1   **Testing the maintenance of natural responses to survival-relevant calls in the conservation**

2   **breeding population of a critically endangered corvid (*Corvus hawaiiensis*)**

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8

9   **Abstract**

10   Vocal communication serves an important role in driving animals' social interactions and

11   ultimately their survival. However, both the structure of and responses towards natural vocal

12   behavior can be lost or subject to alteration under human care. Determining if animals in

13   conservation breeding programs exhibit and respond appropriately to species-specific

14   vocalizations is therefore important for ensuring their survival post-release. We tested whether

15   endangered 'alalā (*Corvus hawaiiensis*), which are extinct in nature, have retained their natural

16   responses to vocal calls that would be linked to survival and reproduction in the wild. We

17   conducted our studies on breeding populations derived from a small number of founding 'alalā

18   maintained under human care since their extinction in the wild in 2002. We presented pairs of

19   'alalā with alarm, territorial intrusion, and two types of control playback calls (a non-threatening

20   territorial maintenance call and a novel heterospecific call). 'Alalā were significantly more likely

21   to approach the speaker following alarm call playback than other call types, and were more likely

22   to respond to territorial intrusion calls with the same aggressive territorial calls. Males were more

23   likely to make these aggressive calls than females, mirroring their roles in territory defense. We

24   also found individual consistency in the level of vocal behavior response across all call types,

25   indicating that some individuals are more vocal than others. These results are encouraging,

26   showing that 'alalā exhibit relevant, species-specific behaviors despite generations under human

27   care. They do illustrate, however, that not all individuals respond appropriately, so vocal response

28   may be an important factor to consider in determining the release suitability of individuals.

29

30   **Significance Statement**

31   Effective communication is crucial to the survival of many animals, but can erode

32   without natural selection. Therefore, testing the flexibility and maintenance of communication

33   and vocal responses in contexts where animals are isolated from conspecifics or from survival

34   consequences, such as in conservation breeding centers, can help determine species'

35   susceptibility to communication loss. We used playbacks of survival-related conspecific calls to

36   test if 'alalā (*Corvus hawaiiensis*), retained species-specific responses to these calls after

37   generations under human care. We found that birds maintained a species-level natural response,

38   however these natural responses were not consistent across individuals, suggesting that some

39   birds may not be well equipped to survive in the wild without additional training or care.

40

41   **Keywords** anti-predator, captive breeding, communication, corvid, translocation, territory

42   defense, vocalization

43

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50

51   **Declarations**

52   **Funding**

56   **Conflicts of Interest**

57   The authors have no conflicts of interest to declare that are relevant to the content of this article.

58   **Availability of data and material**

59   Data and code are available on the OSF repository at: https://osf.io/pu5fb/. Pre-print available at:

60   https://www.biorxiv.org/content/biorxiv/early/2021/05/25/2021.05.24.445466.full.pdf

61   **Code availability**

62   Data and code are available on the OSF repository at: https://osf.io/pu5fb/.

63   **Ethics approval**

64   This work using animal subjects was approved by San Diego Zoo Wildlife Alliance's IACUC

65   committee (No. 16-009). Several permits were issued for the conservation breeding of 'alalā

66   (USFWS Native Endangered Species Recovery Permit TE060179-5, State of Hawaii Protected

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68

69   **Introduction**

70   Animals have evolved mechanisms for communication that facilitate survival and

71   reproduction. For primarily vocal species, how individuals respond to conspecific vocal cues and

72   signals can have survival-relevant consequences. For instance, failing to respond to an alarm call

73   could result in predation, or failing to adequately broadcast territorial calls could cause a loss of

74   territory, mates, or offspring. Whether or not animals exhibit appropriate species-specific

75   communication is particularly important in conservation breeding programs. Animals that have

76   spent generations under human care need to retain their natural behaviors for successful return to

77   the wild (Rabin 2003; McPhee 2004 a, b). However, behavioral erosion is a common byproduct

78   when animals are held under human care, resulting in the loss of survival-relevant behaviors,

79   deviations from wild-type behavior in form, or the expression of behaviors in inappropriate

80   contexts. These alterations in behavioral phenotype can occur developmentally in a single

81   generation or genetically/epigenetically across generations, and have been documented across a

82   wide variety of vertebrate species for many functional categories of behavior, including

83   antipredator, locomotory, foraging, refuge use, and reproductive, competitive and other social

84   behaviors (Frankham 2008; Grueber et al. 2015; Grueber et al. 2017). The loss of survival-relevant

85   behaviors (McPhee and Carlstead 2010; Shier 2016), including losses in vocal diversity (Corfield

86   et al. 2008; Digby et al. 2013) and dialect drift (Lewis et al. 2021) can alter call functionality and

87   compromise reintroduction programs using animals bred under human care. A combination of

88   forces from evolution, culture (Brakes et al. 2019), and direct experience interact to shape animals'

89   vocal behaviors (Hollén and Radford 2009; Bradbury and Vehrencamp 2011), all of which may

90   be impacted by the altered environment in conservation breeding facilities. Management of

91   animals under human care, therefore, faces the challenge of providing opportunities for animals to

92   maintain and express these behaviors in preparation for release into the wild (Greggor et al. 2018).

93   Among the vocal signals that animals need to retain, anti-predator signals (alarm calls) are

94   particularly important because they are a component of an animal's defense against predators

95   (Hollen and Radford 2009). Developmentally, they may be more canalized, with alarm call

96   production and responses emerging in development without direct experience with predators.

