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Title	Attraction of other species by Bluethroat Luscinia svecica song playback during autumn migration: an experimental test using bird-ringing data
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Publication date	2016-10-01
Original Citation	de la Hera, I., Fontanilles, P., Delalande, L., Glad, A. and Sarraude, T. (2016) 'Attraction of other species by Bluethroat Luscinia svecica song playback during autumn migration: an experimental test using bird-ringing data', Ardeola, 64(1), pp. 91-99. doi:10.13157/arla.64.1.2017.sc4
Type of publication	Article (peer-reviewed)
Link to publisher's version	https://doi.org/10.13157/arla.64.1.2017.sc4 - 10.13157/arla.64.1.2017.sc4
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Download date	2026-11-08 08:30:36
Item downloaded from	https://hdl.handle.net/10468/7003

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HETEROSPECIFIC ATTRACTION BY BLUETHROAT *LUSCINIA SVECICA* SONG PLAYBACKS DURING AUTUMN MIGRATION: AN EXPERIMENTAL TEST USING BIRD-RINGING DATA

ATRACCIÓN DE HETEROSPECÍFICOS POR REPRODUCCIONES DEL CANTO DEL PECHIAZUL *LUSCINIA SVECICA* DURANTE LA MIGRACIÓN OTOÑAL: UN TEST EXPERIMENTAL EMPLEANDO DATOS DE ANILLAMIENTO

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Short title: Heterospecific attraction by Bluethroat playbacks

Key words: Adour, conspecific attraction, lme4, tape-lure, Turdidae.

Word count: 2491 (main text without summaries)

SUMMARY.- Playback use of a particular avian species is well-known to have the potential to bias the composition of conspecifics (with regard to sex, age or body condition) that can be captured during mist-netting sessions. However, the possibility that playbacks affect heterospecific captures has been less explored and lacks solid experimental evidence. In this study, we explicitly tested through an experimental approach whether the use of a Bluethroat *Luscinia svecica* playback altered the overall number of heterospecifics captured during autumn migration in a wetland located in southwestern France. We found that playback use increased the capture rates of species that were not the direct target of the playback. This heterospecific attraction effect should be assessed and carefully considered when designing any avian monitoring program.

RESUMEN.- El uso de reproducciones sonoras de una especie de ave concreta es bien conocido que tiene el potencial de sesgar la composición de conespecíficos (con relación al sexo, edad o condición corporal) que pueden ser capturados durante las sesiones de anillamiento con redes japonesas. Sin embargo, la posibilidad de que los reclamos sonoros afecten a las capturas de heterospecíficos ha sido menos explorada y carece de evidencias experimentales sólidas. En este estudio testamos explícitamente si el uso de reclamos de Pechiazul *Luscinia svecica* alteraba el número total de heterospecíficos capturados durante la migración otoñal en una zona húmeda localizada en el suroeste de Francia. Nuestros resultados muestran que el uso de reproductores sonoros incrementa las tasas de capturas de especies que no fueron el objetivo directo del reproductor. Este efecto de atracción por heterospecíficos debería ser evaluado y considerado cuidadosamente a la hora de diseñar cualquier programa de seguimiento de aves.

Animals make use of different sensorial (e.g. acoustic, visual) stimuli coming from other individuals to take behavioural decisions (Maynard-Smith & Harper, 2003). In birds, the existence of conspecific and heterospecific acoustic interactions is well known and is an important research area within social information theory (Nocera *et al*., 2006; Valone, 2007; Magrath *et al*., 2009). Interestingly, avian bioacoustics also has a large potential in practical applications, such as promoting the settlement of birds in particular areas (Ward & Schlossberg, 2004; De Jong *et al*., 2015), deterring species that are conflictive for human activities (Ribot *et al*., 2011) or increasing capture rates of rare species in monitoring programs (Julliard *et al*., 2006; Jiguet *et al*., 2011). However, our knowledge of how birds respond to different artificial aural attractants is still very sparse and biased towards particular avian groups (e.g. colonial seabirds) and mostly during reproduction (Ahlering *et al*., 2010).

