



# Does complexity of conspecific song influence reproductive decisions and investment in European Common Reed Warblers: an experimental playback approach?

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## Abstract

The beginning of reproduction is usually accompanied by a series of decisions including where to establish a territory, attract a mate, to lay eggs or give birth. These decisions might be influenced by the quality of potential partners but also conspecific neighbours. In birds, the quality of an individual can be signaled by song features, like song complexity. Besides choosing the right location for reproduction and mate attraction, we propose here that song may also influence the reproductive investment, in particular early maternal investment, which is known to be a sensitive determinant reflecting different allocation of females in relation to male quality and attractiveness. Here, we examined whether song quality of conspecific neighbours can stimulate various reproductive parameters in European Common Reed Warblers (*Acrocephalus scirpaceus*). Therefore, we performed field playback experiments advertising song of high and low complexity and examined their influence on settlement decisions, early maternal investment, and breeding success. The number of nests and start of egg laying did not differ between the two playback types but nests were significantly closer to high complex song playbacks. Furthermore, in the high complex song group, a significantly higher proportion of eggs was fully dark pigmented than in the low complex playback group. Nests near high complex playbacks were significantly more successful and significantly more offspring were produced. The results are discussed in relation to whether males use song to stimulate female investment and whether establishing a breeding location near a high-quality neighbour may be an additional aspect in settlement decisions.

**Keywords** *Acrocephalus scirpaceus* · Song complexity · Reproductive parameters · Playback experiment · Maternal investment

## Zusammenfassung

**Beeinflusst die Komplexität des Gesangs benachbarter Artgenossen Fortpflanzungsentscheidungen und Investitionen bei europäischen Teichrohrsängern: ein experimenteller Ansatz mit Hilfe von Gesangsplaybacks?**

Zu Beginn der Fortpflanzung sind in der Regel eine Reihe von Entscheidungen zu treffen wie z. B., wo ein Territorium etabliert, ein Partner angelockt, oder ein idealer Brutplatz zur Verfügung steht. Diese Entscheidungen können von der

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Qualität potenzieller Partner, aber auch von anderen Artgenossen in der Umgebung beeinflusst werden. Bei Vögeln können z. B. Gesangsmerkmale wie die Komplexität des Gesangs die Qualität eines Individuums signalisieren. Neben der Wahl des richtigen Ortes für die Fortpflanzung und dem Anlocken von Partnern vermuten wir, dass der Gesang auch den reproduktiven Aufwand eines Individuums beeinflussen kann. Insbesondere die frühe mütterliche Investition, ist ein sensibler Faktor der z. B. auch durch die Qualität und Attraktivität der Männchen beeinflusst werden kann und sich in der Verteilung mütterlicher Ressourcen unter den Nachkommen widerspiegelt. In diesem Zusammenhang untersuchten wir beim Teichrohrsänger (*Acrocephalus scirpaceus*), ob auch die Gesangsqualität von Nachbarn der gleichen Art, reproduktive Parameter stimulieren kann. Dazu führten wir im Freiland Playbackexperimente mit Gesängen, die hohe und niedrige Komplexität widerspiegeln durch, und untersuchten ihren Einfluss auf Ansiedlungsentscheidungen, frühe mütterliche Investition und Bruterfolg. Die Ergebnisse zeigten keinen Unterschied in der Anzahl der Nester und dem Beginn der Eiablage zwischen Standorten, die mit den beiden Playbacktypen beschallt wurden, aber die Nester lagen deutlich näher bei Standorten bei denen hochkomplexe Gesänge präsentiert wurden. Darüber hinaus war in der Gruppe mit hochkomplexem Gesang ein signifikant höherer Anteil der Eier vollständig dunkel pigmentiert als in der Gruppe mit weniger komplexem Gesang. Außerdem war der Bruterfolg von Nestern in der Nähe von hochkomplexen Gesängen signifikant höher und es wurden signifikant mehr Nachkommen produziert. Die Ergebnisse werden im Hinblick darauf diskutiert, ob Männchen ihren Gesang, neben dem Anlocken von Weibchen, auch dazu nutzen können, um die mütterlichen Investitionen ihrer Partner zu beeinflussen, und ob in diesem Zusammenhang ein Brutplatz in der Nähe eines Nachbarn mit hoher Gesangsqualität ein zusätzlicher Aspekt bei Ansiedlungsentscheidungen sein könnte.

## Introduction

In birds for each individual, the start of reproduction means a few necessary decisions to be taken, like where to establish a territory, attract a mate, to lay eggs or give birth (Emlen and Oring 1977; Greenwood 1980). These decisions are influenced by a variety of environmental factors, including e.g., i) the availability of resources comprising food or shelter, ii) the quality of display sites influencing the likelihood to attract a mate, iii) the existence of proper breeding sites, and iv) protection from predation (Cody 1985; Danchin et al. 1998; Morris 2003; Schmidt et al. 2010; Szymkowiak and Kuczyński 2015). Mainly for female birds also, the quality of the potential partner plays an important role in this decision-making process. Whereby the relative importance of habitat and mate choice may vary between individuals, region or species (Jennions and Petrie 1997; Price 1998; Chalfoun and Schmidt 2012). In some species habitat quality is essential and to occupy a high-quality territory may significantly influence a male's mating success. In male Corn Buntings (*Emberiza calandra*) or Great Reed Warblers (*Acrocephalus arundinaceus*) for example, the number of attracted females and hence the degree of polygyny depends on the quality of their territory (Leisler 1985; Hartley et al. 1995; Leisler and Wink 2000). Female corn buntings solely choose their breeding site based on habitat (territory) quality, whereas in other species also, or mainly male quality are important (Andersson 1994).

Thus, habitat choice and mate choice can be considered as the two most important activities that must be done at the onset of reproduction (Andersson 1994). However, beside

territory and male quality also, the social environment can influence reproductive decisions. In this context, one important function of the social environment is related to habitat copying (Delgado et al. 2009). E.g., young males may be less experienced in evaluating habitat parameters properly and, therefore, an alternative would be to consult help regarding choice of a breeding site (Höglund and Alatalo 1995; Kentie et al. 2014). In this respect, social attraction or habitat copying has been demonstrated to be a useful mechanism (Szymkowiak et al. 2016; Buxton et al. 2020; Luepold et al. 2023). Higher quality conspecifics usually occupy better territories (Johnson 2007) and may, therefore, also positively influence the breeding success of the copying individual. Thus, when relying on conspecifics, individuals may not just copy their habitat choice but even integrate information about their intrinsic quality into their own decision-making process. Another benefit of social aggregation could be related to communal defence strategies against nest predators (Krams and Krama 2002).

