground and so is the recording equipment and the battery.

Table S1 | Overall nest attendance in relation to male nest attendance

Response	Effect	Estimate	95% CI	
			Lower	Upper
Overall nest attendance	Intercept	0.817	0.799	0.835
(proportion of monitored time)	Proportion of male incubation	0.318	0.224	0.412

The posterior estimates (medians) of the effect sizes with the 95% credible intervals (CI) from a posterior distribution of 5,000 simulated values generated by the 'sim' function in R¹. Included are only nests with at least two days of incubation data and days with at least 90% of recording ($N = 60$ nests). Results of this Gaussian model were weighted by square root number of monitoring days.

Table S2 | Nest attendance in relation to responsibility and sex of the parent

Response	Effect type	Effect	Estimate	95% CI	
				Lower	Upper
Nest attendance (proportion per parent)	Fixed	Intercept	0.212	0.168	0.257
		Sex	-0.213	-0.259	-0.166
		Incubation responsibility	0.618	0.564	0.672
		Sex x Incubation responsibility	0.302	0.218	0.385
	Random (variance)	Nest (Intercept)	4%		
		Residual	96%		

The posterior estimates (medians) of the effect sizes with the 95% credible intervals (CI) from a posterior distribution of 5,000 simulated values generated by the 'sim' function in R¹. Variance components were estimated by the 'lmer' function in R². Included are only nests with at least two days of incubation data and days only days monitored for more than 90% of day ($N = 120$ parents from 60 nests). Results of this Gaussian model were weighted by square root number of monitoring days.

Table S3 | Daily nest attendance in relation to male nest attendance, incubation period and season.

Response	Effect type	Effect	Estimate	95% CI	
				Lower	Upper
Nest attendance (proportion per day)	Fixed	Intercept	0.867	0.857	0.876
		Day of incubation	0	-0.009	0.009
		Start of incubation	0.002	-0.008	0.011
		Male attendance	0.043	0.033	0.053
		Day of incubation x Start of incubation	0.003	-0.006	0.012
	Random (variance)	Nest (Intercept)	12%		
		Male attendance	19%		
		Residual	69%		

The posterior estimates (medians) of the effect sizes with the 95% credible intervals (CI) from a posterior distribution of 5,000 simulated values generated by the 'sim' function in R¹. Variance components were estimated by the 'lmer' function in R². Included are only days monitored for more than 90% of day ($N = 191$ days from 78 nests). Continuous predictors and the random slope were z-transformed (mean-centred and divided by SD). The Gaussian model was weighted by square root of monitored time within day (proportion). Note that a model containing also random slope of 'Day of incubation' period did not converge. However, including incubation period as random slope instead of male attendance yielded similar estimates (that is the lack of 'Incubation period' random slope does not seem to overestimate current results).

Table S4 | Hourly nest attendance in relation to time of day.

Response	Effect type	Effect	Estimate	95% CI		
				Lower	Upper	
Nest attendance (proportion per hour)	Fixed	Intercept	0.718	0.7	0.736	
		Sex (M)	-0.571	-0.596	-0.545	
		Sin (24 time)	-0.012	-0.027	0.003	
		Cos (24 time)	0.218	0.196	0.242	
		Sin (12 time)	0.021	0.008	0.035	
		Cos (12 time)	0.027	0.01	0.044	
		Sin (24 time) x Sex (M)	0.029	0.008	0.05	
		Cos (24 time) x Sex (M)	-0.357	-0.39	-0.325	
		Sin (12 time) x Sex (M)	-0.039	-0.058	-0.019	
		Cos (12 time) x Sex (M)	-0.071	-0.095	-0.047	
		Random (variance)	Nest (Intercept)	3%		
			Sin (24 time)	3%		
			Cos (24 time)	6%		
		Sin (12 time)	4%			
		Cos (12 time)	6%			
		Sex within nest (Intercept)	7%			
		Sin (24 time)	2%			
		Cos (24 time)	10%			
		Sin (12 time)	1%			
		Cos (12 time)	4%			
	Day in season (Intercept)	0%				
	Sin (24 time)	0%				
	Cos (24 time)	0%				
	Sin (12 time)	0%				
	Cos (12 time)	0%				
	Residual	53%				

The posterior estimates (medians) of the effect sizes with the 95% credible intervals (CI) from a posterior distribution of 5,000 simulated values generated by the 'sim' function in R¹. Variance components were estimated by the 'lmer' function in R². As female and male attendance within given hour are not independent, for each hour we randomly sampled the sex, whose nest attendance we then used as a Gaussian response ($N = 7933$ fully monitored hours from 113 nests; 3902 hours of female nest attendance and 4031 hours of male attendance). This procedure also dramatically reduced temporal autocorrelation of residuals. To further account for non-independence of data points, 'Sex' nested in 'Nest' (i.e. specifying bird ID) and day in season were fitted as random intercepts and 'time' as a random slope. 'Time' was transformed to radians ($2 \times \text{time} \times \pi / \text{period of interest} - 12$ or 24h) and fitted as sine and cosine of radians. Note that we tested not only for circadian 24h-rhythmicity, but also for 12-hour rhythmicity in nest attendance, as based on our observations sex-specific nest attendance changes after sunrise and before sunset. Also, simplifying the random structure of the model by omitting random slopes yielded similar estimates.

Table S5 | Length of incubation bouts in relation to male nest attendance, incubation period and season.

Response	Effect type	Effect	Estimate	95% CI	
				Lower	Upper
Bout length (hour)	Fixed	Intercept	4.7	4.56	4.84
		ln (Length of previous bout)	0.062	0.018	0.108
		Start of incubation	-0.137	-0.271	0.002
		Day of incubation	-0.065	-0.161	0.036
		Sex (M)	-1.346	-1.46	-1.228
		Sin (time)	-0.261	-0.369	-0.15
		Cos (time)	0.8	0.651	0.953
		Cos (time) × Start of incubation	-0.104	-0.228	0.019
		Sin (time) × Start of incubation	0.018	-0.083	0.112
		Sin (time) × Day of incubation	-0.047	-0.122	0.028
		Cos (time) × Day of incubation	-0.06	-0.165	0.041
		Sin (time) × Sex (M)	0.391	0.282	0.493
		Cos (time) × Sex (M)	-0.791	-0.966	-0.611
	Start of incubation × Sex (M)	0.023	-0.056	0.103	
	Day of incubation × Sex (M)	-0.14	-0.216	-0.061	
	Random (variance)	Day in season (intercept)	2%		
		Sin (time)	1%		
		Cos (time)	3%		
		Nest (intercept)	10%		
	Sin (time)	5%			
	Cos (time)	3%			
	Day of incubation	0%			
	Residual	76%			

The posterior estimates (medians) of the effect sizes with the 95% credible intervals (CI) from a posterior distribution of 5,000 simulated values generated by the 'sim' function in R¹. Variance components were estimated by the 'lmer' function in R². Only complete incubation bouts were used ($N = 3184$ bouts from 107 biparentally incubated nests). To approach normality of residuals, 'Bout length' was ln-transformed and fitted as Gaussian response. Continuous predictors were z-transformed (mean-centred and divided by SD). 'Time' was transformed to radians ($2 \times \text{time} \times \pi / 24\text{h}$) and fitted as sine and cosine of radians. To eliminate temporal autocorrelation in residuals, we also fitted 'Length of previous bout'. To further account for non-independence of data points, 'Nest' and 'Day in season' were fitted as random intercepts and 'time' and 'Day of incubation' (within 'Nest') as random slopes. Note that models containing also random intercept for 'Sex' nested in 'Nest' (i.e. specifying bird ID) did not converge.

Table S6 | Length of female night bout in relation to length of the night.

Response	Effect type	Effect	Estimate	95% CI	
				Lower	Upper
Bout length (hour)	Fixed	Intercept	0.026	-3.919	3.946
		Night length	1.351	0.899	1.815
	Random (variance)	Nest (Intercept)	97%		
		Night length	1%		
		Residual	1%		

The posterior estimates (medians) of the effect sizes with the 95% credible intervals (CI) from a posterior distribution of 5,000 simulated values generated by the 'sim' function in R¹. Variance components were estimated by the 'lmer' function in R². For each female, we included her longest incubation bout during given night, and only bouts with at least 60% of their length in night; night defined as sun being $> 6^\circ$ under the horizon ($N = 133$ bouts from 55 nests). Bout length was fitted as Gaussian response. To account for non-independence of data points, 'Nest' was fitted as random intercept and 'Night length' as random slope.

Table S7 | Median bout length of a parent in relation to sex and nest attendance of a particular parent.

Response	Effect type	Effect	Estimate	95% CI	
				Lower	Upper
Median incubation bout of a parent [h]	Fixed	Intercept	2.81	1.329	4.265
		Sex (M)	0.032	-1.534	1.651
		Nest attendance	2.34	0.328	4.461
		Sex (M) × Nest attendance	2.488	-0.629	5.507
	Random (variance)	Nest (Intercept)	18%		
		Residual	82%		

The posterior estimates (medians) of the effect sizes with the 95% credible intervals (CI) from a posterior distribution of 5,000 simulated values generated by the 'sim' function in R¹. Variance components were estimated by the 'lmer' function in R². Included are only nests monitored during both day and night time ($N = 71$ nests). 'Median incubation bout of a parent' was ln-transformed and fitted as Gaussian response. As female and male bouts from the same nest are unlikely independent, we fitted 'Nest' as random intercept. Note, the results of this Gaussian model are weighted by square root number of monitoring days.

Table S8 | Exchange gap length in relation to sex, time of day, incubation period, and season.

Response	Effect type	Effect	Estimate	95% CI	
				Lower	Upper
Exchange gap (min)	Fixed	Intercept	0.802	0.7	0.908
		Sex (M)	-0.213	-0.308	-0.118
		Start of incubation	-0.064	-0.165	0.034
		Day of incubation	-0.04	-0.145	0.068
		Sin (time)	-0.1	-0.175	-0.028
		Cos (time)	-0.257	-0.391	-0.121
		Cos (time)x Sex (M)	-0.139	-0.283	0.002
		Sin (time) x Sex (M)	-0.005	-0.094	0.083
		Day of incubation x Sex (M)	-0.026	-0.089	0.04
		Start of incubation x Sex (M)	0.015	-0.051	0.08
	Random (variance)	Nest (Intercept)	6%		
		sin(rad)	1%		
		cos(rad)	10%		
		Day of incubation	8%		
		Residual	75%		

The posterior estimates (medians) of the effect sizes with the 95% credible intervals (CI) from a posterior distribution of 5,000 simulated values generated by the 'sim' function in R¹. Variance components were estimated by the 'lmer' function in R². Same dataset as for analysis of incubation bout length (Table S5) was used ($N = 3184$ exchange gaps from 107 biparentally incubated nests). Response variable 'Exchange gap' was ln-transformed and fitted as Gaussian response. Continuous predictors (except for 'time') were z-transformed (mean-centred and divided by SD). 'Time' was transformed to radians ($2 \times \text{time} \times \pi / 24\text{h}$) and fitted as sine and cosine of radians. To further account for non-independence of data points, we fitted 'Nest' as a random intercept and 'time' as a random slope. Note that models containing also random intercept of 'Sex' nested in 'Nest' (i.e. specifying bird ID) did not converge.

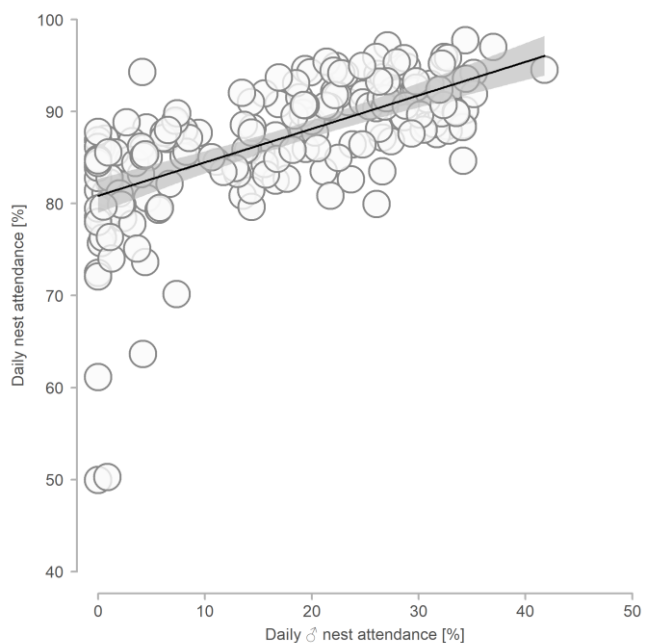


Figure S1. Overall daily nest attendance in relation to male daily nest attendance. Circles indicate individual days ($N = 191$ days from 78 nests; included are only days monitored for more than 90% of day). Line with shaded area indicates model prediction with 95% credible intervals based on the joint posterior distribution of 5,000 simulated values based on the model output (Table S3) and generated by the 'sim' function in R¹.

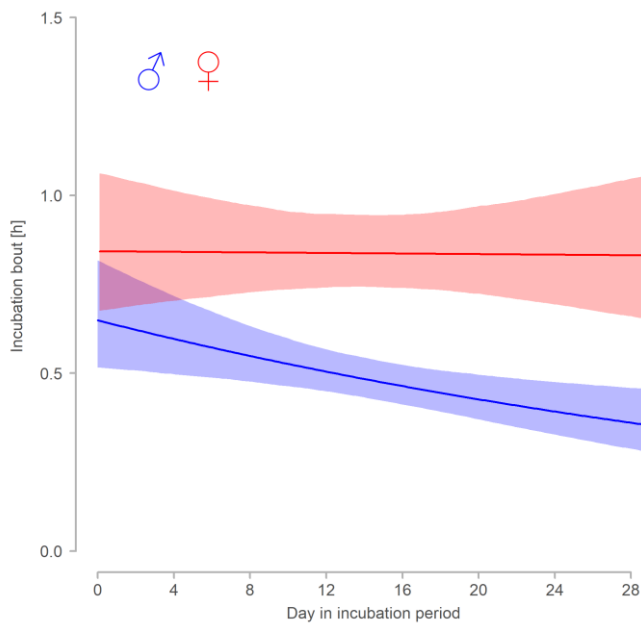


Figure S2. Mean incubation bout length in relation to incubation period and sex. Colour indicates sex (females in red, males in blue), lines with shaded areas indicate model prediction with 95% credible intervals based on the joint posterior distribution of 5,000 simulated values based on the model output (Table S5) and generated by the 'sim' function in R¹ ($N = 3184$ bouts from 107 biparentally incubated nests; only complete bouts were used).

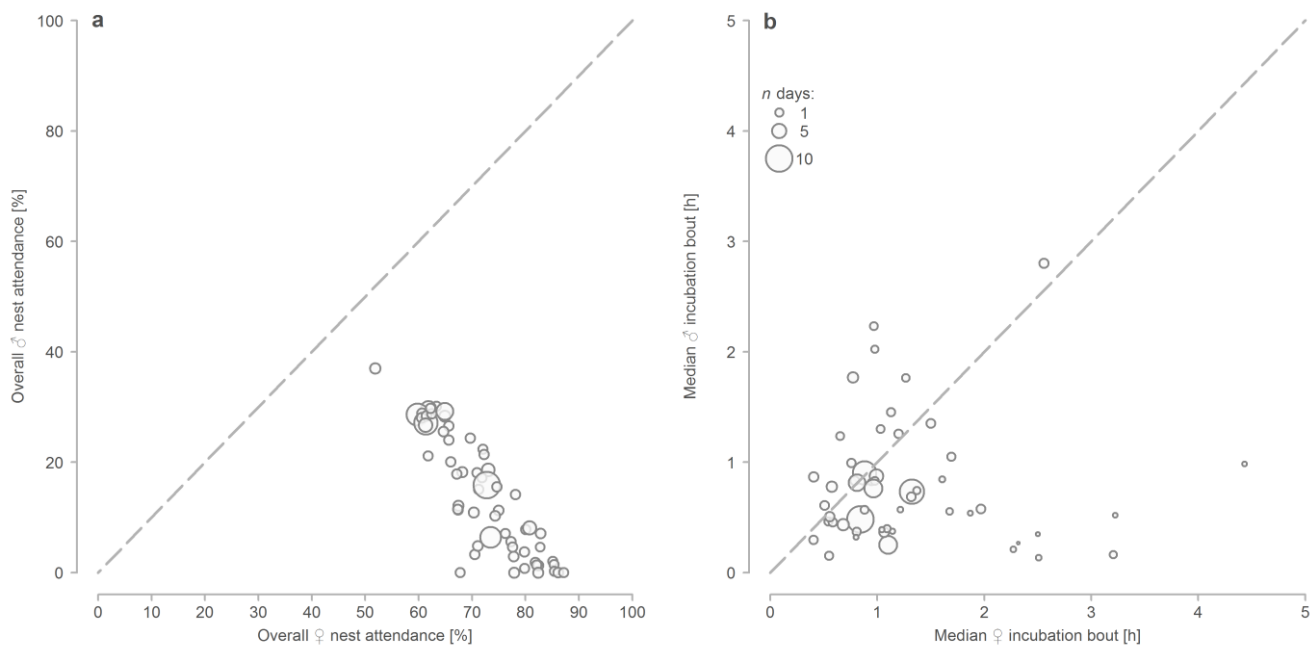


Figure S3. Relationship between female and male incubation. a, Female nest attendance in relation to male nest attendance. **b,** Median length of female incubation bout in relation to median length of male incubation bout (note that only daylight incubation data are included). **a, b,** Circles represent individual nests and their size number of days with incubation data; included are only nests with at least two days of incubation data and days with at least 90% of recording ($N = 60$ nests). Dashed line indicates perfect positive correlation, points above the line nests with male bouts longer than those of females and points below the line nests with female bouts longer than those of males.

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Chapter 2

Unexpected diversity of incubation rhythms of shorebirds.

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The behavioural rhythms of organisms are thought to be under strong selection, influenced by the rhythmicity of the environment. Behavioural rhythms are well studied in isolated individuals under laboratory conditions, but in free-living populations, individuals have to temporally synchronize their activities with those of others, including potential mates, competitors, prey and predators. Individuals can temporally segregate their daily activities (e.g. prey avoiding predators, subordinates avoiding dominants) or synchronize their activities (e.g. group foraging, communal defence, pairs reproducing or caring for offspring). The behavioural rhythms that emerge from such social synchronization and the underlying evolutionary and ecological drivers that shape them remain poorly understood. Here, we address this in the context of biparental care, a particularly sensitive phase of social synchronization where pair members potentially compromise their individual rhythms. Using data from 729 nests of 91 populations of 32 biparentally-incubating shorebird species, where parents synchronize to achieve continuous coverage of developing eggs, we report remarkable within- and between- species diversity in incubation rhythms. Between species, the median length of one parent's incubation bout varied from one to 19 hours, while period length – the cycle of female and male probability to incubate – varied from six to 43 hours. The length of incubation bouts was unrelated to variables reflecting energetic demands, but species relying on crypsis had longer incubation bouts than those that are readily visible or actively protect their nest against predators. Rhythms entrainable to the 24-h light-dark cycle were less likely at high latitudes and absent in 18 species. Our results indicate that even under similar environmental conditions and despite 24-h environmental cues, social synchronization can generate far more diverse behavioural rhythms than expected from studies of individuals in

captivity^{5-7,9}. The risk of predation, not the risk of starvation, may be a key factor underlying the diversity in these rhythms

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Unexpected diversity in socially synchronized rhythms of shorebirds

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The behavioural rhythms of organisms are thought to be under strong selection, influenced by the rhythmicity of the environment^{1–4}. Such behavioural rhythms are well studied in isolated individuals under laboratory conditions^{1,5}, but free-living individuals have to temporally synchronize their activities with those of others, including potential mates, competitors, prey and predators^{6–10}. Individuals can temporally segregate their daily activities (for example, prey avoiding predators, subordinates avoiding dominants) or synchronize their activities (for example, group foraging, communal defence, pairs reproducing or caring for offspring)^{6–9,11}. The behavioural rhythms that emerge from such social synchronization and the underlying evolutionary and ecological drivers that shape them remain poorly understood^{15–7,9}. Here we investigate these rhythms in the context of biparental care, a particularly sensitive phase of social synchronization¹² where pair members potentially compromise their individual rhythms. Using data from 729 nests of 91 populations of 32 biparentally incubating shorebird species, where parents synchronize to achieve continuous coverage of developing eggs, we report remarkable within- and between-species diversity in incubation rhythms. Between species, the median length of one parent's incubation bout varied from 1–19 h, whereas period length—the time in which a parent's probability to incubate cycles once between its highest and lowest value—varied from 6–43 h. The length of incubation bouts was unrelated to variables reflecting energetic demands, but species relying on crypsis (the ability to avoid detection by other animals) had longer incubation bouts than those that are readily visible or who actively protect their nest against predators. Rhythms entrainable to the 24-h light–dark cycle were less prevalent at high latitudes and absent in 18 species. Our results indicate that even under similar environmental conditions and despite 24-h environmental cues, social synchronization can generate far more diverse behavioural rhythms than expected from studies of individuals in captivity^{5–7,9}. The risk of predation, not the risk of starvation, may be a key factor underlying the diversity in these rhythms.

Incubation by both parents prevails in almost 80% of non-passerine families¹³ and is the most common form of care in shorebirds¹⁴. Biparental shorebirds are typically monogamous¹⁵, most species lay three or four eggs in an open nest on the ground¹⁵ and cover their eggs almost continuously¹³. Pairs achieve this through synchronization of

their activities so that one of them is responsible for the nest at a given time (an incubation bout). Alternating female and male bouts generate an incubation rhythm with a specific period length (cycle of high and low probability for a parent to incubate).

We used diverse monitoring systems (Methods and Extended Data Table 1) to collect data on incubation rhythms from 91 populations of 32 shorebird species belonging to 10 genera (Fig. 1a), extracted the length of 34,225 incubation bouts from 729 nests and determined the period length for pairs in 584 nests (see Methods, Extended Data Figs 1, 2).

We found vast between- and within-species variation in incubation bout length and in period length (Figs 1–3 and Extended Data Fig. 3). Different species, but also different pairs of the same species, adopted notably different incubation rhythms, even when breeding in the same area (see, for example, incubation rhythms in Barrow, Alaska, represented by '1' in Fig. 1b, c; incubation rhythms for each nest can be found in the supplementary actograms of ref. 16). In some pairs, parents exchanged incubation duties about 20 times a day (Fig. 2a; for example *Charadrius semipalmatus*, Fig. 1b), whereas in others a single parent regularly incubated for 24 h (Fig. 2a; for example *Limnodromus scolopaceus*, Fig. 1b), with exceptional bouts of up to 50 h (supplementary actograms of ref. 16). Similarly, incubation rhythms of pairs in 22% of nests followed a strict 24-h period (Fig. 2b; for example *Tringa flavipes*, Fig. 1b), whereas the rhythms of others deviated markedly from a 24-h period (Fig. 2b) resulting in ultradian (<20 h in 12% of nests; for example *Numenius phaeopus*; Fig. 1b), free-running-like (for example *Calidris alpina*; Fig. 1b) and infradian rhythms (>28 h in 8% of nests), with some having period lengths up to 48 h (for example *Limnodromus scolopaceus*; Fig. 1b). This variation in period length partly related to the variation in bout length (Fig. 3). In the suborder Scolopaci, period length correlated positively with median bout length, but in the suborder Charadrii species with 24-h periods had various bout lengths, and species with similar bout lengths had different period lengths.

Despite substantial within-species variation, we found a strong evolutionary signal for both bout and period length with a coefficient of phylogenetic signal λ close to 1 (Extended Data Table 2). This is consistent with the notion that biological rhythms are largely genetically determined and conserved among related species^{8–10}. However, the phylogenetic effect seems unevenly distributed over the taxonomic level. Suborder explained 33% of the phenotypic variance in both bout

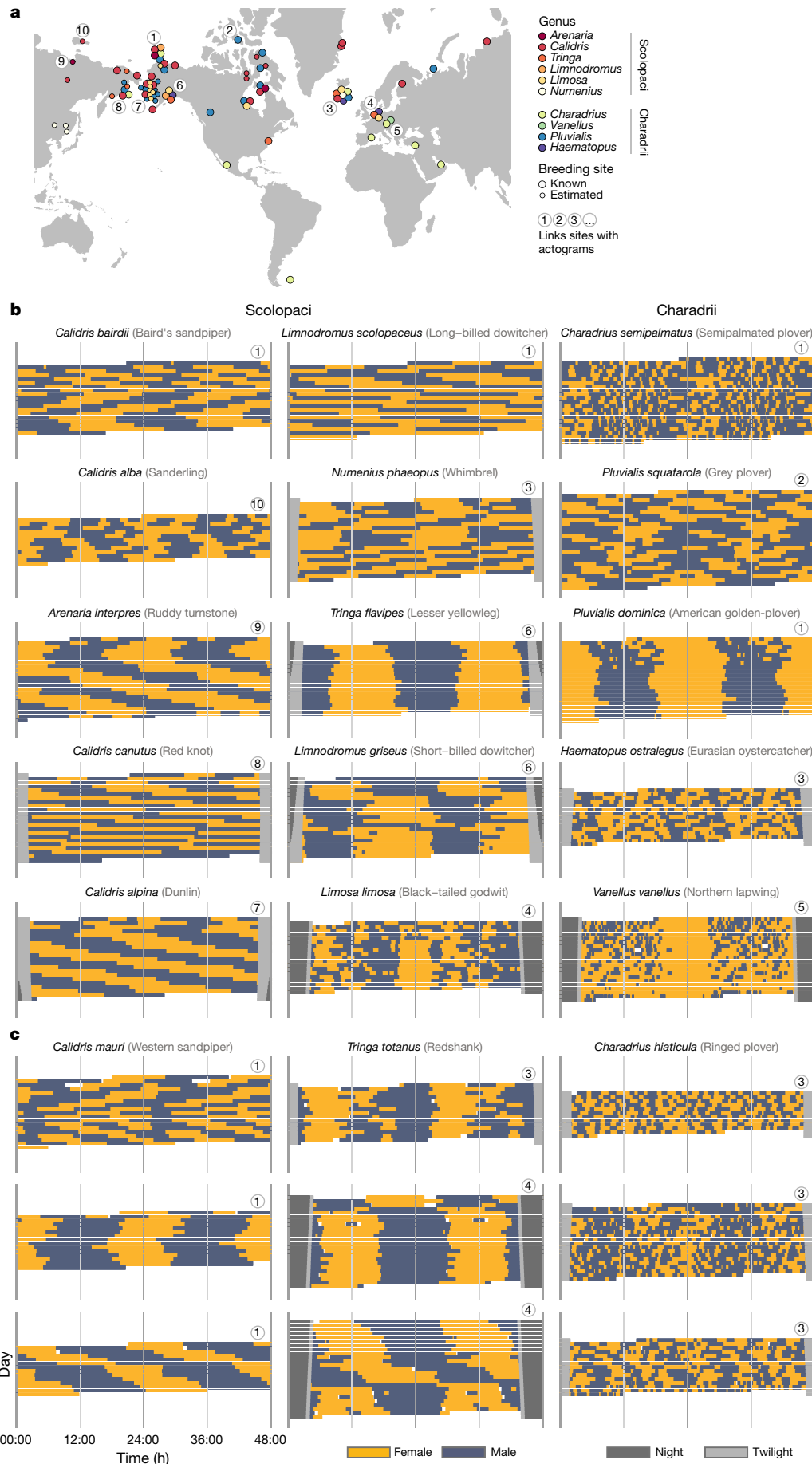


Figure 1 | Map of studied breeding sites and the diversity of shorebird incubation rhythms. **a**, Map of breeding sites with data on incubation rhythms. The colour of the dots indicates the genus (data from multiple species per genus may be available), the size of the dots refers to data quality (large dots, exact breeding site known; small dots, breeding site estimated; see Methods). For nearby or overlapping locations, the dots are scattered to increase visibility. Contours of the map were made with Natural Earth, <http://www.naturalearthdata.com>.

b, c, Illustrations of between-species diversity (**b**) and within-species diversity (**c**; note that the three rhythms for *Calidris mauri* and *Calidris hiaticula* come from the same breeding location). Each actogram depicts the bouts of female (yellow) and male (blue) incubation at a single nest over a 24-h period, plotted twice, so that each row represents two consecutive days. If present, twilight is indicated by light grey bars and corresponds to the time when the sun is between 6° and 0° below the horizon, night is indicated by dark grey bars and corresponds to the time when the sun is $>6^\circ$ below the horizon. Twilight and night are omitted in the centre of the actogram (24:00) to make the incubation rhythm visible. The circled numbers (1–10) indicate the breeding site of each pair and correspond to the circled numbers on the map in **a**.

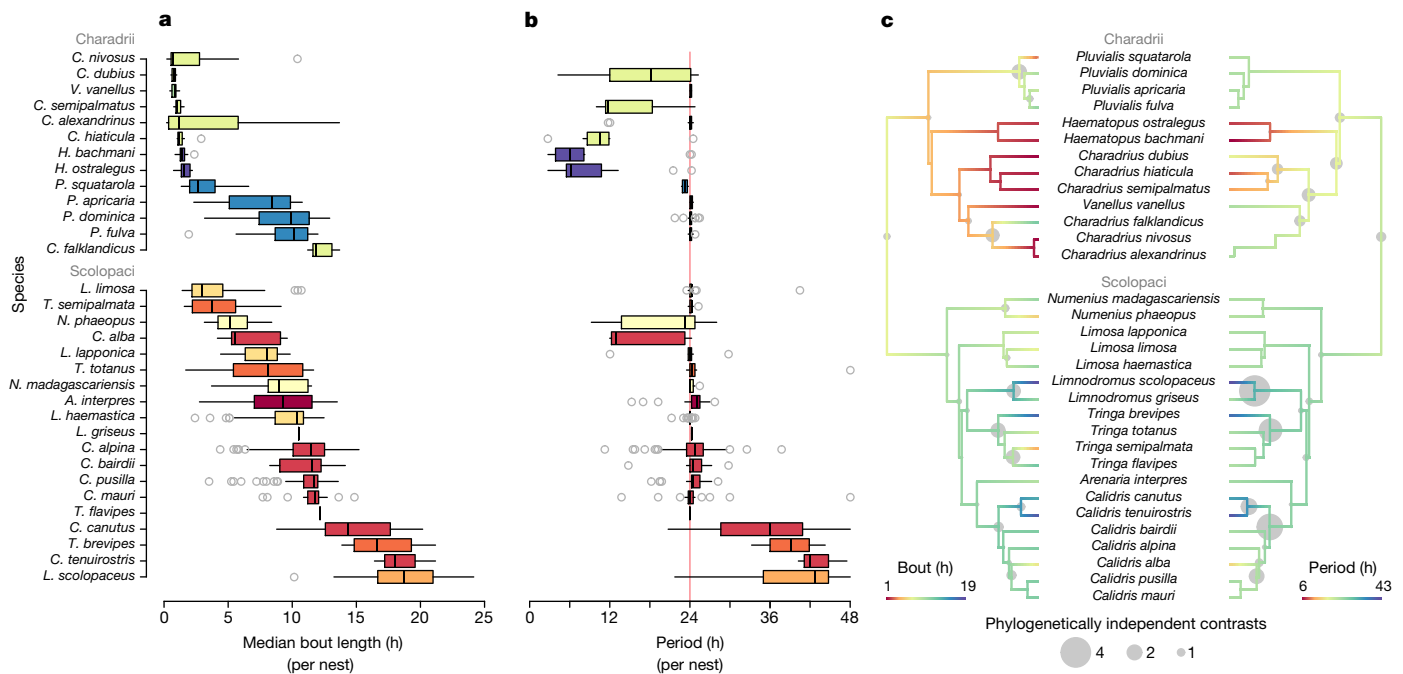


Figure 2 | Variation in incubation rhythms and its estimated evolution. **a, b,** Box plots are ordered by species (within suborder) from the shortest to the longest median bout length, and depict the genus (colour as in Fig. 1a), median (vertical line inside the box), 25–75th percentiles (box) 25th and 75th percentiles minus or plus 1.5× interquartile range, respectively, or the minimum and maximum value, whichever is smaller (box) outliers (circles). $n_{\text{median bout length}} = 729$ and $n_{\text{period}} = 584$

nests. **b,** The red vertical line indicates a 24-h period. **c,** Observed and reconstructed incubation bout and period length visualized (by colour) on the phylogenetic tree²⁹ using medians of each species (based on medians of each population) and 1 out of 100 sampled trees (see Methods). The grey circles represent phylogenetically independent contrasts³⁰ and hence emphasize the differences at each tree node.

and period length, with the Scolopaci having longer incubation bouts and periods than the Charadrii (Extended Data Table 3 and Figs 2, 3). Species explained 41% of the phenotypic variation in bout length and 46% in period length, but genus explained little (<1% in both bout and period length; Extended Data Table 3), suggesting that despite a strong phylogenetic signal, these traits can rapidly diverge (Fig. 2c).