Playback use is a common practice in scientific mist-netting (Redfern & Clark, 2001). It is often accepted that this method increases the number of captures of target species (conspecific attraction), although sound experimental studies are still scarce (e.g. Mukhin *et al*., 2008; Arizaga *et al*., 2015). Much less understood, and often overlooked, is the potential impact of playbacks on capture rates of non-target species through heterospecific attraction. The possibility that this effect occurs during migration has been raised by several authors (Herremans, 1990; Wojczulanis-Jakubas *et al*., 2016). However, it has not been explicitly tested experimentally, let alone using a design that accounts for the spatial and temporal variation in bird abundance and capture rates. Thus, most relevant studies on this matter have been carried out without alternating treatments between study plots (i.e. overlooking spatial variation, e.g. Mukhin *et al*., 2008) or without overlapping in time treatment and control captures (i.e. ignoring temporal variation; e.g. Wojczulanis-Jakubas *et al*., 2016). Formally, the

52 robustness of this type of experimental designs would be compromised since observed
53 patterns can be alternatively explained by, for example, differences between plots in
54 habitat features or by temporal differences in the influx of migrants stopping over at the
55 study site.
56 The Bluethroat (*Luscinia svecica*) is a migratory species whose stopover ecology has
57 recently attracted much attention in North Western Europe (Arizaga *et al.*, 2013; 2015).
58 Thus, some ringing stations use audio-players of this species to maximize its captures
59 during migration, but the consequences of this methodology on the catchability of other
60 species remains unknown. In order to clarify this issue, we performed a playback
61 experiment in a wetland located in southwestern France, where studies on the stopover
62 ecology of Bluethroats and other species are carried out using aural attractants.
63 This study took place in Villefranque, Quartier-bas (43°27'N, 01°28'W, France), a
64 locality situated in the lower basin of Nive river, near the Adour mouth in Bayonne city.
65 The study site is a wetland dominated by reed bed (*Phragmites australis*) and other less
66 common plants associated with damp meadows, and scattered by some native (mostly
67 Willows *Salix* spp.) and exotic trees (mainly Elder *Acer negundo*) (see Fontanilles *et al*.,
68 2014). We placed three triplets of mist-nets (triplet A, B and C) in the area, separated
69 each other by more than 70 m. Each triplet consisted of three 12-m mist-nets positioned
70 in straight line (36 m of overall length per triplet) and always in the same fixed
71 positions. We made 21 ringing sessions (i.e. 21 ringing days) between August 18th and
72 October 2nd, 2015, when migrating Bluethroats stopover in this region during their post-
73 breeding migratory period. During each session, we used two playbacks, each one
74 placed in two of the three triplets, while the triplet without playback was used as a
75 control. Both playbacks had the same characteristics and consisted in an mp3 (Intenso
76 music walker) containing a male Bluethroat song connected to an amplifier (18W Kemo

#M033) and a speaker (DB Sonic 6010). The playback installed in one triplet rarely could be heard from the other triplets. We used exactly the same commercial male song used in the ringing campaigns performed in our study site and other areas (Arizaga *et al*., 2015), which was extracted from Roché (2009). This song recording was played repeatedly from approximately 30 minutes before dawn (when mist-nets were open) until the end of the ringing session (when mist-nets were furled). Playbacks were located at the midpoint of the corresponding 36-m mist-net line at volume 28 out of the 32 levels of the mp3. Whether the control triplet was on triplet A, B or C (see Fig. 1) was selected randomly when possible, but it was conditional on two requirements. First, each one of the three triplets was the control once every three ringing sessions and, second, we avoided that the same triplet was used as control in two consecutive sessions. This decision was taken in order to have each experimental set-up homogeneously distributed over time. According to this, each triplet was the control triplet seven days throughout the study period.