A second function of the social environment is related to mating behaviour. E.g., the quality of a conspecific neighbour could also influence the mating success of a male (Schjørring et al. 1999; Kokko and Rankin 2006). A more attractive neighbour may attract more potential mating partners which consequently may increase the likelihood to meet and attract potential mating partners, when settling nearby (Westneat and Sherman 1997; Kokko and Rankin 2006). Settling near a high quality, even already mated male could be also beneficial for females as they may benefit by increasing the likelihood of extrapair fertilizations and consequently increasing the genetic quality of their offspring or future mating chances (Wagner 1992, 1998). Thus, although for different goals, both sexes may have reasons to carefully

select the social neighbourhood and settle near conspecific males of high quality and/or being highly attractive.

Here, we propose a third function in relation to the social environment namely, that attractiveness or quality of the neighbour also influences the reproductive investment, in particular early maternal investment of a reproducing female. Maternal investment is known to be a sensitive determinant reflecting different allocation of females in relation to male quality (attractiveness) (Mosseau and Fox 1998). To gather information about individual quality, female birds frequently use parameters like body and ornaments size, coloration intensity or fighting ability (Gosler et al. 1998; Keyser and Hill 2000; Fülöp et al. 2022). In particular, male song could be an ideal candidate in relation to maternal investment. Besides being an important criterion in mate choice, song can also directly stimulate female investment (Mota and Depraz 2004).

There is indication for egg investment in relation to song quality like egg yolk testosterone concentration (Gil et al. 2004), production of heavier eggs (Soma and Okanoya 2013) or bigger clutches (Darolová et al. 2012). Furthermore, song structure can be related to breeding success in terms of hatching (Woodgate et al. 2012) or nesting success (Conner et al. 1986). However also behavioural aspects can be influenced by song quality (Hoi-Leitner et al. 1993; Hofstadt et al. 2002; Darolová et al. 2012; Bartsch et al. 2015). There is indication that song stimulates reproductive activities, e.g., Mota and Depraz (2004) could demonstrate in a field experiment on European Serins (*Serinus serinus*), that male song can stimulate female nesting behaviour. Their playback experiments suggest that presentation of a song of any male, additional to the male mate, has a stimulating effect.

Assuming that song serves to stimulate female investment, we hypothesise here that, also song quality of conspecific neighbours may influence early maternal investment and consequently breeding success. To examine our hypothesis, we choose the Common Reed Warbler (*Acrocephalus scirpaceus*) as our model species. Specifically, we want to answer the question whether song complexity could, beyond mate choice, also play a role in stimulating female investment into offspring.

The Common Reed Warbler is ideal for this investigation because (i) the importance of song quality in mate choice, (ii) indication for maternal investment dependent on song quality, (iii) their socially monogamous mating system mediated by the occurrence of extra-pair paternity.

Ad (i) Song complexity seems to be an important feature of female choice and song seems to be an indicator of individual quality which is well documented in *Acrocephalus* warblers in general (Catchpole 1980; Hasselquist et al. 1996; Marshall et al. 2003; Catchpole and Slater 2008), and the Common Reed Warbler in particular (Krištofík et al. 2014). Ad (ii) In a previous study on Common Reed Warblers, we

found that egg yolk weight as well as egg yolk testosterone concentration increased when their mates sang faster and repeated syllables more often (Krištofík et al. 2014). Ad (iii) Common Reed Warblers are mainly socially monogamous as in our study area (Leisler and Schulze-Hagen 2011), although there seems variation in the mating system with some populations exhibiting polygyny (Halupka et al. 2014, 2024). Common Reed Warblers also tend to exhibit moderate (Davies et al. 2003) to high levels of extra-pair paternity (Hoi et al. 2013).

Thus, if males use song as a tool to stimulate female investment (Mota and Depraz 2004), we predict that establishing a breeding location near a male or several males singing highly complex, may be also beneficial for a male in terms of stimulation of early maternal investment of its female partner and, therefore, could be an additional aspect in settlement decisions. In this context, Garcia-Fernandez et al. (2010) found indication that female canaries eavesdrop on the song of other males, specifically song interactions between males which subsequently influence their partners' maternal investment. Based on this information, we investigate the role of song quality of conspecific neighbours and early female investment and breeding success in the European Common Reed Warbler. We would predict that at sites with high song complexity presented: (i) more females should settle and, therefore, more nests to be found, (ii) nests to be closer to the simulated song posts, (iii) laying to start earlier, iv) higher early maternal investment, and v) higher reproductive success. Therefore, we used song playbacks of male Common Reed Warblers to experimentally simulate territory owners singing with high and low complexity. By broadcasting several singing males from one site, we tried to simulate song hot spots (for details see "Methods") to amplify the stimulus presented towards females. Subsequently, we determined nest site location, start of egg laying, early maternal investment in terms of clutch size and egg quality and breeding success in terms of hatched and fledged nestlings in relation to playback type.

## Methods

### Study species and site

The Common Reed Warbler is a seasonally and socially monogamous species without visual or morphological sexual dimorphism (Glutz von Blotzheim and Bauer 1991). Common Reed Warblers start to arrive in our study area at the beginning of April and immediately start to produce their complex and continuous song (Catchpole 1980; Glutz von Blotzheim and Bauer 1991; Krištofík et al. 2014). Their typical breeding localities are reed stands, usually less diverse and monotone environments with not much variation in

vegetation structure. Males arrive prior to the females in the breeding area and establish a territory (Hoi et al. 1991; Ille et al. 1996). These multi-purpose territories are used to attract a female, deter competitors, to some degree for foraging and offspring feeding (Leisler and Schulze-Hagen 2011) and to ensure a sufficient interterritory distance, to reduce density dependent nest predation (Hoi and Winkler 1998) and opportunities for extra-pair copulations and extra-pair paternity (Currie et al. 1998). Shortly after mating, females begin to build their nests usually near a male's song posts. The distance between nest and song post is about 8–11 m (unpublished data). Given that territories are small and homogenous and similar regarding habitat structure (Hoi et al. 1991; Ille et al. 1996), especially the very complex male song is very likely most important in female choice.

