

Response of reed warbler and sedge warbler to acoustic playback in relation to age, sex, and body condition

Katarzyna Wojczulanis-Jakubas¹ · Jakub Wietrzykowski¹ · Dariusz Jakubas¹

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Abstract Acoustic playback has been shown to substantially increase the number of birds trapped. However, other effects of playback have rarely been investigated; the few studies that have been published strongly suggest that when playback is applied some groups of birds may be captured more easily than others, leading to biased results. In this study, we evaluated the experimental effect of acoustic playback (by comparing days when playback was used and was not used) at a stop-over site in central Europe during the post breeding period and the autumn migration. We examined the influence of playback on numbers of birds captured, i.e., two reedbed passerines—the reed warbler *Acrocephalus scirpaceus* (RW) and the sedge warbler *A. schoenobaenus* (SW)—with respect to their age, sex and body condition. We found that playback increased the number of birds captured 2.5 times in RW and 3.6 in SW. We did not find evidence for a differentiated response to the acoustic stimuli between adults and immatures. The sex ratio in RW was not affected by acoustic playback, but we did record significant male-bias in SW. The body condition of lured and non-lured birds was similar in both species. The sex bias revealed in one species but not in the other, clearly shows that great caution should be exercised when using playback to attract various species.

Keywords Playback · Sex ratio · Migration · Stop-over

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✉ Katarzyna Wojczulanis-Jakubas
biokwj@univ.gda.pl

¹ Department of Vertebrate Ecology and Zoology, University of Gdańsk, Wita Stwosza 59, 80-308 Gdańsk, Poland

Zusammenfassung

Die Antwort von Teich- und Schilfrohrsängern auf akustisches Playback in Hinsicht auf Alter, Geschlecht und Körperkondition

Es ist gezeigt worden, dass akustisches Playback die Anzahl gefangener Vögel deutlich erhöht. Andere Effekte von Playback sind jedoch kaum untersucht worden; die wenigen hierzu veröffentlichten Untersuchungen deuten stark darauf hin, dass mit Hilfe von Playback einige Vogelgruppen leichter gefangen werden können als andere, was zu verfälschten Ergebnissen führt. In dieser Studie haben wir in einem mitteleuropäischen Rastgebiet in der Zeit nach der Brut und während des Herbstzuges den experimentellen Effekt akustischen Playbacks durch das Vergleichen von Tagen mit und ohne Playback untersucht. Wir haben den Einfluss von Playback auf die Anzahl gefangener Teich- (*Acrocephalus scirpaceus*; RW) und Schilfrohrsänger (*A. schoenobaenus*; SW) in Hinsicht auf Alter, Geschlecht und Körperkondition analysiert. Wir fanden heraus, dass Playback die Anzahl gefangener RW um das 2,5-fache und die gefangener SW um das 3,6-fache erhöhte. Wir fanden keine Hinweise darauf, dass Altvögel und immature Vögel auf die akustischen Stimuli unterschiedlich reagieren. Das Geschlechterverhältnis gefangener RW wurde durch das Playback nicht verschoben, während wir bei SW mit Playback signifikant mehr Männchen fingen. Die Körperkondition von mit und ohne Playback gefangenen Vögeln war bei beiden Arten ähnlich. Dass bei einer der beiden untersuchten Arten (nicht jedoch bei der anderen) ein Geschlecht häufiger mit Hilfe von Playback gefangen wurde als das andere, zeigt deutlich, dass man Vorsicht walten lassen sollte, wenn man Playback einsetzt, um verschiedene Vogelarten anzulocken.

Introduction

Acoustic playback has been extensively used at many ringing stations to increase the number of trapped birds. This method does indeed increase the number of captures many times, even in unsuitable habitats (Herrmans 1990; Mukhin et al. 2008; Jiguet et al. 2011). This is especially important when the number of individuals captured without any playback is not enough to solve certain problems statistically. However, despite the method's great popularity, few systematic studies have been carried out to examine its effects on capture structure; there is some evidence suggesting that playback may lead to biased samples (e.g., Furness and Baillie 1981; Figuerola and Gustamante 1995; Schaub et al. 1999; Shekerman 1999; Brotons 2000; Lecoq and Catry 2003).