Two ecological factors may explain the observed variation in bout length. First, the ‘energetic demands hypothesis’ stipulates that the length of an incubation bout depends on the energetic state of the bird^{13,17}. This predicts that large species will have longer incubation bouts than smaller species, because they radiate less body heat per unit of mass and that incubation bouts will shorten with increasing breeding latitude, because—everything else being equal—energy

stores will deplete faster in colder environments (Extended Data Fig. 4a, b shows latitudinal cline in summer temperatures). However, bout length was unrelated to body size (Fig. 4a) and correlated positively (instead of negatively) with latitude (Fig. 4b). These correlational results across populations and species support recent experimental findings within species¹⁸ and suggest that in biparentally incubating shorebirds energetic demands are not an important ecological driver underlying variation in bout length.

An alternative explanation for variation in the length of incubation bouts relates to anti-predation strategies. Those species that rely primarily on parental crypsis (Extended Data Fig. 5a) benefit from reduced activity near the nest, because such activity can reveal the location of the nest to potential predators^{19,20}. Thus, in these species, selection will favour fewer change-overs at the nest and therefore longer incubation bouts. By contrast, species that are clearly visible when sitting on the nest or that rely on active anti-predation behaviour (Extended Data Fig. 5b), including having a partner on the watch for predators, leaving the nest long before the predator is nearby and attacking or distracting the predator¹⁵, obtain no advantage from minimizing activity. For these species, bout length can shorten, which may be advantageous for other reasons (for example, reduced need to store fat). We quantified anti-predation strategy as the distance at which the incubating parent left the nest when approached by a human (escape distance), because cryptic species stay on the nest longer (often until nearly stepped upon)¹⁵. Despite the large geographical distribution of the studied species, with related variability in the suite of predators and predation pressure²¹, and even when controlling for phylogeny (which captures much of the variation in anti-predation strategy, Extended Data Fig. 6), escape distance negatively correlated with the length of incubation bouts (Fig. 4c). This result suggests that bout length co-evolved with the anti-predation strategy.

Under natural conditions, most organisms show 24-h rhythmicity, but during the summer, when most shorebirds breed, the 24-h variation in light decreases with latitude leading to continuous polar daylight

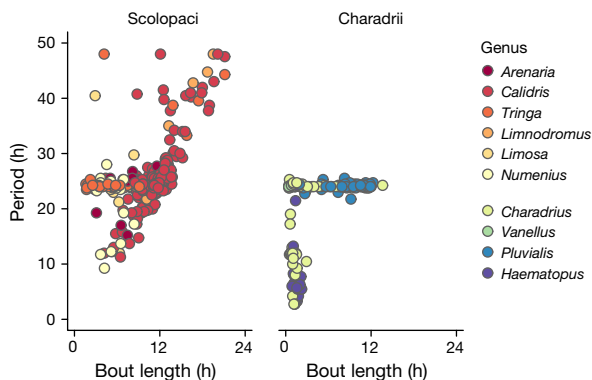


Figure 3 | Relationship between bout and period length. Each dot represents a single nest ($n = 584$ nests), colours depict the genus. In the suborder Scolopaci the median bout length and period length correlate positively ($r_{\text{Spearman}} = 0.56$, $n = 424$ nests); in the suborder Charadrii, periods longer than approximately 24 h are absent, and there is no simple relationship between bout and period length ($n = 160$ nests). For species-specific relationships see Extended Data Fig. 3.

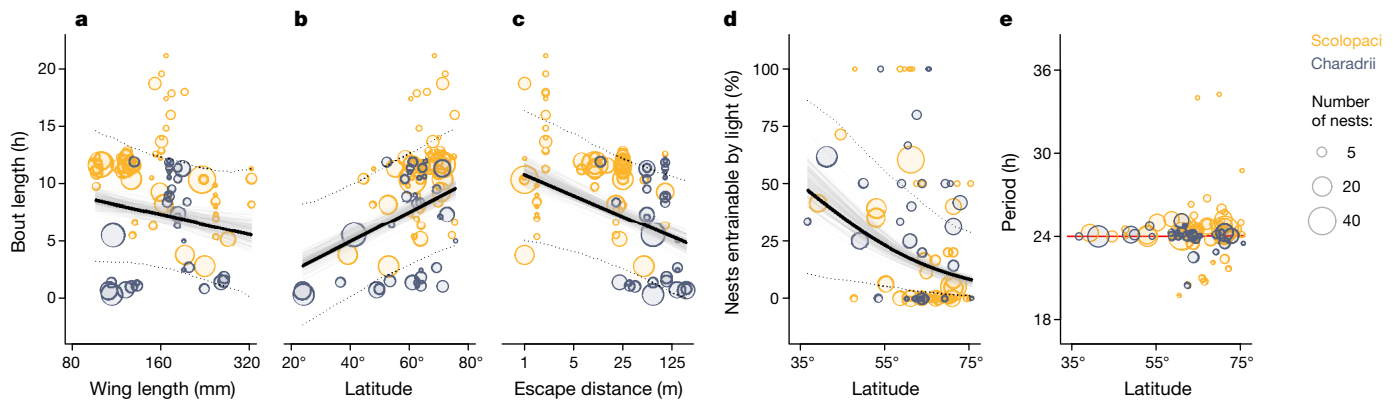


Figure 4 | Predictors of variation in incubation rhythms.

a–c, Relationships between bout length and body size, measured as female wing length (**a**), breeding latitude (**b**) and anti-predation strategy, quantified as escape distance (**c**) for $n = 729$ nests from 91 populations belonging to 32 species. **d**, The relationship between the proportion of nests with a period length that is entrainable by the 24-h light–dark cycle (that is, period lengths: 3, 6, 12, 24, or 48 h) and breeding latitude ($n = 584$ nests from 88 populations belonging to 30 species). **e**, The distribution of period length over latitude. The period was standardized to 24 h so that all 24-h harmonics are depicted as 24 h (red line) and respective deviations from each harmonic as deviations from 24 h (for example, a period of 12.5 h is depicted as 25 h). **a–e**, Each circle represents the population

in the northern-most breeding grounds²² (Extended Data Fig. 4c, d). Such reduced variation in 24-h light intensity may cause a loss of 24-h rhythmicity^{23–25}. As a consequence, circadian behavioural rhythms should exhibit a latitudinal cline²². As predicted, incubation rhythms with periods that do not follow the 24-h light–dark cycle, such as free-running-like patterns (left column in Fig. 1b), occurred more often in shorebirds breeding at higher latitudes (Fig. 4d). The absolute deviations of periods from 24 h and 24-h harmonics also increased with latitude (Fig. 4e and Extended Data Table 4). Although this supports the existence of a latitudinal cline in socially emerged behavioural rhythms²², we found a substantial number of rhythms that defy the 24-h day even at low and middle latitudes (Fig. 4d–e).

Many shorebirds predominantly use tidal habitats, at least away from their breeding ground¹⁵. To anticipate tidal foraging opportunities, these species may have activity patterns with a period length resembling the tidal period. Because changing to a different rhythm is costly²⁶, these tidal activity patterns might carry over to incubation. Although half of our species are tidal away from their breeding grounds, and some forage in tidal areas also during breeding (approximately 12% of populations), in only 5% of nests did pairs display a period length that can be entrained by the tide. Moreover, tidal species had period lengths similar to, not longer than, non-tidal ones (Extended Data Table 4). Hence, unlike the 24-h light–dark cycle, tidal life-history seems to play at best a negligible role in determining incubation rhythms.

Three main questions arise from our results. First, is variation in incubation bout length in cryptic species related to the actual predation pressure? This can be tested by comparing bout length between populations of a particular species that are exposed to different predator densities, or between years that differ in predation pressure. Second, it remains unclear how the diverse social rhythms emerge. Are these rhythms a consequence of behavioural flexibility, or a ‘fixed’ outcome of synchronization between the circadian clocks of the two individuals involved? An experimental study on ring doves (*Streptopelia risoria*) suggests that parents may even use two timers—circadian oscillation and interval timing—to determine when to incubate²⁷. Parents rapidly adjusted their schedules to phase-shifted photoperiods and their incubation rhythm ‘ran free’ in constant dim illumination (implying a circadian mechanism), whereas an experimental delay in the onset of an incubation bout did not change the length of the bout because the incubating parent refused to leave the nest until its incubation bout reached the ‘typical’ duration

(implying interval timing). Third, what are the fitness consequences for the parents of having a certain incubation rhythm? For example, the costs of having a particular incubation rhythm may be unevenly distributed between the two parents (for instance, because one parent is on incubation duty when food is more readily available, or because one parent ‘enforces’ its own rhythm at a cost to the other parent). In conclusion, our results reveal that under natural conditions social synchronization can generate much more diverse rhythms than expected from previous work^{5–7,9,28}, and that these rhythms often defy the assumption of entrainment to the 24-h day–night cycle. Risk of predation, rather than risk of starvation, seems to have a key role in determining some of the variation in incubation rhythms. We describe this diversity in the context of biparental incubation, but diverse behavioural rhythms may also arise in many other social settings (for example, in the context of mating interactions²⁵ or vigilance behaviour during group foraging). Essentially, the reported diversity suggests that the expectation that individuals within a pair (or group) should optimize their behavioural rhythms relative to the 24-h day may be too simplistic, encouraging further study of the evolutionary ecology of plasticity in circadian clocks.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Contributions M.B. and B.K. conceived the study. All authors except B.H. collected the primary data (see <https://osf.io/sq8gk>, ref. 16). M.B. coordinated the study and managed the data. M.B. and M.V. developed the methods to extract incubation. M.B. extracted bout lengths and with help from A.R. and M.V. created actograms. M.B. analysed the data with help from M.V. M.B. prepared the supporting information. M.B. and B.K. wrote the paper with input from the other authors. Except for the first, second and last author, the authors are listed alphabetically by their first name.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to M.B. (bulla.mar@gmail.com) and B.K. (b.kempenaers@orn.mpg.de).

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METHODS

Recording incubation. Incubation data were obtained between 1994 and 2015, for as many shorebird species ($n = 32$) and populations ($n = 91$) as possible (that is, no statistical methods were used to predetermine sample size), using six methods (for specifications of the equipment see Extended Data Table 1). (1) In 261 nests, a radio frequency identification reader (RFID) registered the presence of tagged parents at the nest. The passive integrated tag was either embedded in a plastic flag^{31,32}, with which the parents were banded, or glued to the tail feathers³³. In 200 nests the RFID was combined with a temperature probe placed between the eggs. The temperature recordings allowed us to identify whether a bird was incubating even in the absence of RFID readings; an abrupt change in temperature marked the start or end of incubation³¹. (2) For 396 nests, light-loggers were mounted to the plastic flag or a band that was attached to the bird's leg^{34,35}. The logger recorded maximum light intensity (absolute or relative) for a fixed sampling interval (2–10 min). An abrupt change in light intensity (as opposed to a gradual change caused, for example, by twilight) followed by a period of low or high light intensity marked the start or end of the incubation period (Extended Data Fig. 2). (3) For nine nests a GPS tag, mounted on the back of the bird, recorded the position of the bird³⁶. The precision of the position depends on cloud cover and sampling interval³⁶. Hence, to account for the imprecision in GPS positions, we assumed incubation whenever the bird was within 25 m of the nest (Extended Data Fig. 2b). (4) At three nests automated receivers recorded signal strength of a radio tag attached to the rump of a bird; whenever a bird incubated, the strength of the signal remained constant²⁴ (supplementary actograms; pages 257–259 of ref. 16). (5) At 53 nests video cameras were used to identify the incubating parents. (6) Eight nests were continuously observed. In (5) and (6) parent identification was based on plumage, colour rings or radio tag. In one of the populations, three different methods were used, in seven populations representing seven species two methods were used. In one nest, two methods were used simultaneously (Extended Data Fig. 2b).

Extraction of incubation bouts. An incubation bout was defined as the total time allocated to a single parent (that is, the time between the arrival of a parent at and its departure from the nest followed by incubation of its partner). Bout lengths were only extracted if at least 24 h of continuous recording was available for a nest; in such cases, all bout lengths were extracted. For each nest, we transformed the incubation records to local time as UTC time + (longitude of the nest/15). Incubation bouts from RFIDs, videos and continuous observations were mostly extracted by an R script and the results verified by visualizing the extracted and the raw data^{16,31,37,38}; otherwise, M.B. extracted the bouts manually from plots of raw data^{39,40} (plots of raw data and extracted bouts for all nests are in the supplementary actograms of ref. 16; the actograms were generated by the ggplot and xyplot functions from the ggplot2 and lattice R-packages^{41–43}). Whenever the start or end of a bout was unclear, we classified these bouts as uncertain (see next paragraph for treatment of uncertain bouts). In case of light-logger data, the light recordings before and after the breeding period, when the birds were definitely not incubating, helped to distinguish incubation from non-incubation. Whenever an individual tagged with a light-logger nested in an environment where the sun was more than 6° below the horizon for part of a day (that is, night), we assumed an incubation bout when the individual started incubating before the night started and ended incubating after the night ended. When different individuals incubated at the beginning versus at the end of the night, we either did not quantify these bouts or we indicated the possible time of exchange (based on trends in previous exchanges), but classified these bouts as uncertain (see supplementary actograms¹⁶). In total, we extracted 34,225 incubation bouts.

The proportion of uncertain bouts within nests had a distribution skewed towards zero (median = 0%, range, 0–100%, $n = 729$ nests), and so did the median proportion of uncertain bouts within populations (median = 2%, range, 0–74%, $n = 91$ populations). Excluding the uncertain bouts did not change our estimates of median bout length (Pearson's correlation coefficient for median bout length based on all bouts and without uncertain bouts: $r = 0.96$, $n = 335$ nests with both certain and uncertain bouts). Hence, in further analyses all bouts were used to estimate median bout length.

Note that in some species sexes consistently differed in bout length (Fig. 1b, for example, *Vanellus vanellus*). As these differences are small compared to the between-species differences and because in 27 nests (of 8 species) the sex of the parents was unknown, we used median bout length independent of sex in this study.

Extraction of period length. The method used for extracting the period length of incubation rhythm for each nest is described in the Extended Data Fig. 1.

Extraction of entrainable periods. We classified 24-h periods and periods with 24-h harmonics (that is, 3, 6, 12, 48 h) as strictly entrainable by 24-h light fluctuations ($n = 142$ nests out of 584). Including nearest adjacent periods (± 0.25 h) increased the number of nests with entrainable periods ($n = 277$), but results of statistical analyses remained quantitatively similar. We consider periods and

harmonics of 12.42 h (that is, 3.1, 6.21, 12.42, 24.84 h) as strictly entrainable by tide. However, because the periods in our data were extracted in 0.25-h intervals (Extended Data Fig. 1), we classified periods of 3, 6.25, 12.5, 24.75 h (that is, those closest to the strict tide harmonics) as entrainable by tide ($n = 32$ nests out of 584). Including also the second nearest periods (that is, 3.25, 6, 12.25, 25) increased the number of nests entrainable by tide to $n = 55$.

Population or species life-history traits. For 643 nests, the exact breeding location was known (nests or individuals were monitored at the breeding area). For the remaining 86 nests (from 27 populations representing 8 species, where individuals were tagged with light-loggers at the wintering area), the breeding location was roughly estimated from the recorded 24-h variation in daylight, estimated migration tracks, and the known breeding range of the species^{44–51}. One exact breeding location was in the Southern Hemisphere, so we used absolute latitude in analyses. Analyses without populations with estimated breeding-location or without the Southern Hemisphere population generated quantitatively similar estimates as the analyses on full data.

For each population, body size was defined as mean female wing length⁵², either for individuals measured at the breeding area or at the wintering area. In case no individuals were measured, we used the mean value from the literature (see open access data for specific values and references⁵³).

Anti-predation strategy was assessed by estimating the escape distance of the incubating bird when a human approached the nest, because species that are cryptic typically stay on the nest much longer than non-cryptic species, sometimes until nearly stepped upon^{48,54}. Escape distance was obtained for all species. Forty-four authors of this paper estimated the distance (in metres) for one or more species based on their own data or experience. For ten species, we also obtained estimates from the literature⁴⁸. We then used the median 'estimated escape distance' for each species. In addition, for 13 species we obtained 'true escape distance'. Here, the researcher approached a nest (of known position) and either estimated his distance to the nest or marked his position with GPS when the incubating individual left the nest. For each GPS position, we calculated the Euclidian distance from the nest. In this way we obtained multiple observations per nest and species, and we used the median value per species (weighted by the number of estimates per nest) as the true escape distance. The species' median estimated escape distance was a good predictor of the true escape distance (Pearson's correlation coefficient: $r = 0.89$, $n = 13$ species). For analysis, we defined the escape distance of a species as the median of all available estimates.

For each species, we determined whether it predominantly uses a tidal environment outside its breeding ground, that is, has tidal versus non-tidal life history (based on refs 48, 50, 51). For each population with exact breeding location, we scored whether tidal foraging habitats were used by breeding birds for foraging (for three populations this information was unknown)⁵³. For all populations with estimated breeding location we assumed, based on the estimated location and known behaviour at the breeding grounds, no use of tidal habitat.

Statistical analyses. Unless specified otherwise, all analyses were performed on the nest level using median bout length and extracted period length.

We used phylogenetically informed comparative analyses to assess how evolutionary history constrains the incubation rhythms (estimated by Pagel's λ coefficient of phylogenetic signal^{55,56}) and to control for potential non-independence among species due to common ancestry. This method explicitly models how the covariance between species declines as they become more distantly related^{55,57,58}. We used the Hackett⁵⁹ backbone phylogenetic trees available at <http://birdtree.org> (ref. 60), which included all but one species (*Charadrius nivosus*) from our dataset. Following a subsequent taxonomic split⁶¹, we added *C. nivosus* to these trees as a sister taxon of *C. alexandrinus*. Phylogenetic uncertainty was accounted for by fitting each model with 100 phylogenetic trees randomly sampled from 10,000 phylogenies at <http://birdtree.org> (ref. 60).

The analyses were performed with Bayesian phylogenetic mixed-effect models (Fig. 4 and Extended Data Tables 2, 4) and the models were run with the MCMCglmm function from the R package MCMCglmm⁶². In all models, we also accounted for multiple sampling within species and breeding site (included as random effects). In models with a Gaussian response variable, an inverse-gamma prior with shape and scale equal to 0.001 was used for the residual variance (that is, variance set to 1 and the degree of belief parameter to 0.002). In models with binary response variables, the residual variance was fixed to 1. For all other variance components the parameter-expanded priors were used to give scaled F -distributions with numerator and denominator degrees of freedom set to 1 and a scale parameter of 1,000. Model outcomes were insensitive to prior parameterization. The MCMC chains ran for 2,753,000 iterations with a burn-in of 3,000 and a thinning interval of 2,500. Each model generated approximately 1,100 independent samples of model parameters (Extended Data Tables 2, 4). Independence of samples in the Markov chain was assessed by tests for autocorrelation between samples and by using graphic diagnostics.

First, we used MCMCglmm to estimate Pagel's λ (phylogenetic signal) for bout and period length (Gaussian), and to show that our estimates of these two incubation variables were independent of how often the incubation behaviour was sampled ('sampling' in min, ln-transformed; Extended Data Table 2). Hence, in subsequent models, sampling was not included.

Then, we used MCMCglmm to model variation in bout length and period length (Extended Data Table 4). Bout length was modelled as a continuous response variable and latitude (in degrees, absolute), female wing length (mm, ln-transformed) and approach distance (m, ln-transformed) as continuous predictors. Predictors had low collinearity (at nest, population and species level; all Pearson or Spearman correlation coefficients $|r| < 0.28$). To test for potential entrainment to 24-h, period length was modelled as a binary response variable (1 = rhythms with period of 3, 6, 12, 24, or 48 h; 0 = rhythms with other periods) and latitude as a continuous predictor. To test how circadian period varies with latitude or life history, the period was transformed to deviations from 24 h and 24-h harmonics and scaled by the time span between the closest harmonic and the closest midpoint between two harmonics. For example, a 42-h period deviates by -6 h from 48 h (the closest 24-h harmonic) and hence -6 h was divided by 12 h (the time between 36 h—the midpoint of two harmonics—and 48 h—the closest harmonic). This way the deviations spanned from -1 to 1 with 0 representing 24 h and its harmonics. The absolute deviations were then modelled as a continuous response variable and latitude as continuous predictor. The deviations were also modelled as a continuous response and species life history (tidal or not) as categorical predictor.

In all models the continuous predictor variables were centred and standardized to a mean of zero and a standard deviation of one.

We report model estimates for fixed and random effects, as well as for Pagel's λ , by the modes and the uncertainty of the estimates by the highest posterior density intervals (referred to as 95% CI) from the joint posterior distributions of all samples from the 100 separate runs, each with 1 of the 100 separate phylogenetic trees from <http://birdtree.org> (ref. 60).

To help interpret the investigated relationships we assessed whether incubation rhythms evolved within diverged groups of species by plotting the evolutionary tree of the incubation rhythm variables (Fig. 2c), as well as of the predictors (Extended Data Fig. 6).

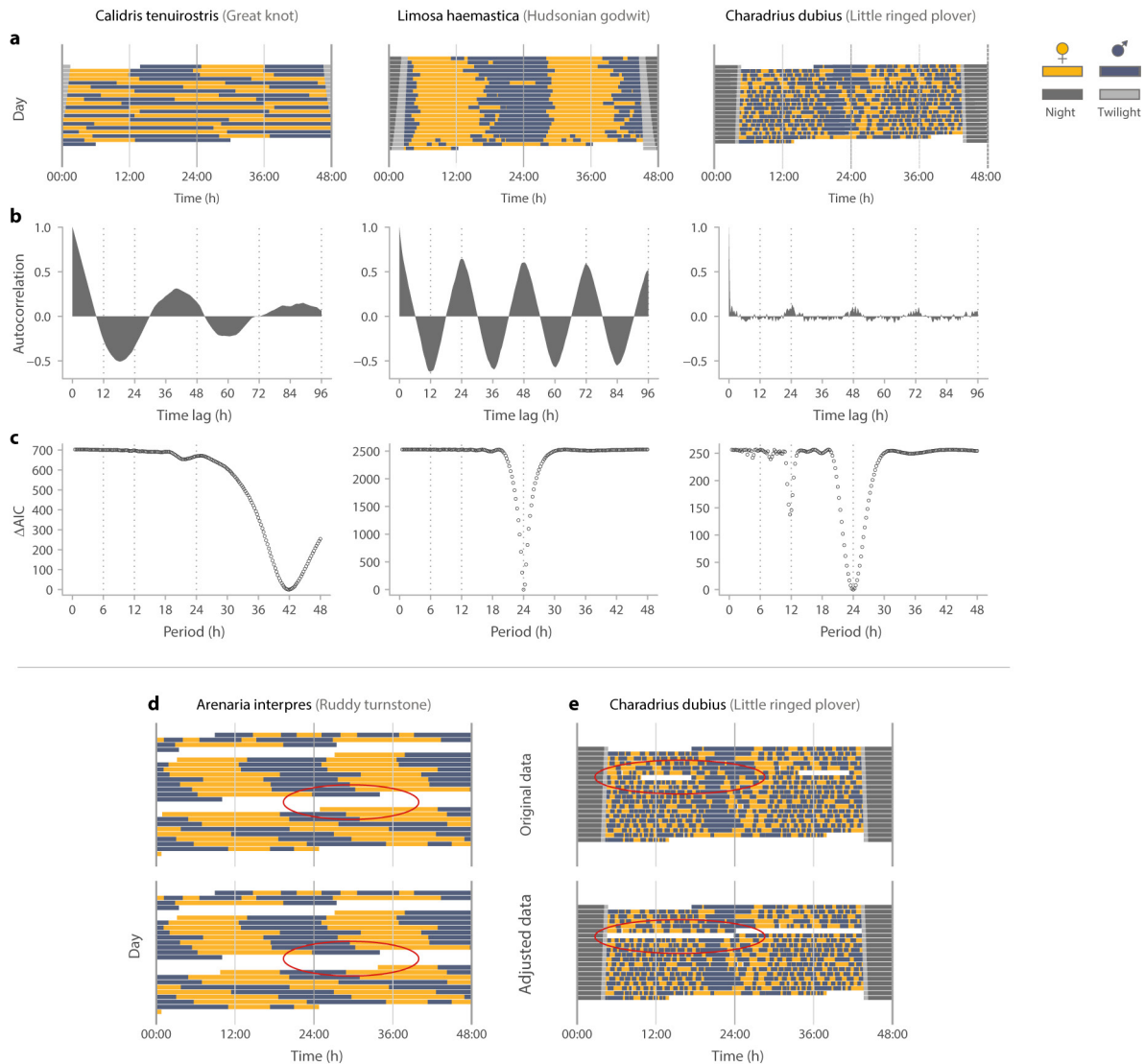
The source of phylogenetic constraint in bout and period length was investigated by estimating the proportion of phenotypic variance explained by suborder, genus and species (Extended Data Table 3). The respective mixed models were also specified with MCMCglmm⁶² using the same specifications as in the phylogenetic models. Because suborder contained only two levels, we first fitted an intercept mixed model with genus, species, and breeding site as random factors, and used it to estimate the overall phenotypic variance. We then entered suborder as a fixed factor and estimated the variance explained by suborder as the difference between the total variance from the first and the second model. To evaluate the proportion of the variance explained by species, genus and breeding site, we used the estimates from the model that included suborder.

R version 3.1.1 (ref. 63) was used for all statistical analyses.

Code availability. All statistical analyses, figures, and the supplementary actograms are replicable with the open access information, including computer software and code for R, available at the Open Science Framework, <https://osf.io/wxufm/> (ref. 16).

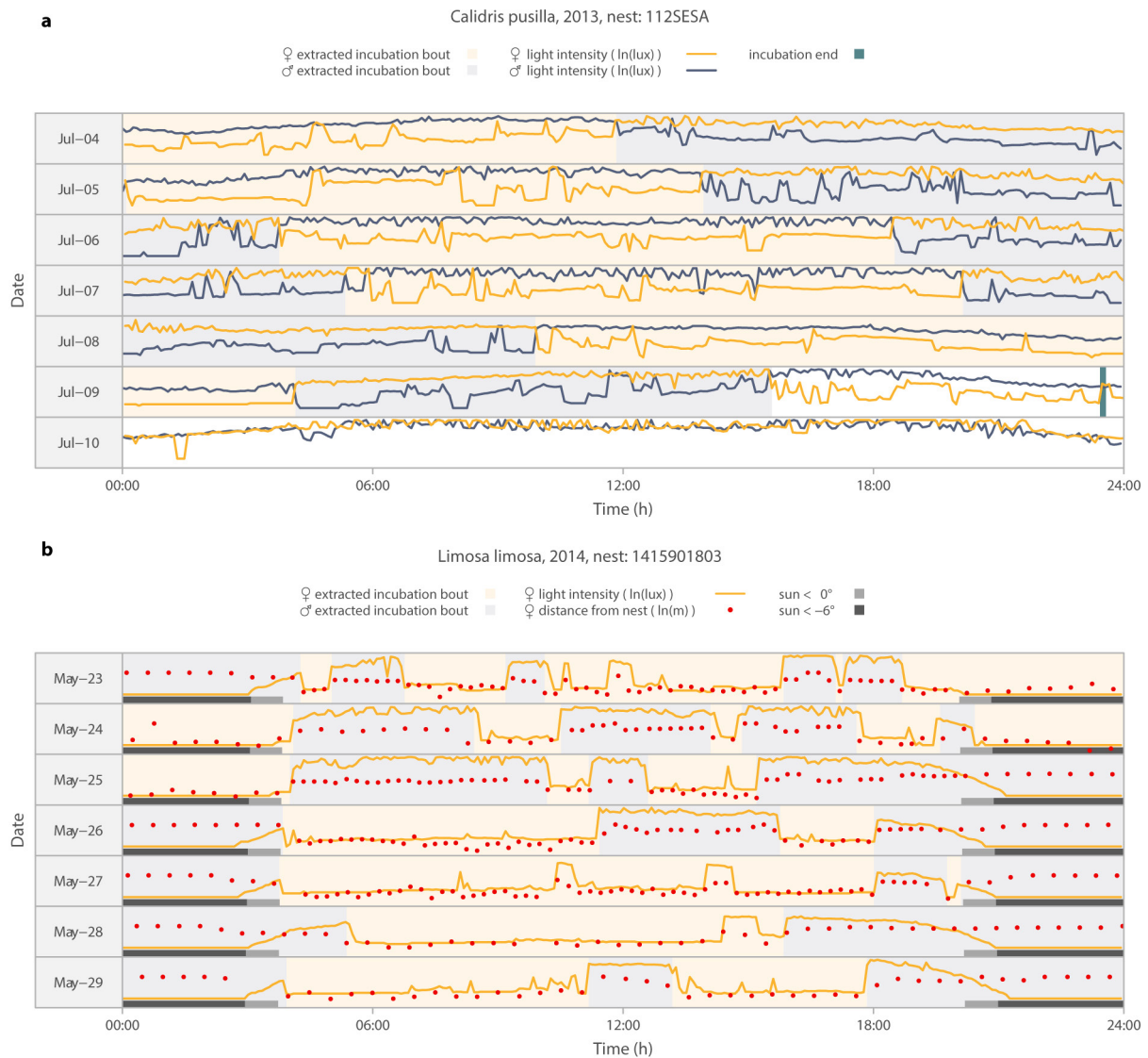
Data availability. Primary and extracted data that support the findings of this study are freely available from the Open Science Framework, <https://osf.io/wxufm/> (ref. 16). Source data for Figs 1–4 are provided with the paper.

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Extended Data Figure 1 | Extracting period length of incubation rhythms. **a–c,** Each column represents an example for a specific nest with long, intermediate and short incubation bouts. **a,** From the extracted bout lengths we created a time series that indicated—for each nest and for every 10 min interval—whether a specific parent (female, if sex was known) incubated or not. Exchange gaps (no parent on the nest) had to be <6 h to be included (for treatment of exchange gaps >6 h see **d, e**). **b,** We then estimated the autocorrelation for each 10-min time-lag up to 4 days (R ‘acf’ function⁶³). Positive values indicate a high probability that the female was incubating, negative values indicate that it was more likely that the male was incubating. We used only nests that had enough data to estimate the autocorrelation pattern ($n = 584$ nests from 88 populations of 30 species). The visualized autocorrelation time series never resembled white or random noise indicative of an arrhythmic incubation pattern. To determine the period (that is, cycle of high and low probability for a parent to incubate) that dominated the incubation rhythm, we fitted to the autocorrelation estimates a series of periodic logistic regressions. In each regression, the time lag (in hours) transformed to radians was represented by a sine and cosine function $f(t) = a_0 + b\left(\cos \frac{2\pi t}{T}\right) + c\left(\sin \frac{2\pi t}{T}\right) + e$, where $f(t)$ is the autocorrelation at time-lag t ; a_0 is the intercept; b is the

slope for sine and c the slope for cosine, T represents the length of the fitted period (in hours), and e is an error term. We allowed the period length to vary from 0.5 h to 48 h (in 15 min intervals, giving 191 regressions). **c,** By comparing the Akaike’s information criterion⁶⁴ (AIC) of all regressions, we estimated, for each nest, the length of the dominant period in the actual incubation data (best fit). Regressions with ΔAIC ($\text{AIC}_{\text{model}} - \text{AIC}_{\text{min}}$) close to 0 are considered as having strong empirical support, while models with ΔAIC values ranging from 4–7 have less support⁶⁴. In 73% of all nests, we determined a single best model with $\Delta\text{AIC} \leq 3$ (**c**, middle ΔAIC graph), in 20% of nests two best models emerged and in 6% of nests 3 or 4 models had $\Delta\text{AIC} \leq 3$ (**c**, left and right ΔAIC graphs). However, in all but three nests, the models with the second-, third- and so on best ΔAIC were those with period lengths closest to the period length of the best model (**c**, left and right ΔAIC graphs). This suggests that multiple periodicities are uncommon. **d, e,** The extraction of the period length (described in **a–c**) requires continuous data sets, but some nests had long (>6 h) gaps between two consecutive incubation bouts, for example because of equipment failure or because of unusual parental behaviour. In such cases, we excluded the data from the end of the last bout until the same time the following day, if data were then available again (**d**), or we excluded the entire day (**e**).