This study was performed in the year 2019 at fishponds near Veľké Blahovo, West Slovakia (48.052433°N, 17.598156°E). The area included marsh stands, mainly consisting of *Phragmites australis* and partly *Typha angustifolia* and *Carex* spp. around three fishponds, covering an area of approximately 70 ha. Common Reed Warblers usually breed in dense reed stands but also on the edge between reed or sedges and open water (Catchpole 1983; Glutz von Blotzheim and Bauer 1991).

### Design of the playback experiments

Based on the experience of previous years we selected ten suitable playback spots (breeding activities observed in the previous years) and randomly assigned each spot to a playback type (low or high complexity playback). To avoid acoustic interference between playback spots, the distance between playbacks was at least 100 m, a distance from which playbacks could not be heard.

Playback experiments were performed daily between April 5 and June 10 and the daily time window for the playbacks to be performed stretched from 6:00 a.m. to 13:00 p.m. After playback presentation, all speakers were removed from the poles and recharged overnight. Thus, all speakers were newly set up every following day but at the same site.

For the song presentations, we used ten black playback speakers (Technaxx MusicMan Mini BT-X2, 5 × 5 × 5 cm) protected from above by a green plastic roof and fixed on poles at a height of 1.5 m, which approximately corresponds to the singing height of Common Reed Warblers (Heuwinkel 1990; Leisler and Schulze-Hagen 2011). Playback setup was done by two persons (AD, JK), each being responsible for the same five playback locations throughout the experimental period.

### Playback preparation and procedures

For the playbacks, we used the songs of Common Reed Warblers which had been recorded at the same locality and already analyzed in previous years. This data set consisted of recordings of 74 males. For song recording, we used an M-Audiotrack digital recorder connected to a condenser microphone mounted on a Grampian parabolic reflector using a sampling rate of 44.1 kHz and a bit depth of 24 bit, an FFT length of 256 points on a flat-top window type for precise magnitude measurements, and in order to achieve a higher temporal resolution, a 50% overlap. For song analyses, we used Avisoft Bioacoustics software (Avisoft Bioacoustics, Glienicke, Germany). The 30 s of song of each male was printed and subsequently analyzed by AD. Following Krištofík et al. (2014), for the analyses, recordings were further divided into 6-s intervals to determine structural parameters over the course of the song and the reliability of using 30 s of male song. This interval length was chosen based on early studies on Common Reed Warblers (Krištofík et al. 2014).

For the playback experiments, we used only recordings of good quality, without disturbance by con- and heterospecifics. To generate (by LR) the playbacks, we used the program Raven Pro. The sound pressure level was kept the same for all experiments (within the natural range), otherwise recordings have not been manipulated. Based on the variable “new syllable types”, a parameter which has been found to be a very suitable measure for song complexity in Common Reed Warblers (Krištofík et al. 2014), we chose song recordings of 15 males with extreme song complexity values and assigned them either to the highest ( $n = 8$  males) or lowest complexity group ( $n = 7$  males).

Different song features may differently contribute to song complexity, differ between males, and may represent different aspects of a male's song. Therefore, we further evaluated whether the parameter we used to preselect songs with low and high complexity (Krištofík et al. 2014) sufficiently describes overall differences in complexity. Thus, we used in total eight song parameters, which confirmed the original division (see below and results). These eight song parameters included (i) song speed, (ii) syllable types, (iii) new syllables, (iv) new syllable types, (v) repeated syllables, (vi) repeated syllable types, (vii) immediately repeated syllables and (viii) syllable switches. (i) Song speed reflects the number of syllables a male produces/30 s. (ii) Syllable types represent the number of different syllable types a male produce in 30 s based on 5–6-s intervals. (iii) New syllables represent the number of new syllables in 30 s, based on their appearance at every 6-s interval. (iv) New syllable types represent the number of new syllable types in 30 s based on their appearance at every 6-s interval. (v) Repeated syllables represent the total number of syllable repetitions during 30 s of



**Table 1** Song parameters of song playbacks with low and high complexity

Song parameters	Song complexity	
	Low	High
	Mean (SE)	Mean (SE)
Song speed	169.25 (2.5)	184.78 (3.14)
Syllable types	53.35 (1.47)	93.15 (3.1)
New syllables	81.90 (3.54)	143.60 (2.16)
New syllable types	25.55 (0.64)	69.30 (2.80)
repeated syllables	154.00 (2.79)	151.90 (5.82)
Repeated syllable types	41.10 (1.94)	46.90 (1.17)
Switches	81.00 (3.62)	106.80 (4.86)
Immediately repeated syllables	116.75 (4.63)	116.75 (5.45)

Given are means and in parenthesis standard errors

**Table 2** Factor loadings of the different song parameters on component I and II

Song parameters	Component	
	I	II
Song speed	<b>0.884</b>	0.300
Syllable types	<b>0.899</b>	-0.390
New syllable	<b>0.905</b>	0.126
New syllable types	<b>0.925</b>	-0.336
Repeated syllables	-0.005	<b>0.919</b>
Repeated syllable types	0.462	0.413
Syllable switches	0.656	-0.617
Immediately repeated syllables	-0.037	<b>0.945</b>

The model selected was based on a principal component analysis after rotation using Kaiser Varimax normalization (rotation merge after three iterations). Each male entered only once in the analyses. High correlation coefficients are indicated in bold

song, based on 5–6-s intervals. (vi) Repeated syllable types represent the total number of syllable types repeated during 30 s of song, based on 5–6-s intervals. (vii) Immediately repeated syllables represent the number of syllables that are immediately repeated over 30 s of song, based on 6-s intervals. Finally, (viii) Syllable switches represent the number of switches/30 s song based on 5–6-s intervals. For details regarding song parameters, see also Křištofík et al. (2014).

### Song differences between playback types

To get a more complete picture regarding the complexity of a male's song structure, we included the above-mentioned parameters (see also Table 1) in a principal component analysis which confirmed the preselection based on new syllable types (Table 1). Based on these analyses also including the other parameters, two components could be extracted, explaining together 81.4% of the total variance

(Table 2). After a Varimax rotation, high factor loadings of the variables, song speed, number of syllable types, new syllables, and new syllable types (Table 2) suggest component I (explaining 51.2% of variance) to represent a component describing song complexity. On the other hand, highly positive factor loadings of the variables number of repeated syllables and immediately repeated syllables and negative loadings of the variable syllable switches (Table 1) with component II (explaining 30.2% of variance) suggests this component to represent structural simplicity.