97   While learning is undoubtedly important for fine-tuning production and responses to antipredator

98   calls (Seyfarth and Cheney 1980; Griffin et al. 2000), selection should favor individuals that

99   respond appropriately to alarm calls without direct experience associating alarm calls with

100   predation, therefore they should be relatively resistant to loss in comparison to other call types.

101   However, some antipredator behavior responsiveness can be lost over an individual's lifetime in

102   the absence of predator exposure (Muralidhar et al. 2019). The importance of antipredator alarm

103   calling for conservation breeding and translocation programs is underscored by the finding that

104   predation is one of the primary causes of mortality for animals after release to the wild, across

105   taxonomically diverse species (Fischer and Lindenmayer 2000; Moseby et al. 2011; Berger-tal et

106   al. 2020).

107   Another important set of signals helps animals avoid conspecific conflict by alerting others

108   to their shared or defended resources. Territorial species, for instance, have evolved multiple forms

109   of communication that broadcast an intent to defend their territory. From ornamental displays, to

110   physical demonstrations (e.g., Decourcy and Jenssen 1994), or auditory signals (Greenfield and

111   Minckley 1993), there are a variety of ways that animals communicate territory occupancy to avoid

112   unnecessary conflict. These calls often serve to communicate motivation and a level of severity of

113   the territorial threat (Mager et al. 2012), ranging from maintenance calls to aggressive intrusion

114   calls. The ability to efficiently set up and defend a territory can be critical to breeding and later

115   reproductive success (Hiebert et al. 1989). Therefore, for territorial species that rely on vocal

116   communication, having an ability to understand and respond to different territorial calls may be

117   crucial for survival and reproduction in the wild. Any divergence in the production or perception

118   of these important calls from wild phenotypes may challenge social integration and the success of

119   translocation outcomes (Lewis et al. 2021).

120   Here we examine the communication system of the endangered ʻalalā (*Corvus hawaiiensis*)

121   within conservation breeding facilities, with the goal of evaluating their responses to major

122   categories of conspecific vocal signals. Ultimately, we aim to determine if they have retained

123   important components of natural behaviors that will facilitate successful reintroduction. The ʻalalā,

124   or Hawaiian crow, is the last remaining corvid species of the Hawaiian islands (Banko et al. 2002).

125   They were a keystone species for the Hawaiian wet and mesic forests, and the only known seed

126   disperser for a number of native plants (Culliney et al. 2012). They went extinct in the wild in

127   2002, after decades of rapid decline due to disease, habitat loss/fragmentation and invasive

128   predators, today surviving only in conservation breeding facilities (USFWS 2009). Efforts are

129   ongoing to reintroduce ʻalalā back into the wild, yet the birds still face a long road to recovery and

130   have not yet demonstrated successful breeding in the wild.

131   There have been but a few studies on ʻalalā vocal behavior, yet we know they have a diverse

132   vocal repertoire (Tanimoto et al. 2017a). Based on the complexity of vocal communication in other

133   corvids (Enggist-Dueblin and Pfister 2002) and the role it plays in their social lives (Clayton and

134   Emery 2007), we would expect ʻalalā calls to broadcast varying vocal signals, containing

135   information about predators or conspecific territorial intrusions, with potentially important fitness

136   consequences. ʻAlalā are fiercely territorial as adults, but as juveniles they form flocks, and

137   associate with members of both sexes. Historically, breeding pairs, and especially males, would

138   make a number of different, frequent territorial broadcast calls on the edges of their large territories

139   (Banko et al. 2002); Additionally, like many species, 'alalā are known to employ a range of alarm

140   calls to warn others of danger (Tanimoto et al. 2017a; Greggor et al. 2021); although it is currently

141   unknown if the structural differences between 'alalā alarm call types are used to distinguish

142   functional call categories. While comparatively little was known about 'alalā calls when the

143   species became extinct in the wild, evidence suggests that the frequency and type of some calls

144   they make in the conservation breeding centers differ from their historical vocal behavior in the

145   wild, including in the categories of territorial and alarm calls (Tanimoto et al. 2017b). It is unclear

146   whether these changes are due to the erosion of natural behavior in the conservation breeding

147   facilities, or due to a lack of context for expressing wild-type calls, for example, a lack of predation

148   pressure resulting in a reduced need for alarm calls. Additionally, given that they are housed at

149   much higher densities than wild 'alalā and suffer reproductive consequences from close proximity

150   of conspecifics (Flanagan et al. 2020), it is possible that individuals in the conservation breeding

151   population have become desensitized to their social surroundings, and no longer respond

152   appropriately to these important signals, especially given that corvids have been shown to ignore

153   unreliable callers (Wascher et al. 2015). Thus far there have been no studies examining behavioral

154   responses to experimental playbacks of vocal calls in 'alalā, beyond a preliminary pilot that

155   identified alarm call types (i.e. Greggor et al. 2021). Given how little is known about the vocal

156   behavior of 'alalā in the wild before they went extinct, any future insight into call types and

157   function must study the population under human care, however, we acknowledge that

158   interpretations of the current fitness consequences of call function should be interpreted cautiously

159   in light of these knowledge gaps.