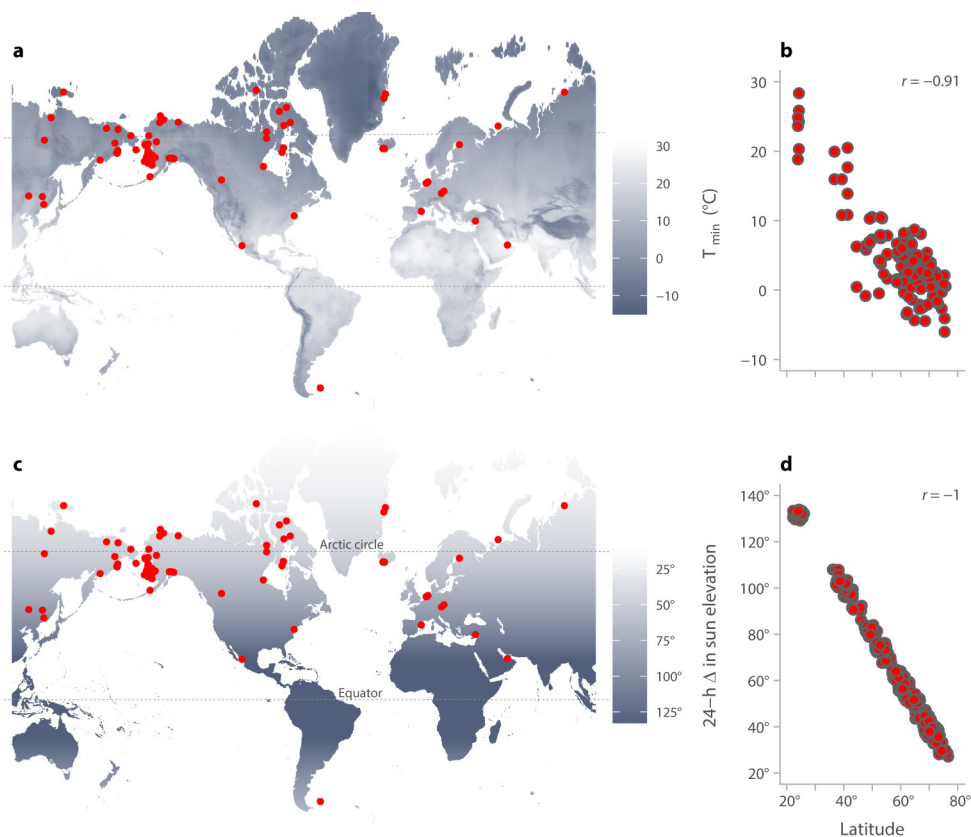


Extended Data Figure 2 | Extracting incubation bouts from light-logger data. **a**, An example of a nest with a light intensity signal from both parents (yellow line, female; blue line, male). The incubation bouts for a given parent reflect periods dominated by lower light values compared to those of the partner. Note the sharp drop in the light levels at the beginning of each incubation bout and the sharp increase in the light levels at the end. Change-overs between partners occur when the light signal lines cross. Such pronounced changes in light intensity detected by the logger were used to assign incubation even when only a single parent was tagged. Note that after the chicks hatch and leave the nest (9 July, vertical bar), the light intensity signals from both parents remain similar. **b**, An example

of a nest where one incubating parent was simultaneously equipped with a light-logger and with a GPS tag. The yellow line indicates light levels, red dots indicate the distance of the bird to the nest. As expected, low light levels co-occur with close proximity to the nest, and therefore reflect periods of incubation. Although light levels decrease during twilight (light grey horizontal bar), the recordings were still sensitive enough to reflect periods of incubation, that is, the light signal matches the distance (for example 25 May: female incubated during dawn, but was off the nest during dusk). **a**, **b**, Rectangles in the background indicate incubation bouts (female, light yellow polygon; male, light blue polygon).

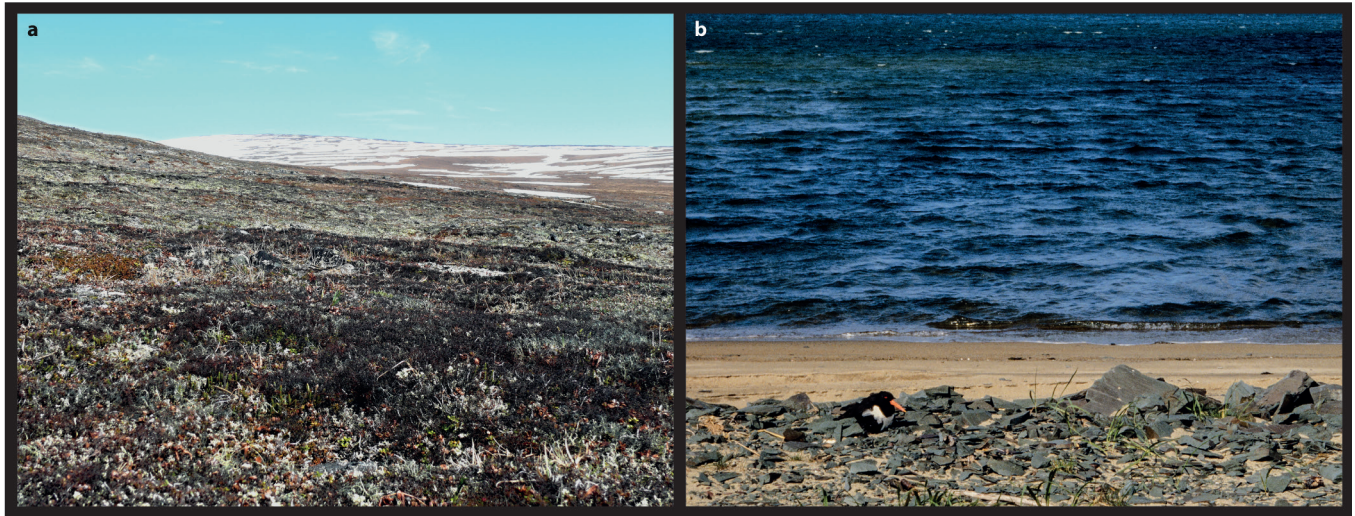


Extended Data Figure 3 | Relationship between bout and period length for 30 shorebird species. Each dot represents one nest ($n = 584$ nests), colours indicate the genus.



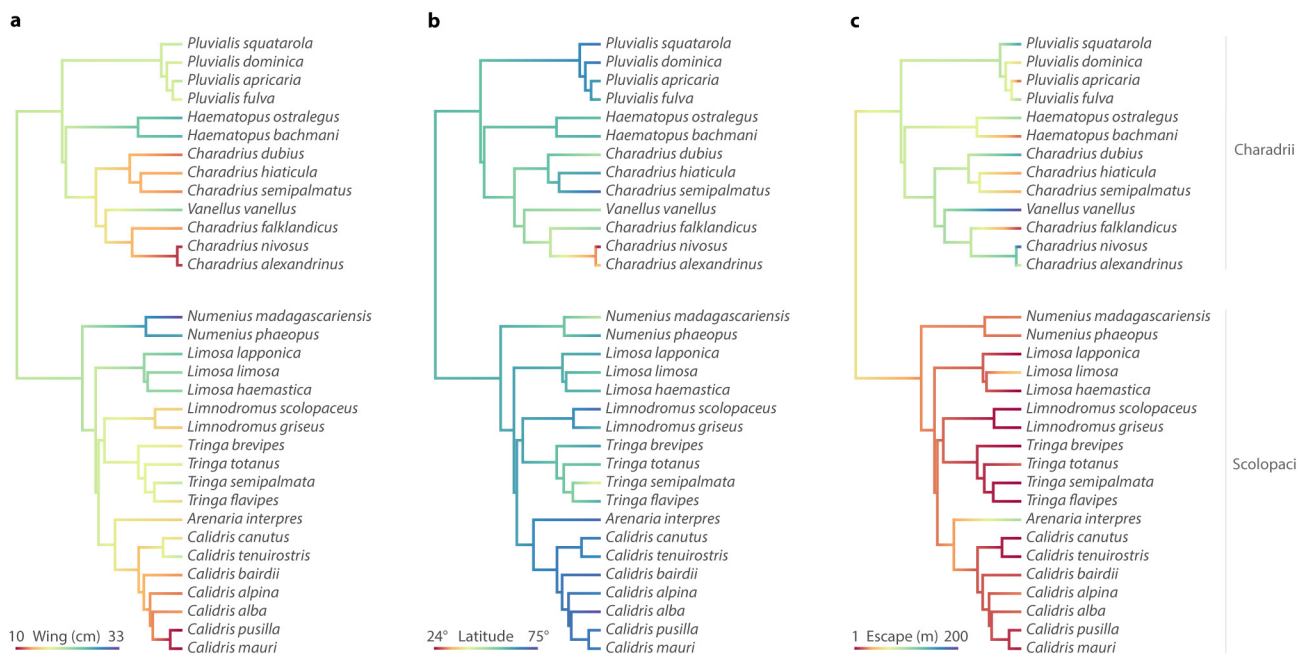
Extended Data Figure 4 | Ecological correlates of latitude. **a**, Variation in minimum temperature across the globe represented by mean minimum June temperature for the Northern Hemisphere and mean minimum December temperature for the Southern Hemisphere. **b**, Correlation between absolute latitude and the mean minimum temperature of the month ($n = 729$ nests). For each nest we used the month that contained most of the incubation data. For maximum temperature the correlation was the same ($r = -0.91$, $n = 729$ nests). **c**, Daily variation in sun elevation (that is, in light conditions) are represented as the difference between the

noon and midnight sun elevation for the summer solstice in the Northern Hemisphere and the winter solstice in the Southern Hemisphere. **d**, Correlation between absolute latitude and daily variation in sun elevation for mid-day of incubation data for each nest ($n = 729$ nests). The points are jittered, as otherwise they form a straight line. **a**, **c**, Red points indicate the breeding site for each population ($n = 91$). **a**, **b**, The minimum and maximum monthly temperature data were obtained from <http://www.worldclim.org> using the raster R-package⁶⁵. **c**, **d**, Sun-elevation was obtained by the 'solarpos' function from the maptools R-package⁶⁶.



Extended Data Figure 5 | Between-species variation in parental crypsis during incubation. **a, b,** Shorebirds vary in how visible they are on the nest while incubating. The nearly invisible great knot (*Calidris tenuirostris*; **a**; central and facing right) sits tight on the nest when approached by a

human until nearly stepped upon. In contrast, the conspicuous Eurasian oystercatcher (*Haematopus ostralegus*; **b**) is visible on the nest from afar and when approached by a human leaves the nest about 100 m in advance (Credits: **a**, M. Šálek; **b**, J. van de Kam).



Extended Data Figure 6 | Phylogenetic relationships for predictors. **a**, Body size, estimated as female wing length. **b**, Latitude (absolute). **c**, Escape distance. **a–c**, We visualized the evolution of these traits^{29,67} using the median (**a**, **b**; based on population medians), estimates of escape distance for each species (**c**) and one of the 100 sampled trees (see Methods).

Extended Data Table 1 | Incubation monitoring methods and systems

Method	Model	Company	www	Year	Sampling interval (min)	<i>n</i> populations	<i>n</i> nests	<i>n</i> both parents tagged
RFID + tag embedded in the flag ^{31,32}	tag: 9.0 × 2.1 mm, 0.087 g, 134.2 kHz	Biomark	http://www.biomark.com/	2011-2014	0.08	19	200	157
	RFID: custom made	Calima Engineering, & Max Planck Institute for Ornithology	http://www.calima.de http://www.orn.mpg.de/en					
	temperature probe: TinyTag Talk PB-5005-0M6 (ø 2.5 mm) with Talk 2 logger TK-4023 or MSR® (12 × 6 mm) with MSR® 145 logger	Gemini Data Loggers Ltd	www.tinytag.info					
	MSR® (12 × 6 mm) with MSR® 145 logger	MSR® Electronics GmbH	http://www.msr.ch/					
RFID + tag glued to the tail ³³	tag: 11.5 × 2.12 mm, 0.1 g, 125 kHz	Trovan®	http://www.trovan.com	2007	5	1	3	3
	RFID: custom made	EID Aalten BV	http://www.dorset.nu/en/ www.tinytag.info					
	temperature probe: TinyTag Talk PB-5005-0M6 (ø 2.5 mm) with Talk 2 logger TK-4023	Gemini Data Loggers Ltd	www.tinytag.info					
	tag: 11.5 × 2.12 mm, 0.09g, 128 kHz	Trovan®	http://www.trovan.com	2005-2008	1.7-5.5	1	34	34
Light logger ^{34,35,68}	RFID: LID650 with extended memory, driver TM613, Antenna ANT614							
	tag: TIRIS 12 × 6 × 3 mm, 0.4 g, 134.2 kHz	Texas Instruments	http://www.ti.com	1997	1/3	1	6	6
	RFID: TIRIS Micro-reader							
	TIRIS tag: 12 × 6 × 3 mm, 0.4 g, 134.2 kHz	Texas Instruments	http://www.ti.com	2006	1/3	1	18	18
GPS-tracker ³⁶	RFID: custom made	Francis Scientific Instruments Limited	https://www.duedil.com/comp-any/01964877/francis-scientific-instruments-limited					
	MK10, 12, 14 18, 20: 0.7-1.4g, light scale 0-64 (64 ≥ 100 lux)	British Antarctic Survey, currently Biotrack	http://www.biomark.com/	2008-2013	2-10	48	261*	55
Radio-transmitter ²⁴	Intigeo W65A9RK: 0.7g, absolute light levels (lux)	Migrate Technology Ltd	http://www.migratetech.co.uk	2012-2014	5-10	23	136*	11
	UvA-BiTS 4C: 62 × 30 × 12, 14 g	University of Amsterdam	http://www.uva-bits.nl/	2010	10-30	1	8	8
Video ⁶⁹⁻⁷²	UvA-BiTS 2CDse: 52 × 22 × 9, 7.5 g			2014	20	1	1**	0
	iTag: 26 × 15 × 9 mm, 4 g	e-obs GmbH	http://www.e-obs.de/	2009	0.07	2	3	3
Observations				2005-2006	1/3	1	28	-
				2005-2011	constant	3	25	-
Observations				1994	30	1	5	-
				2011	constant	1	3	-

For details about methods used in each populations, see supplementary data of ref. 53.

*At one nest a bird with a MK logger was recaptured and the logger exchanged for an Intigeo logger. This nest appears in *n* for both logger types.

**Simultaneously equipped with light-logger (Intigeo). This nest appears in *n* for both GPS-tracker and Intigeo.

Extended Data Table 2 | Effects of phylogeny and sampling on bout length and period length

Response	Effect type	Effect	Posterior mode	95% CI		N (range)
				Lower	Upper	
Median bout [h]	Fixed	Intercept	7.2	1.04	12	1100 (924-2079)
		Sampling	0.16	-0.2	0.61	1100 (809-1644)
	Random (variance)	Phylogeny	25.33	4.6	59.6	1100 (753-1383)
		Species	0.01	0	12.1	1100 (779-1636)
		Breeding site	2.13	0.96	4.28	1100 (808-2242)
	Residual		5.04	4.51	5.61	1100 (838-1444)
		Page's λ	1	0.5	1	1100 (814-1316)
Period [h]	Fixed	Intercept	21.94	12.8	30.67	1100 (765-1392)
		Sampling	0.13	-0.41	0.65	1100 (741-1468)
	Random (variance)	Phylogeny	66.22	14.3	153	1100 (729-1638)
		Species	0.06	0	29.36	1100 (729-1435)
		Breeding site	0.01	0	0.88	1100 (814-1378)
	Residual		14.87	13.3	16.84	1100 (884-1460)
		Page's λ	1	0.54	1	1100 (740-1523)

The posterior estimates (modes) of the effect sizes with the highest posterior density intervals (95% CI) and the median and range of the effective sample sizes (N (range)) come from the joint posterior distribution of 100 separate runs each with 1 of 100 separate phylogenetic trees from <http://birdtree.org>. $n_{\text{bout}} = 729$ nests from 91 populations belonging to 32 species. $n_{\text{period}} = 584$ nests from 88 populations belonging to 30 species. Sampling (how often the incubation behaviour was sampled) was ln-transformed and then mean-centred and scaled (divided by s.d.). For procedures and specifications related to phylogenetic Bayesian mixed models see Methods. Estimating Page's λ on the species level ($n_{\text{bout}} = 32$ species, $n_{\text{period}} = 30$ species) with phylogenetic generalized least-squares using the function 'ppls' from the R package *caper*⁷³ gave similar results (median (range) $\lambda_{\text{bout}} = 0.73$ (0.63–1) and $\lambda_{\text{period}} = 0.95$ (0.64–1), based on 100 estimates each for 1 of the 100 trees).

Extended Data Table 3 | Source of phylogenetic signal

Suborder included	Response	Effect type	Fixed effects	Posterior mode	95% CI		N
					Lower	Upper	
No	Bout [h]	Fixed	Intercept	7.69	4.11	10.76	1100
			Random (variance)	Genus	9.54	0.02	46.6
		Species		11	4.84	25.77	1100
		Breeding site		2.25	1.06	4.5	1100
		Residual	5.03	4.55	5.61	1100	
Yes	Bout [h]	Fixed	Intercept (Charadrii)	4.48	1.05	7.66	1100
			Suborder (Scolopaci)	6.07	1.52	10.44	1100
		Random (variance)	Genus	0.09	0	21.35	1100
			Species	11.29	5.53	23.99	1275
			Breeding site	2.31	0.98	4.28	1100
			Residual	5.04	4.52	5.58	1100
No	Period [h]	Fixed	Intercept	23.46	18.22	27.39	1100
			Random (variance)	Genus	19.05	0	97.79
		Species		41.02	17.04	72.66	1100
		Breeding site		0.01	0	0.9	1100
		Residual	14.94	13.35	16.76	1100	
Yes	Period [h]	Fixed	Intercept (Charadrii)	18.61	13.12	23.98	940
			Suborder (Scolopaci)	8.92	0.6	15.4	990
		Random (variance)	Genus	0.33	0	59.09	769
			Species	34.41	16.91	68.14	1142
			Breeding site	0.01	0	0.84	1100
			Residual	15.01	13.34	16.86	891

The posterior estimates (modes) of the effect sizes with the highest posterior density intervals (95% CI) and the effective sample sizes (N) come from a posterior distribution of 1,100 simulated values generated by MCMCglmm in R⁶². $n_{\text{bout}} = 729$ nests from 91 populations belonging to 32 species. $n_{\text{period}} = 584$ nest from 88 populations belonging to 30 species.

Extended Data Table 4 | Effect of latitude, body size, escape distance and life history on biparental incubation rhythms in shorebirds

Response	Effect type	Fixed effects	Posterior mode	95% CI		N (range)
				Lower	Upper	
Bout [h]	Fixed	Intercept	7.45	2.65	12	1100 (804-1496)
		Wing length	-0.78	-2.5	1.05	1100 (839-1638)
		Latitude	1.72	0.63	2.65	1100 (850-1642)
		Escape distance	-1.68	-3.3	-0.25	1100 (634-2046)
	Random (variance)	Phylogeny	0.19	0	45	1100 (803-1875)
		Species	0.07	0	14.4	1100 (695-1580)
		Breeding site	1.4	0.59	3.02	1100 (833-1480)
		Residual	5.02	4.53	5.64	1100 (516-1916)
Page1's λ		0.72	0.13	1	1100 (731-1407)	
Light entrainable rhythm [1,0] on binomial scale	Fixed	Intercept	-1.62	-3.19	-0.13	1100 (731-1633)
		Latitude	-0.56	-1.15	-0.07	1100 (765-1575)
	Random (variance)	Phylogeny	0.05	0	5.54	1100 (883-1371)
		Species	0.02	0	2.68	1100 (965-2246)
		Breeding site	0	0	0.63	1100 (605-1304)
	Page1's λ		0.74	0.02	1	1100 (932-1498)
Absolute deviations from 24-h	Fixed	Intercept	0.17	-0	0.35	1100 (459-1501)
		Latitude	0.03	-0	0.07	1100 (777-1488)
	Random (variance)	Phylogeny	0	0	0.07	1100 (786-1393)
		Species	0	0	0.03	1100 (861-1412)
		Breeding site	0	0	0	1100 (826-1860)
	Residual	0.03	0.03	0.04	1100 (948-2039)	
Page1's λ		0.74	0.02	1	1100 (843-1471)	
Deviations from 24-h	Fixed	Intercept (non-tidal)	0.02	-0.04	0.09	1100 (851-1742)
		Life history (tidal)	-0.02	-0.1	0.04	1100 (702-2257)
	Random (variance)	Phylogeny	0	0	0.01	1100 (806-1692)
		Species	0	0	0	1100 (692-1601)
		Breeding site	0	0	0.01	1100 (656-1490)
	Residual	0.07	0.06	0.08	1100 (760-1563)	
Page1's λ		0.77	0.01	1	1100 (864-1451)	

The posterior estimates (modes) of the effect sizes with the highest posterior density intervals (95% CI) and the median and range of the effective sample sizes (N (range)) come from the joint posterior distribution of 100 separate runs each with 1 of the 100 separate phylogenetic trees from <http://birdtree.org>. $n_{\text{bout}} = 729$ nests from 91 populations belonging to 32 species. For models on light-entrainable rhythm, absolute deviations and deviations from 24 h: $n = 584$ nests from 88 populations belonging to 30 species. Latitude (in bout model: absolute value), wing length (ln-transformed), and escape distance (ln-transformed) were mean-centred and scaled (divided by s.d.). The estimates for the light-entrainable rhythm are on a binomial scale. For procedures and specifications related to phylogenetic Bayesian mixed models see Methods.

Parental incubation exchange in a territorial bird species involves sex-specific signalling

Martin Sládeček, Eva Vozabulová, Kateřina Brynychová & Miroslav E. Šálek

Background

Effective communication between sexual partners is essential for successful reproduction. Avian parents with biparental incubation need to know how to negotiate, when and who will incubate, and how to harmonize partner exchange at the nest. Although considerable effort has been dedicated to studies of incubation rhythms, few studies have investigated how behavioural signals serve to tighten cooperation between parents. Moreover, existing studies are almost exclusively restricted to species in which long distances between incubating and non-incubating parents prevent continuous communication during incubation. Thus, the most frequently described parental exchange system is a simple model characterized by the return of the non-incubating parent to the nest itself. Here, we propose more complex parental exchange behaviour in the Northern Lapwing (*Vanellus vanellus*), a territorial species capable of continuous partner communication during incubation and with a highly variable male contribution to incubation.

Results

Northern Lapwing females regularly vocalized shortly before departing from the nest, while males mostly left the nest quietly. Responsiveness of the male to female vocalization, perhaps in combination with her flying away from the nest, helped to synchronize incubation care by increasing the probability of exchange, and also by shortening the exchange gaps. In contrast, a male-to-female exchange gap most often occurred after the male quietly flew away from the nest. The frequency of female vocal signalling was not correlated with the male incubation effort on a between-nest scale, but the highest probability of a female-to-male exchange occurred after vocal signalling by females with the most nest-attentive males. Conversely, lowered effort by females to vocalize in the night was accompanied by lower willingness of males to incubate.

Conclusions

Our results suggest that (1) that the incubating parent can communicate with the non-incubating partner using sex-specific behavioural signals, and this helps to synchronize parental exchange on the nest, (2) this signalling may combine acoustic and visual cues, and (3) the efficiency of this signalling might influence the overall nest attendance. The presumption that the repertoire of behavioural signals during reproduction will be much more complex in territorial species that are capable of

continuous communication between the partners during the incubation period should be further tested.

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RESEARCH

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Parental incubation exchange in a territorial bird species involves sex-specific signalling



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Abstract

Background: Effective communication between sexual partners is essential for successful reproduction. Avian parents with biparental incubation need to know how to negotiate, when and who will incubate, and how to harmonize partner exchange at the nest. Although considerable effort has been dedicated to studies of incubation rhythms, few studies have investigated how behavioural signals serve to tighten cooperation between parents. Moreover, existing studies are almost exclusively restricted to species in which long distances between incubating and non-incubating parents prevent continuous communication during incubation. Thus, the most frequently described parental exchange system is a simple model characterized by the return of the non-incubating parent to the nest itself. Here, we propose more complex parental exchange behaviour in the Northern Lapwing (*Vanellus vanellus*), a territorial species capable of continuous partner communication during incubation and with a highly variable male contribution to incubation.

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Keywords: Biparental incubation, Incubation rhythms, Parental care, Shorebirds, Nest relief, *Vanellus vanellus*, Waders

Background

Effective communication between sexual partners is essential for successful reproduction. In biparental species, in particular, acoustic and visual communication between the partners can tackle issues of sexual conflict [1, 2] and also issues of tighter cooperation [3, 4]. In many avian species, both parents take part in incubating the eggs [5], and this increases the demands on

communication between incubating and non-incubating partners. A variety of incubation patterns have been described, ranging from exchanges between partners at the nest every few minutes [6] to incubation sessions lasting several weeks [7–9]. However, a question remaining almost unstudied concerns how parents communicate on the scale of particular exchanges.

Most studies targeting the question of partner exchange at the nest have been carried out on species in which the non-incubating parent spends its off-duty time far from the nest [9–12], and thus the parents are

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unable to communicate continuously. The only feasible way to make a synchronous partner exchange in these cases is therefore probably for the off-duty parent to return to the nest itself [8]. In seabirds, such as albatrosses [9], penguins [13] and skuas [14] with extremely long incubation bouts and hundreds of kilometres long foraging trips, the incubating bird waits until the partner returns. Any failure in this return can therefore lead to a critical decline in the body condition of the incubating bird, and even to abandonment of the nest [7, 8, 11]. However, even in species with much more frequent nest relief, the exchanges usually take place while both parents are present at the nest. This is frequently accompanied by some kind of displays [15] or by other rituals, such as allopreening [3, 16].

There is much more opportunity for communication between the partners and for negotiating about the timing of their exchange on the nest in species where the non-incubating parent spends most of its off-duty time near to the nest, or if it frequently visits the nest even during its off-duty time. Multiple visits preceding an exchange were observed in captive ringed doves (*Streptopelia risoria*) [3]. These regular contacts enable tight cooperation between the parents. Only 13% of nest reliefs were initiated by nest abandonment by the incubating bird before the partner returned. Similarly, in zebra finches (*Taeniopygia guttata*) such regular visits are accompanied by repeated acoustic duets, through which the sitting bird signals its need to be exchanged [4, 17]. In these cases, both birds are probably involved in the negotiation process about when it is time to exchange incubation duties. This can help in achieving tight coordination of incubation care [3, 4, 18].

However, in many species it is not unusual for the incubating parent to leave the nest before the arrival of its partner, and thus the incubation sessions are separated by so-called “exchange gaps” [19, 20]. It is undesirable for the exchange gaps to be too long, because they may increase the risk of nest depredation [21] or cooling of the unattended eggs [22]. Even species that have exchange gaps as a regular part of their incubation schedule should therefore use some request signalling for nest relief. However, the mechanisms for communication between the partners in these species aimed particularly at motivating the non-incubating parent to return to the nest and engage in incubation duties are poorly understood.

The Northern Lapwing (*Vanellus vanellus*) is a biparentally incubating shorebird with a highly variable male contribution to incubation [23–26], and with irregular frequency of parental exchanges [25–27]. The male contribution to incubation is ordinarily smaller than the contribution of the female. The male contribution peaks during the day, while it is almost totally absent in the

night [26, 28]. The Lapwing has intermittent incubation, with about 13% of the time when the nest is not attended by either parent [26]. However parental exchange occurs only during a relatively small part of the incubation recesses (Actograms in: [25], this paper). Northern Lapwings are territorial, and the birds spend most of the time in their territories, usually in open habitats [29, 30], which enables continuous contact and communication between partners [30].

In this paper, we analyse behavioural patterns associated with incubation gaps in breeding Northern Lapwings. We hypothesized that the incubating parent communicates with the non-incubating partner using behavioural signals, and that this helps to synchronize parental exchange on the nest. Specifically, and based on our direct observations, we suggest that when intending to exchange with the partner, the incubating parent vocalizes briefly (i.e. for a few seconds) before departing from the nest. The urgency of this signal can be reinforced by flying away from the nest, a more pronounced action than walking away. If this is true, we would expect that 1) partner exchange will occur more probably during the recesses after the departure of the on-duty parent, after issuing a vocalization signal, perhaps reinforced by flying away; 2) there will be shorter recesses accompanied by nest relief coming after these signals (i.e. the signals increase partner synchronization); 3) if the off-duty parent ignores the signal, the subsequent recess will be longer than the recesses without signalling, as a result of partner disagreement within the negotiation process.

Based on the fact that the male contribution to incubation varies strongly among the nests [23–26], we further investigated whether the variation in the male contribution to incubation 1) is predicted by the vocal signalling effort made by the female, or 2) reflects the efficiency of these signals (i.e. more incubating males exchange the female more probably after her signalling). Similarly, because the male contribution to incubation shows strong daily rhythmicity, being highest during the day (with peaks after sunrise and before sunset) and is almost absent in the night [25, 26], we further tested: 3) whether the effort put into signalling by the female changes in the course of the day, and 4) whether the signalling efficiency (i.e. male willingness to exchange) changes in the course of the day.

Methods

General field procedure and data extraction

We monitored the incubation of Northern Lapwings in the České Budějovice basin, Doudlebia, Czech Republic (49.25°N, 14.08°E), on approximately 40 km² of agricultural landscape, during April and May 2016. We searched for nests by thoroughly scanning fields and

meadows with telescopes, or by walking through areas with high nest densities. We monitored incubation with a small camera (\varnothing 2 cm, length 4 cm) placed approximately 1.5 m from the nest in a southward direction, in order to minimize the time that the lens faced the sun (which would have overexposed the videos and made individuals hard to recognize). The digital recorder stored videos at 10 frames per second with 640×480 -pixel resolution. The system was powered by a 12-V, 44-Ah battery buried together with the recorder under the ground. The target was to obtain ~ 2 – 3 days of recordings from each nest.

We extracted the incubation behaviour using AVS Media Player (<http://www.avs4you.com/AVS-Media-Player.aspx>). First, we determined each arrival or departure of incubating birds with precision of 1 second. The sex of the birds was determined on the basis of sex-specific plumage traits, such as crest length and the extent of the melanin ornaments on the breast and on the face [31]. Then, we thoroughly scanned the last 5 seconds before each departure in order to identify whether or not the incubating bird had vocalized. Vocalization was clearly identifiable on the videos by specific head movements and by bill opening. As two of the video sets that were used were additionally provided with a small microphone, we were able to validate the linking of specific head and bill movements with vocalization.

For each departure from the nest, we scored vocalization as a binomial variable (1 = at least one call; 0 = without a call), and we noted whether the bird flew away or walked away. Because the recordings from some nests were damaged or ended early due to nest depredation, we excluded from the analysis any nests with less than 10 scored incubation recesses.

We defined an ‘**incubation recess**’ as any period of time for which the nest was unattended by either of the parents. Subsequently, we classified the incubation recess as a ‘**break**’ (the same parent came back and continued incubation) or as an ‘**exchange gap**’ (parents exchanged during the incubation recess) [19]. In order to relate female vocal signalling with the between-nest variation in the male contribution to incubation, we introduced a term ‘**male incubation effort**’, calculated as the ratio of male nest attendance at the nest to the overall time for which the nest was attended by either of the parents (i.e. excluding all incubation recesses). ‘**Female vocalization effort**’ was then defined as the proportion of female departures accompanied by vocalization (per particular nest/hour), and ‘**female vocalization efficiency**’ was defined as the probability that the male will come to incubate after female vocalization.