Thus, comparing factor loadings derived by the eight parameters of songs with low and high complexity revealed statistically significant differences between these two groups (see results Table 2) and suggest together with the high explanatory values and correlation of song variables with the components, that playbacks are robust against individual variation.

After determining song recordings with low and high complexity, all recordings of a group were compiled in a single playback file and used to simulate either song spots with low or high complexity. Each male recording lasted for 120 s and was interrupted by 30 s of silence before the following male song recording started. The selection of strophe length and breaks is based on natural observations, inspection of song recordings, and described by Glutz von Blotzheim and Bauer (1991). Furthermore, in the highly complex song, each male ( $n=8$ ) appeared two times over the course of the whole playback, whereby always different parts of a male song recording have been included. For songs with low complexity seven males were available, therefore, one male appeared three times during the playback. Thus, the total playback time was 40 min for playbacks with low and high complexity respectively, and songs were advertised in a loop for nine times. Thus, the total time of the experiment was 6 h. For high and low complexity song types, we generated three playbacks, respectively, where the order of presentation of song of individual males varied and which were alternately changed daily for presentation.

In this way, we wanted to create song hot spots, with not only a single but a cluster of males advertising extreme songs, either highly complex or of very low complexity. Furthermore, with this design, we can reduce pseudo-replication and the influence of individual specific features or peculiarities of single playback recordings on settlement decisions.

### Field work

To determine the number and location of nests as well as the start of egg laying, after playback presentation and birds have been observed nest building, we started to search for nests in the surroundings, within 25 m of each playback, because within this distance, audibility of the reproduced

song was guaranteed. Nest search was done by three persons (JK, AD, LR). All nests in the survey area around the loudspeakers were found without eggs during their construction. For each nest, we measured its distance from the playback. We further monitored all nests and recorded clutch and brood size, and hatching and fledging success, as well as nest predation.

To determine egg quality, we collected the third egg of each clutch. In order to distinguish the third egg of a clutch, which was removed in the morning it was laid, the first two eggs were marked with a tiny spot on the shell surface using a black permanent marker. The third egg was replaced by a dummy egg made of epoxide. No brood was deserted because of our manipulation. The removed egg was immediately put into a cooling box (4 °C) for transportation and, after arrival in the laboratory, was measured and frozen at –80 °C.

### Laboratory egg quality procedure

To determine egg quality, we measured the length, width and weight of the freshly laid egg, the weight of the egg yolk and white, and as an additional egg quality parameter, we measured the antimicrobial activity of the egg white lysozyme. Lysozyme is the best known antimicrobial protein in egg white which is effective against Gram-positive bacteria which can significantly influence hatching success and embryo viability (Pellegrini et al. 1992; Javůrková et al. 2015; Ragland and Criss 2017).

Egg size (egg length and maximal width) was measured with electronic calipers to the nearest 0.01 mm immediately after egg removal. Fresh egg weight was determined with an electronic balance to the nearest 0.0001 g, at the day of sampling in the afternoon in the laboratory. Eggs were frozen to –80 °C and analyzed after fieldwork was completed. For the analyses, eggs were defrosted, and egg yolks were carefully dissected from thawing albumen. Frozen yolks were weighed, and both egg white and yolk were then stored at –80 °C until lysozyme analyses. Eggshells were rinsed under warm distilled water and dried at room temperature for later measurements in relation to eggshell coloration.

To determine the antimicrobial activity of lysozyme, we prepared the albumen samples, which were collected into pre-weighed 2.0-mL reagent tubes. Each tube with an albumen sample was weighed, and the albumen was lyophilized. Lyophilized powder was subsequently dissolved in 200 ml of distilled water, and the obtained albumen solution was used to determine antibacterial activity. A radial diffusion assay was used to evaluate the lysozyme concentration of albumen samples. Briefly, one bacterial colony of overnight agar plate culture of *Micrococcus luteus* was suspended in phosphate-buffered saline (PBS), and the turbidity of suspension was adjusted to 108 CFU/mL. A 100- $\mu$ L aliquot of suspension

was inoculated to 10 mL of melted LB broth (Luria broth) containing 0.9% (w/v) agar preheated at 48 °C and poured into 90-mm Petri dishes. After solidification, 5-mm diameter wells were punched into LB agar, and 5  $\mu$ L of sample was added to each well. The antibacterial activity of examined samples was compared based on the radius of a clear inhibition zone around the well, against standard solutions of a chicken egg white lysozyme (Sigma-Aldrich, Munich, Germany) after 24 h of incubation at 37 °C. Antibacterial activity of albumen samples was expressed as concentration of egg white lysozyme (lg/g of egg white) (Majtan et al. 2012). The results given represent mean values from four measurements of each egg (Křištofík et al. 2014).

To describe eggshell colour, we used three parameters: (1) Surface pigmentation cover: thereby, we distinguished two types (categories). (i) Areal dark surface pigmentation covering the egg to such an extent that the greenish (blueish) biliverdin-based background color could not be seen, (ii) dark spot surface pigmentation: i.e., dark pigments concentrated in distinct spots surrounded by the background color. Thus, for the analyses, we used categorical data to separate the two types (fully covered vs spotted).

(2) Spot distribution: dark pigment spots can appear as (i) clusters at the end or slightly in front (coronal ring) of the blunt pole, and (ii) spots more evenly distributed over the eggshell surface. Thus, for the analyses, we used categorical data for the two possible characteristics.

(3) Spot size: for this purpose, we determined the length (mm) of the three largest spots located outside the blunt pole and used their average length in the analyses. Spots concentrated at the blunt pole are usually very dense and overlapping, which prevents exact measurements.

### Statistical analyses

A principal component analysis was used to compare the differences between song playbacks used for song of low and high complexity. For this purpose, we used eight parameters described above and in Table 1. The final model selected was rotated, using a Kaiser Varimax normalization (rotation merged after 3 iterations). Each male entered only once in the analyses. A binomial test was used to compare the number of nests established near songs of low and high complexity and the proportion of successful versus unsuccessful nests in relation to playback type.