160   We conducted an investigation of 'alalā responses to vocal signals to better understand

161   vocal communication and behavior of this near-extinct species. Specifically, we examined whether

162   'alalā still respond to species-specific calls in ways that indicate a retained meaning, despite their

163   generations removed from nature, or whether their responses to conspecific call playbacks are

164   indicative of a loss of vocal signals under human care, a call for concern that portends poor social

165   integration and antipredator defense upon release. We presented birds with recorded playbacks of

166   alarm and aggressive territory intrusion calls, alongside control calls and sounds, and measured

167   how likely birds were to approach the calling sound and to respond to it with the same call type or

168   a different call type (Table 1). We chose these two call types in the context of preparing birds for

169   survival alongside predators and the maintenance of social skills necessary for setting up and

170   defending territories from conspecifics. We also presented 'alalā with two control calls to rule out

171   the effect of the social novelty and auditory novelty of hearing a call outside of their aviary.

172   Namely, we played a non-threatening 'alalā territorial maintenance call, which birds routinely

173   make from their existing territories, and should therefore not broadcast any threat, and a call from

174   a novel species that 'alalā have never heard.

175   If the environment of the conservation breeding facility has reduced birds' responses to

176   auditory stimuli generally (i.e. desensitization), we would expect that 'alalā produce no response

177   to any of the call types (neither approaching nor making calls of their own), suggesting that the

178   birds are over-stimulated. Responding naturally to one category, but not all of them, could indicate

179   that some calls have lost their referential meaning either due to a lack of context for expression or

180   as an artefact of generations of conservation breeding. Meanwhile, if birds have retained their

181   natural responses to alarm and territorial intrusion calls, we would expect to see differences

182   between them and control call types. Specifically, birds should respond to territorial intrusion calls

183   with their own aggressive territorial intrusion calls (Bradbury and Vehrencamp 2011), and this

184   effect should be most pronounced in males, due to the larger role males historically played in

185   territorial defense (Banko et al. 2002). For alarm calls, the natural response is more complicated.

186   We would expect birds to approach the source of the call to investigate the potential source of

187   danger, and may respond with alarm calls (Hill 1986; Manser et al. 2002). Meanwhile the birds

188   should not show any clear response to the general territorial maintenance call, since it does not

189   denote a threat, and may show signs of neophobia, or a hesitancy to approach, the novel call, since

190   'alalā are highly neophobic in other contexts (Greggor et al. 2020).

191

192   **Methods**

193   We conducted the experiment on 28 breeding pairs, i.e. 62% of the entire 'alalā breeding

194   population, at the Keauhou Bird Conservation Center (KBCC; n = 24 pairs) and the Maui Bird

195   Conservation Center (MBCC; n = 4 pairs) between September and November 2018. The birds at

196   both facilities are currently several generations removed from the wild. Birds were tested in their

197   home enclosure with their breeding partner. Each pair was housed in an outdoor aviary, with

198   covered areas for shelter and feeding, and had daily ad libitum access to food and water.

199   Throughout the facility, interaction with people is minimized to reduce the effects of human care

200   (see Greggor et al. 2018). The birds have auditory access to other breeding pairs and most do not

201   have visual access. For the few buildings with more than one pair in adjacent aviaries we only

202   tested one pair per building to minimize subjects' prior exposure to the experimental setup. It is

203   possible that birds could see the experimental setup at adjacent aviaries, so we put as much time

204   as possible between trials at neighboring aviaries. At KBCC there was an average of 25 days

205   between trials at neighboring aviaries, though due to scheduling constraints the range was

206   somewhat large, between 5-53 days between trials at neighboring aviaries. At MBCC due to time

207   constraints at this facility there were only two pairs of neighboring aviaries, and these trials were

208   done 2 and 3 days apart. Four pairs were physically separated from each other for husbandry

209   reasons, but both had visual access to each other during all of their trials.

210   *Experimental design of playback study*

211   We designed the playback study to determine how 'alalā respond to alarm and other social

212   and non-social acoustic stimuli. We hypothesized that responses to different call types will vary

213   according to the context in which they are used and putative call meaning (Tanimoto et al. 2017a).

214   The experiment entailed playback of four distinct acoustic stimuli: (1) conspecific alarm call, (2)

215   conspecific territorial intrusion call, (3) conspecific non-threatening territorial maintenance call,

216   and (4) heterospecific call from a novel bird species (Table 1). Alarm calls are high-pitched calls

217   given to warn other birds of danger (Tanimoto et al. 2017a), which are typical of corvid vocal

218   repertoires (e.g. Marzluff et al. 2010; Bila et al. 2016; McIvor et al. 2018). By contrast, territorial

219   intrusion calls are given when birds are actively and aggressively defending their territory, in

220   situations that can escalate into physical aggression. Meanwhile, the non-threatening, territorial

221   maintenance calls that 'alalā make routinely on the edges of their territories in the absence of any

222   direct threat or aggression were included as a conspecific control stimuli.

223   We employed a repeated-measures design in which each subject received playback of each

224   call type in a balanced, random order ensuring that each type of call was played first, second, third,

225   or fourth an equal number of times across all trials. Individual and temporal differences in

226   responsiveness to the experimental setup and acoustic playback can be a source of response

227   variation that adds statistical noise in playback studies. To address this possibility, we included a

228   pre-trial period before call playbacks in which subjects were exposed to ambient forest noise

229   playback (details below). The same forest noise was used as a post-playback stimulus for all trials,

230   so that conditions were constant for comparisons of observed behavior in pre- and post-call

231   playback periods. We used this comparison to evaluate behavioral changes in the aftermath of call

232   playbacks.