Validation of the assumptions, to avoid confounding effects

In order to correctly interpret the results of this study, we first explored the vocalization pattern of incubating

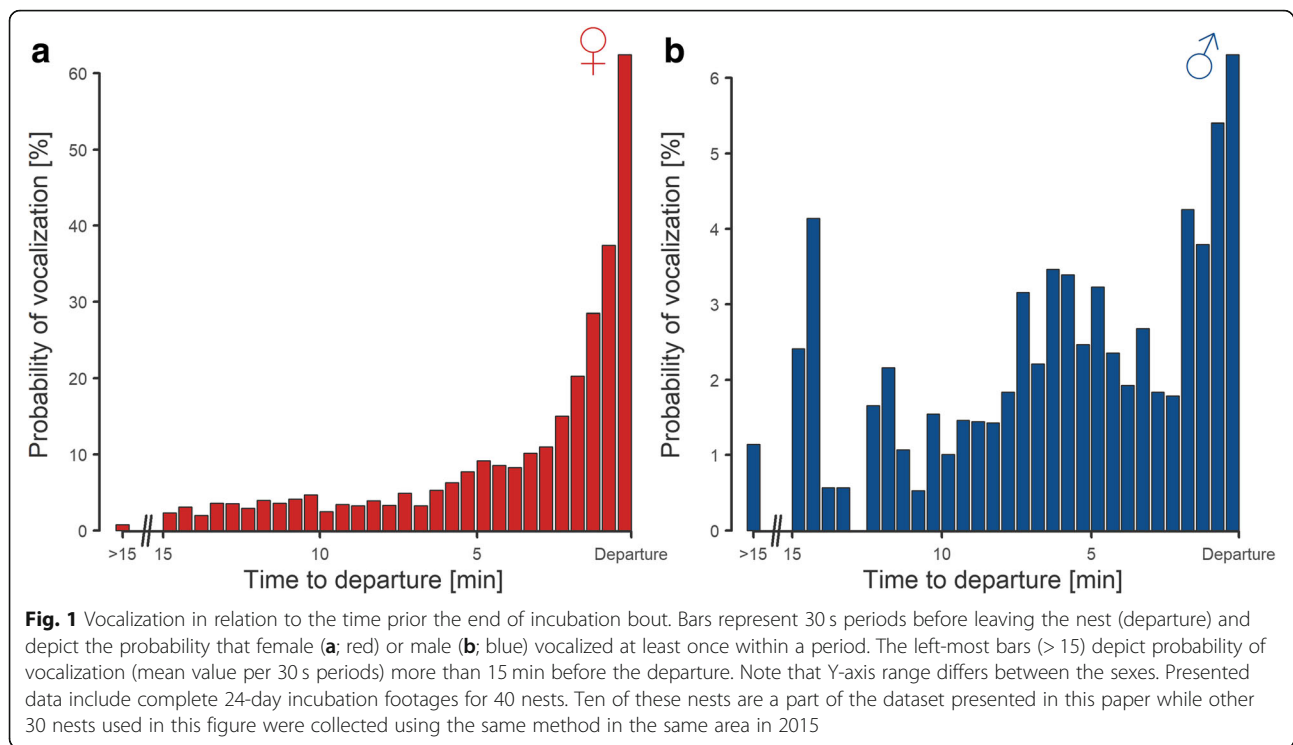
Northern Lapwings with a particular focus on the context of departure from the nest. We investigated whether vocalization can occur frequently at any time during incubation (and might thus confound our interpretation of partner behaviour) or whether it is concentrated just before departure from the nest (as predicted for the purposes of this study). We therefore specifically analysed a subset of 40 nests (~ 960 h) with 1 day of continuous (i.e., completely uninterrupted) videotaping, which enabled us to determine in detail all vocal sessions throughout a one-day incubation course. The set consisted of 30 nests collected in another study in 2015, and a subset of 10 nests from 2016 that were included in this paper.

We found that although vocalization events could take place at any time during the incubation bouts in both sexes, the frequency steeply increased in few minutes prior to departure. Whereas in males the pattern is weak, in females it is much more pronounced. The vocalization of females peaks immediately before the departure, with more than 60% probability of vocalization during the last 30 s. It contrasts with strongly decreasing probability up to 1.3% (mean probability of vocalization for any thirty-second interval five or more minutes prior to departure; Fig. 1a, b). Secondly, using this dataset, we investigated whether more attentive males (i.e. those that made a greater incubation effort) could have been (positively) assortatively mated with more vocal females, which would confound our interpretation of female vocal signalling efficiency. We observed no positive correlation, and we conclude that the incubation effort in males is not directly positively associated with the vocalization frequency of their female mates (Additional file 1: Figure S1, Table S1).

Statistical analysis

All statistical analyses were performed in R version 3.3.0 [32]. For the model-based parameter estimates (or for the contrasts between these estimates) we report the effect sizes as medians and Bayesian 95% credible intervals (95%CrI) represented by the 2.5 and 97.5 percentiles from the posterior distribution of 5000 simulated values obtained by the ‘sim’ function from the ‘arm’ R package [33].

Binomial response variables were fitted with generalized mixed-effect models with a binomial error structure and the logit link function, using the ‘glmer’ function from the ‘lme4’ R package [34]. In particular, in order to explain the probability of an exchange gap (i.e. the probability of nest relief during an incubation recess) we used three binomial predictors: “sex”, “vocalization” (yes or no) and “departure type” (“flight” or “walk”). All these effects were used both as main effects and in interactions (including three-way interaction). To explain the probability of vocalization before departure, we also used



“sex” and “departure type” as predictors in terms of main effects and in interaction.

In order to test the daily rhythmicity in the female vocalization effort, we also used vocalization before female departure (yes or no) as a response in the model, with time as a predictor. We used time transformed to radians ($2 \cdot \text{time} \cdot \pi / \text{period of interest}$) and subsequently fitted it as the sine and the cosine of the radians. We used 24 h as a period of interest and, due to the obvious bimodality of the response variable, with peaks in the morning and in the late afternoon, we also used 12 h as a period of interest. Similarly, the “departure type” binomial response was fitted with time (24-h rhythmicity) in interaction with sex.

The length of the incubation recesses was fitted with the mixed-effect model with a Gaussian error structure using the “lmer” function from the “lme4” R package [34]. The response variable was log-transformed to approach the normality of the model residuals. Binomial variables “sex”, “vocalization” (yes or no) and “departure type” (“flight” or “walk”) were used as predictors in the model. We fitted nest identity as a random intercept in all the models described above, and in models using temporal information as a predictor we also fitted time (sine and cosine) as random slopes [35].

To analyse the between-nest differences in female vocalization effort, we used the male incubation effort as a response variable. Female vocalization effort and vocalization efficiency were then z-standardized

(centered and mean-divided [36]), and were used as predictors in a general linear model fitted using the “lm” function [32]. The model was weighted by the square-rooted number of analysed female departures from the nest.

Because of the overall scarcity of male incubation in the night (and thus the small sample size of exchange gaps in the night), we were unable to use models to test the night efficiency of female vocalization or the male responsiveness to these signals. We therefore divided all incubation recesses into those started during the dark part of the day (i.e. when the sun was more than 6° below the horizon) and those started during daylight. We then tested 1) whether female vocalization in the night raised the probability of nest relief, and 2) whether the probability that the male would comply with the signalling is the same for both day and night. We tested these hypotheses using the Boschloo test, a technique from a group of unconstrained exact tests for two binomial proportions, which is suitable for use when small expected values occur. This approach using the p -value from Fisher’s exact test as a test statistic is explicitly recommended by Mehrotra et al. [37] as convenient in cases of unbalanced designs. In particular, we used the “exact.test” function from the “Exact” R package [38].

Results

A total of 63 nests were monitored for 2854 h (12 to 116 h; median = 41.37, sd = 18.2) and 5033 nest

departures were scored (23 to 242 from particular nests; median = 77, sd = 36.4). Females departed in 3367 cases (66.8%) and males departed in 1666 cases (33.1%). Overall, an exchange gap occurred in 25.6% of incubation recesses (CrI: 22–30%), and was on an average 17% (CrI: 14–20%) more likely after male departures (710 out of 1666; 37.6%; CrI: 34–41%) than after female departures (719 out of 3367; 20.3%; CrI: 17–24%).

Patterns of nest departures and vocalization

The use of departure types (flight or walk) and also the probability of vocalization before departure differed between the sexes and varied with the time of day. Males flew away (1415 cases; 87.1% of flight departures; CrI: 84–89%) more often than females (2317 cases; 70.4%; CrI: 67–74%), and females accompanied their departures with vocalization much more often (1385 cases; 41.5%; CrI: 37–46%) than males (193 cases; 10.3%; CrI: 8–12%). Females (but not males) vocalized much more frequently when they flew away from the nest than when they walked away (52 vs. 18%; Fig. 2a, Additional file 1: Table S2). In the daily pattern of females, flight departures prevailed during the night, while they dropped to less than 50% around midday (Additional file 1: Figure S2a, Table S3). In males, this drop was less pronounced, albeit still significant (Additional file 1: Figure S2b, Table S3). The daily pattern of female vocalization during nest departures was bimodal, with peaks after sunrise and before

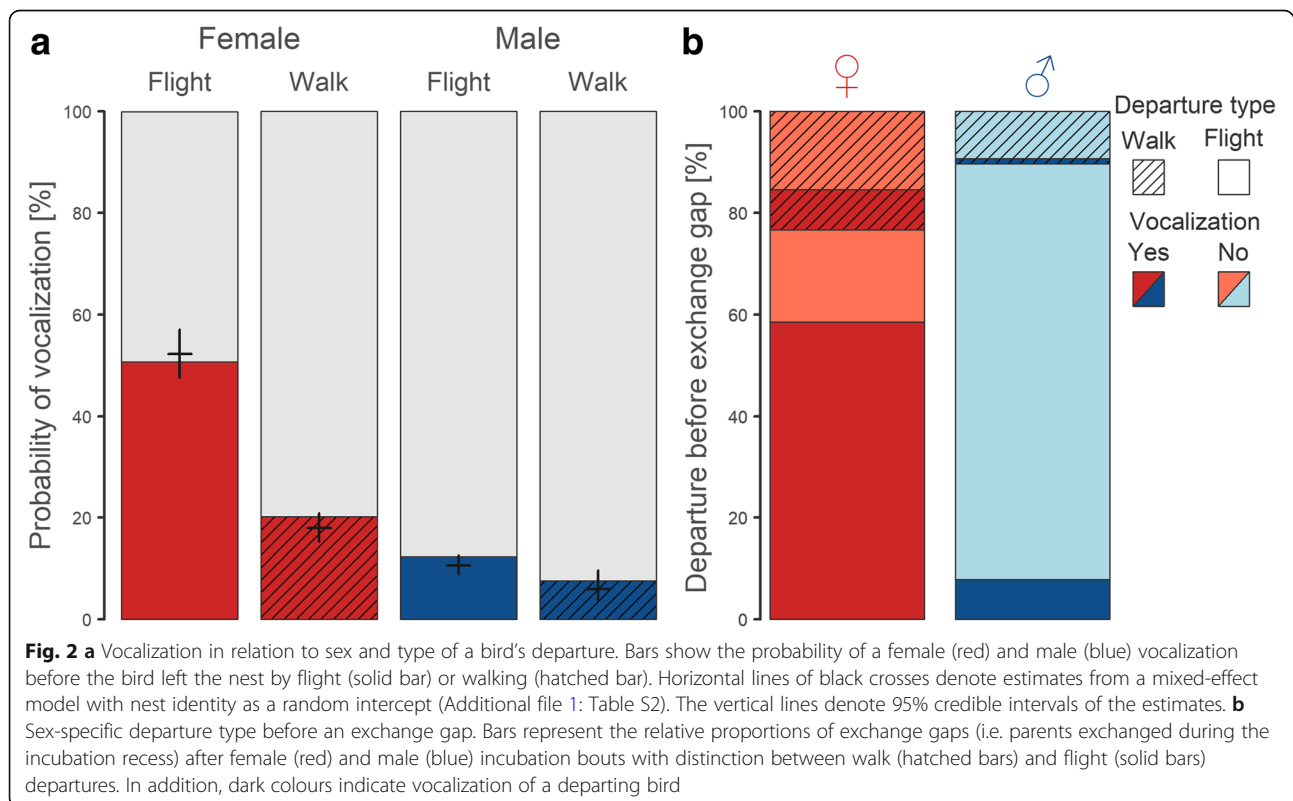
sunset, and followed the ratio of the male contribution to incubation (with the minimum during the night; Fig. 3, Additional file 1: Table S4).

Probability of exchange gaps with sex-specific signalling

The probability of parental exchange after an incubation recess was associated with vocalization by an incubating female, but not male. In females, the probability of being exchanged by a male was enhanced by previous vocalization, both when the female flew away (36% vs 9% without vocalization; Fig. 4, Table 1) and when she walked away (26% vs 12% without vocalization; Fig. 4, Table 1). In addition, an exchange after female vocalization was more likely after she flew away than after she walked away (see non-overlapping CrIs in Table 2). Nevertheless, female flight departure itself (i.e. without vocalization) did not increase the probability of an exchange gap. Out of 719 exchange gaps after female incubation, 478 (i.e. 66%, Fig. 2b) were preceded by female vocalization, and of these 421 (58%, Fig. 2b) were also followed by flight departures. In contrast, in males the vocalization before flight departure decreased the probability of male-to-female exchange (Fig. 4, Table 1).

Effect of vocalization on the synchronization of exchange gaps

Female vocalization before departure from the nest helped to synchronize the exchange gaps, since the



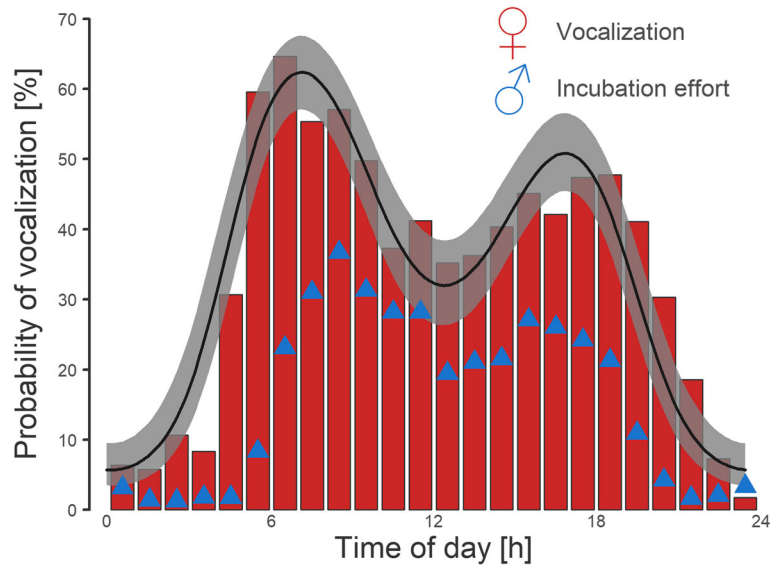


Fig. 3 Daily pattern of female vocalization before leaving the nest and male incubation effort. Red bars depict real proportions of female departures accompanied by her vocalization for a particular hour of the day. The curve with shaded area indicates the model prediction with a 95% credible interval (Additional file 1: Table S4). Blue triangles illustrate the proportion of male contribution to incubation in our dataset for a particular hour of day

exchange gaps coming after female incubation bouts were better synchronized (i.e. they were 1.25 min shorter; CrI: 0.85–1.71 min., Fig. 5) after vocalization than without vocalization. The opposite was true if the recess resulted only in a break (i.e. if the male did not come to exchange the female). The breaks coming after female departure accompanied by vocalization were 1.29 min longer (CrI: 0.93–1.68 min.) than those without vocalization (Fig. 5, Tables 3 and 4). Conversely, the incubation recesses of males were generally shorter than those of females, and the length

of the exchange gaps coming after male incubation bouts was not affected by whether or not the male vocalized.

On a between-nest scale, the male contribution to incubation in a particular nest was not enhanced by the female vocalization effort (i.e. the proportion of departures accompanied by vocalization per particular nest/hour). However, in nests with a higher male contribution to incubation, the males were more likely to come and incubate after female vocalization (Fig. 6, Additional file 1: Table S5).

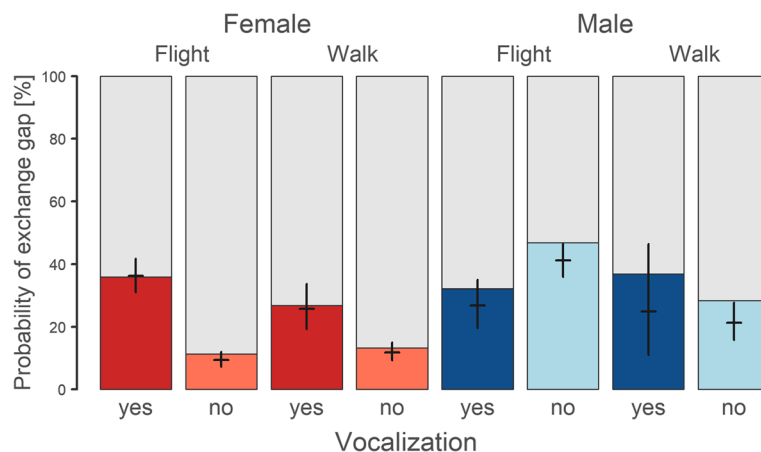


Fig. 4 The probability of an exchange gap during an incubation recess. Bars show the probability that a female (red) or male (blue) is exchanged by the partner. Dark colour indicates, that the departing bird vocalized before the departure. Labels above the plot distinguish if the nest was left by flight or walking. The horizontal lines of black crosses denote estimates from the mixed effect model with nest identity as a random intercept (Table 1). The vertical lines indicate 95% credible intervals of the estimates

Table 1 Probability of exchange gap during incubation recess

Level	sex	Vocalization	Type of departure	Estimate	95% CrI	
					Lower	Upper
1	F	YES	FLIGHT	0.36	0.31	0.42
2	F	NO	FLIGHT	0.09	0.07	0.12
3	F	YES	WALK	0.26	0.19	0.34
4	F	NO	WALK	0.12	0.09	0.15
5	M	YES	FLIGHT	0.27	0.2	0.35
6	M	NO	FLIGHT	0.41	0.36	0.47
7	M	YES	WALK	0.25	0.11	0.47
8	M	NO	WALK	0.21	0.16	0.28

Daily pattern in vocalization efficiency

Although the overall frequency of female vocalization in the night was very low (10.7% of departures) and there were only 17 subsequent exchange gaps from 8 nests, female vocalization before departure strongly increased the probability of her being exchanged even in the night (Boschloo test; $p < 0.001$). Nonetheless, the efficiency of female vocalization signalling (i.e. the probability that a male will come after female vocalization) was significantly lower in the night than in daytime (Boschloo test; $p = 0.017$).

Table 2 Probability of exchange gap during incubation recess

Contrast	Estimate	95% CrI	
		Lower	Upper
1–2	0.27	0.22	0.31
1–3	0.1	0.03	0.17
3–4	0.14	0.08	0.21
2–3	–0.16	–0.24	–0.1
2–4	–0.02	–0.05	0.01
5–6	–0.14	–0.21	–0.07
5–7	0.02	–0.2	0.18
7–8	0.03	–0.11	0.25
6–7	0.16	–0.05	0.3
6–8	0.2	0.14	0.25
1–5	0.1	0.02	0.17
2–6	–0.32	–0.36	–0.27
3–7	0.01	–0.21	0.16
4–8	–0.09	–0.16	–0.04

The posterior estimates (medians) of the effect sizes with the 95% credible intervals (CrI) from a posterior distribution of 5000 simulated values generated by the 'sim' function in R [33]. Variance components were estimated by the 'glmer' function for binomial errors with logit link function [34]. **1)** Estimates for particular factor combination levels (see Fig. 4). **2)** Estimates for selected contrasts (number in column "contrast" refers to level number in Table 1). Note that presented values were back-transformed. Those contrasts whose 95% credible intervals do not contain 0 are highlighted in bold

Discussion

In this study, we have revealed several aspects of partner communication in the Northern Lapwing during the incubation period: 1) females (but not males) combine acoustic and motion signals in an attempt to ask the partner for nest relief, and these signals, together with male willingness to exchange with the female, shape the length of the incubation recesses; 2) scarcity of male incubation at night is associated with a lower female vocalization effort, and also with lower male readiness to incubate; 3) the between-nest differences in male incubation effort are shaped by the willingness of the male to provide nest relief, rather than by the female vocalization effort. We discuss these topics below.

Use of signals

Unlike many other related species with biparental incubation [19, 39, 40], Northern Lapwings have an incubation rhythm that is characterized by frequent but relatively short incubation recesses, only a minority of which (i.e. 25% in our sample) serve as an exchange gap (Fig. 4). Some of the incubation recesses without nest relief therefore have other functions, e.g. leaving the nest unattended during a disturbance or a predator approach (and relying on nest crypsis) [41], a direct predator attack [41, 42], or just a short foraging break. For example, females often took a break around the noon, walked and foraged nearby the nest (our direct observations both in video recordings and in the field).

In addition to the reasons mentioned above, we suggest that a proportion of incubation breaks can also result from failures of the negotiation process about partner exchange at the nest [3]. We show that females had a far higher probability of being exchanged by a male when they vocalized shortly before departing from the nest, and this pattern was more obvious when the female flew away (though the pattern could still be observed when she walked away). This suggests that female vocalization could serve as a signal to the male partner requesting an exchange of incubation duties. The patterns in the length of incubation recesses were also consistent with our predictions; exchange gaps were shortened (i.e. better synchronized) whereas breaks (i.e. recesses without parent exchange) were prolonged when there was female vocalization. Thus, we can assume that when the male does not fulfil the female's exchange request, the female waits within the negotiation process for a considerably longer period, then returns to continue in incubation.

However, an alternative explanation can be put forward, at least in some events, i.e. that prolonged breaks after female vocalization can occur in cases when the female signals a perceived danger, such as an approaching predator, rather than a need to be exchanged. At the

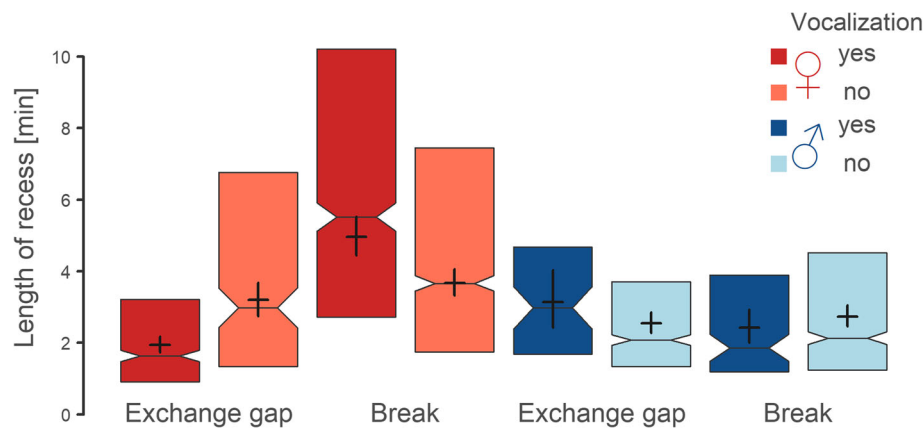


Fig. 5 Length of incubation recess in relation to sex, vocalization and type of a bird's departure. The boxplots summarize lengths of the recesses after female (red) and male (blue) incubation bouts, colour intensity indicates whether the bird vocalized before leaving the nest (dark colours) or did not (light colours). Recesses are classified either as an "Exchange gap" (parents exchanged during the incubation recess) or as a "Break" (the same parent returned and continued incubation). The median length of the recess is depicted by the vertical line inside the box, its 95% confidence interval by the notch, and the 25–75% quantiles by the box. The horizontal lines of black crosses denote estimates from the mixed effect model with nest identity as a random intercept (Table 2). The vertical lines indicate 95% credible intervals of the estimates

same time, the voice activity of the female often graduates for several minutes before she leaves the nest (see Fig. 1a), and such conspicuous behaviour in the presence of a predator could be counterproductive in terms of nest protection. Moreover, long female breaks after a disturbance (accompanied by vocalization), contrasting with really short female-to-male exchange gaps on the nest in the same situations, seem to be cumbersome and difficult to explain (Fig. 5). Finally, it seems improbable that there would be a rapid female-to-male exchange after a disturbance when the male-to-female exchange is slower, in a species where the main role of a male is to protect the territory from predators and the male participates considerably less than the female in incubation care (Fig. 5). There is a need for further studies to determine the roles of both alternatives suggested here, and their effects on the length of incubation recesses in avian incubation.

Table 3 Length of recess

Level	sex	Vocalization	Type of gap	Estimate	95% CrI	
					Lower	Upper
1	F	YES	EXCHANGE	1.94	1.74	2.17
2	F	NO	EXCHANGE	3.19	2.75	3.7
3	F	YES	RECESS	4.96	4.42	5.52
4	F	NO	RECESS	3.67	3.33	4.04
5	M	YES	EXCHANGE	3.15	2.45	4.03
6	M	NO	EXCHANGE	2.54	2.28	2.86
7	M	YES	RECESS	2.42	2.02	2.93
8	M	NO	RECESS	2.73	2.45	3.05

We documented also a considerable proportion of exchange gaps (33%; Fig. 2b) after female departure without previous vocalization. We cannot rule out that vocalization occurred in these cases immediately after leaving the nest, when the female was already out of camera view. On the other hand, it might indicate that the negotiation process also involves other signals, made away from the nest, but note that these exchanges were

Table 4 Length of recess

Contrast	Estimate	95% CrI	
		Lower	Upper
1–2	−1.25	−1.71	−0.85
1–3	−3.02	−3.46	−2.6
3–4	1.29	0.93	1.68
2–4	−0.48	−0.88	−0.03
5–6	0.6	−0.1	1.47
5–7	0.72	−0.07	1.65
7–8	−0.31	−0.74	0.17
6–8	−0.19	−0.46	0.07
1–5	−1.21	−2.05	−0.52
2–6	0.65	0.23	1.11
3–7	2.53	1.96	3.1
4–8	0.93	0.67	1.21

The posterior estimates (medians) of the effect sizes with the 95% credible intervals (CrI) from a posterior distribution of 5000 simulated values generated by the 'sim' function in R [33]. Variance components were estimated by the 'lmer' function in R [34]. **3**) Estimates for particular factor combination levels (see Fig. 5). **4**) Estimates for selected contrasts (number in column "contrast" refers to level number in Table 3). Note that response variable was log-transformed in the model, but presented values were back-transformed. Those contrasts whose 95% credible intervals do not contain 0 are highlighted in bold

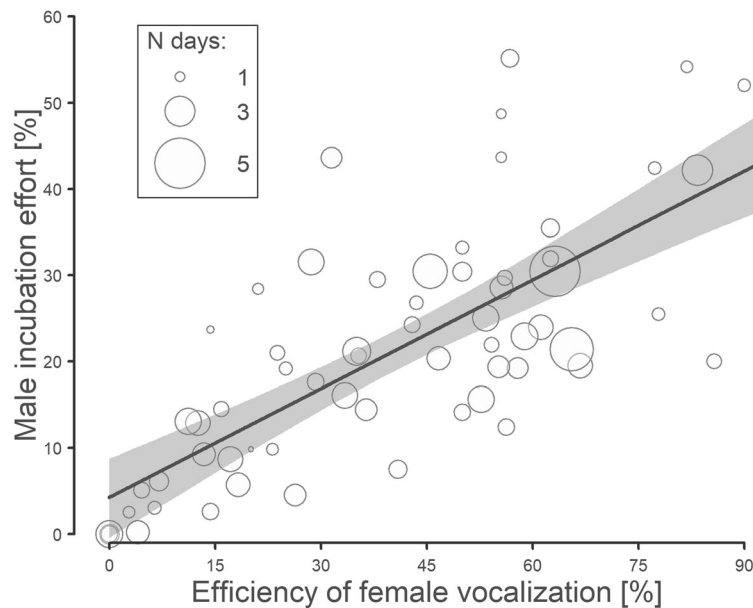


Fig. 6 The relationship between male incubation effort and the efficiency of female vocalization. The male incubation effort is taken as the ratio of male nest attendance at the nest to the overall time, for which the nest was attended by either of the parents. The efficiency of female vocalization is the proportion of female departures accompanied by her vocalization after which the male came to incubate (i.e. “Exchange gap” took place). Circles represent the individual nests and their size the number of days with incubation data. The line with shaded area indicates the model prediction with a 95% credible interval (Additional file 1: Table S5)

generally worse coordinated (Fig. 5). Some less common alternative ways of communicating, or failures of usual patterns regarding the exchange process, could exist in the Northern Lapwing, as is also found in other species. For example, although regular nest reliefs in Ringed Doves and Herring gulls (*Larus argentatus*) take place in the presence of both parents on the nest, it has been documented that some smaller proportion of the nest reliefs in these species are accompanied by exchange gaps [3, 20], even though such exchange gaps can be accompanied by a severely enhanced risk of egg depredation [21].

We observed different signalling patterns in males than in females. Vocalization was observed in only 11.6% of males, and was even accompanied by a decrease in the probability of an exchange gap. We suggest several possible explanations for this different pattern. Firstly, males may not need any specific requesting signal to negotiate an exchange with the female partner. As parental exchange occurs much more often after male departure than after female departure, the departure of a male who generally incubates less than the female can itself serve as a signal for the female to negotiate an exchange, even without a male call. Furthermore, Lapwing male acoustic signalling during incubation may serve primarily as a warning in response to an approaching predator [43]. We know that Lapwings avoid incubating in the presence of a predator, leaving the nest for the

necessary period of time and relying on egg crypsis [41]. The male behaviour described here may therefore be seen as an aspect of the key role of the male in guarding the nest against predators. This could explain why males more frequently fly away from the nest than walk away from it, which would enable the male to attack the predator faster and more effectively [43].

Our findings could suggest that, in contrast with most of the previously studied species [3, 4, 7, 12, 20], the timing of nest reliefs in Northern Lapwings might be induced by the bird that is currently incubating, particularly by females. However, revealing who really initiates the exchange on the nest would require simultaneous recording of both partners (on the nest and away from it), which is a topic requiring further observational research.

Night incubation

Females greatly lowered their vocalization effort before departing from the nest in the night. This could be because male incubation in the night is very rare in the Northern Lapwing [25, 28, 44], and thus the possibility of being exchanged can be negligible for a female. However, despite the overall scarcity of male night incubation in our sample (17 cases), the probability of an exchange gap after female vocalization during nest departure was still almost 20% (in comparison with 35% during the day), while it was reduced to only 1.6% after a “silent

departure” (in comparison with 15% during the day). Thus, although the males showed significantly lowered willingness to provide night nest relief, there was still a substantial chance for a female to get male help on the nest in the night after vocalization signalling.

So, why did the females lower their vocalization efforts so much in the night? We suggest that this pattern could mirror the response to increased predation pressure during the night, when mammalian predators are most active ([45, 46]; own observation). This explanation is justified by the observation that the nests of Northern Lapwings are depredated almost solely by nocturnal mammals ([47]; all 11 cases of known depredations in the study population). Firstly, vocalization during the night can attract nest predators, and females may face a trade-off between sitting quietly for most of the night and loudly highlighting the position of her nest. Our results indicate that most females probably prefer to bear the incubation bout for a whole night in order to be as inconspicuous as possible. Secondly, it could be more beneficial for females to leave the vigilant males to guard the nest in the night, rather than to ask for exchange. In future research, we therefore propose to test the significance of acoustic cues, such as bird calling, on mammal predator orientation in the night. We also need to describe Northern Lapwing male behaviour in the night, with respect to their ability to warn the sitting female about the approach of a predator, which is a strong characteristic feature of Lapwing males during the day [29, 42].

Between-nest differences in male incubation attendance

As can be found elsewhere [24, 26, 28], the male contribution to incubation is a strong predictor of overall nest attendance in the Northern Lapwing. This could be because of female energy limitations to fully compensate reduced male care [48], or it could be a result of negotiations over parental care [1]. Predictions from theoretical models assume that an evolutionarily stable strategy in response to the reduced parental effort of one partner is for the other partner to compensate to some extent ([1, 49, 50], but see: [51]). This explanation has also been supported by empirical data [52, 53]. Our study suggests a possible extending of this previous knowledge with a new finding in the behaviour of partners in this mechanism: it was found that better incubating males were more willing to come and incubate after the female had signalled her departure from the nest, but that the female signalling effort itself did not affect the extent of male care in a particular nest. This finding, together with the fact that the subsequent recess is longer if a female “exchange request” is not fulfilled by the male, suggests that it is the negotiation process associated with the fine-tuning between the partners that can influence the

total nest attendance, rather than an energetic constraint [1]. On the basis of our data, we are not able to quantify the importance of this partnership mechanism and to compare it with the effect of energetic constraints. However, the negotiation process resulting from tuning and compliance between the partners appears to be a possible proximate mechanism that modifies the overall incubation attendance in biparentally nesting birds.