We used parametric tests throughout the study except for data on nest position and start of egg laying for high and low complexity song, which did not follow a normal (Kolmogorov–Smirnov test for both;  $p < 0.008$ ) or equal distribution (Kolmogorov–Smirnov test for both groups  $p < 0.001$ ). In this case, we used nonparametric alternatives, e.g., to represent the data we used median and interquartile ranges and a Mann–Whitney U test to compare nest distances from the

playback speaker between playback types and start of egg laying between playback types.

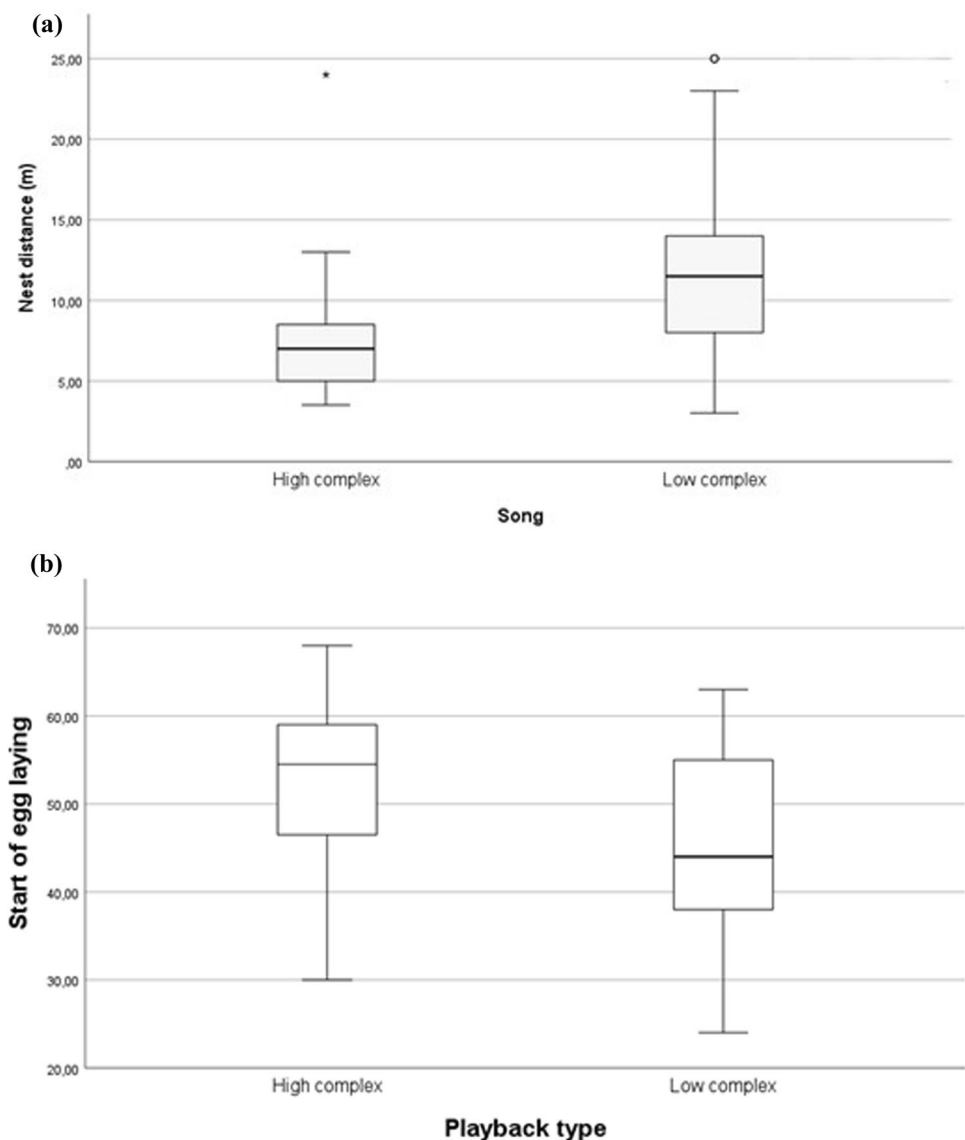
A MANOVA was used to investigate the role of song complexity for early maternal investment. For this purpose, egg length,—width,—weight, egg yolk and egg white weight and lysozyme were used as dependent variables. Playback type (low or high complexity) was used as an independent factor and as a covariate we included start of egg laying. Similarly, a MANOVA was used to determine the role of song complexity on breeding success. For this purpose, we used clutch size and the number of fledglings as dependent variables, playback type (low or high complexity) as an independent factor and start of egg laying as a covariate.

## Results

### Is the number of nests, nest position and start of egg laying influenced by playback type?

In total, 40 nest have been found in the surroundings (> 25 m) of the ten playback speakers, whereby the number of nests did not differ between the two playback types (binomial test:  $z=0.63$ ,  $p>0.53$ ). 18 nests (3.6 nests/playback site) have been found near highly complex song playbacks and 22 nests (4.4 nest/playback site) near the song playbacks with low complexity. The distance of the nest position from playbacks significantly differed (Mann–Whitney  $U$  test:  $U=2.1$ ,  $p=0.036$ ), whereby nests have been found nearer to the speakers broadcasting high complexity (median:

**Fig. 1** **a** Nest distance (m) from the playback and, **b** start of egg laying, in relation to the song type presented (high complex or low complex song)



7 m, interquartile ranges 5–9 m) than low complexity song (median 11.5 m; interquartile ranges 7.5–16.25 m; Fig. 1a).

Start of egg laying did not differ between playback types (high complexity song playback median: 54.5; interquartile ranges 45.25–58.5,  $n = 17$ ; low complexity song playback median: 44, interquartile ranges 34–55,  $n = 20$ ,  $U$  test:  $U = 69.5$ ,  $p = 0.152$ ; Fig. 1b).

### Is female egg investment related to playback type?

Examining eggshell color parameters (see methods) revealed a significant difference in eggshell pigmentation cover between the experimental groups. In the high complexity playback group, a significantly higher proportion of eggs (54.5%) was fully dark pigmented in comparison to the low complexity playback group (8.3%; binomial test:  $z = -2.4$ ,  $p = 0.016$ ).

Spot distribution in terms of proportion of spots at the blunt pole versus spots evenly distributed over the eggshell surface did not significantly differ between playback groups (binomial test:  $z = -1.22$ ,  $p > 0.21$ ) and also spot size did not differ between playback groups (ANOVA:  $F = 3.55$ ,  $p > 0.13$ ).