A sample bias in captures with acoustic playback may arise if some groups of birds respond to the stimulus differently, and are thus attracted to the capture site with different intensity. Firstly, adult birds may be attracted more strongly than immatures as a result of their prior experience and familiarity with the songs of breeders in suitable habitats. Alternatively, naïve immatures may use every possible cue to locate sites and food, so they may be more susceptible to such stimulation. Both scenarios have been found to be the case (Brotons 2000; Mukhin et al. 2008; Jiguet et al. 2011). Secondly, males and females may differ in their response to acoustic playback, as males are usually more vocal than females. As such, males may be more strongly attracted by acoustic playback (Shekerman 1999; Lecoq and Catry 2003). In some species, however, both sexes were found to respond similarly to acoustic stimuli (James 1983; Figuerola and Gustamante 1995; Scebba 2001). Finally, birds in poor body condition for some species (Furness and Baillie 1981; Figuerola and Gustamante 1995; Fransson et al. 2008) but not for others (Brotons 2000) were found to be more readily attracted to conspecific songs.

All these possible biases have to be borne in mind when data acquired with the support of acoustic playback are used, as these biases may compromise the responses to some biological questions. Since there are few studies focusing on this subject and results vary between species (see above), it is difficult to draw any inference regarding the general mechanisms leading to bias; consequently, it is difficult to predict a priori the effect of the acoustic playback on a given species. Hence, there is a great need to test the effect of the acoustic playback on various species, especially those which are commonly captured using this method.

In this study, we investigated the response to the acoustic playback in two long-distance migrants—reed warbler *Acrocephalus scirpaceus* (hereafter RW) and sedge warbler *A. schoenobaenus* (SW)—with respect to the birds'

age, sex and body condition. Both species are small passerines that live exclusively in reedbed habitats (Bairlein 1983; Cramp 1998). Two studies have examined the influence of acoustic playback on the capture of RW. One showed that adult RW tended to be attracted by acoustic playback more often than immatures (Mukhin et al. 2008). The other showed that acoustic playback could increase the probability of the birds' recapture (Schaub et al. 1999). Both studies demonstrated that acoustic playback increased the number of captures. To our knowledge, no systematic study of this subject has ever been conducted for SW. Neither have sex and body condition of the two species ever been examined in this context.

We investigated the response of the birds to the acoustic playback by comparing the number of captures of a given characteristic (age, sex, body condition) between days with and without the stimuli. We carried out the experiments over a longer period of time, comprising both the post-breeding period and part of the autumn migration. We selected this period as it is the time when the two species are frequently trapped using acoustic playback. We lured the birds only during the hours of daylight. Since both species are nocturnal migrants (Cramp 1998), such an approach allowed us to focus solely on the effect of playback on attracting the birds to the netting site (not to the stop-over site, which would have been the case if nocturnal playback had been applied).

In the light of the results of other studies (e.g., Herrmans 1990; Mukhin et al. 2008; Jiguet et al. 2011), exceptionally consistent in this respect, we expected that playback would increase the overall number of captures in both species. Based on the results of Mukhin et al. (2008), we expected an adult-biased response to acoustic stimuli in RW. For SW, we expected a like bias given the similarity of the biological traits of the two species as well as their close relatedness. With regard to sex, we expected males to be more responsive than females in both RW and SW, as this seems to be the case in another *Acrocephalus* species, the aquatic warbler *A. paludicola* (Wojczulanis-Jakubas et al. 2013). Finally, we expected that birds in poorer body condition would be strongly attracted to the mist-nets, as birds of the genus *Acrocephalus* seem to use acoustic cues to find suitable habitats for landing during migration (Mukhin et al. 2008). Thus, birds being in great need of restoring their body reserves should be more responsive to playback than those in better body condition.