Conclusion

To conclude, we have documented that, in a territorial species capable of continuous communication between the partners during incubation, vocal and motion signals could be used for better synchronization of nest relief. Because it seems that the effectiveness in negotiating about exchanging parental duties influences the length of incubation recesses, we have also suggested how the negotiation process could influence overall nest attendance. Since we found vocalization signalling only in females, we suggest that behavioural signals serving parental cooperation and negotiation in birds can be sex-specific.

Additional file

Additional file 1: Figure S1. Frequency of female hourly vocalization in relation to male incubation effort. **Figure S2.** Daily pattern of flight away from the nest during a bird's departure. **Table S1.** The relationship between male incubation attendance and female vocalization effort during the incubation. **Table S2.** Patterns of probability of vocalization. **Table S3.** The probability of flight away during departure. **Table S4.** Circadian pattern of female exchange requesting. **Table S5.** Between nest differences in male contribution to incubation. (DOCX 132 kb)

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Availability of data and materials

The raw data and R codes that enabled replications of all analyses performed in this paper, and that were also used for generating all of the figures, are available from Open Science Framework: <https://osf.io/zmd8g/>.

Authors' contributions

MS, EV and MŠ collected the data; MS EV and KB extracted the incubation from videos, MS analysed the data and wrote the paper, with input from MŠ. All authors read, commented on and approved the final manuscript.

Ethics approval and consent to participate

The study was conducted in accordance with the current laws of the Czech Republic, where it was performed under the ringing licence (1082) of the Czech Ornithological Society.

Competing interests

The authors declare that they have no competing interests.

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SUPPLEMENTARY INFORMATION for

Parental incubation exchange in a territorial bird species involves sex-specific signalling

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Figure S1.

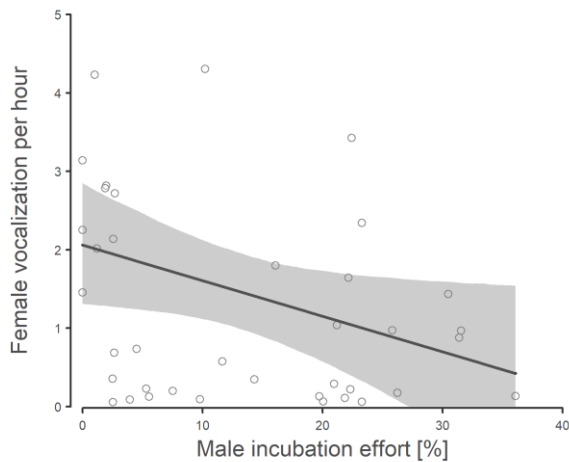


Figure S1 Frequency of female hourly vocalization in relation to male incubation effort. Male incubation is taken as the proportion of male nest attendance from the overall time for which the nest was attended by any parent (i.e. excluding all incubation recesses). Frequency of female vocalization is a mean number of female “vocal sessions” per hour of female incubation. Calls separated by at least 30 seconds of silence are considered as two discrete sessions. Presented data include complete 24h-day of incubation footage for 40 nests. Ten out of these nests are a part of other data presented in this paper, while 30 of them were recorded using the same method within the same area in 2015. Line with shaded area indicates the model prediction with 95% credible intervals (Tab. S1). Circles represent the individual nests.

Figure S2.

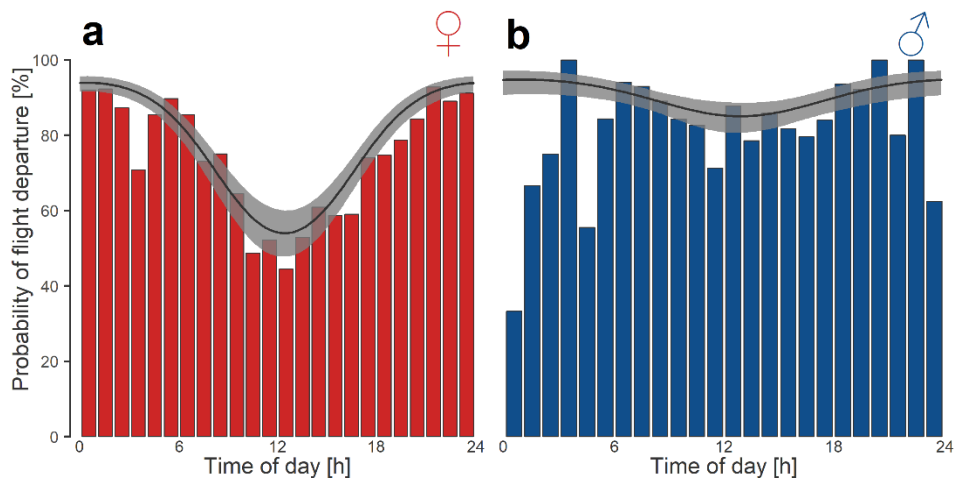


Figure S2 Daily pattern of flight away from the nest during a bird's departure. Bars depict the real probability that female (red) or male (blue) left the nest by flight (i.e. not by walking away), within a particular hour. Lines with shaded areas indicate the model prediction with 95% credible intervals (Table S3).

Supplementary table S1 | The relationship between male incubation attendance and female vocalization effort during the incubation.

Response	Effect	Estimate	95% CrI	
			Lower	Upper
Female vocalization effort	Intercept	2,075	1,319	2,863
	Male incubation effort	-4,695	-9,332	-0,05

The posterior estimates (medians) of the effect sizes with the 95% credible intervals (CrI) from a posterior distribution of 5,000 simulated values generated by the 'sim' function in R[1]. Variance components were estimated by the 'lm' function in R. Estimates whose 95% credible intervals do not contain 0 are highlighted in bold. Male incubation is taken as the proportion of male nest attendance at the nest to overall time for which the nest was attended by any parent (i.e. excluding all incubation recesses). Female vocalization is then taken as mean number of female "vocal sessions" per hour of female incubation. As two discrete vocal sessions are taken the calls separated by at least 30 s without calling. Presented data include complete 24 day incubation footage for 40 nests. 10 out of these nests are also a part of other data presented in this paper, while 30 of them were recorded with the same method and within the same area during 2015.

Supplementary table S2 | Patterns of probability of vocalization

a

Level	sex	Type of departure	Estimate	95% CrI	
				Lower	Upper
1	F	FLIGHT	0.52	0.48	0.57
2	F	WALK	0.18	0.15	0.21
3	M	FLIGHT	0.11	0.09	0.13
4	M	WALK	0.06	0.04	0.1

b

Contrast	Estimate	95% CrI	
		Lower	Upper
1-2	0.34	0.31	0.38
1-3	0.42	0.38	0.46
3-4	0.05	0.01	0.08
2-4	0.12	0.08	0.16

The posterior estimates (medians) of the effect sizes with the 95% credible intervals (CrI) from a posterior distribution of 5,000 simulated values generated by the 'sim' function in R[1]. Variance components were estimated by the 'glmer' function for binomial errors with logit link function and nest identity as a random intercept [2]. **a)** Estimates for particular factor levels combinations (see Fig. 1a). **b)** Estimates for selected contrasts (number in column "contrast" refers to level number in Table 2a). Note that presented values were back-transformed; contrasts whose 95% credible intervals do not contain 0 are highlighted in bold.

Supplementary table S3 | The probability of flight away during departure

Response	Effect type	Effect	Estimate	95% CrI	
				Lower	Upper
Probability of flight departure	Fixed	Intercept	1.450	1.264	1.635
		Sex (M)	0.870	0.598	1.143
		Sin (24 time)	0.171	0.022	0.328
		Cos (24 time)	1.278	1.029	1.519
		Sex(M) x Sin (24 time)	-0.025	-0.261	0.215
		Sex (M) x Cos (24 time)	-0.717	-1.103	-0.335
	Random (variance)	Nest (Intercept)	32%		
		Sin (24 time)	13%		
		Cos (24 time)	54%		

The posterior estimates (medians) of the effect sizes with the 95% credible intervals (CrI) from a posterior distribution of 5,000 simulated values generated by the 'sim' function in R[1]. Variance components were estimated by the 'glmer' function for binomial errors with logit link function[2]. Variable 'time' was transformed to radians ($2 \cdot \text{time} \cdot \pi / \text{period of interest: 24h}$) and fitted as sine and cosine of radians. Note that presented estimates are logit-transformed.

Supplementary table S4 | Circadian pattern of female exchange requesting

Response	Effect type	Effect	Estimate	95% CrI	
				Lower	Upper
Exchange request effort	Fixed	Intercept	-0.576	-0.770	-0.387
		Sin (24 time)	0.448	0.297	0.593
		Cos (24 time)	-0.607	-0.902	-0.306
		Sin (12 time)	0.230	0.104	0.352
		Cos (12 time)	-0.653	-0.792	-0.515
	Random	Nest (Intercept)	26%		
	(variance)	Sin (24 time)	9%		
		Cos (24 time)	65%		

The posterior estimates (medians) of the effect sizes with the 95% credible intervals (CrI) from a posterior distribution of 5,000 simulated values generated by the 'sim' function in R[1]. Variance components were estimated by the 'glmer' function for binomial errors with logit link function[2]. Variable 'time' was transformed to radians ($2 * \text{time} * \pi / \text{period of interest: 12 or 24h}$) and fitted as sine and cosine of radians. Note that presented estimates are logit-transformed. Estimates whose 95% credible intervals do not contain 0 are highlighted in bold.

Supplementary table S5 | Between nest differences in male contribution to incubation

Response	Effect	Estimate	95% CrI	
			Lower	Upper
Proportion of male nest attendance	Intercept	0.204	0.183	0.225
	Exchange request effort	0.017	-0.006	0.040
	Exchange request efficiency	0.100	0.077	0.122

The posterior estimates (medians) of the effect sizes with the 95% credible intervals (CrI) from a posterior distribution of 5,000 simulated values generated by the 'sim' function in R[1]. Variance components were estimated by the 'lm' function in R. Both predictors were z-transformed (mean-centred and divided by SD). The model was weighted by square root of monitored time. Estimates whose 95% credible intervals do not contain 0 are highlighted in bold.

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Chapter 4

Daily rhythmicity of female self-maintenance activities during parental care is affected by predation risk and incubation attendance by male in a biparental shorebird

Kateřina Brynychov, Martin Sldeek, Eva Vozabulov & Miroslav E. řlek

Reproduction in birds is a highly time-consuming period leading to the trade-off between benefits from increased parental care and the predation risk as well as the costs of limited time for individual's self-maintenance behavior. In many bird species the incubation includes cooperation of both parents whose tuning of incubation rhythm and mutual assistance can affect reproduction output. Although incubation rhythms themselves were subject of considerable attention, the rhythmicity of self-maintaining activities remains almost unstudied, the rather in a context of predation risk. Yet, it can be subject of strong selection with far-reaching fitness consequences for incubating parents. Using continuous video-recording of incubating parents we investigated daily rhythmicity of sleeping and body care (i.e. "preening") by females of the Northern Lapwing (*Vanellus vanellus*), a wader species with high variation in contribution of males to incubation as well as predation pressure on nests. We suggested that sleeping leads to a decrease of susceptibility to predators and preening can decrease the crypsis of female on the nest (i.e. make the nest more detectable by predators), so the females try to avoid these activities during the time of increased predation risk. Consistently, both activities were inhibited during the night, when high risk of predation occurs. Furthermore, we tested whether higher male contributions will lead to lower occurrence of female self-maintenance activities during incubation (i.e. as a consequence of increased time space for pursuing both activities during incubation gaps). This was true for preening, but we found opposite relationship between male incubation effort and female sleeping. We conclude that predation risk strongly shapes rhythmicity of self-maintaining activities during incubation, which enhances the importance of male contribution to incubation. Thus, the consequences of being paired with less care-giving males could be more far-reaching than simply reducing of provided parental care.

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Daily rhythmicity of female self-maintenance activities during parental care is affected by predation risk and incubation attendance by male in a biparental shorebird

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Abstract

Reproduction is a highly time-consuming period leading to the trade-off between benefits from increased parental care and costs due to predation risk and limited time for own self-maintenance care. In many bird species the incubation includes cooperation of both parents whose tuning of incubation rhythm and mutual assistance can affect reproduction output. Although incubation rhythms themselves were subject of considerable attention, the rhythmicity of self-maintaining activities remains almost unstudied, the rather in a context of incubation. Yet, it can be subject of strong selection with far-reaching fitness consequences for incubating parents. Using continuous video-recording of incubating parents we investigated daily rhythmicity of sleeping and body care by females of the Northern Lapwing (*Vanellus vanellus*), a wader species with high variation in contribution of males to incubation as well as predation pressure on nests. We suggested that sleeping leads to a decrease of susceptibility to predators and preening can decrease the crypsis of female on the nest making the nest more detectable, so the females should avoid these activities during the time of increased predation risk. Consistently, both activities were inhibited during the night, when high risk of predation occurs. Furthermore, we tested whether higher male contributions will lead to lower occurrence of female self-maintenance activities during incubation (i.e. as a consequence of increased time space for pursuing both activities during incubation gaps). This was true for preening, but we found opposite relationship between male incubation effort and female sleeping. We conclude that predation risk strongly shapes rhythmicity of self-maintaining activities during incubation, which enhances the importance of male contribution to incubation. Thus, the consequences of being paired with less care-giving males could be more far-reaching than simply reducing of provided parental care.

Introduction

Behavior of wild animals is thought to be a subject of strong rhythmicity with respect to diverse external conditions (Dunlap et al. 2004, Bulla et al. 2016a). In addition, this rhythmicity often needs to be synchronized among the partners during reproduction. Particularly in birds, the reproduction usually includes time-consuming egg incubation consisting in long-lasting sitting on the nest. But at the same time, the parents must address self-maintenance behavior such as foraging,

sleeping and body care (Cotgreave and Clayton 1994). As these activities may increase risk of depredation, the most prevalent cause of nest failures (Ricklefs 1969), incubating parents have to solve the trade-offs associated with care for offspring and self-maintenance requirements. Potential drivers which can mitigate the predation risk arising from these trade-offs is to subordinate the behavioral rhythms on nests to the rhythm of predation pressure if it exists and to rely on partner's assistance. Although there are many studies showing high proportion of the time spent with self-maintaining activities pointing to their importance in animal kingdom (Amlaner and Nigel 1983, Cotgreave and Clayton 1994, Roth et al. 2006), studies of rhythmicity in self-maintenance behaviors during incubation in relation to timing of predation risk and parental assistance does not exist. If such rhythms and relationships exist, we have to assume that they influence animal reproduction and should not be thus overlooked in complex studies aimed in indicators of individual fitness.

Self-maintenance behavior including sleep and preening addresses necessary animal life requirements (Van Iersel and Bol 1957, Steinmeyer et al. 2010). From behavioral viewpoint, sleep is a temporary and rapidly reversible state of reduced susceptibility to surrounding stimuli having important restorative function (Siegel 2003, Lima et al. 2005). Sleeping birds suffer from a decreased attention, often with adverse consequences resulting in higher vulnerability to predation (Lima et al. 2005, Lima and Rattenborg 2007). On the other hand, sleeping birds are inconspicuous, which leads in some species in increasing of sleeping effort in reaction to perceived predation risk (Zimmer et al. 2011). Sleep fluctuates widely from more than half of a day to a few hours (Roth et al. 2006), although there are also extreme cases of adaptive temporary sleep loss (Lesku et al. 2012). In fact, various species substantially differ in the extent, to which they are flexible in timing of sleep (e.g. Hamilton et al. 2002, Chudzinska et al. 2013). Birds also need to spend a big proportion of time by preening of feathers by bill (Delius 1988, Cotgreave and Clayton 1994, Włodarczyk 2017), which is necessary for feather maintenance, preen oil distribution and checking ectoparasites (van Rhijn 1977, Delius 1988). Performing preening supposedly reduces vigilance of an individual and makes it much more visible to predators (Smith et al. 2012). Timing of preening is probably very flexible (Delius 1988) which could enable individuals to time the preening to the periods when it is less risky.

Parental duties are time consuming and energetically demanding (Thomson et al. 1998) and force the parents to deal with the trade-offs between investments into the offspring and own needs. In biparentally incubating birds, a common form of avian parental care (Deeming 2002), both parents need to synchronize their activities in order to minimize the time for which the nest is left unattended (Sládeček et al. 2019a). At the same time, however, they are restricted in time for pursuing self-maintaining activities. Since a big proportion of the off-nest time of each partner is necessary to spend by foraging, there can be hard decision making about which activities pursue during incubation (and when) and which during the

off-nest time. Consequently, when a substantial variation in division of incubation duties between the parents exists, the contribution of a partner (typically a male) can play an important role in this process. Particularly, higher rate of male contribution can enable female to pursue more sleep and preening time during the off-nest time, and thus stay more vigilant and inconspicuous during incubation.

To our knowledge, a study revealing a possible rhythmicity of self-maintenance activities of incubating birds and linking it with rhythmicity of predation risk and parenting effort has not yet been conducted on wild animals. Generally, studies of rhythmicity of self-maintenance activities are overall scarce, the more describing these patterns in incubating birds. Also, continuous observations of more individuals through the whole 24-hour day are lacking. In this study, we analyzed behavioral patterns from continuous video-recordings of incubating Northern Lapwings (*Vanellus vanellus*), a biparentally incubating wader species with great variability in contribution of male to incubation among the nests (Sládeček et al. 2019b). Consequently, there are females sitting on the nest only around 50% of their time while others have nest attendance close to 90%. Given the huge between-females differences in time schedules it is justified to assume that high female incubation attendance will prevent females to satisfy their sleeping or preening needs during their off-nest time. Lapwings breeds visibly in open fields, where they are able to efficiently deteriorate visually oriented predators (Elliot 1985a, Kis et al. 2000) . On the contrary, as a ground-nesting species, Northern Lapwings are highly vulnerable in the night due predation risk by medium-sized mammals as Red Fox (*Vulpes vulpes*) or martens (*Martes* sp.) (Seymour et al. 2003). Due to this fact and because the night incubation is almost exclusively female activity (Jongbloed et al. 2006, Sládeček and Bulla 2018, Sládeček et al. 2019b), we may expect different behavioral patterns of incubating females during daytime and night. In addition, a lack of male care should be accompanied by increased amount of time spent with self-maintenance behavior, potentially increasing predation risk.

First, we questioned whether sleeping and preening are subject to an apparent daily rhythm or whether they are incidental during incubation. Second, we predicted that timing of these activities will be controlled by the risk of depredation, that is that females will pursue sleeping and preening mostly during the periods with lower predation pressure (i.e. during daylight). Third, we hypothesized that females paired with more care-giving males are able to better maximize their vigilance on the nest and thus endeavor to reduce these activities during the incubation bouts. Thus, we predicted that duration of both activities on the nest will be negatively correlated to the male incubation effort.

Methods

During 2016-2016 (March – June), we monitored 55 nests of Northern Lapwing in the area of České Budějovice basin, Czech Republic (49° 15'N, 14° 05'E). Using small cameras placed approximately 1.5 m from the nest, we obtained continuous video recording which allowed us detail analysis of behavior of incubating birds (Sládeček et al. 2019b). For the purpose of this paper, we choose one day of complete record (i.e. 24 hours of recording without any interruption) for each nest.

We analyzed all target behavioral traits from recordings using software Boris version 6.3 (Friard and Gamba 2016), with precision on one second. First, we determined beginnings and ends of all incubation bouts, taken as the time when bird stands by both legs in the nest. Within the pair we identified the sex of incubating bird, using set of sex-specific plumage traits, such as the crest length, and the extent of melanin-based ornaments on the face and breast which are well identifiable features. (Meissner et al. 2013, Schonert et al. 2014).

Second, we record beginnings and ends of all sleeping bouts. Out of the two sleeping postures described elsewhere (Amlaner and Nigel 1983, Dominguez 2003, Gauthier-Clerc and Tamisier 2012), birds predominantly slept with head turned backward and partly hidden between shoulder coverts, but rarely (i.e. ~ 2% of sleeping bouts) also with drooping head and bill forward. Note, that while staying in sleeping position, bird often opened one eye for short time and scanned the surrounding. Although we didn't use EEG, this behavior is very probably connected to unihemispheric slow-wave sleep, frequently reported in birds elsewhere (Rattenborg et al. 1999, 2000, Roth et al. 2006). Thus, these periods were included into the sleeping bouts.

Third, we recorded feather preening, defined as rapid bill movements towards the plumage and preen gland (van Rhijn 1977). Since this activity is often done in clusters of many one or several seconds long bouts, intermittent by the breaks of similarly short lengths, we recorded this behavior as point events, separated at least by 30 seconds. That is, that when we recorded preening, another record has been done at least 30 seconds after that.

To assess the daily patterns of predation pressure, we merged data from all known depredation events in study area with known timing within the day. This included all predation events shot on cameras (n = 21), and recordings from temperature dataloggers (n = 23), or RFID dataloggers (n = 6), where we assume that approximate time of depredation is the time of last recorded incubation. Because we assume, that pattern of predation pressure will be similar in the second studied species, the Little Ringed Plover (*Charadrius dubius*), we strengthen our dataset with 17 known predation events from nests of this species. Unfortunately, similar datasets of known predation time of bird nests are extremely rare elsewhere, but the resulting pattern is in approximate agreement with known patterns of

predation on Northern Lapwing nests (Macdonald and Bolton 2008, Mason et al. 2018).

Statistical analysis

All procedures were performed in R version 3.5.0 (R-Core-Team 2017). General linear mixed-effects models were fitted using the 'lmer' function from the 'lme4' R library (Bates et al. 2015). For all model-based parameter estimates we report the effect sizes as a median and Bayesian 95% credible interval (95%CrI) based on the posterior distribution of 5000 values simulated by the 'sim' function from the 'arm' R library (Gelman et al. 2016).

In all models, we used similar set of predictors. To test for the daily rhythmicity in a response, we transformed the time to radians ($2 \cdot \text{time} \cdot \pi / \text{period}$ of supposed rhythmicity) and fitted sine and cosine of radians (Bulla et al. 2016). As a period of rhythmicity, we used either 24, or 12-hour cycle, based on obvious visualized pattern and controlled with the model AIC. As a measure of male contribution to incubation we used the ration of male nest attendance to the overall time for which the nest has video recorded. That is, that this proportion is made from longer time period, than the data about sleeping and preening, since only subset of the video recordings was used for detail analysis of these features. We used the whole period in order to make better estimates of real male contribution to incubation. However, note that male incubation effort is highly repeatable among days (Sládeček et al. 2019b). As a proxy of predation pressure, we used simply the number of depredation events known from our datasets during a particular hour of day. In all models, we include also the interaction between the male incubation effort and a time of day. All predictors (except for time) were z-transformed (mean centered and divided by standard deviation) (Schielzeth 2010). In all models, we fitted nest identity as a random intercept, and time predictors, as well as predation rate also as a random slopes (Schielzeth and Forstmeier 2009).

To explain the variation in females sleeping behavior, we used two mixed-effect models. In first, we explained a ratio of the female sleeping to the overall time for which she attended the nest within the particular hour ("**sleeping effort**"). That is, that hours when female does not incubate were not included into this analysis. We weighted the model by square root of the female incubation time during particular hour. Note also, that an alternative way, explaining the absolute time of sleeping, regardless the female nest attendance during the hour gives similar results (supplementary table S1). In the second model, we used the length of sleeping bouts as an explanatory variable.

As an explanatory variable for describing variation of preening effort, we used number of preening records, divided by the female incubation attendance within particular hour ("**preening effort**"). Since the new record of preening has been taken when this behavior occurs at least 30 second after previous record, this measure approximately corresponds to the probability that some preening occurs within 30 seconds of female incubation. For this analysis we used only hours with

more than 10 minutes of female incubation, and we weighted the model by square root of the female incubation time during particular hour.

Test for potential confounding effects

In order to avoid misinterpretations of results, we first define and test the effect of potentially confounding effects of timing of the nest within the breeding season and timing of video-recordings within the incubation period. Both predictors have no substantial effect neither on sleeping behavior (Supplementary table S2), but the timing within the incubation period has positive effect on the frequency of preening (Supplementary table S3).

Results

Variation in samples

A total of 55 nests with a complete record day were monitored. Within this time females spent incubating 17.8 +/- 2.7 hours (mean +/- SD, range: 6.5 - 21.8 hours), while males 2 +/- 1.6 hours (mean +/- SD, range: 0 – 6.4 hours). Thus, nests were unattended by either of parents for 4.1 +/- 2.3 hours (mean +/- SD , range: 0.8 – 14.7 hours) (for detail description of Lapwing incubation pattern see (Sládeček et al. 2019b)).

We collected timing of 50 depredation events from our study area. Predation events were much more frequent during the night hours, while almost absent during the midday and afternoon (Fig 1c). The identified predators detected from video recordings were the Red Fox (*Vulpes vulpes*) (n = 15), Stone Marten (*Martes foina*) (n = 4), European badger (*Meles meles*) (n=1) and Wild Boar (*Sus scrofa*) (n = 1).

Sleeping

In general, females slept 3.0 +/- 1.8 hours (mean +/- SD, range: 13 minutes – 7.0 hours) which corresponds to 17.5% of their incubation time (mean, range: 1.2% – 45%). However, this time has been divided mostly into very short sleeping bouts, with median length only 1.7 minutes (range: 3 seconds – 1 hour). Sleeping effort showed strong bimodal rhythmicity during the day, with maximums during morning and late afternoon, and minimums during the midday and especially during the night (Table 1; Figure 1a,b, Supplementary Figure S1). In addition, intensity of predation pressure lowered the occurrence of sleeping (Table 1; Figure 1c). Bimodality of female sleeping effort has been connected to the male contribution to incubation. Contrary to our expectations, females with higher help from their males slept more, and the bimodal nature of their sleeping rhythm has been stronger, than in females with low, or no help (Table 1; Figure 1b, Supplementary Figure S1).

In contrast with the sleeping effort, lasting of particular sleeping bouts shows unimodal rhythmicity with maximum during the night and minimum during midday

(Table 2; Figure 2). This pattern was unaffected by male incubation, with no clear additive effect of predation pressure (Table 2).

Table 1 | Circadian pattern of female sleeping

Response	Effect type	Effect	Estimate	95% CI	
				Lower	Upper
Female sleeping effort	Fixed	Intercept	0.182	0.157	0.209
		M incubation	0.056	0.032	0.082
		Sin (12 hours)	0.038	0.008	0.067
		Cos (12 hours)	-0.089	-0.115	-0.063
		Predation	-0.037	-0.063	-0.011
		M incubation : Sin (12 hours)	0.036	0.006	0.066
		M incubation : Cos (12 hours)	-0.033	-0.058	-0.008
		Random (variance)	Nest (Intercept)	11	
		Sin (12 hours)	13		
		Cos (12 hours)	7		
		Predation	11		
	Residual	58			

The posterior estimates (medians) of the effect sizes with the 95% credible intervals (CI) from a posterior distribution of 5,000 simulated values generated by the 'sim' function in R (Gelman et al. 2016). Variance components were estimated by the 'lmer' function (Bates et al. 2015). Response variable was relative proportion of sleeping within the incubation time, during particular hour of day. Time was taken as "hour of day" transformed to radians ($2 \times \text{hour} \times \pi / \text{period of interest} - 12\text{h}$) and fitted as sine and cosine of radians. Continuous predictors and the random slope (except for time) were z-transformed (mean-centered and divided by SD). Model was weighted by square root of female incubation time during the hour. Estimates whose 95% credible intervals don't contain 0 are highlighted in bold.

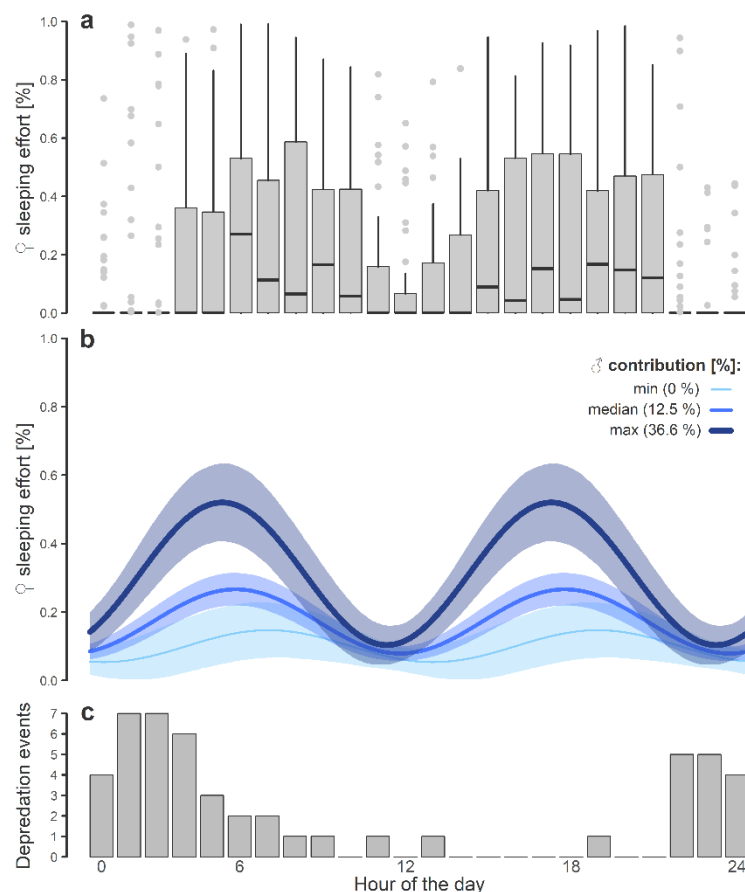


Figure 1. Daily changes in female sleeping effort. **a**) Variation in female sleeping effort during the day. Boxplots depict median (vertical line inside the box), 25–75th percentiles (box), 25th and 75th percentiles minus or plus 1.5× interquartile range, respectively, or the minimum and maximum value, whichever is smaller (whiskers) and outliers (circles). **b**) Predicted daily rhythm of female sleeping effort with respect to the male contribution to incubation. Curves with shaded areas indicate model prediction with 95% credible intervals based on the joint posterior distribution of 5,000 simulated values from model outputs (Table 1) and generated by the 'sim' function in R (Gelman and Hill 2007). **c**) Daily pattern of predation pressure. bars depict the number of predation events known from our study area during the particular hour of the day.

Table 2 | Circadian pattern in length of female sleeping bouts

Response	Effect type	Effect	Estimate	95% CI	
				Lower	Upper
Length of sleeping bouts	Fixed	Intercept	0.071	0.060	0.082
		M incubation	0.004	-0.007	0.014
		Sin (24 hours)	0.014	0.007	0.021
		Cos (24 hours)	0.03	0.017	0.042
		Predation	0.008	0	0.016
		M incubation : Sin (24 hours)	0.002	-0.004	0.007
	M incubation : Cos (24 hours)	-0.001	-0.012	0.01	
	Random (variance)	Nest (Intercept)	17		
		Sin (24 hours)	4		
		Cos (24 hours)	17		
		Predation	4		
		Residual	58		

The posterior estimates (medians) of the effect sizes with the 95% credible intervals (CI) from a posterior distribution of 5,000 simulated values generated by the ‘sim’ function in R (Gelman et al. 2016). Variance components were estimated by the ‘lmer’ function (Bates et al. 2015). Response variable was relative proportion of sleeping within the incubation time, during particular hour of day. Time was taken as “hour of day” transformed to radians ($2 \times \text{hour} \times \pi / \text{period of interest} - 12\text{h}$) and fitted as sine and cosine of radians. Continuous predictors and the random slope (except for time) were z-transformed (mean-centered and divided by SD). Estimates whose 95% credible intervals don’t contain 0 are highlighted in bold.