Our study was performed within a radio-tracking-based project studying the spatial ecology of Bluethroats stopping over in the area during their autumn migration, so that the ringing dates selected to perform the experiment and the duration of each ringing session was constrained by the monitoring necessities of this more general and priority project. Thus, the number of days elapsed between consecutive ringing sessions (range: 1-11 days) and the duration of each ringing session differed (range: 150-360 min). However, the opening and closing time of each triplet was virtually the same within each day, so that differences in the patterns of captures observed in this study cannot be attributed to methodological biases.

Each bird captured during a ringing session was ringed, and its species, age and sex were determined based on available literature (Svensson, 1992; Jenni & Winkler, 1994).

We decided to not consider the re-traps of individuals previously captured within the same day in order to avoid pseudo-replication. Playbacks might affect each species differently depending on their phylogenetic or trophic similarity to the Bluethroat (DeJong *et al.*, 2015). Likewise, playbacks might affect the proportion of age and sex groups captured for a particular species (Arizaga *et al.*, 2015). Our sample size was too small to test all these specific effects reliably. Consequently, we opted for restricting our analyses to: the overall number of captures (excluding Bluethroats), the number of captures of species not included in *Muscicapidae* (the family the Bluethroat belongs to) and the closely-related family *Turdidae* (see Gill & Donsker, 2016), and specific analyses for Bluethroats and for the most commonly trapped species in this study (the Reed Warbler *Acrocephalus scirpaceus*).

We used Generalized Linear Mixed models (GLMM) with Poisson error distribution to test the hypothesis that the overall number of captures per triplet per day (either, among and within triplets) would be larger when using the aural attractant. In this analysis, we included the overall number of captures (excluding captures of Bluethroats) per triplet as dependent variable, triplet (three levels: A, B and C), playback (whether a particular triplet had playback on a particular day or not) and their interaction as fixed effects. The day was included as a random factor, since the number of captures can greatly vary between days depending on the prevailing meteorological conditions and other factors that affect landing decisions of migrating birds. We performed the same model using the number of captures of species not belonging to the families *Turdidae* or *Muscicapidae* (see Table 1 to identify these species) and the captures of the Reed Warbler as dependent variables. Finally, a similar model with binomial error distribution was performed for the capture or not of at least one Bluethroat in each particular triplet per day, with the purpose of exploring the existence of conspecific

attraction in this species. All statistical tests were performed using the *R* package *lme4* (Bates & Maechler, 2010) and considering a threshold of $P = 0.05$.

We made a total of 574 captures from 21 different avian species during our 21 ringing sessions (Table 1). The Reed Warbler was by far the most commonly mist-netted species, followed in numbers by the Cetti's Warbler and the Sedge Warbler. All other species, including the Bluethroat, accounted for less than 5% of the overall number of captures (Table 1). Mean number of captures per day excluding the Bluethroats was 26.6 birds (range= 8-144; ± 6.4 se). We detected a significant effect of the Bluethroat playback on the number of heterospecifics captured (Table 2), as it can also be noted in Figure 1, where –for illustrative purposes– the percentage of captures made in each triplet per day was represented under the three different experimental set-ups (note also that the experimental set-up was not an effect to be estimated in the statistical tests; see Table 2). The effect of the playback did not differ between triplets as it can be deduced from the lack of statistically significant interactions between the terms 'playback' and 'triplet' (see Table 2). Results were qualitatively the same when only captures of individuals belonging to species not included within the families *Turdidae* or *Muscicapidae* were analysed ('playback' effects: Estimate = 0.64 ± 0.19 se, *Z-value* = 3.33, $P < 0.001$). Additionally, this pattern does not seem to be only mediated by the most commonly trapped species, since the effect of the playback on the number of Reed Warblers trapped was marginally non-significant ('playback' effects: Estimate = 0.46 ± 0.25 se, *Z-value* = 1.82, $P = 0.069$).