Methods

We carried out the study out in the southern part of the "Lake Druzno" reserve (54°05'N, 19°27'E) in northern Poland, during the annual autumn trapping of small

passerines, carried out there regularly since 1990. Druzno is a large, shallow lake, much overgrown with reed beds, which makes it an attractive place for breeding and migrating RWs and SWs (Jakubas et al. 2002; Nitecki 2013). Typically, the birds are captured in mist-nets, with no playback, according to a daily schedule from dawn to dusk, between the last week of July and the end of August, i.e., during the post-breeding period and the first half of the autumn migration of *Acrocephalus* species (Chernetsov 1998; Chernetsov and Manukyan 2000; Kozłowska et al. 2009). Although it is impossible to delineate an exact borderline between the post-breeding and migration periods owing to the mixture of local individuals and birds in transit, the number of adults decreased considerably (Fig. 1), and correlation between proportion of recaptures and Julian date became insignificant in both species after 1 August (i.e., 213th Julian day, Spearman’s rank correlation, $P > 0.05$ for species), indicating an onset of migration.

In 2013, birds were trapped and ringed between 25 July and 1 September; the same number of mist-nets was used all the time. We performed the acoustic playback (hereafter experimental days) every 3–6 days, starting from 28 July. To control for the effect of the acoustic playback, we established the control day, without playback, 2–4 days after each experimental day. On the control days, all the procedures applied to the birds on the experimental days

were followed except for the acoustic playback. We aimed to trap birds on both experimental and control days (hereafter sessions) once every 5 days of the whole trapping period (Fig. 1), under stable weather conditions (no rain, and only little, if any, wind). In all, we managed eight sessions, all with roughly similar weather conditions on the experimental and control days.

There were three playback points located among the mist-nets (within 3–30 m of the nearest three nets) so that the sound from the loudspeakers was well audible at all 22 net sites. Each playback set-up consisted of an mp-3 player (SU-YOSD06, China) with a built-in loudspeaker (6 W) of 150–18,000 Hz frequency range, an amplifier capable of increasing the power of the loudspeaker up to 40 W, and portable batteries allowing for continuous operation of the whole set-up for ca 10 h in the given conditions. The breeding songs of RW (0.93 min) and SW (1.05 min) recorded in Europe were played in mp-3 format alternately and continuously at each playback point starting from 30 min before the sunrise until noon on the experimental days. Thus, the birds were lured continuously for 7–8 h.

All RWs and SWs trapped from dawn until noon on both experimental and control days were subject to the standard procedure applied to the trapped birds at the Lake Druzno ringing station, i.e., they were ringed individually, aged [as immatures or adults according to Svensson (1992)],