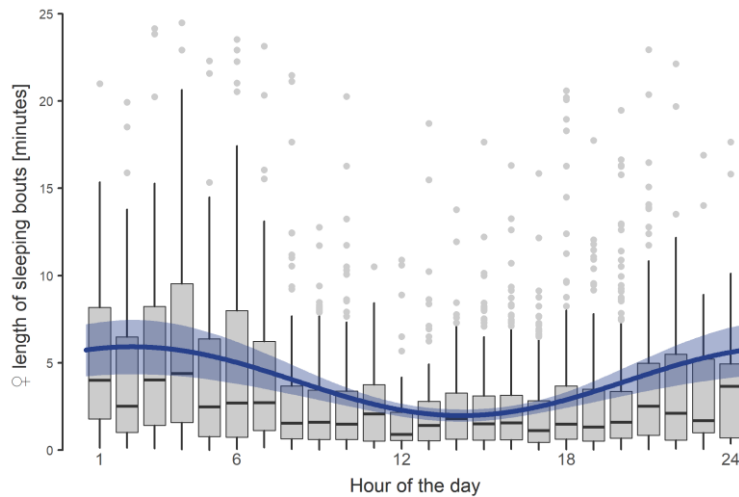


Figure 2. Daily changes in the length of female sleeping bouts. Boxplots depict median (vertical line inside the box), 25–75th percentiles (box), 25th and 75th percentiles minus or plus 1.5× interquartile range, respectively, or the minimum and maximum value, whichever is smaller (whiskers) and outliers (circles). The curve with shaded area indicates model prediction with 95% credible intervals based on the joint posterior distribution of 5,000 simulated values from model outputs (Table 2) and generated by the ‘sim’ function in R (Gelman and Hill 2007).

Preening

The median frequency of preening was 5.5 per hour of incubation, however it ranged from 0 to more than 50. This frequency shows unimodal rhythm, with maximum during midday and minimum during the night (Table 3; Figure 3a,b). In contrast with the sleeping, the more male helped the female with incubation, the less frequency of preening the female had during incubation (Table 3, Figure 3b, Supplementary Figure S2). Also, the daily pattern was more prone in females with lower assistance from male (Table 3, Figure 3b, Supplementary Figure S2). Similarly like in sleeping effort, we found additive negative effect of predation pressure on the frequency of preening (Table 3). Note, that controlling the model on the day of incubation period (potential confounding effect identified from methods; Supplementary table S3) yield quantitatively similar results.

Table 3 | Circadian pattern of female preening

Response	Effect type	Effect	Estimate	95% CI	
				Lower	Upper
Female preening frequency	Fixed	Intercept	7.932	6.762	9.095
		M incubation	-1.522	-2.655	-0.391
		Sin (24 hours)	-0.245	-1.319	0.765
		Cos (24 hours)	-4.547	-5.856	-3.232
		Predation	-1.04	-1.843	-0.223
		M incubation : Sin (24 hours)	0.262	-0.466	0.986
	M incubation : Cos (24 hours)	1.65	0.581	2.69	
	Random (variance)	Nest (Intercept)	21		
		Sin (24 hours)	13		
		Cos (24 hours)	17		
		Predation	5		
Residual		45			

The posterior estimates (medians) of the effect sizes with the 95% credible intervals (CI) from a posterior distribution of 5,000 simulated values generated by the 'sim' function in R(Gelman et al. 2016). Variance components were estimated by the 'lmer' function(Bates et al. 2015). Response variable was taken as frequency of female preening extrapolated to the whole hour. Only hours with at least 10 minutes of female incubation has been taken into account. Time was taken as "hour of day" transformed to radians ($2 \cdot \text{hour} \cdot \pi / \text{period of interest} - 24\text{h}$) and fitted as sine and cosine of radians. Continuous predictors and the random slope (except for time) were z-transformed (mean-centered and divided by SD). Model was weighted by square root of female incubation time during the hour. Estimates whose 95% credible intervals don't contain 0 are highlighted in bold.

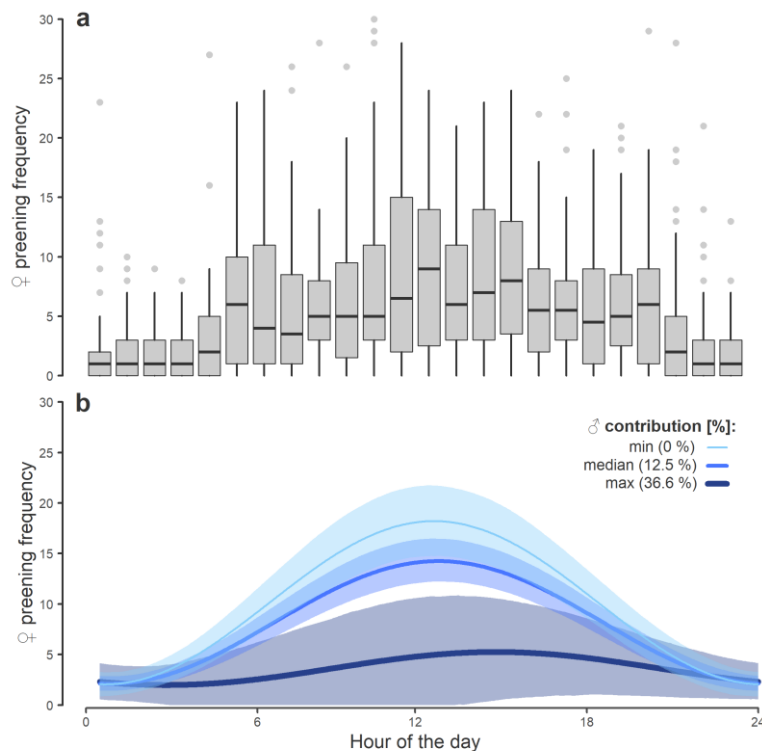


Figure 3. Daily variation in frequency of female preening during incubation. a) Variation in female preening during the day. Boxplots depict median (vertical line inside the box), 25–75th percentiles (box), 25th and 75th percentiles minus or plus 1.5× interquartile range, respectively, or the minimum and maximum value, whichever is smaller (whiskers) and outliers (circles). b) Predicted daily rhythm of female preening with respect to the male contribution to incubation. Curves with shaded areas indicate model prediction with 95% credible intervals based on the joint posterior distribution of 5,000 simulated values from model outputs (Table 1) and generated by the 'sim' function in R (Gelman and Hill 2007).

Discussion

Using continuous video-recordings of breeding Northern Lapwings, we confirmed a strong rhythmicity of female self-maintenance behavior during incubation. In addition to the clear patterns in accordance with the alternation of day and night, we revealed two factors playing a crucial role in shaping this rhythmicity. First, we showed that both activities were suppressed particularly during those hours of the night that are characteristic with a high risk of nest and adult predation by night-active mammalian predators. Second, we showed that male contribution to incubation affected intensity of female self-maintenance behavior on the nest. Specifically, increased male incubation effort led to increased female sleeping time but, in contrast, to reduced time spent with preening.

Diversity of behavioral rhythms

The three measured behavioral traits, the preening effort, sleeping effort and length of sleeping bouts, demonstrated three different rhythms. Both preening effort and sleeping effort achieved maximum during the day, but the sleeping bouts were longest during the night. Whereas the sleeping effort peaked in the early morning and before sunset, the preening effort had a unimodal pattern with the maximum around the noon. This particularly means that the both preening and sleeping were suppressed during the night, a time with the highest predation pressure, in our as well as other Lapwing populations (Seymour et al. 2003, Eglinton et al. 2010). Thus, we assume that nightly incubating females of Northern Lapwings endeavor to be as vigilant as possible consistently with our premise.

It is not easy to compare our findings about rhythms of the measured behavioral traits with the patterns found elsewhere, as the time-budget studies and particularly those involving analyses of daily rhythmicity are lacking in incubating birds. In particular, we are not aware of any study reporting the daily patterns of female preening on the nest. To our knowledge, however, the only work reporting the sleeping effort of incubating mallards (*Anas platyrhynchos*) found a higher sleeping intensity of females during the night (Javůrková et al. 2011), which is the period of higher risk of adult depredation by night mammalian predators. Similarly, the experimentally tested non-incubating ducks in captivity (Zimmer et al. 2011) and wintering Great Tits (*Parus major*) (Stuber et al. 2014) showed increasing time of sleeping as a response on a perceived predation risk. It is thus possible, that the sleeping birds can rely on sound stimuli produced by an approaching predator depending on the actual circumstances. Indeed, relying on sound stimuli can be appropriate for the ducks breeding in dense vegetation as well as for passerines overnighting in nest boxes. On the contrary, Northern lapwings breed in open areas with a good view of surroundings, bare grounds or low and lively vegetation, where early visual detection of silently approaching predators during the night may be much more reliable to reduce predation risk than a reliance on only bland acoustic cues. The sleep rhythm of Northern Lapwings (and ecologically similar species) may be thus specifically subordinated to the ground-nesting in open habitats. This

assumption of habitat influence on the sleeping rhythm should be further tested across various bird species.

A particular question is why there are two peaks in sleeping effort, after sunrise and before sunset, and why the females sleep little around the noon. We can only speculate that females need to replenish their energy stores after and before the long night incubation accompanied by a high vigilance. Alternatively, and mutually not exclusively, female sleeping on the nest during the morning and afternoon can be just another perspective of their antipredator behavior. Some studies (Bayne and Hobson 1997) document a higher activity of visually oriented nest predators as corvids just after sunrise and before sunset. In addition, it is reasonable to assume that also other predators which pose a danger to parents themselves exhibit increased activity in the morning and late afternoon. Birds of prey like Hawks (genus *Accipiter*), occasionally preying upon the adult Lapwings (Cramp and Simmons 1983; own unpublished observations), are certainly stepping up their efforts to find food for their nestlings after a night break as well as before the oncoming sunset. At the same time, minimizing of movement on nests is referred to be a good tactic how reduce the attention of visually oriented predators searching for prey (Martin et al. 2000, Muchai and Du Plessis 2005), which may be either bird egg or adult. Thus, limiting activity through sleep at the time of peak predator activity may be a proper antipredator tactic combined with self-maintenance behavior. Although the frequency of second self-maintenance activity, preening on the nest, is rather continuous throughout the daylight, it peaked around the noon, the time with a certain predation release. This may be also a reason why this activity coincides with a general drop of incubation attendance (Sládeček et al. 2019b). However, it should be also taken into account that the rhythms of female activities are probably synchronized with the rhythms of their male partners, i.e. with regard on when the males sleep or guard the territory, which may partially contribute to the explanation of female behavior on the nests. Unfortunately, awareness of the activity patterns of individuals outside the nests remain completely unknown. In each case, prioritization of visual or acoustic perception of nest surroundings can decide about the daily pattern of particular rhythms such as sleep, vigilance or preening in breeding animals.

On the contrary to the sleeping effort, the night sleeping bouts, although generally rare, were paradoxically somewhat longer (median ~ 4 minutes) than the daylight sleeping bouts (median ~ 2 minutes). From the physiological viewpoint, they could partially compensate for very long passages of demanding vigilance but, as we suggest, still without substantial increase of predation risk. Although we showed that the Northern Lapwing relies mainly on visual perception at night as it remains vigilant most of the nighttime, it probably may use rarely also acoustic cues to compensate these rare vigilance breaks. As most of Northern Lapwings breed in loose colonies, in the study area (Šálek and Šmilauer 2002) we suggest that during rare sleeping breaks the birds may rely on neighbors' reactions rather than on own eyesight. The awareness of mammalian predators at night may be perceived

through breeding neighbors who are vigilant at this moment. This perception may even be hundreds of meters which is certainly more efficient than the own night vision, and may be thus the reason for longer, though rare, sleeping bouts. In fact, the increase in the length of night sleeping bouts to 4 minutes in average is still not too long time to lose awareness of what is happening in the wider surroundings of the nest site.

Effect of male incubation effort

This is the first study convincingly documenting that male contribution to incubation influences not only the overall incubation attendance in birds but also self-maintenance behavior of their incubating female partners. We assumed that higher male contribution to incubation allows females to keep the self-maintaining activities out of the nest because these activities are associated with higher predation risk. This was found to be true for preening, since incubating females with more care-giving males had much lower frequency of this behavior. However, we found the opposite pattern for the sleeping effort of females. Specifically, we showed that the females paired with more care-giving males slept more on the nests.

According to our prediction, the females paired with more care-giving males spent less time with preening. Unfortunately, there is no study, which we can confront our finding with. We suggest that the females more supported by male incubation are able to reduce moves on the nest caused by the preening and perform this risky but important self-maintaining activity at the time out of the nest. An alternative explanation is that such females have lower need for preening at all (e.g. due to lower ectoparasite load), but we consider this as improbable as we do not find support in published literature.

However, why the females paired with more care-giving males slept more, i.e. opposite to our prediction? There is a way to interpret this behavior by the other aspect of male's partner role during the reproduction. The Northern Lapwing males are active in the territory defense, consisting of vigilance, warnings and direct attacks of approaching predators (Cramp and Simmons 1983, Elliot 1985b, a, Kis et al. 2000). If this male defensive activity is correlated with the willingness to incubate, then the females paired with more territory-defending males could feel safer while incubating and could afford to be less attentive. Relationship between territorial aggressiveness and parental care has been studied throughout the animal kingdom, often with contradictory conclusions (Redondo 1989, McDonald et al. 2001, Ros et al. 2004, Cain and Ketterson 2013, Szász et al. 2019). In fact, this relationship might represent a form of better social synchronization in the incubation rhythm a topic that is rarely well described in wild animal populations (Bulla et al. 2016, Leniowski and Węgrzyn 2018).

Sleep deprivation can negatively influence attention, motivation, memory (Rolls et al. 2011, Vyazovskiy et al. 2011) or reproductive output (Potdar et al. 2018), often with a negative impact on female fitness. Therefore, the described positive

relationship between male incubation effort and female sleeping on the nest would be another and not yet proven advantage of being paired with a more care-giving male. Although we have no data about how the females can compensate a lack of sleep during their off-nest time, we suppose that this will be marginal compared to the sleeping time during incubation. First, the females with small (or none) help from their partners can hardly sleep out of the nest (as they have only 10-15% of time for all out-of-nest activities including foraging). Second, the negligible proportion of off-nest time spent by sleeping in the Northern Lapwing had been suggested by (Grønstøl 2003), although using small and non-continuous dataset. Moreover, the Grønstøl's (2003) study indicates a slightly more time off-nest sleeping of the females paired with more incubating males. Similarly, the Semipalmated Sandpiper (*Calidris pusilla*) spent by sleeping 28% of incubation time (Ashkenazie and Safriel 1979) but slept very little during the off-nest time (Bulla et al. 2015).

We estimated that the overall sleep load during the day was about 3 hours, which is similar to sleep loads found elsewhere in other shorebirds (Ashkenazie and Safriel 1979, Amlaner and Nigel 1983) and also other birds (Amlaner and Nigel 1983, Roth et al. 2006). However, there was truly extreme variation among females, ranging from 14 minutes to 7 hours (or alternatively 1-45% of incubation time). Therefore, as we analyzed always one randomly selected day from the incubation period of each nest, we cannot exclude that females with the detected extremely low sleep load slept more during the previous or the subsequent days. However, we consider that the individual rhythms are generally more or less stable for three reasons. First, incubation patterns, such as incubation attendance and male contribution remain almost consistent in the course of incubation period and are highly repeatable among days (Sládeček et al. 2019b). Second, sleeping effort was not influenced by the incubation stage and does not change within the season. Third, also in other species the biological rhythms such as sleeping effort remain more or less repeatable (Steinmeyer et al. 2010, Stuber et al. 2015, 2016).

Conclusion

Apart from that sunrise and sunset are the most commonly suggested phenomena associated with behavioral rhythms, using the example of self-maintenance behavior of incubating females we revealed diverse patterns of daily rhythms among seemingly related behavioral components. Although the studied Northern Lapwing belongs to the species with polyphasic sleep and a great flexibility in sleep phasing patterns, we showed that sleeping on the nest as strongly rhythmic, driven by a temporal distribution of predation risk. In addition to self-maintenance role, the sleeping under clearly defined conditions is a tactic of incubating birds to save nests and themselves against a wide spectrum of predators. The study demonstrates how two different self-maintaining activities may act opposite and additionally in their circadian pattern. The rhythmicity of behavioral components is thus probably a more general phenomenon than previously expected also in such

behavioral traits as self-maintenance including preening that may seem to be freely distributed throughout the day. Beyond the predation, the intensity of rhythms can be modified also by the partner's help in biparental species, the phenomenon not described satisfactorily up to date. Predation, habitat and social synchronization of the partners are thus important drivers shaping behavioral components of life histories in birds. Well-tuned rhythms can probably contribute to reproductive success and thus need to be studied more thoroughly to better understand variation in individual fitness. However, a huge among-individual variation enhances the need for studies based on sufficient number of individuals observed during the whole 24-hours day. Insight into complexities and contexts is possible only by means of full-day continuous recordings of more individuals, which requires use of technologies able to collect consistent and sufficiently large samples beyond anecdotal observations typical for most published behavioral studies.

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Availability of data and materials

The raw data and R codes that enabled replications of all analyses performed in this paper, and that were also used for generating all of the figures, are available from Open Science Framework: <https://osf.io/r8tztg/>.

Authors' contributions

M.S, K.B and M.Š. conceived the study, M.S and E.V. collected the incubation data and extracted incubation behavior from recordings, K.B. extracted other behavioral data from recordings, M.S. analyzed the data, M.S., K.B. and M.Š. wrote the manuscript.

Ethical approval

The study was conducted in accordance with the current laws of the Czech Republic.

Competing interests

The authors declare that they have no competing interests.

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SUPPLEMENTARY Information FOR

Daily rhythmicity of female self-maintenance activities during parental care is affected by predation risk and incubation attendance by male in a biparental shorebird

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Table S1 | Circadian pattern of female sleeping (absolute time within hour)

Response	Effect type	Effect	Estimate	95% CI	
				Lower	Upper
Female sleeping time	Fixed	Intercept	0.098	0.062	0.135
		M incubation	0.352	0.129	0.567
		Sin (12 hours)	-0.001	-0.043	0.042
		Cos (12 hours)	-0.051	-0.089	-0.012
		M incubation : Sin (12 hours)	0.258	-0.002	0.519
		M incubation : Cos (12 hours)	-0.262	-0.507	-0.02
	Random (variance)	Nest (Intercept)	10		
		Sin (12 hours)	11		
		Cos (12 hours)	9		
		Residual	70		

The posterior estimates (medians) of the effect sizes with the 95% credible intervals (CI) from a posterior distribution of 5,000 simulated values generated by the ‘sim’ function in R(Gelman et al. 2016). Variance components were estimated by the ‘lmer’ function(Bates et al. 2015). Response variable was proportion of sleeping during particular hour of day. Time was taken as “hour of day” transformed to radians ($2 \times \text{hour} \times \pi / \text{period of interest} - 12\text{h}$) and fitted as sine and cosine of radians. Estimates whose 95% credible intervals don’t contain 0 are highlighted in bold.

Table S2 | Sleeping effort in relation to incubation period and season.

Response	Effect	Estimate	95% CI	
			Lower	Upper
Sleeping effort (proportion per day)	Intercept	0.117	-0.153	0.388
	Start of incubation	0.001	-0.002	0.003
	Day of incubation	-0.002	-0.007	0.003

The posterior estimates (medians) of the effect sizes with the 95% credible intervals (CI) from a posterior distribution of 5,000 simulated values generated by the ‘sim’ function in R(Gelman et al. 2016). Variance components were estimated by the ‘lm’ function. Response variable was proportion of sleeping during the female incubation throughout the whole analyzed period of 24 hours. Predictors are the day when the breeding attempt has started (number within the year) and day within the incubation period, for which the recordings has been analyzed. Estimates whose 95% credible intervals don’t contain 0 are highlighted in bold.

Table S3 | Preening effort in relation to incubation period and season.

Response	Effect	Estimate	95% CI	
			Lower	Upper
Preening effort (frequency per day)	Intercept	86.965	-116.384	301.109
	Start of incubation	-0.166	-2.059	1.613
	Day of incubation	4.064	0.283	7.879

The posterior estimates (medians) of the effect sizes with the 95% credible intervals (CI) from a posterior distribution of 5,000 simulated values generated by the ‘sim’ function in R(Gelman et al. 2016). Variance components were estimated by the ‘lm’ function. Response variable was proportion of sleeping during the female incubation throughout the whole analyzed period of 24 hours. Predictors are the day when the breeding attempt has started (number within the year) and day within the incubation period, for which the recordings has been analyzed. Estimates whose 95% credible intervals don’t contain 0 are highlighted in bold.

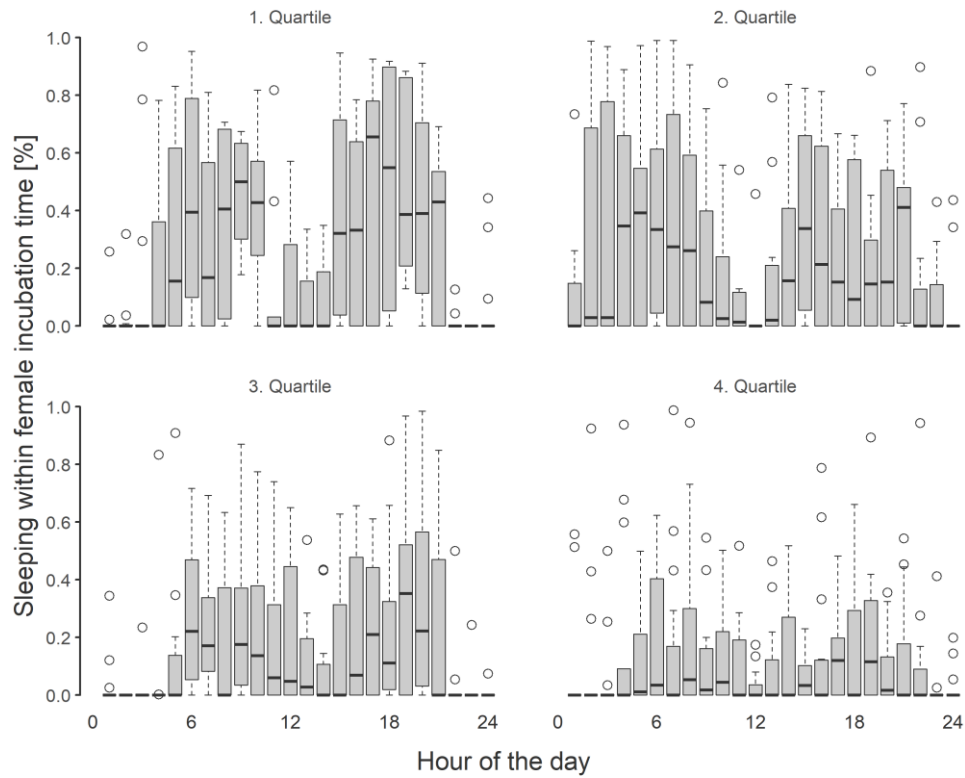


Figure S1. The proportion of female incubation time female spent with sleeping in relation to the time of day and male contribution to incubation. The boxplots represent the real proportions in our dataset and are separated by the quartiles of male contribution to incubation (Q1: 0 – 4.7 %; Q: 4.8 – 12.5 %; Q3: 12.6 – 20.9 %; Q4: 21 – 36.6 %). Boxes depict median (vertical line inside the box), 25–75th percentiles (box), 25th and 75th percentiles minus or plus 1.5× interquartile range, respectively, or the minimum and maximum value, whichever is smaller (whiskers) and outliers (circles).

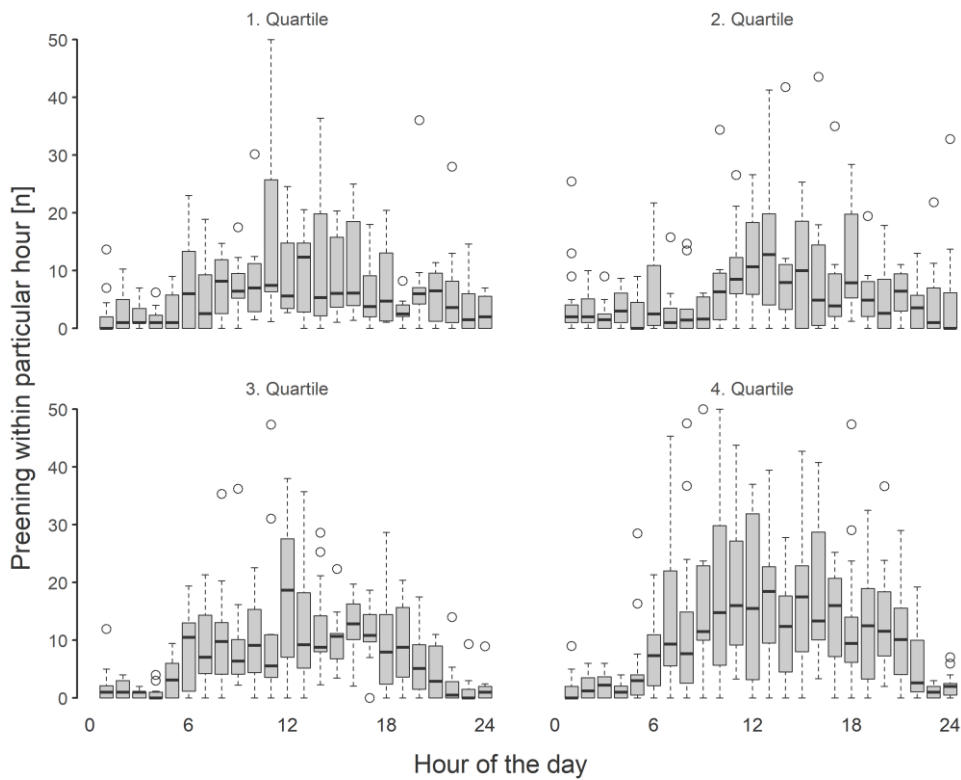


Figure S2. Frequency of female preening during incubation in relation to the time of day and male contribution to incubation. Presented data represent the real frequencies in our dataset and are separated by the quartiles of male contribution to incubation (Q1: 0 – 4.7 %; Q: 4.8 – 12.5 %; Q3: 12.6 – 20.9 %; Q4: 21 – 36.6 %). Boxes depict median (vertical line inside the box), 25–75th percentiles (box), 25th and 75th percentiles minus or plus $1.5 \times$ interquartile range, respectively, or the minimum and maximum value, whichever is smaller (whiskers) and outliers (circles).

Chapter 5

Great variability in nest lining size: support for thermoregulation but not for anti-predatory adaptation hypothesis

Vojtěch Kubelka, Martin Sládeček & Miroslav E. Šálek

Nest lining is a key component in nests of many bird species. Among ground-nesting birds with open nests, it usually consists of dry sticks and stalks creating a thermoregulatory insulating layer for the eggs. However, a bigger nest lining can attract predators and increase nest mortality. The factors influencing behavioural plasticity in birds facing the trade-off between nest lining thermoregulation and conspicuousness for predators have remained poorly understood. The Northern Lapwing *Vanellus vanellus*, a visibly incubating shorebird with an active nest defence against potential predators, demonstrates great variability in the size of nest lining and, at the same time, is subject to a high frequency of nest predation. We analysed the variability of nest-lining size across time and space in 915 measurements of 601 lapwing nests in South Bohemia, Czech Republic, during 2010–2015. We show that lapwing nests placed closer to small water pools with generally cooler microclimates had bigger nest lining. The size of nest linings also reflected the availability of nest lining material in the vicinity of the nest. On the other hand, there was no effect of nest position within the breeding association and distance to the nearest perch as a possible stand for predators on nest lining size. Furthermore, nest lining size did not predict nest predation rate. Our findings suggest that lapwings adjust the size of their nest lining to local microclimate conditions rather than potential predation risk which is in concordance with the thermoregulation hypothesis of the nest size in birds.

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Great variability in nest lining size: support for thermoregulation but not for anti-predatory adaptation hypothesis

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Abstract

Nest lining is a key component in nests of many bird species. Among ground-nesting birds with open nests, it usually consists of dry sticks and stalks creating a thermoregulatory insulating layer for the eggs. However, a bigger nest lining can attract predators and increase nest mortality. The factors influencing behavioural plasticity in birds facing the trade-off between nest lining thermoregulation and conspicuousness for predators have remained poorly understood. The Northern Lapwing *Vanellus vanellus*, a visibly incubating shorebird with an active nest defence against potential predators, demonstrates great variability in the size of nest lining and, at the same time, is subject to a high frequency of nest predation. We analysed the variability of nest-lining size across time and space in 915 measurements of 601 lapwing nests in South Bohemia, Czech Republic, during 2010–2015. We show that lapwing nests placed closer to small water pools with generally cooler microclimates had bigger nest lining. The size of nest linings also reflected the availability of nest lining material in the vicinity of the nest. On the other hand, there was no effect of nest position within the breeding association and distance to the nearest perch as a possible stand for predators on nest lining size. Furthermore, nest lining size did not predict nest predation rate. Our findings suggest that lapwings adjust the size of their nest lining to local microclimate conditions rather than potential predation risk which is in concordance with the thermoregulation hypothesis of the nest size in birds.

Keywords Ground-nesting bird · Microclimate · Nest predation · Nest site moisture · Northern lapwing · Shorebirds · *Vanellus vanellus*

Zusammenfassung

Hohe Variabilität im Ausmaß der Nestschulterung: Belege sprechen für die Thermoregulations-, nicht aber für die Feindvermeidungshypothese.

Die Nestschulterung ist ein wichtiger Bestandteil der Nester vieler Vogelarten. Bei Bodenbrütern mit offenen Nestern besteht diese normalerweise aus trockenen Stöckchen und Halmen, welche eine thermoregulatorische Isolationsschicht für die Eier bilden. Eine stärkere Nestschulterung kann jedoch Prädatoren anlocken und somit die Nestlingsmortalität erhöhen. Die Faktoren, welche die Verhaltensplastizität bei Vögeln im Konflikt zwischen thermoregulatorischer Nestschulterung und der Auffälligkeit für Prädatoren beeinflussen, sind bisher kaum erforscht. Der Kiebitz *Vanellus vanellus*, eine offen brütende Limikolenart, welche ihre Nester aktiv gegen Prädatoren verteidigt, weist eine starke Variabilität bezüglich des Ausmaßes der Nestschulterung auf und unterliegt zugleich einer hohen Nestprädationsrate. Wir untersuchten die zeitliche

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und räumliche Variabilität im Ausmaß der Nestpolsterung anhand von 915 Maßen von 601 Kiebitznestern aus Südböhmen (Tschechische Republik) aus den Jahren 2010–2015. Wir konnten zeigen, dass näher an kleinen Gewässern mit allgemein kühlerem Mikroklima platzierte Kiebitznester stärker ausgepolstert waren. Die Stärke der Nestpolsterung spiegelte außerdem die Verfügbarkeit von Nistmaterial in Nestnähe wider. Andererseits gab es keinen Einfluss der Nestposition innerhalb der Brutgemeinschaft oder des Abstands zur nächsten Sitzwarte als möglichem Ansatz für Prädatoren auf die Stärke der Nestpolsterung. Des Weiteren ließ das Ausmaß der Nestpolsterung keinen Aufschluss auf die Nestprädatorenrate zu. Unsere Ergebnisse legen nahe, dass Kiebitze die Stärke ihrer Nestschicht eher an das lokale Mikroklima anpassen als an ein potenzielles Prädatorenrisiko, was im Einklang mit der Thermoregulationshypothese der Nestgröße bei Vögeln steht.

Introduction

The majority of bird species build open cup nests lined with dry plant material and/or soft feathers (Hansell and Deeming 2002; Gill 2007; Deeming and Reynolds 2015). The principal function of the nest lining is thermoregulation of the clutch (Reid et al. 2002; Tulp et al. 2012; Heenan 2013), even though protection of eggs against mechanical damage as well as attracting sexual partners have been suggested to play a role in some species (del Hoyo et al. 1996; Hansell and Deeming 2002; Deeming and Mainwaring 2015; Bidle et al. 2018). Incubation temperature and the amount of heat supplied at certain stages to embryo development may dramatically affect metabolic processes and contribute to the quality of hatchlings (Starck and Ricklefs 1998). Moreover, a bigger nest lining can significantly reduce the energetic costs of incubation faced by adults (Reid et al. 2002; Tulp et al. 2012).

On the other hand, a bigger nest lining with good thermoregulatory function may increase nest detectability for visually oriented predators (Mayer et al. 2009; Mainwaring et al. 2015). Indeed, some studies found that bigger nests are more likely to be depredated in comparison with smaller ones (Grégoire et al. 2003; Antonov 2004; Biancucci and Martin 2010). Therefore, in deciding how to construct their open nest, breeding adults face a trade-off between providing a substantial lining to strengthen the thermoregulatory function of the nest or choosing a scanty lining to reduce the risk of nest predation (Ricklefs 1983; Deeming and Reynolds 2015). This decision may also be influenced by environmental conditions at breeding grounds because, e.g., wetter places tend to have cooler microclimate (e.g. Reid et al. 2002; Rogers et al. 2006; Yang et al. 2013).