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235

236   Table 1 Explanation of stimuli types and predicted responses

Call type	Experimental design function	Predicted behavioral responses
Alarm	Evaluate whether 'alalā retain anti-predator responsiveness to conspecific calls indicating danger or predation threat	Vigilance, alarm calls, cautious approach (predicted to coordinate social anti-predator response)
Territorial Intrusion	Discriminate between response to an alarming social stimulus versus an alarming antipredator stimulus	Aggressive approach, territorial intrusion calling
Territorial maintenance	Control: non-alarming conspecific stimulus	No specific call response predicted, but 'alalā could show interest and possible social attraction

Novel Control	Control: non-alarming heterospecific stimulus	No call response predicted; perhaps hesitancy to approach, based on neophobia.

237

238   *Call recording and playback methods*

239   We collected all audio recordings with a Roland R-05 acoustic recorder and directional

240   boom microphone. Conspecific calls and forest noise controls were recorded at the KBCC. We

241   collected calls opportunistically but tried to capture certain situations where we expected birds to

242   make the calls in question. For example, for territorial intrusion calls we recorded when males

243   were being moved between aviaries for husbandry purposes, as this is a time when we would

244   expect these territorial intrusion calls. Additionally, the alarm calls collected were verified against

245   calls collected at times of recapture at the facility and predatory exposure (e.g. Greggor et al. 2021).

246   To address pseudoreplication, one set of calls was collected from each of three males, resulting in

247   three unique sets of conspecific calls. A follow up analysis revealed no difference in birds'

248   responses between the three sets of calls (see Supplementary Materials). We assigned 'alalā call

249   types initially by social context and subjective perception, then confirmed assignment by

250   examining spectrograms. Calls from unfamiliar species were collected opportunistically at the

251   Panaewa Zoo in Hilo, Hawaii from medium- or large-bodied birds that breeding center 'alalā

252   would never have experienced (peacock *Pavo cristatus*, cockatoo *Cacatua moluccensis*, and toucan

253   *Ramphastos toco*).   Since several species of corvids are able to distinguish characteristics of

254   conspecifics from calls (Boeckle et al. 2012; Mates et al. 2015; Woods et al, 2018), each pair

255   received all three call exemplars from the same male to avoid individual differences in donor birds

256   influencing responses to call types. As breeding center birds are likely able to hear most

257   conspecifics housed at the same facility, all subjects were likely somewhat familiar with individual

258   calls from nearby conspecifics. However, to minimize familiarity with calls we selected playback

259   calls from individuals that were not housed in adjacent aviaries and did not repeat call playback

260   sets at adjacent aviaries.

261   We ensured that calls in the same category from different males had similar delta frequency

262   and average power using Raven Pro, version 1.5. In order to standardize the duration and number

263   of calls between call types with different durations and pauses, we ascertained that each 30-second

264   stimulus contained 6-7 seconds of sound and between 6-20 individual calls, ensuring the natural

265   spacing that the bird used between individual sounds to the extent possible. Therefore, we did not

266   edit the calls themselves but did edit the spacing between calls and the number of calls to keep the

267   total sound duration to 6-7 seconds for all kinds of stimuli. We measured sound duration and

268   conducted all audio editing in Audacity. We also checked that all sounds in the same category

269   were played from the speaker (omni jacket ultra, Altec Lansing) at the same minimum and

270   maximum decibel level using the Decibel X app for iphones.

271   All playback trials were conducted between 9:30am and 11:30am, when morning

272   husbandry-related disturbance had finished. Also, this time window is not within the species-

273   specific period of peak vocal activity that have been historically reported (0600 to 0900 and 1500

274   to 1800; Banko et al. 2002), to attempt to avoid times where there could be background noise from

275   other aviaries that might otherwise cause a distraction. For each playback trial we placed the

276   speaker on the ground in a standardized location on the side of the aviary, along an opaque wall

277   on the outside of the aviary. While the birds may have known a person was outside the aviary, they

278   were unable to directly see the observer placing the speaker. We allowed the subjects a 5-min

279   habituation period after the observer placed the speaker and moved to the observation location.

280 Each of four trials consisted of three different observation periods: pre-trial (3-min playback of

281 forest noise to control for general response to sound playback), stimulus presentation (0.5-min call

282 playback), and post-trial (3-min forest noise playback repeated). Following a 19-min intermission,

283 we repeated the three-period trial using a different call type. This playback routine was repeated

284 for all four calls in balanced, randomized order across individuals. This resulted in 30 minutes

285 between stimulus presentations. The observer scored trials live through a small window at the front

286 of the aviary and recorded trials by setting up a video camera at each window. Because the stimuli

287 were auditory and trials were scored live, it was not possible to record data blind. The birds likely

288 heard the observer enter, but once the 5-minute habituation began, the observer stayed quiet and

289 out of view as much as possible. Therefore, it is possible that the birds were aware of the presence

290 of the observer and video camera, but we tried to minimize disturbance as much as possible. We

291 scored trials live but consulted video recordings to improve data accuracy. We recorded the

292 following behaviors across all three trial periods: (1) approach (bird approaches playback speaker,

293 measured as a binary variable with any movement of the bird in the direction of the speaker

294 counting as an approach); (2) latency to approach (time at which bird first moved in the direction

295 of the stimulus); (3) number and (4) type of all calls made by each bird. Call types were classified

296 into four categories: alarm, territorial intrusion, subordinate begging and other. Territorial

297 maintenance calls were never made in response to the stimuli so they were not included in the

298 models.

299 *Analysis*

300 All analyses were conducted in R version 3.4.1 (R core team 2017). In order to determine

301 if our pre-trial conditions were consistent for each stimulus, we ran a generalized linear mixed

302 model (GLMM) with a negative binomial distribution (as data were zero inflated; Bliss et al. 1953)

303 and a log link function, using the package glmmTMB (version 0.2.3; Brooks et al. 2017), with the

304 number of all calls made during the pre-trial as the response variable. The initial model included

305 the main effects of stimulus type and order as well as the interactions between stimulus type and

306 order. We also included the random effect of bird ID.