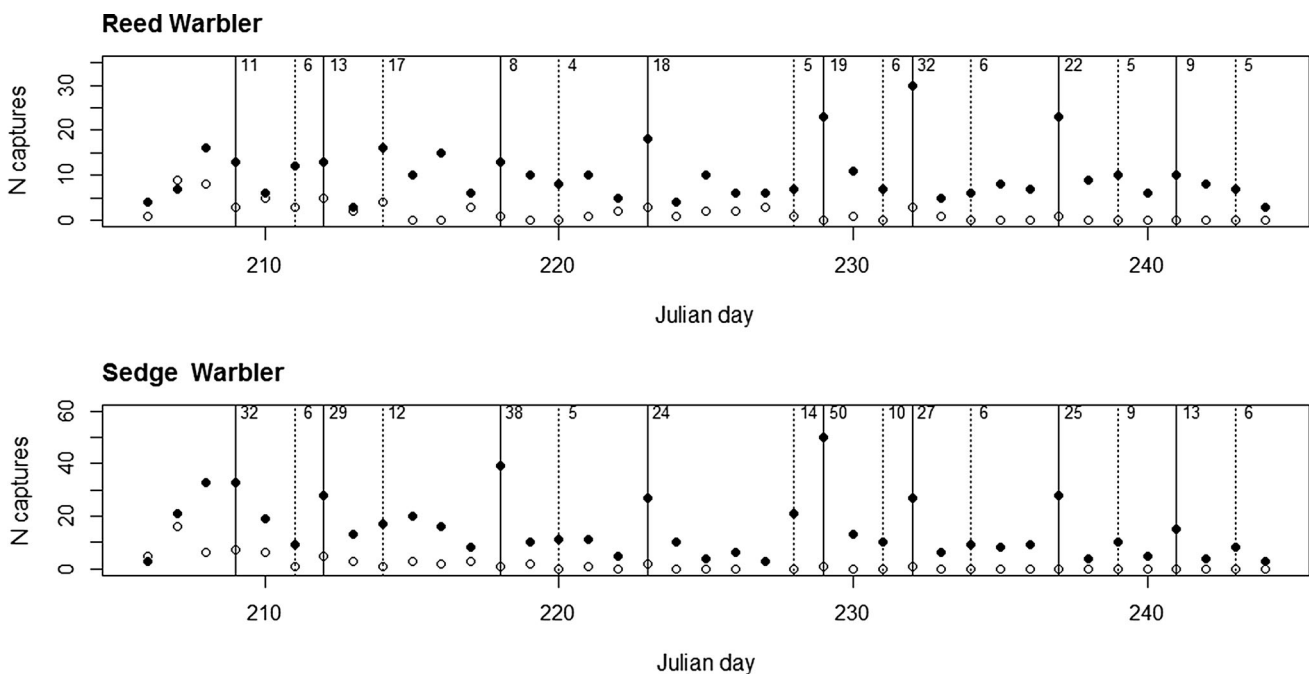


Fig. 1 Dynamics of capturing adults (white circles) and immatures (black circles) of reed warbler and sedge warbler at Lake Druzno (N Poland) in 2013. The vertical solid and dashed lines indicate the days when the experiments with acoustic playback and controls were performed, respectively. The numbers on the right of the vertical lines

indicate the number of birds newly captured until noon of the given experimental and control day (i.e., during the hours when playback was applied and birds were sampled for the purpose of the present study)

measured (flattened wing length, with a ruler accurate to 1 mm) and weighed (on an electronic balance accurate to 0.1 g, OHAUS, China). Also, the birds' fat reserves were assessed using Busse's (2000) 0–8 fat score system. Additionally, for the purpose of the present study, 5–7 body feathers were taken from each bird for molecular sexing, as the sexes in both species are indistinguishable based on external features (Ellrich et al. 2010; Wojczulanis-Jakubas and Jakubas 2011). In total, we trapped 218 RWs and 335 SWs on the experimental and control days.

We extracted DNA for molecular analysis from the proximal tip of the feathers using the Sherlock AX kit for biological tracks (A&A Biotechnology, Gdynia, Poland). We used the P2 and P8 primer pair to amplify a 390-bp fragment on the W chromosome (in females only), and a 370-bp fragment on the Z chromosome (in both sexes), with a 50 °C annealing temperature in the PCR (Griffiths et al. 1998). This sex difference in PCR products was clearly visible in UV-light when they were separated on 3 % agarose gel stained with Midori Green Advance (Nippon, Germany). The protocol was used successfully in the previous studies of birds from the genus *Acrocephalus* (e.g., Jakubas and Wojczulanis-Jakubas 2010; Wojczulanis-Jakubas and Jakubas 2011). We were able to sex 94 % of the birds sampled, with no failure bias for any age, sex, or date groups.

Data analysis

We examined the effect of the acoustic playback on (1) the number of birds trapped, (2) the age and sex proportions in the trapped birds, and (3) the birds' body condition. We performed all the analyses separately for each species, focusing only on birds that had been captured on the experimental or control day for the first time that resulted in a total of 186 RWs and 306 SWs.