Despite decades of intensive research on nest morphology (Deeming and Mainwaring 2015; Mainwaring et al. 2015; Martin et al. 2017), the extent to which individual birds can use environmental cues to fine-tune the morphology of their nest is still unclear (Healy et al. 2015) and further research is needed on how nest size interacts with nest location and parental behaviour (Mainwaring et al. 2015). The aforementioned scenario presents an opportunity to investigate the context of the trade-off between thermoregulation and predation risk, as well as quantify effects of external factors on

the behavioural plasticity of individual breeding birds that may have consequences for nesting success, productivity and inevitably species population dynamics.

Nest lining is characteristic for the ground-nesting shorebird, Northern Lapwing (*Vanellus vanellus*). This species breeds across the Eurasian temperate zone in moderate climates where it builds open nests, predominantly on bare ground with sparse or no vegetation in agricultural landscape (Cramp and Simmons 1983; Nethersole-Thompson and Nethersole-Thompson 1986; Shrubbs 2007) and is subject to a high risk of nest predation (e.g. Cramp and Simmons 1983; MacDonald and Bolton 2008a; Roodbergen et al. 2012). High nest predation was recorded also in our target lapwing population in South Bohemia, Czech Republic, where nest position within the breeding association (semi-colony or loose colony) may influence the nest predation rate (Šálek and Šmilauer 2002) and visually oriented predators are active (Šálek and Zámečník 2014). Northern Lapwing nest lining size is highly variable, ranging from sparse to very large (Cramp and Simmons 1983; Shrubbs 2007) and consists of plant material, predominantly dry stalks of cereals and grasses. The building of each nest continues with the progressive filling of the excavated scrape with plant material, particularly during pre-laying and laying periods by both male and female birds (Cramp and Simmons 1983; Nethersole-Thompson and Nethersole-Thompson 1986; Shrubbs 2007) and may thus function as a part of display ritual (Cramp and Simmons 1983). However, environmental factors affecting nest lining size and possible consequences for nest survival are unknown.

The aim of this study is to investigate factors influencing the variability in nest lining size and test whether there is evidence for the thermoregulation or anti-predatory adaptation hypothesis. Specifically, using the Northern Lapwing as the model species, we ask: (1) What is the variability of nest lining size? (2) Which factors (nest site moisture, ambient temperature, position within breeding association, distance to the nearest perch for potential predators, incubation start date, incubation stage and nest linings material availability) predict the nest lining size? Bigger nest linings in wet nesting sites with a cooler microclimate or during spells of lower ambient temperature would represent support for thermoregulation hypothesis, whereas bigger nest linings in the

middle of the breeding association or further away from the nearest perch for potential predators would suggest influence of anti-predatory adaptation; (3) is a bigger nest lining more obvious for potential predators and does nest predation rate increase with nest lining size?

Materials and methods

Study area and fieldwork

We searched for the Northern Lapwing nests in České Budějovice basin in the centre of South Bohemia (49.0°N, 14.4°E) in the Czech Republic during 2010–2015. The study area consists of circa 60 km² of agricultural landscape with prevailing arable land of altitude 380–420 m, for more details, see Šálek and Šmilauer (2002) and Zámečník et al. (2018). We searched for nests in areas with breeding lapwings using binoculars and scopes, or direct physical investigation of dense breeding colonies during the breeding season (end of March to end of May).

We recorded nest GPS coordinates and assigned each nest into one of three categories according to habitat structure (Table 1). We determined the start of incubation for each nest with use of the flotation method (van Paassen et al. 1984) or according to known egg-laying sequence [incubation start = a day when the third egg was laid; Shrubbs (2007), for two-egg clutches the date of second egg laying was used]. During every visit, we took a digital picture of the nest from 1 m directly above the nest with 35-mm optical distance (35-mm lens) to capture the nest and close surroundings up to 1 m from the nest so as to be able to evaluate the size of the nest lining. During 2014–2015 we also took an additional picture from 2 m directly above the nest with 35-mm optical distance to capture wider surroundings up to 2 m from the nest for the purpose of nest lining material availability description. We followed the fate of each nest and determined it as successful (hatched or surviving particular period), depredated or failed for other reason (e.g. agriculture machinery). Every nest where at least one chick hatched was regarded as successful. A hatched nest was recognised according to tiny eggshell fragments remaining in the nest scrape from the hatching process (Green et al. 1987). Clutches with infertile eggs with present parents

which had not been depredated over expected egg-laying and incubation period were regarded as successful ones for the purpose of predation analyses. Only complete nest depredations were included in the depredated nests category (partial egg loss were omitted) because partial egg loss does not necessarily mean depredation. While using nest video surveillance, we have repeatedly recorded that egg loss can be the result of accidental egg damage and subsequent egg-removal by incubating parent. Partial egg loss accounts up to 7% of nests in our lapwing population (own unpublished data). Depredated nests were recognised according to the absence of all eggs before estimated hatching with no marks suggesting another cause of failure or according to remnants of depredated eggs in the nest or close vicinity.

Data processing

Three authors of this study independently scored nest lining size from digital nest images according to prepared scale into one of eight categories: 0.5–4 from very sparse to large nest lining (see examples in Fig. 1). The arithmetic mean of these three scores was used for each nest at each nest visit in subsequent analyses. Repeatability among evaluators estimated by function “rpt” (Nakagawa and Schielzeth 2010; Gaussian model) using 1000 bootstraps was 72% (95% CI: 69–75%). To assess whether nest lining size corresponds with nest lining magnitude at the bottom of the nest as an important parameter of thermoregulation capacity, we temporarily removed eggs for a small number of randomly selected nests and carefully measured the nest lining thickness at the bottom of active nests by a vernier calliper (in mm). Indeed, lining thickness strongly correlated with the lining size scored from photographs (Spearman’s rank correlation; $r_s = 0.78$, $P < 0.001$, $n = 18$ active nests in 2014); therefore, the nest size scored from photographs were used in all analyses as reliable (and for visually oriented predators obvious) predictor of the nest lining magnitude in the bottom as well as at the sides of the nest scrape. Furthermore, V. K. determined the nest site moisture up to 1 m from the nest with use of digital nest images into three categories 0—dry nest site (structurally more diversified substrate, small clods separated, light soil); 1—moist nest site (compacted substrate without small clods, dark soil); 2—open water (pool) present up to 1 m from the nest. Because the site moisture

Table 1 Habitat categories distinguished in the study

Category	Description of physiognomy	Crops merged
1	Structurally uniform bare field without or with little vegetation	Freshly harrowed or sown spring cereal, maize, bean
2	Structurally diversified plot without or with mosaic vegetation	Ploughed field, sparsely overgrown fallow, stubble
3	Continuously vegetated areas	Winter cereal, grassland, oilseed, clover



Fig. 1 The scale of Northern Lapwing (*Vanellus vanellus*) lining size variability. South Bohemia, Czech Republic. Note that these are four examples out of eight possible categories of nest lining size 0.5–4. **a** Nest with nest lining size scored as 1: sparse nest lining. **b** Nest with nest lining size scored as 2: obvious nest lining but not meaningfully

extend the nest scrape rim. **c** Nest with nest lining size scored as 3: distinct nest lining well extended beyond the nest scrape rim. **d** Nest with nest lining size scored as 4: large nest lining. Photographs credits: **a–c** Vojtěch Kubelka, **d** Vladimír Štorek

category was clearly obvious from the picture, only one person was involved in this process. Nest site moisture represented a proxy variable for local microclimate characteristics, where wetter nest sites were supposed to have cooler microclimate (e.g. Reid et al. 2002; Rogers et al. 2006; Yang et al. 2013).

Three independent evaluators (see acknowledgement), without knowledge of study questions and the purpose of the assessment, scored the nest lining material availability around the nests from digital nest images according to a prepared scale into one of three categories 0–2 (Fig. 2). The arithmetic mean of these three scores was further used for each nest at each visit in subsequent analyses. Repeatability estimated by function “rpt” (Nakagawa and Schielzeth 2010) using 1000 bootstraps was 61% (95% CI: 55–67%). Every nesting habitat was assigned into one of three categories according to habitat structure (Table 1).

We evaluated the effect of nest position within the breeding association, known to influence nest predation risk (e.g. Šálek and Šmilauer 2002; MacDonald and Bolton 2008b)

directly on nest lining size. We assigned each nest, according to known nest GPS coordinates, into three following categories of the nest position: (1) solitary nest placed outside of any breeding association (at least 200 m from the nearest lapwing nest); (2) edge nest creating a convex polygon of all nests present in a breeding association (loose colony) with less than 200 m distance to the nearest lapwing nest; (3) interior nest placed within the polygon of edge nests in a particular breeding association with less than 200 m distance to the nearest lapwing nest. Furthermore, with use of the ground distance measurement tool in Google Earth (ver. 7.1), we measured in meters the distance of each nest to the nearest potential perch for avian predators higher than 5 m (tree, shrub, high stand, building, pole or power lines).

From the given mean daily ambient temperatures in České Budějovice (Czech Hydrometeorological Institute, pers. comm.) at the edge of study area (altitude of 395 m), we computed mean ambient temperature value in °C from 5 days prior the day of incubation start (excluded) to estimate the general harshness of environmental conditions in

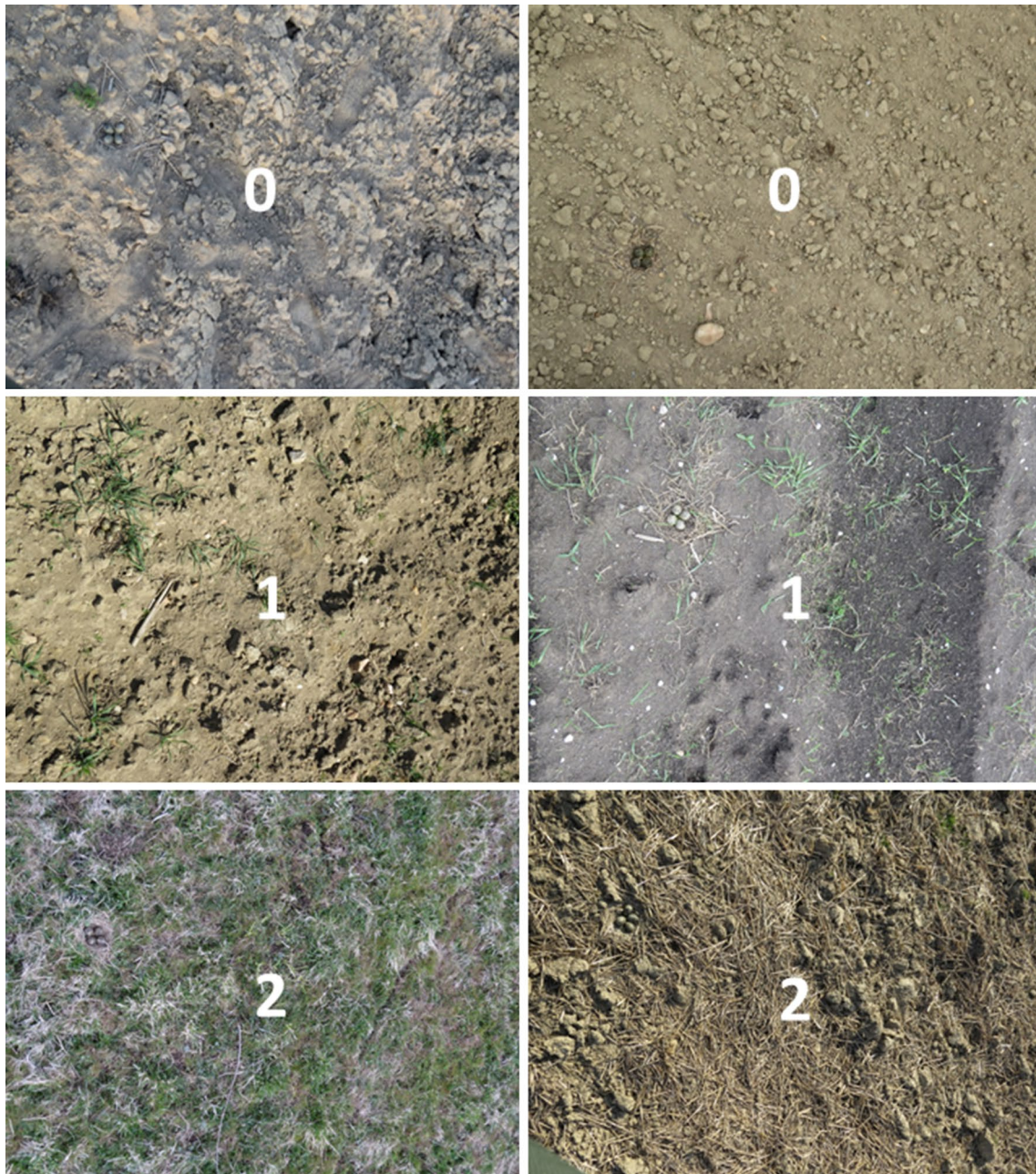


Fig. 2 The scale for nest lining material availability in the vicinity of the nest. (0) nothing or a little; (1) sort of; (2) plenty. Photographs credit: Vojtěch Kubelka

our study area just before and during the egg laying when lapwings predominantly build the nest lining (Cramp and Simmons 1983).

In order to evaluate, whether nest lining size regardless the nest surrounding affects visibility of nests to potential predators in the visible (light) part of electromagnetic spectrum, we conducted experimental enlargement or reduction of nest lining size using ten real nests with big nest lining size and ten nests with small nest lining size. Photographs were taken in 2010 from a height on a 3-m

telescopic pole and nests were randomly situated out of the picture centre. Nest lining was then virtually manipulated on photographs in the software GIMP-2.6.12 using the default cloning function (selective copying using a brush). On each rich-lined nest photo, the majority of the nest lining was covered with small areas selected from the surroundings of the particular nest so that only a clutch with heavily reduced lining remained. In contrast, the lining of the poorly lined nests was virtually enlarged spreading around several small portions of lining up to

the large nest lining size. With this virtual manipulation, a total of 40 nest photos were obtained, of which twenty with large nest lining size and twenty with small nest lining size (half of them manipulated and half of them as control nests in each group). The nests were randomly sorted in the questionnaire so that respondents (not informed in advance about the manipulation and thus unable to distinguish the relationships among the pictures) were asked to score each nest into one of four categories of nest conspicuousness (1: inconspicuous, 2: visible, 3: conspicuous, 4: strikingly visible), considering also the time needed to locate the nest on the picture. Ten original nests with big nest lining size had mean evaluation of 57 respondents 3.4 and ten nests with small nest lining size 1.9. The final scores of 57 respondents evaluating nest visibility were repeatable with 51% (95% CI: 38–61%).

We computed daily nest predation rates according to Mayfield, defined as the number of depredated nests divided by the exposure of all nests in days (Mayfield 1961, 1975). The procedure of computing the exposure for daily nest predation was conducted following Kubelka et al. (2018). The exposure for hatched nests is from the day of finding until known or predicted hatching (e.g. 11 April and 28 April means $28 - 11 = 17$ days of exposure). The exposure for depredated nests lasted from the day of finding until midpoint assumption between last positive and first negative visits of the particular nest, the exposure for failed nests due to any other reason than predation (e.g. agriculture machinery, nest abandonment). For nests with an unidentified fate the exposure lasted from the day of finding until the last positive visit only.

Statistical analyses

Statistical analyses were performed with R, ver. 3.3.3 (R Development Core Team 2017). We used linear mixed-effect models (LME), fitted with the “lmer” function from the “lme4” package (Bates et al. 2015) controlling for all remaining predictors in the model—type III analysis and with a random intercept of the year and locality. Assumptions of models (e.g. normality and homoscedasticity of residuals) were checked visually from diagnostic plots (Crawley 2013). We used the “mixed” function from the “afex” package (Singmann et al. 2018) for *P* values computation. We compared individual categories of nest site moisture and nest lining material availability by post hoc multiple comparisons of means (Tukey contrasts) in the “multcomp” package (Hothorn et al. 2017). We used paired *t* tests for virtual manipulation of nest lining size and generalized linear mixed model (GLMM) with binomial error structure, logit link function and random factor of locality in the analysis of

relationship between daily nest predation rate and nest lining size. All tests were two-tailed.

Results

Nest lining size variability

Nest lining in the given lapwing population was highly variable, ranging between 0.5 and 4 of the mean score, mean = 1.91 ± 0.63 (SD), median = 1.83 and within a reasonable range of 1–25 days of incubation stage it is slightly decreasing over the incubation period (Fig. 3; LME: $F_{1,332} = 50.16$, $P < 0.001$, random factors: year, locality and nest 817 measurements of 557 nests). However the incubation stage did not influence nest lining size in the first measurements of each nest (LME: $F_{1,550} = 1.00$, $P = 0.320$, random factors: year and locality 557 measurements of 557 nests), used in further analyses.

Factors affecting nest lining size

Nest lining size was significantly influenced by nest site moisture and availability of nest lining material (Table 2). Breeding habitat was no longer significant after controlling for the availability of nest lining material. Ambient temperature, incubation start date, incubation stage, position in the breeding association and distance to the nearest perch for potential predators were non-significant predictors of nest lining size. Nest lining size was found to be bigger in wetter nest sites (Fig. 4, Table 2) with the following mean nest lining score values: Dry = 1.69, Moist = 2.05, Water = 2.46 (all categories significantly different: Tukey contrasts; *z* values = 4.79–8.38; P always < 0.001 ; $n = 557$ nests). Nest lining size was found to be bigger at sites with higher nest lining material availability (Fig. 5, Table 2).

Nest visibility and predation rate

Virtual removal of rich nest lining led to a significant decrease of nest visibility (with a change of mean score from 3.4 to 2.0 and the difference in particular nests 1.37 ± 0.16 ; paired *t* test; $t = 8.15$, $df = 9$, $P < 0.001$), representing a mean drop from 84% to 50% of the maximum score (4: strikingly visible). Similarly, the virtual enrichment of poor lining led to a significant increase in nest visibility (change of mean score from 1.9 to 2.9 and the difference in particular nests 1.00 ± 0.14 ; paired *t* test; $t = 6.72$, $df = 9$, $P < 0.001$), representing an increase from 46% to 71% of the maximum score. Nest lining size did not predict daily nest predation rate (GLMM_{binomial}, $z = 0.60$, $P = 0.552$, $n = 590$ nests during 2010–2015).

Fig. 3 Nest lining size variability during incubation. Size of the dots reflects the sample size, error bars = SE, $n=915$ measurements of 601 lapwing nests from 2010–2015 in South Bohemia, Czech Republic. See Methods for scoring procedure and Fig. 1 for nest lining size category examples

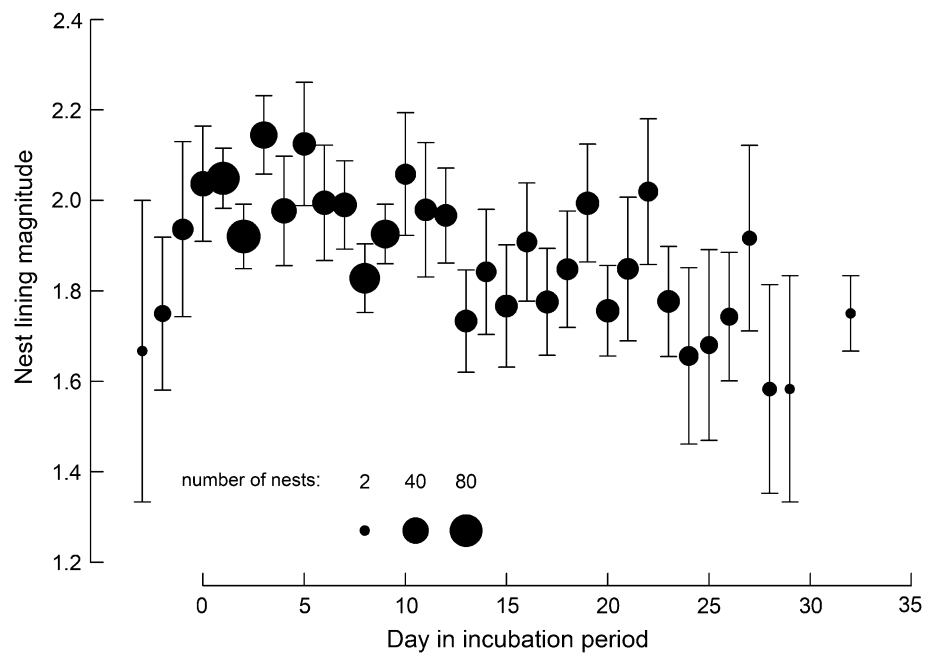


Table 2 Effect of nest site moisture, nest lining material availability, breeding habitat, ambient temperature, incubation start date, position in the breeding association and distance to the nearest perch for potential predators on nest lining size in South Bohemia, Czech Republic

Predictor	<i>df</i>	F	<i>P</i>
(a) Whole dataset 557 nests			
Nest site moisture	2; 249	28.88	< 0.001
Breeding habitat	2; 184	17.72	< 0.001
Incubation stage	1; 543	2.35	0.130
Ambient temperature	1; 400	2.09	0.150
Incubation start date	1; 84	0.46	0.500
(b) Reduced dataset 418 nests			
Nest site moisture	2; 216	17.13	< 0.001
Breeding habitat	2; 197	9.11	< 0.001
Position in the association	2; 404	0.49	0.610
Distance to the perch	1; 195	0.14	0.710
(c) Reduced dataset 206 nests			
Nest lining material availability	1; 182	21.07	< 0.001
Nest site moisture	2; 198	4.97	0.008
Breeding habitat	2; 110	1.40	0.250

Linear mixed effect model with the random effect of year and locality, all predictors were controlled for the effect of remaining ones—type III analysis. All significant predictors are kept in subsequent models. (a) $n=557$ nests during 2010–2015. (b) $n=418$ nests during 2011–2015 with measured position in the breeding association and distance to the perch. (c) $n=206$ nests during 2014–2015 with measured nest lining material availability. We used “mixed” function from ‘afex’ package (Singmann et al. 2018) for *P* values computation. See Methods for more details and Table 1 for habitat descriptions

Discussion

We have shown that Northern Lapwing nest lining size was influenced by nest site wetness and nest lining material availability but not affected by ambient temperature, incubation start date, incubation stage, nest position within the breeding association or nest distance to the nearest perch for potential predators. The effect of breeding habitat was likely driven by a higher nest lining material availability (see more details in Supplementary Appendix). Furthermore, we have shown that the nest lining size does not predict the daily nest predation.

Nests with bigger linings were found at wetter nest sites, especially when an open water pool was closer than one meter from the nest and nest site moisture served as a proxy for local microclimate. Our finding is in line with general assumption that thermoregulatory function of the nest is important (Deeming and Reynolds 2015), especially in ground-nesting species breeding in the extreme climates of Arctic (Tulp et al. 2012) or alpine environments (Camfield and Martin 2009). Thus, Arctic shorebirds prefer to breed on slopes with a milder microclimate (Meltotte et al. 2007) and use specific lining material to reduce heat loss from nests (Reid et al. 2002). Detailed studies on nest design of the Pectoral Sandpiper (*Calidris melanotos*) from areas with permafrost confirmed that the birds reduce the rate of heat loss from the nests using the lining of nest scrapes and that deeper nest cups are more effective in heat retention (Reid et al. 2002). We may assume that just a specific layer of dry stalks containing sufficiently large air cells has effective insulating function against environmental harshness (Deeming and Mainwaring 2015) and could play an important role

Fig. 4 Lining nest size relationship with nest site moisture (a) and breeding habitat (b). Box-plots represent two decades before and six decades after the median of incubation start each year. Medians with 95% CI (notches), quartiles, 1.5 inter-quartile range and outliers are presented, n size = 557 nests during 2010–2015 in South Bohemia, Czech Republic, particular sample sizes in number of nests are follows: Dry = 201, Moist = 296, Water = 60, Bare = 186, Diversified = 212, Vegetated = 159, for more details see Table 1

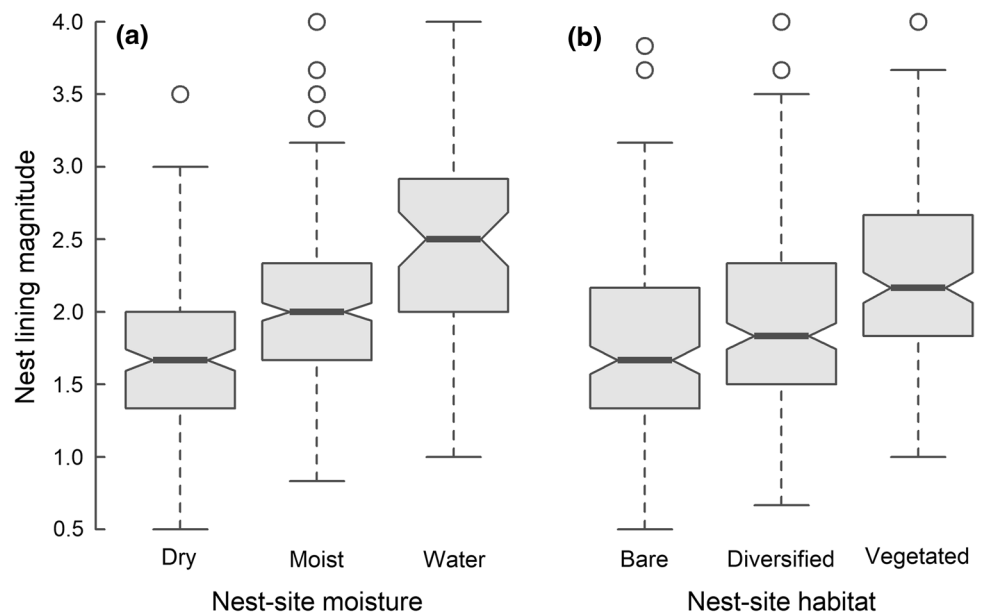
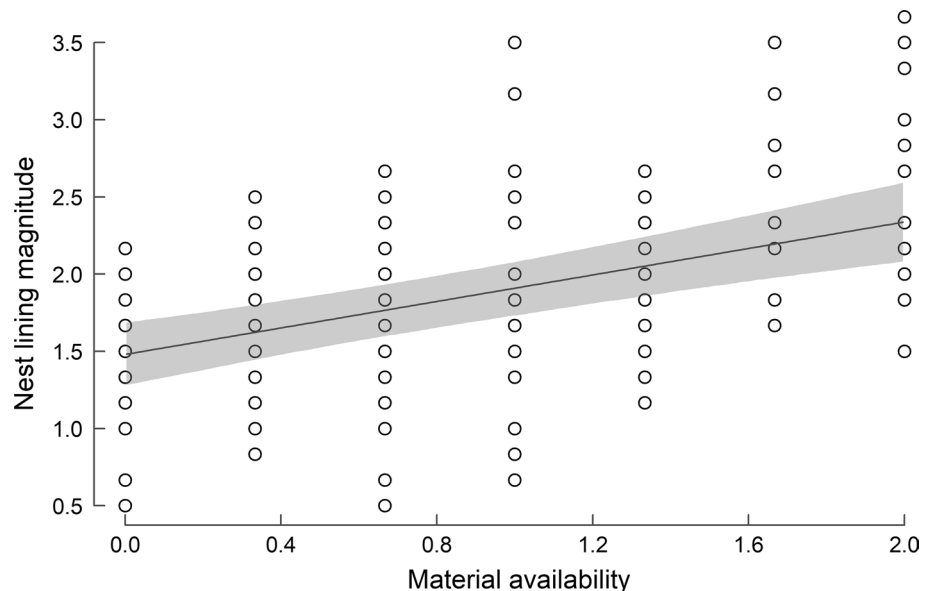


Fig. 5 Effect of nest lining material availability on nest lining size in 206 nests from 2014 and 2015. Line with shaded area indicate model prediction with 95% credible intervals based on the joint posterior distribution of 5 000 simulated values based on model outputs (Table 2) and generated by the “sim” function in R (Gelman et al. 2016)



also for shorebird species breeding in temperate agricultural landscape, particularly in wet places with cooler microclimate (e.g. Reid et al. 2002; Rogers et al. 2006; Yang et al. 2013). No effect of ambient temperature on nest lining size suggests that from the egg insulation perspective, the local microclimate (nest site moisture) plays a more important role than average ambient temperatures over the whole study area. It is important to note that we used the nest site moisture as a proxy variable to characterise the local nest microclimate and direct temperature measurements in the nest surroundings could provide more precise information.

Nest lining size was bigger at places with a higher availability of nest lining material. This finding is not surprising

given the fact that nest lining behaviour performed by both parents can be a part of display ritual in lapwings (Cramp and Simmons 1983; Nethersole-Thompson and Nethersole-Thompson 1986; Shrubbs 2007). This display can be partially ritualized—done also without plant material (Cramp and Simmons 1983); therefore, display intensity does not have to be more intense at sites with more nest lining material availability, but could be more ritualized at the sites with less nest lining material availability. Thus, lapwings are simply using plant material more when it is available in the nest surrounding, which is the case, especially in more vegetated nesting habitat. Similarly, Piping Plovers (*Charadrius melodus*) used more shell fragments in their nest lining when the

nest was located on shelly, rocky, or coarse-sanded beaches (Greenwald 2009). Generally, bird nest lining composition often reflects the nest lining material (quality and quantity) available during nest building (Deeming and Mainwaring 2015). Alternatively, breeding birds could afford bigger nest lining at the places with more nest lining material, because such nests are not so obvious for potential predators in this heterogeneous surrounding (Stevens et al. 2017; Gómez et al. 2018). Disentangling these explanations would need experimental nest lining size manipulation.

While bigger nests are more obvious for potential predators (Grégoire et al. 2003; Antonov 2004; Biancucci and Martin 2010), visually oriented predators are active at lapwing breeding grounds in our study (Šálek and Cepáková 2006; Šálek and Zámečník 2014) and nest lining size was revealed as important factor in determining conspicuousness of lapwing nests for visually oriented predators, there was no significant effect of nest position within the breeding association or nest distance to the nearest perch for potential predators on the nest lining size and no effect of the nest lining on daily nest predation rate. These findings can have several not mutually exclusive explanations: (1) breeding Northern Lapwings are known as aggressive nest defenders (Elliot 1985; Liker and Székely 1999; Kis et al. 2000) and thus can effectively repel potential avian predators from breeding grounds, and therefore, they are not forced to make the nest lining smaller under higher risk or depredation, e.g. nest placement close to perches for predators or outside of the breeding association (Šálek and Šmilauer 2002, MacDonald and Bolton 2008b); (2) visually oriented predators play a minor role in our study population where predominantly mammals, especially Red Fox (*Vulpes vulpes*) and Stone Marten (*Martes foina*), were recognized as nest predators (Kubelka 2015, own unpublished data); (iii) nest lining size per se could not be the important clue for visually oriented predators during nest search, but rather other stimuli, e.g. visibility of incubating parent (Šálek and Zámečník 2014) could play more important role for nest predation risk. Although a bigger nest lining renders lapwing's nest more obvious for potential predators, some ground nesting birds may enhance the crypsis of their eggs by enlarging the stony nest lining (Gómez et al. 2018), therefore the visibility of nests for potential predators should be assessed for each species separately.

The presented study provides several insights into the long-standing debate on the trade-off between nest size thermoregulatory function and conspicuousness for predators (Ricklefs 1983; Deeming and Reynolds 2015). First, using large sample sizes over 6 years, we show that lapwings may adjust their nest lining size to the local microclimate, building larger nest linings in wetter nest locations, thus providing support for thermoregulatory functions of the nest lining. Second, nest lining material availability is also a significant predictor of nest lining size and effects of both predictors can be additive.

Third, nest lining size is not adjusted to presumed nest predation risk and it does not predict nest predation rate, suggesting that lapwings with active defence of their nests are not forced to reduce nest lining size as the anti-predatory adaptation.