We captured more Bluethroats in triplets with ($n = 14$) than in triplets without playback ($n = 2$), but this difference was not significant when tested by a GLMM with binomial error distribution ('playback' effects: Estimate = 0.7 ± 1.49 se, *Z-value* = 0.47, $P = 0.638$).

Our study shows luring effects of Bluethroat song playbacks on other avian species, which increased the overall number of birds trapped during bird-ringing sessions at mist-nets fitted with a playback. Interestingly, observed patterns cannot be explained only by the attraction of individuals phylogenetically more closely related to the Bluethroat, since results did not change qualitatively when captures of birds belonging to the families *Turdidae* and *Muscicapidae* were not considered in the analyses. Thus, our experimental study demonstrates with a proper spatial and temporal control that a specific playback can increase the captures of heterospecific (non-phylogenetically-related) birds.

The heterospecific attraction hypothesis was originally formulated to describe the fact that some migratory bird species use local resident birds from other species as cues to make habitat-selection decisions for breeding (Monkkonen *et al*., 1997; Thomson *et al*., 2003). However, their basic principles might be extrapolated to other periods of the annual cycle of birds (i.e. migration or wintering), as well as to other zoological groups (e.g. amphibians; Pupin *et al*., 2007). Our data support the idea that birds also include the acoustic emissions of other bird species in their decision-making process at their stopover sites. In our study area, birds might be using heterospecific cues in order to track suitable patches for foraging, since avian songs could indicate areas where individuals are in good nutritional condition (Van Hout *et al*., 2012). This interpretation would be supported by the fact that the majority of the birds occurring in our study site are likely to be actively migrating birds that stopped over in the area during their post-breeding migration. This is difficult to elucidate for each particular individual to test this possibility formally, especially for migratory or partial migratory species that show some local breeding pairs (e.g. Reed Warbler, European Robin *Erithacus rubecula*). In any case, it is expected that a high percentage of captured birds corresponded to

177 recently-arrived individuals that were completely unfamiliar with the area and, hence,
178 would make use of available social information (heterospecific songs) to track food
179 resources (Wojczulanis-Jakubas *et al*., 2016).

180 We acknowledge that our study possesses two limitations that lead us to take our
181 results and interpretations with some caution. First, observed patterns might be
182 mediated by the specific nature of the single male song selected to be broadcasted in
183 Villefranque. And, second, we lacked an additional non-natural noisy control (e.g.
184 classic music) that helped us to rule out the possibility that birds were attracted by any
185 type of playback.

186 We failed to detect a significant conspecific attraction effect of Bluethroat playbacks.
187 This was probably mediated by the relatively low number of Bluethroats captured that
188 year, which only allowed us to perform a less powerful GLMM with binomial error
189 distribution to test this hypothesis. However, our rough figures suggest a potential effect
190 since 14 out of the 16 Bluethroats were trapped in triplets with playback (Table 1). If we
191 consider that the null hypothesis would be the capture of twice more Bluethroats in
192 mist-nets with playback (i.e. two triplets with playback versus one triplet without
193 playback each day), we would obtain a marginally non-significant effect using a Chi-
194 Square test (χ_1 =3.15, P = 0.076). A recent study suggests the possibility that Bluethroat
195 playbacks would only increase autumn capture rates of conspecifics at particular
196 locations, probably those having also local breeding populations (Arizaga *et al*., 2015).
197 Breeding Bluethroats have not been recorded in our study site, so individuals of this
198 species in Quartier-bas are considered migrating birds. Consequently, the causes
199 determining the among-site differential response of Bluethroats to their own aural
200 attractant during autumn migration is still an open question.