We used the Wilcoxon rank test to compare the number of birds captured on the experimental and control days, and Fisher exact tests to compare the age and sex proportions between the experimental and control days (all sessions combined). We further analysed the effect of playback on the trapping of birds of given age and sex group using generalized linear models (GLMs). Since factors other than playback can affect the capturing rate of birds of a given age and sex (e.g., Woodrey and Moore 1997), we initially included all possible explanatory variables in the model (hereafter the full model), i.e., playback mode [binary coded (0/1—no playback/playback)], Julian date, and a dummy variable, i.e., sex (0/1—females/males) for the model where age was the response variable, and age (0/1—immatures/adults) for the model where sex was the response variable. We also included all possible interactions between the variables in the full model. We examined

the output from the full model, then averaged the models for which $\Delta AICc < 2$ (Burnham and Anderson 2002), and identified the importance of each variable (Bartoń 2015), including playback mode. The four best models were selected and averaged from the full model in the RW. None of the explanatory variables included in the averaged model (Julian date, playback mode, sex, playback mode \times sex) were significant (all $P > 0.18$; including the playback mode, $Z = 0.30$, $P = 0.76$). The importance of the playback mode was estimated at 57 %. In SW, three models were selected and averaged from the full one. None of the variables included in the averaged model (playback mode, sex) were significant (all $P > 0.69$; the playback mode, $Z = 0.39$, $P = 0.69$). The importance of the playback mode was estimated at 30 %. Given these results, to examine the effect of playback, we performed the analyses with age or sex (response variable) and playback mode (explanatory variable) only included in the model.

To examine the body condition, we used body mass corrected for body size. Several methods have been put forward to correct body mass for body size (e.g., Albrecht et al. 1993; García-Berthou 2001; Schulte-Holstedde et al. 2005; Peig and Green 2009). We employed three different methods: (1) body condition index (BCI) using the formula: $BCI = M/L$, where M is the body mass and L is the linear body measurement of an individual (Albrecht et al. 1993); (2) residuals of a simple linear regression of body mass and linear body measurements following the recommendations of Schulte-Holstedde et al. (2005); and (3) the scaled mass index proposed by Peig and Green (2009) using the formula they suggested. In all the methods we used wing length as a proxy for body size as it was correlated significantly with body mass in both species [Pearson correlations, for immatures only (see explanation below), RW: $r_{151} = 0.34$, $P < 0.001$; SW: $r_{265} = 0.19$, $P = 0.001$]. All these methods delivered qualitatively similar results in terms of the analysis described below. Here, we present the results obtained using the body condition index (BCI), which was the most efficient in correcting body mass for body size.

Using body mass appropriately corrected for body size enables one to apply parametric statistical analysis, which is not possible with fat scores (Benson and Winker 2005). Nevertheless, there was a significant positive relationship between the body mass index and fat scores in both RW (Spearman's rank correlation, $\rho = 0.52$, $n = 151$, $P < 0.001$) and SW ($\rho = 0.16$, $n = 265$, $P = 0.01$). To examine the response of the birds to acoustic playback with respect to their body condition, we performed factorial ANOVA, with body condition index as the response variable, and playback mode, Julian date, and sex with all possible interactions as categorical predictors. The age of birds may also influence their body condition index (e.g.,

Woodrey and Moore 1997). However, one of the groups (adults) was not observed on some days, so the matrix of contrasts failed. Thus, we restricted these analyses to immatures only.

We performed all calculations and analyses in R software (version 3.1.2, Development Core Team 2014), with MASS (Venables and Ripley 2002), and MuMin (Barton 2015) packages.