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Electronic supplementary material

Supplementary appendix

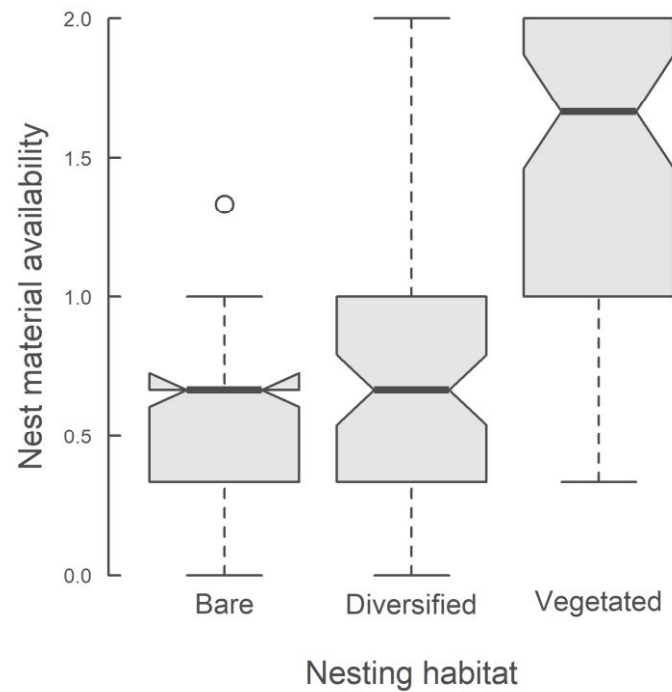


Fig. S1 Lining nest material availability positive relationship with nesting habitat. Box-plots represent two decades before and six decades after the median of incubation start each year. Medians with 95% CI (notches), quartiles, 1.5 inter-quartile range and outliers are presented, n size = 206 nests during 2014–2015 in South Bohemia, Czech Republic, particular sample sizes in number of nests are follows: Bare = 69, Diversified = 68, Vegetated = 54, for more details see Table 1.

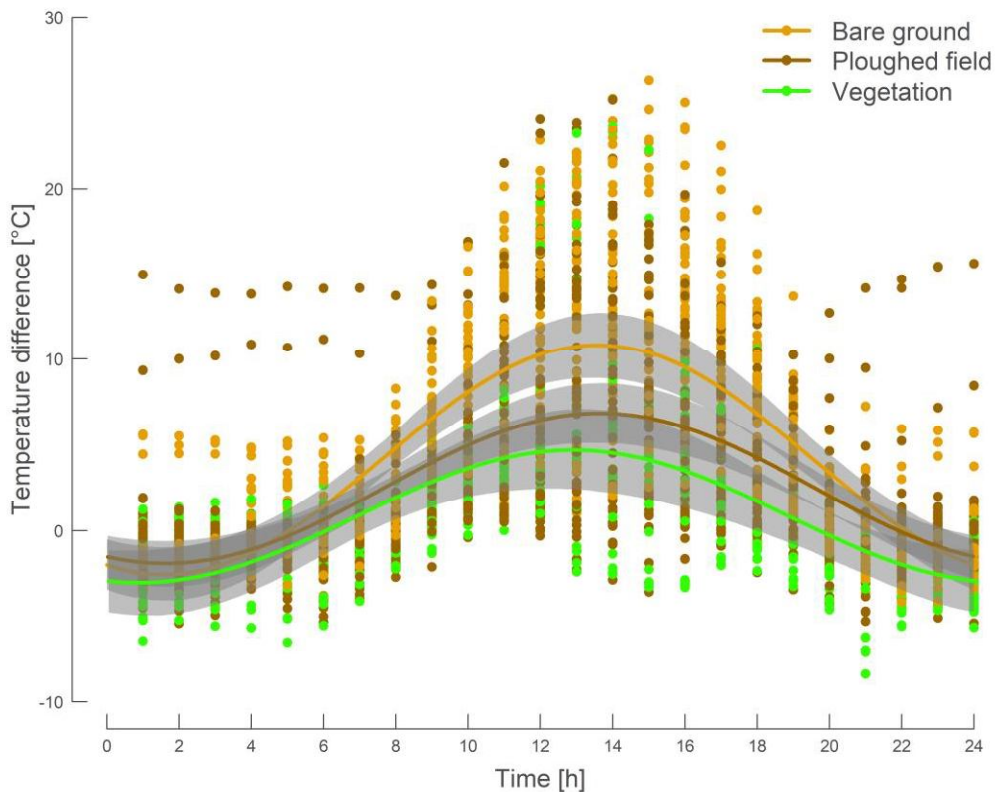


Fig. S2 Differences in ground ambient temperatures among nesting habitat categories, suggesting that vegetated nest sites have more stable and generally colder microclimate. Differences between ambient ground temperature measured in surrounding of 41 nests and meteorological measurement for given hour in nearby city, České Budějovice (ČHMÚ, pers. comm.) were used as a response variable. Measurements were collected between 9 April and 27 May 2016 with temperature data logger placed approximately 1m from the nest. Out of 41 nest-patches, 14 were on bare ground, 18 on ploughed fields and 9 in vegetation. 75 measurements (mean, range 43–193) from particular nest were obtained. Time of day was transformed to radians ($2 \cdot \pi \cdot \text{hour} / 24$) and entered into model as sine and cosine of radians. Both these variables entered into the model (LME: Bates et al. 2015) in interaction with habitat. Moreover, nest identity was fitted as random intercept and sine and cosine of radians as random slopes. Presented are lines with shaded areas indicate model prediction with 95% credible intervals based on the joint posterior distribution of 5 000 simulated values based on model outputs and generated by the ‘sim’ function in R (Gelman et al. 2016).

General discussion

Main findings

This dissertation has revealed in detail several aspects of parental care in the Northern Lapwing, which can be used for a wider generalization of behavior during incubation in birds.

First, a thorough analysis of hundreds of days of video recordings has enabled us to provide a detailed description of the within-nest and between-nest diversity of incubation rhythms (**Chapter 1**). Specifically, we have demonstrated that the incubation rhythms show strong daily rhythmicity but remain stable over the incubation period and during the season. We have further revealed that overall nest attendance varies substantially from nest to nest, and that this variation is strongly affected by male nest attendance. Females compensate for the general lack of male nest attendance only partially.

Second, we have linked the Lapwing data with data from many other species, and we have described the diversity of incubation rhythms among shorebirds (**Chapter 2**). From a comparative perspective, we can see that the Northern Lapwing has rather very short incubation bouts. This fits with the “phylogenetic hypothesis”, because similar lengths of incubation bouts are typical for its related species, the plovers of genus *Charadrius*. However, it also fits with the “antipredation hypothesis”. Lapwings can serve as a typical representative of species that are very well visible while sitting on the nest. They are known to be very aggressive in mobbing predators (Elliot 1985, Kis et al. 2000), and they have long escape distances before a predator or a human approaches. We have shown that this type of species has the shortest incubation bouts among shorebirds (even when controlling for phylogeny). However, our comparative work suggests it is not very probable that the short incubation bouts are related to energetic constraints.

Further, we have demonstrated that continuous video-recordings have the potential to serve as a data reservoir for addressing many other questions. In **Chapter 3** we have shown that the incubating parent can communicate with the non-incubating partner using sex-specific behavioral signals, combining acoustic and visual cues. These signals are probably a part of the negotiation process about parental exchanges on the nest. We have supported this by revealing that the use of these signals helps to synchronize parental exchange on the nest, and that decreasing efficiency of this signaling might have a negative influence on the overall nest attendance.

We then focused on female self-maintenance behavior, particularly sleeping and preening (**Chapter 4**). We found a negative relationship between the male contribution to incubation and the frequency of female preening during incubation. However, the more males incubated, the more time the females spent on sleeping, and the more significant their daily sleeping pattern was.

Finally, we leaved the videotapes and analyzed 915 measurements of the size of the lining of 601 nests (**Chapter 5**). We showed that bigger nest linings are associated with a harsher breeding microclimate, but that they do not correlate with the nest predation rate. Lapwings do not avoid building a big and easily visible nest lining, even in the vicinity of potential predator perches, or when breeding solitarily or on the edge of a breeding association (i.e. in positions with higher risk of depredation; Šálek and Šmilauer 2002, Štorek 2011).

Taken together, our results have pointed out several general implications, which are discussed below.

Variations in the provision of care, and its possible impacts on embryos

The median nest attendance for the Northern Lapwing is approximately 87% (**Chapter 1**, Grønstøl 2003, Lislevand and Byrkjedal 2004, Jongbloed et al. 2006), but the percentage varies greatly from nest to nest. The variation ranged from 67% to 94% in our sample, which represents more than six hours per day of between-nest difference in nest attendance. Moreover, nest attendance is highly repeatable among days (**Chapter 1**). The differences in nest attendance seem to be much larger than those described elsewhere in the literature as having an influence on the reproduction output (Carter et al. 2014, Bueno-Enciso et al. 2017). The important question is therefore how strong an effect this variation has on the individual fitness of Northern Lapwings.

Based on several unpublished observations, we have good reasons to believe that the eggs of the Northern Lapwing have considerable resistance to cooling or overheating. Although it is a curious observation rather than the output of a scientific experiment, we have documented several cases when a vital and healthy chick has been hatched some considerable time after the egg had been abandoned in the nest and then incubated in an artificial hatchery. The most interesting and best documented example showed that (according to the temperature logging in the nest scrape) the last egg had remained more than 3 days without any care from the parents (after partial predation Figure 1). During this time, the egg had been repeatedly exposed to periods of many hours of temperatures below “physiological zero” (i.e. a temperature too low for the development of the embryo), and had also been exposed to temperatures above 40°C. Especially long periods of excessively high temperatures have been shown to be very dangerous

and potentially lethal for avian embryos (Bennet and Dawson 1979, Bennet et al. 1981, Webb 1987). Nevertheless, a healthy chick was hatched after artificial incubation.

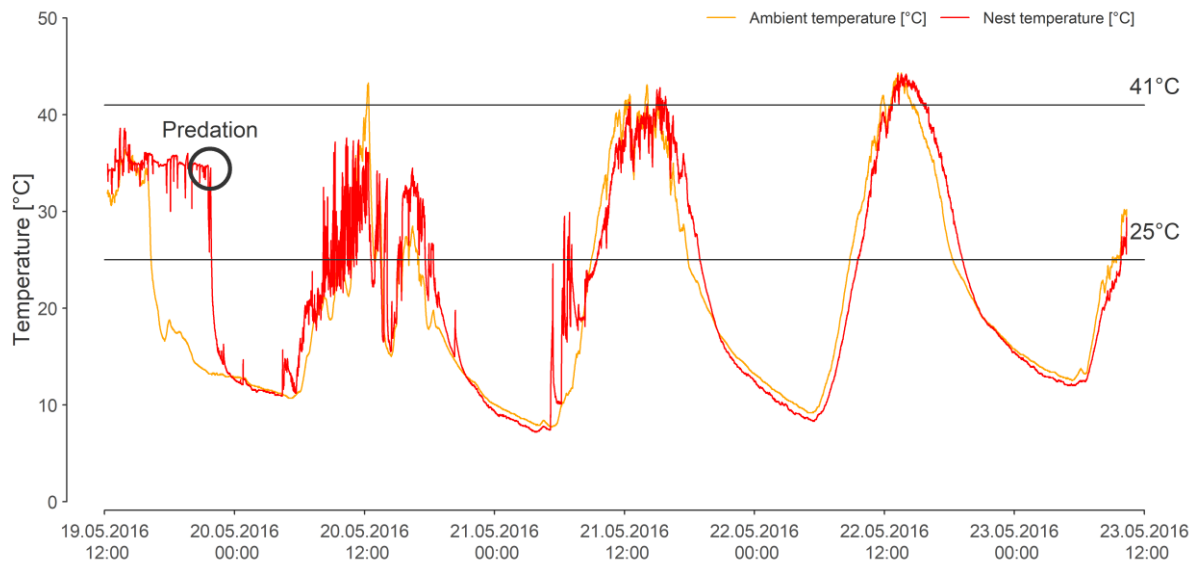


Figure 1. Temperature profile in the partially depredated and subsequently abandoned nest from the day when a predation event occurred to the time when the remaining egg has been collected and put to the artificial hatchery. Red line denotes the temperatures in the nest scrape, orange line in the vicinity of the nest. Black lines denote the approximate range between physiological zero temperature (i.e. temperature when the embryonal development is interrupted) and temperatures potentially harmful temperatures above 41°C.

We believe that it is valuable to report such cases of noteworthy egg resistance. However, they can hardly be proved within standard scientific research, e. g. for ethical reasons, and they cannot be used to assess the real impact of lowered incubation attendance on lapwing fitness. Nonetheless, between-nest differences in incubation attendance have repeatedly been shown to be connected with the length of the incubation period across species (Martin et al. 2007, Carter et al. 2014, Bueno-Enciso et al. 2017), including the Northern Lapwing (Grønstøl 2003). A prolonged incubation period can have a negative influence on the condition of the chick (Olson et al. 2006), can increase pre-hatching mortality (Arnold 1993, Stoleson and Beissinger 1999) and can increase the risk of nest depredation (Mayfield 1961). A negative effect on offspring phenotype has even been documented in species where no correlation was found between incubation attendance and length of the incubation period (Amininasab et al. 2017).

Although the above-mentioned work was carried out on a very small sample of only ten nests, it suggests that there can be a very considerable increase in the length of the incubation period with a decrease in nest attendance (a predicted difference of around 4 days in the length of the incubation period for incubation attendances varying between ~75-92%) (Grønstøl 2003). Unfortunately, we have no further information about the consequences of such a prolongation of the

incubation period on the fitness of Northern Lapwings. However, because the predation pressure on Northern Lapwing nests is very high, the consequences of prolonged exposure to predation risk can be harsh. Given that the daily predation rate in our study population fluctuates around 5%, the real difference in the total predation rate between incubation periods lasting 24 days and lasting 28 days can be more than 5% per breeding attempt. Moreover, the length of the incubation period can have an adverse influence on the condition of the hatchlings (Hepp et al. 1990, Carter et al. 2014). In addition, a strong negative effect of chick condition on chick survival has been documented (Galbraith 1988, Grant 1991). This supports speculations that the fitness costs connected with such huge variations in nest attendance can also be huge.

Unfortunately, we cannot directly test these hypotheses on our current dataset. The reasons are especially the high risk of nest depredation, the inevitable inaccuracy in estimating the beginning of the breeding attempt using the flotation method (Van Paassen et al. 1984), and also the impossibility of making daily nest hatch checks (due to time constraints, and also in order to avoid the negative effects of such frequent disturbances). Given all these problems, we are unable to determine the precise length of the incubation period in the vast majority of the 113 nests in our dataset with estimated nest attendance. Generally, we consider testing these hypotheses to be very challenging in species with the problems that have been mentioned above (i.e. high predation pressure, and the impossibility of making daily nest controls).

The importance of male incubation for parental care

The contribution of the male to parental care is a key component of the variability described throughout this thesis (**Chapters 1,3&4**). The proportion of the time for which the nest is attended by a male varied between nests from 0% to almost 40% (**Chapter 1**, Liker and Székely 1999, Lislevand and Byrkjedal 2004). Females compensate only partially for the lack of male care, increasing their incubation time only by ~ 6.5% with every 10% decrease in male nest attendance (**Chapter 1**). However, we have shown that we can find the consequences of the variability in male behavior in much more aspects of parental care (**Chapters 3&4**).

As was mentioned above, males are responsible for a big part of the variability in overall nest attendance. This raises the question, whether the decrease in total nest attendance is a consequence of the inability of the female to compensate fully for the lack of care from her male partner, or whether it mirrors her unwillingness to carry all the responsibility for the nest. It should be pointed out that even nests with highly nest-attentive males often had nest attendance not higher than 90%, which is fully comparable even with some uniparentally incubating shorebirds (Løfaldli 1985, Kålås 1986). Also, although the effect of the male contribution is

strong and statistically clear (**Chapter 1**, Fig. 2a), the remaining variability has enabled us to see that some females are able to reach higher incubation attendance alone than other females with substantial help from their males. It has also been shown that female body weight remains stable throughout the incubation period (Sládeček 2015), which is a typical pattern in species without strong energetic constraints during incubation (Bryant 1979, Croll et al. 1991, Williams 1996). Thus, in accordance with other recent work (**Chapter 2**, Bulla et al. 2014), it seems improbable that females would lack the energetic capacity to compensate for a lack of male incubation care.

Our findings are in line with the previously described theoretical models which predict partial compensation as an evolutionarily stable response to a decrease in care by the partner. The assumption that the observed decrease in overall nest attendance is, at least partially, an output of negotiation affected by sexual conflict (McNamara et al. 1999, Houston et al. 2005, Harrison et al. 2009, Lessells and McNamara 2012), is also supported by other findings. In **Chapter 3**, we have defined behavioral signals that probably play an important role in the negotiation process. These signals include vocalization practiced shortly before the female departs, and this is in some cases enhanced by her directly flying away from the nest. Consequently, we can see several patterns consistent with the “negotiation hypothesis”. The use of these signals increases the probability that the male will come (i.e. that an exchange will occur during the incubation gap) and also shortens the exchange gap in comparison with events not preceded by female signaling. However, if the male does not fulfill the female’s request for an exchange, the subsequent gap is longer (again, in comparison with gaps that are not preceded by female signaling). Finally, the frequency of female vocal signaling was not correlated with the male incubation effort. However, the more nest-attentive the male was, the bigger chance the female had of being exchanged after signaling.

Based on these findings, we propose that, when the female signals her need for an exchange, she stays away for substantially longer time before she gives up and returns to the nest. We therefore suggest that this may be a proximate mechanism, relating the output from the negotiation to a decrease in incubation constancy. This model might in some cases (e.g. in species with highly unbalanced male contributions to incubation) be better applicable than the earlier repeated-bouts models (Lessells and McNamara 2012, Parker et al. 2014). This is because this model takes into account that one of the parents (i.e. the female) has substantially greater responsibility for the incubation, and that the contribution of the other parent (i.e. the male) is rather optional. Thus, the female can haggle with the male to some extent, but generally she bears responsibility for incubation on her own. This probably leads to a much more asymmetrical negotiation game than has previously been suggested (Schwagmeyer et al. 2002, Parker et al. 2014).

Apart from the matter of incubation attentiveness itself, we have also shown other potential costs when a female chooses a less care-giving male. Females paired with males who rarely incubated spent less time sleeping and more time preening during incubation (**Chapter 4**). We suggest that females with less care-giving males are constrained by the necessity to spend relatively more of their off-nest time on foraging and are therefore to a greater extent forced to maintain their feathers while incubating. However, as will be discussed below, the risk of depredation during the daylight hours is much lower than in the night hours; **Chapter 4**). Thus, since the frequency of both foraging and preening peaked during daylight, we can speculate that the cost of the increased conspicuousness of a self-maintaining female during daylight incubation may also be only minor (**Chapter 2**, Smith et al. 2012).

It is obviously difficult to assess the impact of the fact that the more the male incubates, the more the female can sleep during the period of incubation. We can almost exclude the possibility that solely incubating females sleep more during the incubation gaps (because they probably need almost all the off-nest time for foraging (Grønstøl 2003, Bulla et al. 2015)). Male nest attendance is positively related to the sleeping time of the female. This raises several interesting questions. First, does the lack of sleep have any negative effect on the female? Unfortunately, this has never been studied in Lapwings or in related species, but generally it has been shown that sleep deprivation can cause a range of health problems (Rolls et al. 2011, Vyazovskiy et al. 2011). Second, why does this relationship between the male incubation and the female sleeping effort exist at all? One possible explanation is that better incubating males are also better or more trustworthy in attacking approaching predators, or in giving warnings against the enemy (Elliot 1985). However, this is pure speculation. Further testing is needed, since current evidence for this relationship in other animal taxa is rare and equivocal (Redondo 1989, Cain and Ketterson 2013, Szász et al. 2019).

A very special feature of the incubation rhythms in the Northern Lapwing is the almost complete absence of male incubation during the night (**Chapter 1&2** Sládeček and Bulla 2018). A similar phenomenon has been observed in some plovers of the *Pluvialis* genus (Bulla et al. 2016). However, this is in contrast with the situation found in several related plover species of the genus *Charadrius*, where there is prevalent male night incubation (Thibault and McNeil 1995, Blanken and Nol 1998, St Clair et al. 2010, Ekanayake et al. 2015). The predominance of male night incubation in these plovers has been explained from the antipredator perspective, since the more ornamented males are more conspicuous while sitting on the nest, and are therefore more prone to predation (Ekanayake et al. 2015). However, although Northern Lapwing males are also the more ornamented sex (Meissner et al. 2013), their conspicuous sitting during the day probably has

negligible costs in terms of increased predation risk, because they outweigh this disadvantage by their aggressive mobbing of predators. In addition, it seems improbable that Northern Lapwing males would be less efficient incubators, a phenomenon that is known from other species (Hawkins 1986, Kleindorfer et al. 1995, Voss et al. 2008, Parker et al. 2014) (see supplementary actograms to **Chapter 1**). So why do they so rarely incubate at night? We offer a possible explanation coming from the daily patterns of female vocalizations, the intensity of which decrease steeply during the night (**Chapter 3**). It seems that females probably prefer to incubate the whole night alone, rather than to negotiate about the possibility of exchanging by vocalization, because this negotiation would be costly due to an increase in the depredation risk.

A concluding question about the diversity of male parental care concerns why females so often breed with males that incubate only a little, when other unpaired males are often available (Parish and Coulson 1998, Parish et al. 2001). Sexual selection theory predicts that a female may prefer to breed with a less care-giving male when she obtains some other advantage, such as good genes (Weatherhead and Robertson 1979) or better territory (Berg 1993). It has previously been reported that Northern Lapwing males are not selected for their melanin ornaments (Schonert et al. 2014), a traditionally considered proxy of male quality in the sense of social status (Veiga 1993, Hoi and Griggio 2008, Solberg and Ringsby 2010), competition for resources (Senar et al. 1993, Van den Brink et al. 2012), or territory defense (Quesada and Senar 2007). In addition, the finding that polygynous males usually defend larger territories (Byrkjedal et al. 1997, Parish and Coulson 1998) does not necessarily provide an advantage for the female, since the larger territory is shared with other females. Moreover, many polygynous males are good incubators (Grønstøl 2003). A possible benefit could be that males who incubate only a little make a greater effort to defend their territories (however, see the discussion on female sleeping patterns above). In brief, we did not find any promising answer concerning possible advantages of breeding with little-incubating males, but we have pointed to more aspects of the life of females than are usually considered to be affected by the negative consequences of breeding with such males.

Behavior of incubating Lapwings as an antipredator strategy

How important is predation as a driver shaping the parental behavior of the Northern Lapwing? From a comparative perspective, we found that shorebirds with generally longer flight initiation distance when disturbed by humans generally have shorter incubation bouts (i.e. parental exchange occurs after a shorter period of time) (**Chapter 2**). The Northern Lapwing is a typical representative of such species (Bulla et al. 2016, Sládeček and Bulla 2018). However, when we look at

Northern Lapwing behavior in greater detail, we can observe a wide range of approaches to antipredator strategies, typically contrasting between daylight and night. Consequently, the extent to which lapwings address the risk of predation in their behavioral decisions is remarkably ambivalent.

On the one hand, it seems that Lapwings do not decide about the magnitude of their nest lining on the basis of nest visibility (**Chapter 5**). The size of the nest lining is therefore not related either to the position within the colony (predictor of the nest predation rate, Šálek and Šmilauer 2002), or to the distance to a potential predator perch (Štorek 2011). However, the predation rate does not reflect the magnitude of the nest lining, i.e. there is no substantial risk arising from the conspicuousness of the nest. A similar pattern can be shown on the parent's behavior during incubation. In fact, the behavior of Lapwings on the nest during the day is very conspicuous. Parental exchanges and also gaps in incubation are very frequent (**Chapter 1**, Sládeček et al. 2019). This means that many movements occur around the nest, which can be very helpful as a cue for a predator searching for nests (Šálek and Zámečník 2014). In contrast with many related species (Blanken and Nol 1998, St Clair et al. 2010, Ekanayake et al. 2015), the Lapwing male (the parent with more conspicuous plumage coloration) incubates almost exclusively during daylight (**Chapter 1**, Jongbloed et al. 2006, Sládeček et al. 2019). Moreover, parents actively negotiate about their exchanges on the nest, and this includes conspicuous vocal and movement signaling (**Chapter 3**). The intensity of this signaling is considerably higher during the day than in the night. Finally, during daylight the vigilance of incubating females is disrupted by sleeping and by other self-maintenance activities far more than during the night (**Chapter 4**). All this suggests that the species does not address the risk of predation during the day by reducing the conspicuousness of its presence at the nest or the conspicuousness of the nest itself, with the exception of cryptic egg coloration. The activities performed during incubation can then be subordinated to this perception of the risk of predation during the day, when the main anti-predator tactic consists in early detection of a predator, and its subsequent mobbing.

By contrast, the night incubation behavior is completely different. The females are silent and vigilant (**Chapter 3&4**), and they sit the whole night almost without any interruptions (**Chapter 1**, Sládeček et al. 2019). However, the risk of depredation during the night remains very high (**Chapter 4**). In our study population, the daily predation rate was approximately 5%, which corresponds to an approximately 75% risk that the female will be present when a predator arrives during each breeding attempt (assuming a 27-day incubation period, which is the median length in the study population).

Several conclusions can be drawn from the above paragraphs. First, predation seems to be a very important factor, which needs further attention. It shapes all aspects of parental care, including incubation rhythms and the behavior of the parents at the nest. Second, the extent to which the behavior of incubating parents is shaped by predation can differ greatly, according to the perceived risk of depredation. This means that the importance of predation risk as a driver shaping incubation behavior varies over the course of day. We should therefore distinguish more carefully between species (situations) that are prone to predation by avian predators and those prone to predation by mammalian (olfactory) predators, because the complex of adaptations can be totally different. Future studies should take this into account.

Methodological implications

From a methodological viewpoint, this thesis shows the potential for using continuous video-recordings as a valuable dataset for addressing many aspects of a complex behavioral phenomenon such as incubation care (**Chapters 1-4**). It is true that other methods, such as temperature data-logging and the use of Radio Frequency Identification Device (RFID) transponders enable incubation data to be extracted much more simply and much more quickly, using automated software tools (Bulla et al. 2016). However, they provide information only about the presence/absence of a bird on the nest, and nothing about other aspects of bird behavior. The most universal method - "direct observation" - can provide only observations of limited length. Data based on direct observations usually consists of very short and non-continuous time intervals (Liker and Székely 1999, Bulla et al. 2012). It is therefore improbable that sufficient numbers of less frequent behavioral features will be detected. Moreover, it is usually not possible to make observations during the night. This inevitably leads to bias, since most behaviors show some kind of daily rhythmicity (**Chapters 1-4**, Dunlap et al. 2004).

The use of continuous video recording solves most of the problems mentioned above. We have complete information about focal bird's behavior during both day and night. The informational potential of this kind of dataset reaches far beyond the examples shown within this thesis. By watching such videos, we were able to learn how incubating birds respond to the approach of many animal species (including potential predators, or conspecifics). We can see who repairs and who builds the nest lining, and when. We could also analyze any other specific aspect of the parents' breeding life, including, e. g., exactly how the birds turn the eggs. One specifically important advantage of video recording is that a sufficiently large sample can be obtained even for an analysis of relatively rare and specific features. For example, we were able to make a statistical evaluation of the efficiency of females vocally signaling the need to be exchanged even during the night, when

these signals, and also male incubation, are rare (**Chapter 3**). Finally, extracting the behavior afterwards from the camera footage enabled us to work with a lower error rate than in real-time observations. There were also no errors in reading the data-logging records (own unpublished experiences).

Nevertheless, we have to mention at least one weakness of the method that we used. While we have large sets of information about the incubating parent, we usually have no information about the non-sitting parent, which is a big gap in current knowledge about avian reproduction (Ashkenazie and Safriel 1979, Grønstøl 2003, Bulla et al. 2015). Unfortunately, this shortcoming has limited many of the conclusions that we have been able to draw. For example, when we are describing the vocal signals coming from the departing parent (**Chapter 3**), we have no information about any possible communication from the side of the non-incubating parent, which (we believe) is being requested to incubate (Boucaud et al. 2016, 2017). In addition, we are able to describe the distribution of the self-maintenance activities of females during incubation, but we are missing any information about what they do when they are not incubating (Ashkenazie and Safriel 1979, Bulla et al. 2015).

Although this is surely a key limitation of this thesis, our current work can form a good basis for future work, combining information known from our analysis with some specifically focused direct observations, or (perhaps) with dataloggers such as accelerometers or magnetometers, which can extract the bird behavior automatically (Wilson et al. 2006, Shamoun-Baranes et al. 2012, Williams et al. 2017).

Conclusions

To conclude, our results are based on analyses of several aspects of the parental care provided by the Northern Lapwing. Some of the results deal with phenomena that have been very little investigated in wild animals. These include communication between parents about exchanges on the nest, and the relationship between the division of incubation between parents and the daily rhythmicity of self-maintenance activities.

We have demonstrated that the extent of the male contribution to incubation can not only influence the overall quantity of incubation care but also has an impact on female self-maintenance behavior during incubation. This finding has broadened and throws some light on the potential fitness costs for the female, in connection with mate selection considerations.

We have further shown that the main drivers shaping the behavior of incubating parents can vary according to the daily pattern of predation risk. During the night, the behavior of the parents is highly adapted to cope with the risk of depredation.

In contrast, during the daylight hours, much greater importance is attached to the parents' self-maintenance needs, and there is more conflict about who will incubate the nest.

Our studies have also highlighted several gaps and questions that should be addressed by future research:

- 1) To better understand the different roles of parents during parental care in the Northern Lapwing and ecologically similar species (i.e. biparental, but with highly unbalanced contributions to incubation), we need to know how the ability or the willingness of the male to defend the territory relates to his incubation effort.
- 2) For a better insight into the complex antipredator behavior, we should focus our attention on the role of territory guarding and warning the incubating partner during the night. It would also be useful to investigate how night-operating predators use sound perceptions as a cue in their search for prey. This would help us to finalize the interpretation of the lowered usage of vocal signals during the night (**Chapter 3**).
- 3) From a general perspective, much more attention should be paid to time-budget studies of incubating birds while sitting on the nest and also during their off-nest time (including the rhythmicity of all studied behavioral traits). This would greatly improve our understanding of the complex trade-offs between providing parental care and self-maintaining behavior.
- 4) We should also pay more attention to the patterns and the fitness consequences of between-individual variability in sleep behavior. Specifically, we know almost nothing about the potential fitness costs connected to variations in the time spent sleeping.

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Author contributions

Chapter 1 - Diversity of incubation rhythms in a facultatively uniparental shorebird – the Northern Lapwing: M.S., E.V. and M.Š. collected the data; M.S. and E.V. extracted the incubation data from videos, M.S., M.B. and M.Š. conceived the paper, M.S. and M.B. analyzed and visualized the data, drafted the paper and with input from M.Š. and E.V. wrote the final paper.

Chapter 2 – Unexpected diversity in socially synchronized rhythms of shorebirds: M.B. and B.K. conceived the study. All authors except B.H. collected the primary data (see <https://osf.io/sq8gk>, ref. 16). M.B. coordinated the study and managed the data. M.B. and M.V. developed the methods to extract incubation. M.B. extracted bout lengths and with help from A.R. and M.V. created actograms. M.B. analysed the data with help from M.V. M.B. prepared the supporting information. M.B. and B.K. wrote the paper with input from the other authors. Except for the first, second and last author, the authors are listed alphabetically by their first name. M.S. Extracted incubation data from the video-recordings for the Northern Lapwing and participated on the data collection from the Little Ringed Plover.

Chapter 3 – Parental incubation exchange in a territorial bird species involves sex-specific signaling: MS, EV and MŠ collected the data; MS EV and KB extracted the incubation from videos, MS analyzed the data and wrote the paper, with input from MŠ All authors read, commented on and approved the final manuscript.

Chapter 4 – Daily rhythmicity of female self-maintenance activities during parental care is affected by predation risk and incubation attendance by male in a biparental shorebird: M.S, K.B and M.Š. conceived the study, M.S and E.V. collected the incubation data and extracted incubation behavior from recordings, K.B. extracted other behavioral data from recordings, M.S. analyzed the data, M.S., K.B. and M.Š. wrote the manuscript.

Chapter 5 - Great variability in nest lining size : support for thermoregulation but not for anti-predatory adaptation hypothesis: All authors conceived and planned the study, VK and MS collected the data with the help from MŠ, all authors analyzed the data, VK was the lead writer of the manuscript with input from MS and MŠ.

Declaration of originality

I declare that this thesis has not been submitted for the purpose of obtaining the same or any other academic degree earlier or at another institution. My involvement in the research presented in this thesis is specified in the statement of contributions and it is also expressed through the authorship order of the included publications and manuscript. All relevant literature sources used while writing chapters in this thesis have been properly cited.

Martin Sládeček



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