Regarding egg quality parameters including egg length and width but also egg weight, egg yolk and egg white weight did not significantly differ between playback types (see Table 3 and Fig. 2a, b).

Finally, also egg white lysozyme did not differ between playback types (see Table 3 and Fig. 2c).

**Table 3** Egg size parameters in relation to season and playback type

	Dependent variable	df	F	Significance
Julian date	Egg weight	1	1.816	0.194
	Egg length	1	1.439	0.245
	Egg width	1	1.500	0.236
	Egg yolk weight	1	0.929	0.347
	Egg white weight	1	6.305	<b>0.021</b>
	Lysozyme	1	1.322	0.264
Playback type	Egg weight	1	0.324	0.576
	Egg length	1	0.222	0.643
	Egg width	1	0.364	0.537
	Egg yolk weight	1	0.072	0.791
	Egg white weight	1	0.362	0.554
	Lysozyme	1	0.665	0.425

Results of a MANOVA using different egg size parameters (egg weight, egg length, egg width, egg yolk weight, egg white weight, lysozyme concentration) as dependent variables and song playback type (high and low complexity) as independent factor and start of egg laying as covariate. Start of egg laying is represented as Julian date. Significant value is marked in bold

### Does reproductive success differ between playback types?

Examining basic reproductive parameters revealed no difference in clutch size between playback types (Table 4, Fig. 3). However, breeding success in terms of the number of fledglings significantly differed between playback types, with nests near complex playbacks producing significantly more offspring (binomial test:  $z = 2.87$ ,  $p = 0.004$ ; Table 4, Fig. 3). Ten of twelve (83.3%) broods were successful near the complex song, whereas only 7 out of 22 (31.8%) broods in the low complexity playback group. All unsuccessful nests, namely 2 near the high complexity and 15 near the low complexity playback were predated. No nest was deserted and thus, the difference in breeding success is mainly due to nest predation.

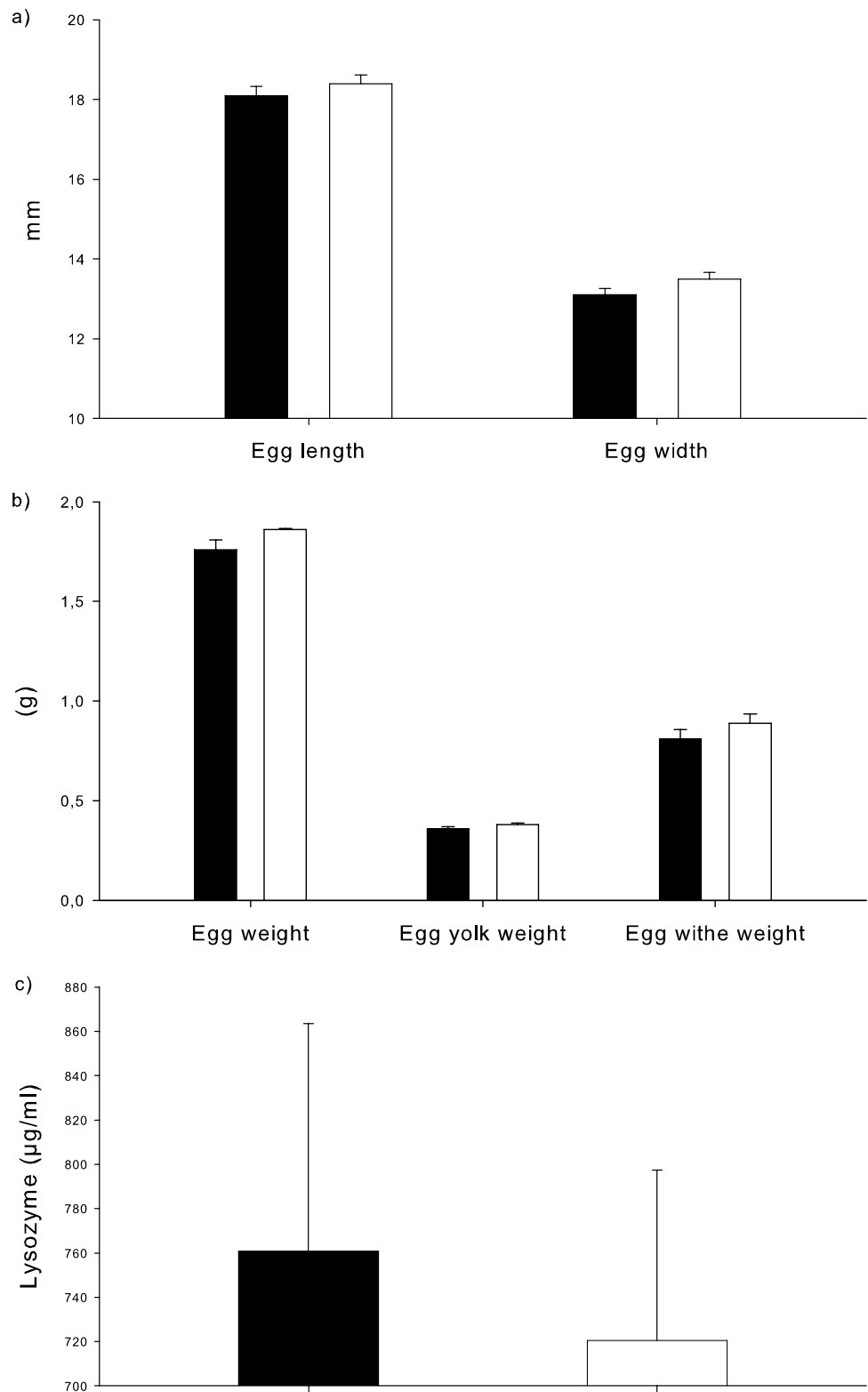
### Discussion

Regarding the behavioural parameters investigated and in line with our predictions only the location of a Common Reed Warbler nest turned out to differ depending on the playback type. As predicted nests are built significantly closer to high complexity playback types, confirming our assumption that complex song signals the higher quality of individuals, which are more attractive. This result could probably be explained by social attraction (Boulinier et al. 2008; Ahlering et al. 2010; Kivelä et al. 2014) or more specifically, given that information about the advertising individual is integrated, by the commodity hypothesis (Wagner et al. 2000). Copying the habitat choice of individuals based on their intrinsic quality could be one possibility. Evidence for breeding site selection based on song features is scarce. Mann et al. (2021) did not find evidence for breeding site selection in Black-and-white Warblers (*Mniotilta varia*) being influenced by a playback treatment, although the number of initially prospecting individuals was higher at playback sites. Similarly, Szymkowiak et al. (2016) found evidence for song quality to influence the choice of a breeding site in Wood Warblers (*Phylloscopus sibilatrix*) but again only at an initial stage.