Results

The number of RW captured on the experimental days was on average 2.5 higher than on the control days (experiment: median = 18.0, Q_1 – Q_3 = 12.75–21.75; control: median = 7.0, Q_1 – Q_3 = 6.0–9.25; Wilcoxon test, $V = 2$, $P = 0.047$). In SW, the number of captured birds on the experimental days was on average 3.5 times higher compared to the control ones (experiment: median = 29.5, Q_1 – Q_3 = 27.5–38.75; control: median = 8.5, Q_1 – Q_3 = 7–11.75; Wilcoxon test, $V = 0$, $P = 0.01$).

Adults constituted 10.6 % ($n = 132$) and 11.1 % ($n = 54$) of RW captures on the experimental and control days, respectively. The proportion of the age groups was similar on both types of days (Fisher's exact test $P = 0.99$). In SW, adults made up 4.6 % ($n = 238$) and 1.4 % ($n = 68$) of the captures on the experimental and control days, respectively, and the proportions were similar on both types of days (Fisher's test $P = 0.48$). Also the results of GLM (only age and playback mode included) showed that the odds of capturing an adult RWs did not increase significantly (only by 1.32) when the playback was applied ($Z = 0.47$, $P = 0.64$). Similarly, the odds of capturing an adult SW did not increase significantly (by 1.58) when the playback was applied ($Z = 0.89$, $P = 0.37$).

Males (both age groups combined) constituted 48 % (CI 5–95 %: 41–56 %, $n = 126$) and 54 % (CI 5–95 %: 42–66 %, $n = 50$) of RW captures on the experimental and control days, respectively. The proportion of the sex groups was similar on both types of days (Fisher's exact test $P = 0.61$). In SW, the proportion of males (both age groups combined) among the trapped birds on the experimental days was higher (59 %, CI 5–95 %: 54–66 %, $n = 228$) than on the control days (44 %, CI 5–95 %: 34–54 %, $n = 64$; Fisher test, $P = 0.03$). The GLM results (only sex and playback mode included) showed that the odds of capturing a male RW did not increase significantly (by only 0.80) when playback was applied ($Z = -0.67$, $P = 0.50$). In contrast, the odds of capturing a male SW increased significantly (by 1.71) with playback ($Z = 2.18$, $P = 0.03$).

The body condition index of immature RWs was related to none of the variables included in the model (all

$P > 0.05$; playback mode: $F_{1,145} = 0.95$, $P = 0.33$) except for the sex ($F_{1,145} = 6.99$, $P = 0.01$), with males having a higher body condition index than females (BCI males: mean = 0.173 ± 0.01 ; BCI females: mean = 0.169 ± 0.01 ; t test, $t = -2.29$, $P = 0.02$). In SW the body condition of immatures was unrelated to any variable included in the model (all $P > 0.05$; playback mode: $F_{1,259} = 0.32$, $P = 0.57$) except for the marginally significant sex \times Julian date interaction ($F_{1,259} = 4.17$, $P = 0.04$).

Discussion

As expected, we found that acoustic playback did affect the trapping of RWs and SWs, increasing the number of captures by 2.5 and 3.5 times, respectively. The increase, however, was not as spectacular as, for instance, in the case of coal tits (*Periparus ater*), when playback increased the number of captures 40-fold (Brotons 2000). It was also slightly lower compared to that reported for the target species by Mukhin et al. (2008; 4.0 in RW and 4.5 in SW). The discrepancy among the results could have resulted from differences in design of the studies, with two factors being likely to affect the number of responding birds. Firstly, we lured the birds during the post-breeding and migration periods using the males' breeding song. These songs serve to attract females, establish territory, and reinforce dominance (Cramp 1998). When the breeding season is over and the attraction of females and territory/dominance are no longer important issues, the birds may be less willing to confront the stimuli, thereby reducing the efficiency of playback in trapping birds. In contrast, when applying an appropriate vocalization under appropriate conditions, the birds' response may be very intense, as was the case with the above-mentioned coal tits (Brotons 2000; contact calls during autumn and winter in species that are quite social at that time of the year). In fact, temporal variation in acoustic playback efficiency in detecting and/or trapping birds has been reported in various species (e.g., Boscolo et al. 2006; Rehm and Baldassarre 2007). Secondly, we lured these nocturnal migrants during the hours of daylight. Such a study design limited the potential number of birds that could be captured to those that spontaneously landed in the study area. In contrast, the study by Mukhin et al. (2008) targeted actively migrating birds aloft, applying playback during the hours of darkness in an unsuitable habitat. Usually, the birds would have overflown a site where they normally do not occur; instead, they landed, lured by the acoustic stimulation. Thus, the number of birds that could be captured by Mukhin et al. (2008) was limited only by the number and receptiveness of birds passing over the area on the experimental nights.