307 To investigate the degree of individual consistency in call responses, we ran an intraclass

308 correlation using the package irr (version 0.84; Gamer et al. 2012) for the number of calls each

309 individual produced during the stimulus and post-trial combined for each stimulus type.

310 In order to determine interest in different stimuli, we ran a cox proportional hazards model

311 using the package survival (version 2.38; Therneau 2015) on the likeliness of birds to approach

312 the stimulus for each stimulus type. This model included stimulus type, sex, and order as main

313 effects as well as the interaction between stimulus type and sex and stimulus type and order. We

314 clustered data around bird ID.

315 To evaluate the circumstances influencing call production, we used generalized linear

316 mixed models (GLMM) with a negative binomial distribution (as data were zero inflated) and a

317 log link function, using the package glmmTMB (version 0.2.3; Brooks et al. 2017) to separately

318 test whether all calls, alarm calls, territorial intrusion calls and begging calls were more likely to

319 occur during the stimulus and post-trial period depending on the playback type. All models initially

320 included the main effects of stimulus type, sex, and trial order as well as the interactions between

321 stimulus type and sex and stimulus type and order. We also included the random effect of bird ID.

322 We used the territorial maintenance control call as the reference group when comparing the

323 different stimulus types as this should represent a conspecific call in a new location, so this

324 accounts for any calls or approaches simply due to the social novelty and not the call itself. The

325   GLMM with the number of territorial intrusion calls made during the stimulus and post-trial would

326   not converge properly when the model included interactions, so only main effects were tested.

327   For all GLMMs except for the model with territorial intrusion calls as the response variable,

328   we first determined if the interaction terms warranted inclusion in the models. Starting with the

329   interaction between stimulus type and order, as this was likely less biologically relevant, and then

330   continuing with the interaction between stimulus type and sex, we removed interaction terms if

331   their inclusion failed to decrease AIC values by > 2. We did not simplify the model past

332   determining which interactions to include, as all remaining effects were important variables we

333   wanted to consider in the final model. See the supplementary materials for the full process of

334   model selection for each response variable. For all GLMMs we also visually inspected binned

335   plots of the expected versus residual values for the final model.

336

337   **Results**

338   *Pre-trial call frequency in response to playback of forest noise*

339   As expected, we found no differences in call number among treatments before stimulus

340   exposure during the pre-trial period (alarm trial vs. territorial maintenance: GLMM, b = -1.008, z

341   = -0.942, P = 0.346; novel control trial vs. territorial maintenance: b = -0.836, z = -0.804, P =

342   0.421; territorial intrusion trial vs. territorial maintenance: b = -0.677, z = -0.665, P = 0.506).

343   Therefore, we have not weighted post-trial data as a function of pre-trial calling rates. However,

344   bird calls significantly increased across trials (b = 0.769, z = 2.179, P = 0.029); therefore, we

345   included trial order in all subsequent models.

346   *Approach latency during stimuli presentation*

347   Birds were more likely to approach after the alarm stimulus than the territorial

348   maintenance stimulus (Fig. 1b; alarm vs. territorial maintenance: Cox Proportional Hazards

349   Model, coefficient = 1.293, z = 2.293, Pr = 0.0218) whereas the other stimuli had no significant

350   effect on the likelihood of approaching compared to the territorial maintenance stimulus (Fig. 1a;

351   novel control compared to territorial maintenance: coefficient = 0.827, z = 1.608, Pr = 0.108;

352   territorial intrusion versus territorial maintenance: coefficient = 0.479, z = 0.892, Pr = 0.372).

353   Trial order was also significant, with birds more likely to approach in later trials (coefficient =

354   0.242, z = 1.997, Pr = 0.0458). Sex did not have a significant effect on the likelihood of

355   approaching (effect of sex: coefficient = 0.115, z = 0.376, Pr = 0.707).
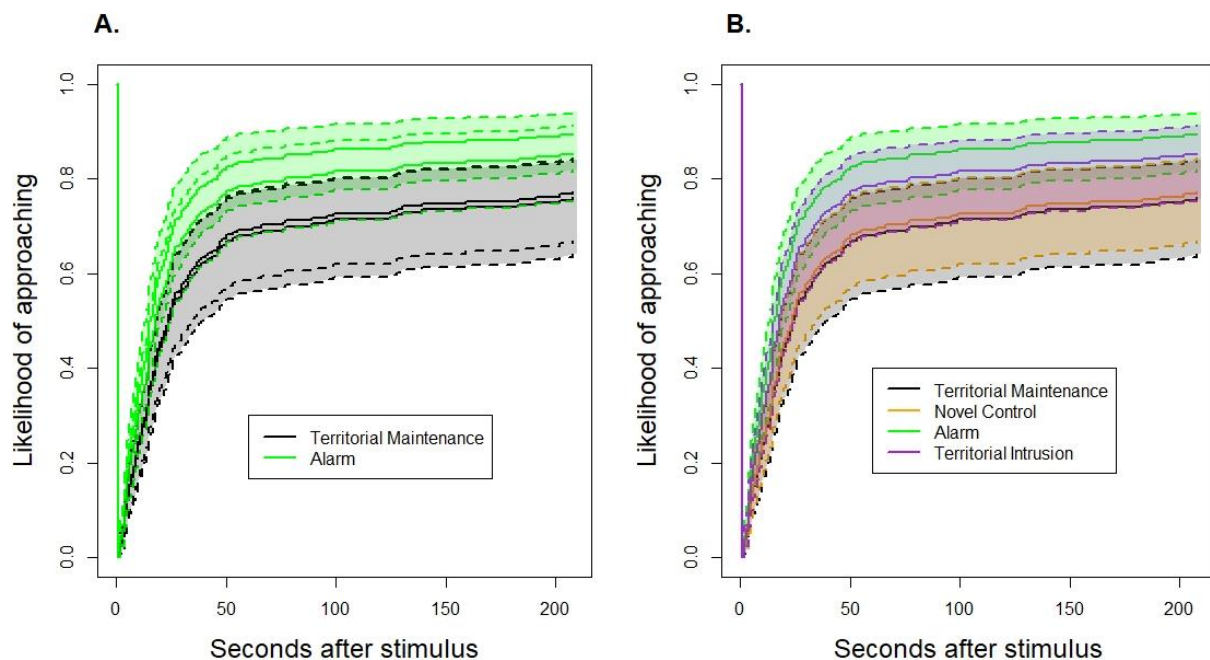
356