Besides an effect on breeding site selection, there is also evidence for an effect of conspecific song on nesting behaviour (Mota and Depraz 2004). In a field experiment on Atlantic Canaries (*Serinus canaria*), they could demonstrate that females spent more time nest building when additionally listening to song playbacks. Different to our study, Mota and Depraz (2004) did not investigate the role of song complexity but rather the amount of song females' experience. What they could clearly demonstrate is that conspecifics can influence different aspects of female nesting behaviour additionally to their male mate. Similarly, Hofstad



**Fig. 2** Egg quality parameters in relation to playback type—high complexity song (filled bars) and low complexity song (open bars). Egg parameters include egg length (mm) and egg width (mm) (upper graph **a**), egg weight (g), egg yolk weight (g) and egg white weight (g) (middle graph **b**) and egg white lysozyme concentration (mg/ $\mu$ l) (lower graph **c**). Given are averages + SE for all



et al. (2002) found no indication for the onset of breeding being influenced by song complexity in a study on Snow Buntings (*Plectrophenax nivalis*), but there seems evidence

that female behaviour of snow buntings is influenced by the song rate of their mates.

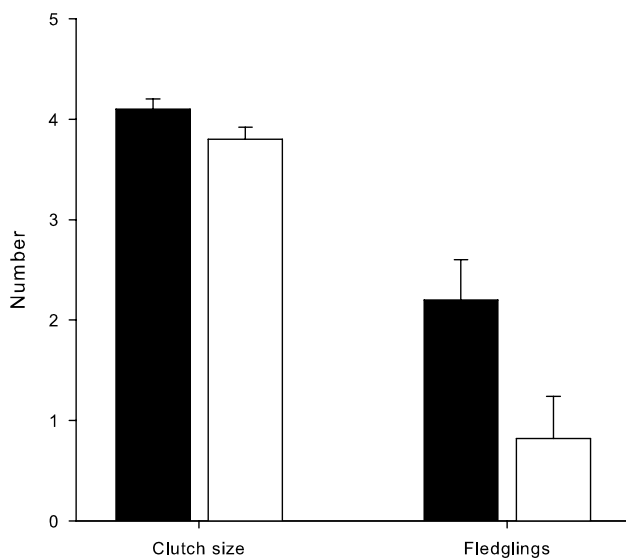
Different and against the prediction the two other behavioural parameters we examined, namely the number of nests

**Table 4** Breeding parameters in relation to playback types

	Dependent variable	df	F	Significance
Date of	Clutch size	1	0.622	0.440
1. egg	Fledged juveniles	1	0.418	0.525
Playback	Clutch size	1	2.066	0.166
Type	Fledged juveniles	1	7.749	<b>0.011</b>

Breeding success in terms of number of eggs (clutch size) and fledgling (fledgling success) as dependent variables in relation to playback type (independent variable) and start of egg laying which was used as covariate. Given are mean of squares,  $F$ ,  $p$ -values and  $df$ . Significant value is marked in bold

Total  $df=34$ ,  $r^2$ (clutch size)=0.101,  $r^2$ (fledged juveniles)=0.328



**Fig. 3** Clutch size and number of fledged nestlings in relation to playback type (high complexity song=filled bars; low complexity song playback=open bars). Given are averages + SE

established near the two playback types as well as the onset of breeding in terms of the start of laying did not differ. Based on these results, a preference towards high or low complexity song cannot be clearly demonstrated and hence a stimulating effect of conspecific song cannot be derived.

Egg investment strategies can be very fine tuned and influenced by a variety of factors (Mousseau and Fox 1998). Female investment into egg compounds can be predominantly influenced by her own intrinsic quality or condition (Martínez-de la Puente et al. 2007; De Coster et al. 2012; Hargitai et al. 2016a). However, another influential factor could be the quality of the male partner (Krištofík et al. 2014; Badás et al. 2017) or even the quality of other conspecifics (García-Fernández et al. 2010). There are in fact several studies indicating a male quality effect on maternal investment. For instance, Indian Peafowl hens (*Pavo cristatus*) paired with more ornamented males produce more

eggs (Petrie and Williams 1993) or larger eggs and deposit higher amounts of testosterone into egg yolk (Loyau et al. 2007). Larger eggs contain more proteins and lipids which can affect embryo survival (Williams 1994). Specifically in relation to song Soma and Okanoya (2013) found that egg weight increased when females were mated to males with longer songs and found indication towards a male-biased sex ratio (more costly sex) in relation to longer song duration of mates. Using song playback experiments Marshall et al. (2005) found that female Atlantic Canaries hearing songs with sexy syllables have higher levels of fecal testosterone but not egg testosterone. Gil et al. (2004) found in a lab experiment that female invested more into egg yolk testosterone when exposed to attractive repertoires, and Mota and Depraz (2004) found evidence of an effect on early maternal investment in follicular development. Finally, we found an effect of song parameters on egg yolk testosterone as well as egg white lysozyme in European Common Reed Warblers (Krištofík et al. 2014).

Based on this result, it could be expected that the singing activity of neighbouring males also affects female egg investment. In this context, we found a significant difference in eggshell pigmentation depending on whether the nest is located near a high or low complexity song playback. Specifically, we found that the proportion of dark pigmented eggs is significantly higher in the high complexity song group.

Therefore, one question arises whether eggshell color production is costly and consequently signals female quality and her maternal investment? In this context, Corti et al. (2018) found a higher hatching success in darker eggs of the Barn Swallow (*Hirundo rustica rustica*), whereas Hargitai et al. (2016b) found that in canaries, females in better body condition laid eggs that had fewer dark pigments but had a more intense blue-green coloration positively reflecting maternal antioxidant availability and body condition. Food availability has also been shown to affect female condition and egg pigmentation. In European Herring Gulls (*Larus argentatus*) (O'Hanlon et al. 2020), darker pigmented eggshells indicate reduced food availability. Similarly, Hargitai et al. (2016a) could show that under unfavorable environmental conditions, female Great Tits (*Parus major*) deposit more of the potentially harmful pro-oxidant protoporphyrin pigment into the eggshell. Also, females laying dark pigmented eggs had more lymphocytes in their circulating blood and had paler yellow breast and lower UV plumage reflectance. This would mean that dark eggs indicate poorer health and individual quality. Thus, following their results females settling near conspecifics producing highly complex song would be in poorer condition. This relationship has to be clarified in future studies.