Despite a slight difference in the number of birds trapped with the help of acoustic playback, the results of both our study and that of Mukhin et al. (2008) clearly show that RW and SW do respond to such a stimulus, even if it is a breeding vocalization broadcast during the post-breeding and migration period. The birds' response to an apparently mistimed stimulus is intriguing. It may be linked, however, with the usage of the acoustic signals, apparently of any kind as a distant cue for habitat recognition in these species (Schaub et al. 1999; Mukhin et al. 2008). Both RW and SW are nocturnal migrants that use fragmented wetlands as stop-over sites (Cramp 1998). It has been suggested that acoustic cues may be of importance to species of naturally fragmented habitats, and to those that make their landfall decision in the darkness when the use of other cues is hampered (reviewed in Chernstov 2006). Moreover, other studies suggest that long-distant migrants often use acoustic cues of foreign species and/or out of the context, which allow them to assess more fully unfamiliar environments during stopover and so reduce the risk associated with lack of information (Németh and Moore 2007, 2014; Nocera et al. 2008).

Contrary to the expectations of Mukhin et al. (2008) and ourselves, we did not find any statistically significant differences in the age proportions between the experimental and control days. However, this result should be treated with caution, as the lack of significance could have been caused by the highly disproportionate sample size for both age groups. The overdominance of immatures could have masked the stronger adult response. Neither can we rule out the possibility that different design of the two studies accounts for the observed discrepancy.

We expected a male-biased sex ratio in the birds captured with acoustic playback, as this has been found in some species (e.g., Shekerman 1999; Lecoq and Catry 2003). Indeed, we did find such a pattern, but only in SW, not in RW. The interspecific difference in male and female responses could be explained by subtle differences in the breeding biology traits of the two species. Although both RW and SW males sing vigorously to attract females during courtship, RW is socially monogamous whereas SW may be socially polygynous (Hasselquist and Langefors 1998). Additionally, male SWs perform some species-specific behaviours such as singing in flight (Cramp 1998; Buchanan and Catchpole 2000) or mate guarding and chasing intruders away from various nests of polygynous individuals. This obviously leads to a higher level of competition between SW males, which in turn may make SW more receptive to acoustic cues. This seems to be plausible, given the fact that in the most promiscuous *Acrocephalus*, the aquatic warbler (Leisler and Wink 2000), the number of immature males captured with

playback exceeded the number of females 2.8-fold (Wojczulanis-Jakubas et al. 2013).

We did not find any difference in body mass in lured and non-lured immatures. Such a pattern has been found in other species (Brotons 2000), but the reverse has also been reported (Furness and Baillie 1981; Figuerola and Gustamante 1995; Fransson et al. 2008). Our results do not support the hypothesis that birds in poorer body condition would be strongly attracted to the mist-nets. We might have obtained a different picture if we had performed the playback during hours of darkness, inducing a landfall of birds with no intention to land, thus with probably greater fat reserves than the ones that landed spontaneously. That, however, would not have supported the hypothesis tested in the present study either.

Summing up, we found that acoustic playback substantially increased capture totals of RW and SW, but it did not affect the proportion of adults and immatures in either species. However, it did alter the sex ratio in SW though not in RW. This interspecific difference shows that even closely related species may respond differently to acoustic stimuli. For that reason, great caution should be exercised when using playback to attract species for which the effects of that method have not been tested.

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