357   Fig. 1 a Inverted survival curves (solid lines) showing the likelihood that birds approach in
358   response to only the two significantly different stimuli, territorial maintenance and alarm
359   (represented by the corresponding colors in the legend) with a 95% confidence interval (dotted
360   lines). b Inverted survival curves (solid lines) showing the likelihood that birds approach each
361   stimulus type (represented by the corresponding colors in the legend) with a 95% confidence
362   interval (dotted lines).

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Individual call consistency during stimuli presentation and post-trial

Birds were individually consistent in the total number of calls they each made during the stimulus and post-trial periods across all different trial treatments (Intraclass correlation coefficient, 0.367, CI = 0.231-0.515, P < 0.001); i,e., birds who made fewer calls in one type of trial also made fewer calls in all trials.

Call responses

Even though birds differed individually in the number of calls they made across all stimuli, no patterns emerged in how many calls birds made between stimuli types during the playback and post-trial period combined (Fig. 2; alarm vs. territorial maintenance: GLMM, b = 0.497, z = 0.737, $P = 0.461$; novel control vs. territorial maintenance: b = 0.271, z = 0.408, $P = 0.683$; territorial intrusion vs. territorial maintenance: b = 1.007, z = 1.471, $P = 0.141$).
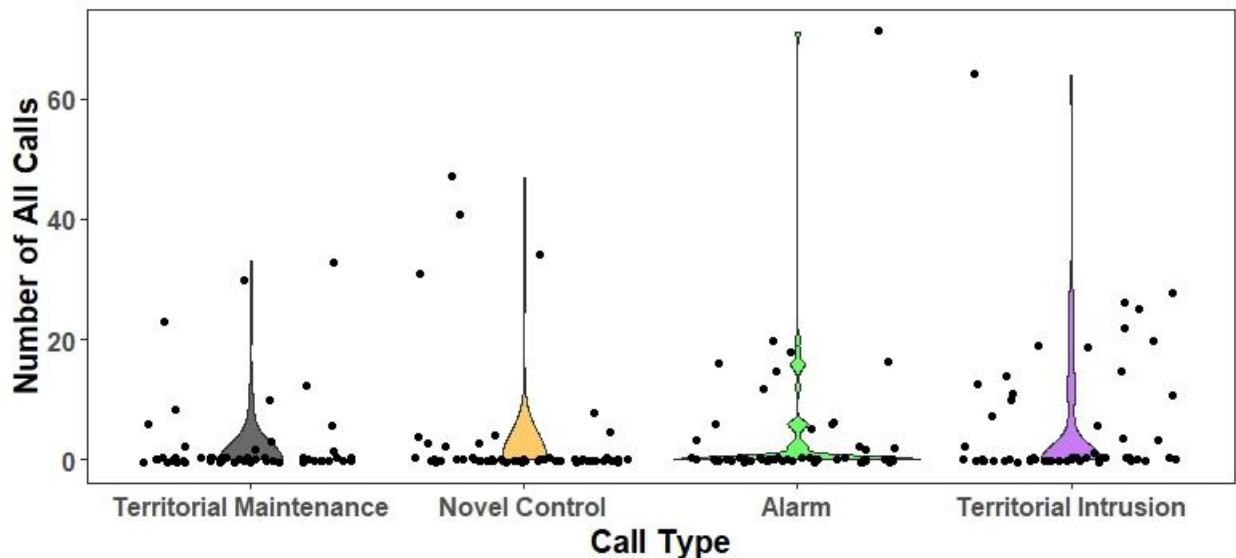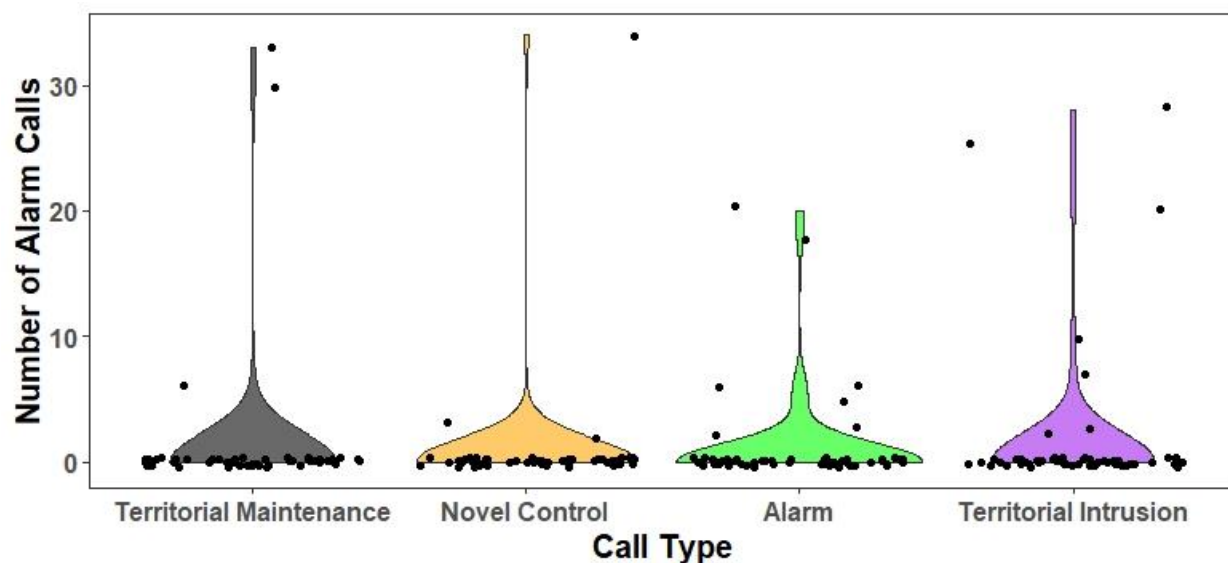