There is increasing evidence suggesting that the information obtained in bird-ringing monitoring programs can be altered by the use of playbacks and, hence, their use is normally prohibited when birds are more sensitive to them (e.g. breeding period; Redfern & Clark, 2001) or within ringing coordinated programs (DeSante *et al*., 2015). The most commonly described alterations are related to modifications in the proportions of the different population groups (i.e. age, sex or birds with different body condition) that can be captured within the target species (Figuerola & Gustamante, 1995; Brotons, 2000; Lecoq & Catry, 2003). Although the potential of playbacks to alter heterospecific captures had been timidly suggested in some studies (Mukhin *et al*., 2008; Wojczulanis-Jakubas *et al*., 2016), this has not been broadly acknowledged. Our study provides substantial empirical evidence of the existence of heterospecific attraction during autumn migration by using a more robust experimental design. Thus, overall captures in the mist-nets equipped with Bluethroat playbacks in our study site are surely overestimated. An interesting remaining question is to address whether this playback effects are homogeneous across species, which could not be tested in our study due to sample size limitations, but would have important implications for bird community comparisons. If the heterospecific attraction effects we found for the Bluethroat also take place with the playbacks of other species, any inference made between sites (or between periods within the same locality) in the capture rates of any species would be compromised if playback protocols are not standardized in time and space. Consequently, the effects of aural attractants on heterospecific captures should be also assessed and carefully considered by ornithologists when designing any avian monitoring program.

ACKNOWLEDGEMENTS.- We are grateful to the funders of the project and all members of OISO association who made this research possible, especially Françis Lartigau and Kevin Sourdrille. This study

was funded by the Conseil Départemental des Pyrénées Atlantiques, Syndicat de la Nive Maritime, and Villefranque council. National Museum of Natural History from Paris authorized the bird-ringing activities. We also want to thank María Torres-Sánchez for her help in the field, and an anonymous reviewer for useful comments on an earlier version of the manuscript.

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Table 1. Number of captures in control and playback triplets and overall number of captures for each of the 21 avian species trapped during the autumn migration period in Quartier-bas (Southwestern France). Species belonging to the *Turdidae* or *Muscicapidae* families are indicated in the first column by '[TURD.]' or '[MUSC.]', respectively. Note that playbacks were used in two out of the three mist-net triplets in each ringing session.

Tabla 1. Número de capturas en tripletes control y con reclamo, así como número total de capturas para cada una de las 21 especies de aves capturadas durante el periodo de migración otoñal en Quartier-bas (Suroeste de Francia). Especies pertenecientes a las familias *Turdidae* y *Muscicapidae* están indicadas en la primera columna como '[TURD.]' o '[MUSC.]' respectivamente. Nótese que los reclamos se emplearon en dos de los tres tripletes de redes en cada jornada de anillamiento.

Species (Scientific name)	*Control captures*	*Playback captures*	*Total captures*
Reed Warbler (*Acrocephalus scirpaceus*)	89	233	322
Cetti´s Warbler (*Cettia cetti*)	7	30	37
Sedge Warbler (*Acrocephalus schoenobaenus*)	5	27	32
European Robin (*Erithacus rubecula*) [MUSC.]	9	16	25
Common Grasshopper Warbler (*Locustella naevia*)	3	20	23
Common Whitethroat (*Sylvia communis*)	2	21	23
Eurasian Blackcap (*Sylvia atricapilla*)	4	17	21
Eurasian Blue Tit (*Cyanistes caeruleus*)	3	15	18
Bluethroat (*Luscinia svecica*) [MUSC.]	2	14	16
Willow Warbler (*Phylloscopus trochilus*)	3	9	12
European Pied Flycatcher (*Ficedula hypoleuca*) [MUSC.]	3	7	10
Garden Warbler (*Sylvia borin*)	1	6	7
Great Tit (*Parus major*)	3	3	6
Melodious Warbler (*Hippolais polyglotta*)	4	1	5
Common Blackbird (*Turdus merula*) [TURD.]	3	2	5
Chiffchaff (*Phylloscopus collybita*)	0	4	4
Eurasian Wryneck (*Jynx torquilla*)	0	3	3
Song thrush (*Turdus philomelos*) [TURD.]	0	2	2
Nightingale (*Luscinia megarhynchos*) [MUSC.]	0	1	1
Whinchat (*Saxicola rubetra*) [MUSC.]	0	1	1

Eurasian Wren (*Troglodytes troglodytes*)	1	0	1
Total	142	432	574

325

326

Table 2. Results of the GLMM effects model that analysed the number of birds captured, excluding Bluethroats.