Another more functional aspect could be that eggshell pigmentation serves to stimulate male parental investment (Walters et al. 2014). Also, spot distribution and spot size

can be indicative of egg and female quality and be influenced by external factors like weather conditions or season (Hargitai et al. 2016c). However, our results regarding spot size and spot distribution in contrast revealed no difference according to playback type.

Regarding the other parameters reflecting early maternal investment we also found no evidence for a stimulating effect of conspecific song differing at least in relation to structural complexity. Size and weight as well as the weight of specific components (yolk and white) did not differ between eggs produced near high or low complexity song of conspecifics. Similarly, the antimicrobial activity of egg white seems not to be influenced by the song of playbacks simulating neighbours that advertise high or low complexity song. This contrasts with our earlier findings in relation to other song parameters like song complexity of the mate, where we could show that maternal lysozyme production is positively correlated to song complexity of the male partner (Krištofik et al. 2014). In addition, Mota and Depraz (2004) found evidence that a similar song can stimulate early maternal investment in terms of follicle development in addition to nesting behaviour. Therefore, regarding maternal investment parameters, our results suggest only some "fine-tuned" effects in females that are or could be influenced by the quality of male song. Our study design also does not allow a simple and unidirectionally interpretation of the importance of song features. A causal relationship is difficult to study which applies generally to neuroendocrine effects and field experiments. Song treatment under a natural setting may have a variety of influential factors and may be hence a result of complex interactions. Song treatment e.g., may affect mate choice of a female. If high-quality females preferentially settle near high complex song the observed maternal investment can be also a result of female intrinsic quality or a composite of female and song quality. Also, habitat quality could interfere in the observed results although, we believe that habitat quality may not play such an important role. Particularly in the Common Reed Warbler habitat features appear to be less important for settling decisions (Hoi et al. 1991; Leisler and Schulze-Hagen 2011). Thus, male song could be important for maternal investment than found in our study but the pathways and mechanism are difficult to establish.

Besides the parameters investigated in relation to early maternal investment, there might be other egg components not considered in this study, which could be influenced by song quality, but which may influence breeding success. Therefore, to examine parameters of breeding success would likely constitute a useful alternative when early maternal investment is partly or totally unknown. Breeding success in fact has been found to be influenced by male song features. For instance, a positive correlation between song complexity and the number of fledglings was found in snow buntings (Hofstad et al. 2002). Song complexity and song

sequencing was also correlated with male feeding rates in Common Nighthawks (*Luscinia megarhynchos*) (Bartsch et al. 2015). Alternatively in Northern Cardinals (*Cardinalis cardinalis*) low song complexity was correlated to increased nesting success (Conner et al. 1986). In southern House Wrens (*Troglodytes aedon chilensis*), males that sang with greater overall syllable type diversity and that had larger song repertoires paired with females that bred earlier and laid more eggs over the course of the breeding season (dos Santos et al. 2018).

Our results revealed a significant effect of song complexity on breeding success. More chicks fledged from nests located near highly complex song. This would be in line with the predictions and the former studies (Krištofik et al. 2014), unfortunately, differences in breeding success in our case is mainly a result of egg predation. Thus, early maternal investment or parental feeding cannot be responsible for this result. Similarly, Woodgate et al. (2012) could show on the one hand that song structure of male zebra finches predicted the number of genetic offspring and hatching success but on the other hand did not predict the number of unrelated foster-offspring or the number or size of eggs in a clutch. Thus, also in this case, an early maternal effect is unlikely to explain the results. Rather, differences in male song predict differences in reproductive success and differences in genetic quality.

Nest predation in the same locality (fish ponds Veřké Blahovo) was under natural conditions about 40% and in an experimentally introduced design with the same nest density about 60% (Darolová et al. 2014). Our results here revealed nest predation to be influenced by singing behaviour. Nest predation near complex song was much lower and near less complex song much higher, which is rather unlikely to be expected by chance. Szymkowiak and Kucziński (2015) could show that fitness consequences of social attraction are predator dependent. Social attraction can lead to breeding clusters which may in turn affect nest predation given that nest predation is density dependent (Hoi and Winkler 1998). Given that internest distances are closer in the complex song group the risk of nest predation should be higher. Consequently, in this group, more care should be given to proper nest site selection and nest protection e.g. nest guarding.

Thus, the differences in nest predation might be due to differences in parental behaviour e.g. nest guarding behaviour during nest site selection and the incubation period. The criteria applied e.g., regarding nest concealment and/or the care in selecting nest sites may differ between individuals or depend on the brood value (Onnebrink and Curio 1991). In this context, song complexity may differently stimulate nesting behaviour e.g., nest guarding or incubation. In the related Moustached Warblers (*Acrocephalus melanopogon*), active nest defense by the incubating parent and high male incubation has been shown to reduce brood predation (Kleindorfer

and Hoi 2010). Also in the Common Reed Warbler, incubation behaviour is flexible and condition dependent (Kleindorfer et al. 1995). Furthermore, Grunst et al. (2016) could show that higher perceived predation risk-induced maternal investment in Song Sparrows (*Melospiza melodia*) and consistent with the good parent hypothesis, males with large song repertoire sizes displayed lower latencies to return to the nest.

In conclusion our results suggest that song quality not only of the male partner but also of conspecific neighbours, can influence reproductive investment and breeding success to some degree. However, concerning early maternal investment parameters, song of conspecific neighbours seems to be less influential and less than the song of the male mate and, therefore, does not represent a significant benefit for male partners. Given the importance of nest predation, it might be interesting in future studies to investigate the role of neighbour song for stimulating behaviours during different reproductive stages.

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**Data availability** The datasets generated and analysed during the current study are available from the corresponding author upon reasonable request.

## Declarations

**Conflict of interest** No potential conflict of interest was reported by the authors.

**Ethical standard** The study complies with the current laws of Slovak republic and was conducted under the permit no. 3320/219-6.3 given by the Ministry of environment of the Slovak republic.

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