Fig. 2 Violin plots and raw data showing the distribution of the number of all calls birds made during the stimulus and post-trial period after each type of stimulus. The points are the raw data for each individual trial, jittered to reduce point overlap.

380   There was also no effect of sex (b = -0.598, z = -1.211, P = 0.226) or order (b = 0.099, z = 0.394,

381   P = 0.693) on the total number of calls birds made during the stimulus and post-trial.

382   When we looked at the production of specific calls, we found that the number of alarm calls

383   birds made during the stimulus and post-trial combined did not significantly vary between any of

384   the different stimuli (Fig. 3; alarm stimulus vs. territorial maintenance: b = -1.584, z = -1.093, P =

385   0.275; novel control stimulus vs. territorial maintenance: b = 0.169, z = 0.097, P = 0.923; territorial

386   stimulus vs. territorial maintenance: b = 0.390, z = 0.312, P = 0.755). All other terms were not

387   significant.

388

389   Fig. 3 Violin plots and raw data showing the distribution of the number of alarm calls birds made
390   during the stimulus and post-trial period after each type of stimulus. The points are the raw data
391   for each individual trial, jittered to reduce point overlap.

392   There was also no difference in the number of begging calls birds made during the stimulus and

393   post-trial between any of the stimuli (alarm stimulus vs. territorial maintenance: b = 0.820, z =

394   0.665, P = 0.506; novel control vs. territorial maintenance: b = -0.193, z = -0.170, P = 0.865;

395   territorial intrusion stimulus vs. territorial maintenance: b = -0.441, z = -0.409, P = 0.683). Birds

396   never made the territorial maintenance call in response to any stimuli.

397   Overall, birds made significantly more territorial intrusion calls during the stimulus and

398   post-trial period in response to the territorial intrusion stimulus than to the territorial maintenance

399   stimulus (Fig. 4; $b = 3.825$, $z = 2.298$, $P = 0.022$) whereas they did not make a significantly

400   different amount of territorial calls during the stimulus and post-trial period in response to any

401   other kind of stimulus (Fig. 4; alarm stimulus vs. territorial maintenance: $b = 2.033$, $z = 1.064$, P

402    $= 0.287$; novel control vs. territorial maintenance: $b = -19.797$, $z = -0.002$, $P = 0.999$).
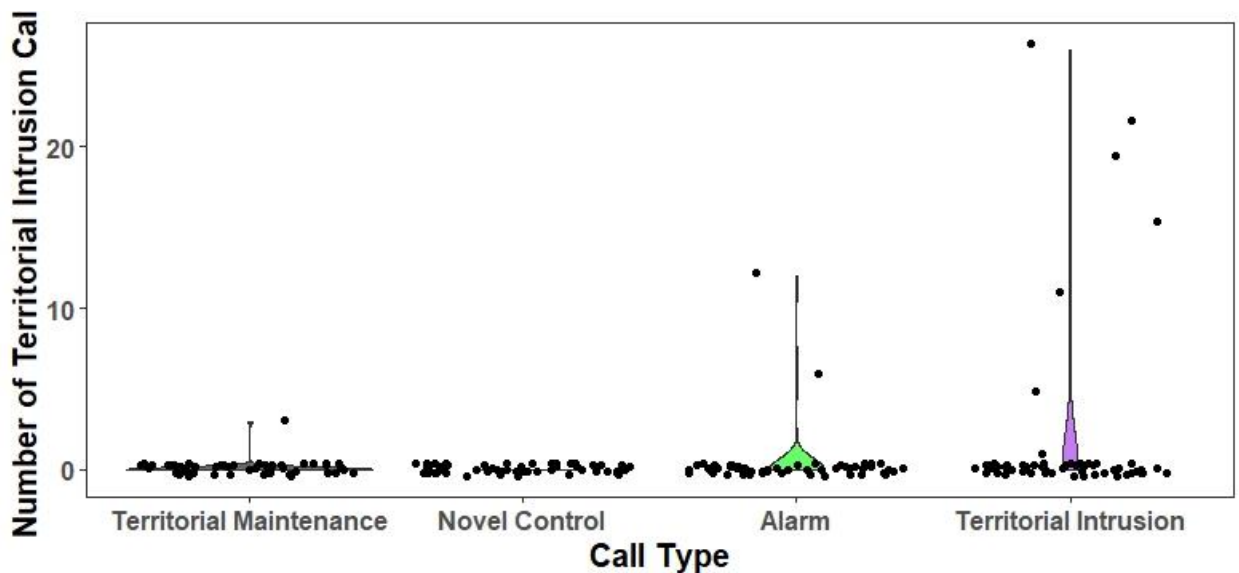
403

404   Fig. 4 Violin plots and raw data showing the distribution of the number of territorial intrusion
405   calls birds made during the stimulus and post-trial period after each type of stimulus. The points
406   are the raw data for each individual trial, jittered to reduce point overlap.