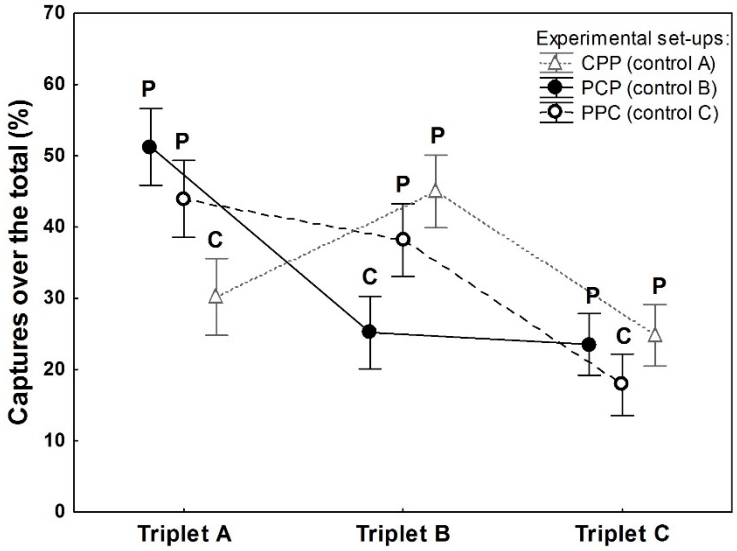
Tabla 2. Resultados del modelo GLMM que analizó el número de aves capturadas una vez se excluyeron las capturas correspondientes al Pechiazul.

Random Effects	*Variance ± Std. Dev.*			
Day	0.497 ± 0.705 (63 observations, 21 days)			
Fixed effects:	*Estimate*	*Std. Error*	*Z value*	*P-value*
Intercept (Triplet A, Control)	1.67	0.21	7.97	<0.001
Triplet B	-0.08	0.24	-0.34	0.733
Triplet C	-0.35	0.28	-1.25	0.213
Playback (Playback)	0.61	0.19	3.28	0.001
Triplet B × Playback	-0.16	0.32	-0.50	0.618
Triplet C × Playback	-0.24	0.35	-0.69	0.488

FIGURE LEGENDS

Figure 1. Percentage of birds captured in each triplet over the total number of daily captures for each of the three experimental set-ups that were possible during each ringing day (control in triplet A, B or C). Whether within each set-up the triplet has a playback or not (control) is indicated in the graph by P or C, respectively. Graph shows means and SE. In all cases the sample size for each bar in the graph is the same (n = 7). Note that the percentage of captures was used for illustrative purposes in order to account for between-day differences in the number of captures, but raw values of captures were used in the statistical analyses (Table 2). Likewise, the experimental set-up was not a main effect to be tested in the statistical analyses, but was also used in this graph to better represent the between and within-triplet variation in capture rates.

Figura 1. Porcentaje de aves capturadas en cada triplete de redes sobre el número total de capturas diarias para cada una de las tres configuraciones experimentales posibles durante cada jornada de anillamiento (control en triplete A, B o C). Si dentro de cada configuración el triplete tenía reclamo sonoro o no (control) se indica en el gráfico como P o C, respectivamente. La gráfica muestra medias y errores estándar. En todos los casos, el tamaño de muestra para cada barra en el gráfico es el mismo (n = 7). Nótese que el porcentaje de capturas sólo se empleó con fines ilustrativos para controlar por las diferencias diarias en el número de capturas, pero fueron los valores originales los utilizados en los análisis estadísticos. Igualmente, la configuración experimental no fue un efecto a testar en los análisis estadísticos (Tabla 2), sino que también se empleó en la gráfica para representar más adecuadamente la variación entre y dentro de tripletes en las tasas de capturas.



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