407   Males made significantly more territorial intrusion calls during the stimulus and post-trial

408   than did females (Fig. 5; $b = 5.087$, $z = 3.106$, $P = 0.002$).

409
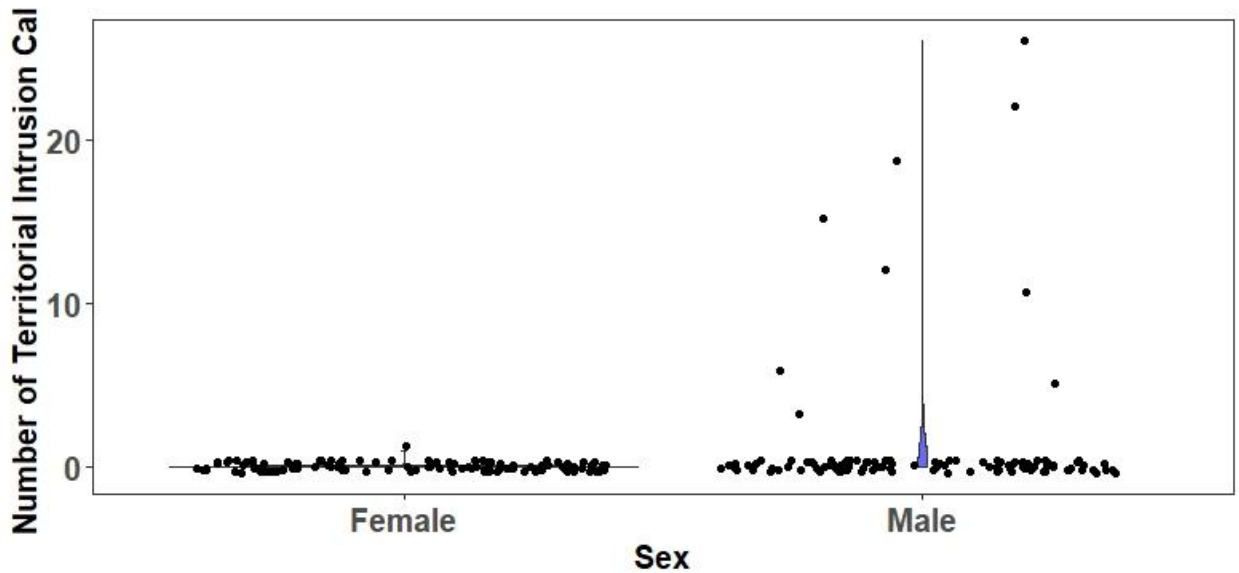
Fig. 5 Violin plots and raw data showing the distribution of the number of territorial intrusion calls males and females made during the stimulus and post-trial period after each type of stimulus. The points are the raw data for each individual trial, jittered to reduce point overlap.

Discussion

Species raised under human care have the potential to lose some of their survival-relevant behaviors (McPhee and Carlstead 2010; Shier 2016), including responses to species-specific vocalizations (Corfield et al. 2008; Digby et al. 2013; Tanimoto et al. 2017b). Conservation breeding programs or managed care may also change selective forces operating across generations or developmental processes operating during individuals' lifetimes, creating new cultural vocal variants and response patterns (Lewis et al. 2021). Disrupting the normal signaler-receiver interplay may result in a breakdown in the communication system, with fitness consequences challenging conservation goals. To address these possibilities, we tested whether pairs of 'alalā have maintained responses to survival-relevant classes of vocalizations. Although the conservation breeding history of the species may have reduced some of their responses, we still saw encouraging signs that 'alalā were able to distinguish between natural call types and demonstrate responses that may be linked to increased survival in the wild. Specifically, we found that 'alalā were more likely

426   to approach in response to alarm calls, indicating they may have been investigating a potential

427   threat or seeking to coordinate a defensive response with conspecifics. Also, we found that males

428   in particular were more likely than females to respond to territorial intrusion calls with territorial

429   intrusion calls of their own, suggesting they were willing to defend their breeding aviary. Finally,

430   our results illustrate that there is some individual consistency in the amount of calling over all

431   treatments, but that individual characteristics (e.g. personality or specific rearing history) does not

432   fully explain the variation in call response to the different treatments. A note of caution in

433   interpreting these results is warranted due to the absence of any quantitative and limited qualitative

434   observations of 'alalā vocal behavior before they went extinct in the wild, so we have few reference

435   points other than comparisons with and generalizations from other species.

436   As a corvid, flexible learning is expected throughout their life (Emery and Clayton 2004),

437   but vocalizations often require exposure during times of parental care or sensitive periods, which

438   may be limited under human care (Corfield et al. 2008; Digby et al. 2013; Tanimoto et al. 2017b).

439   Most 'alalā in this study were puppet-reared by human caretakers (Gregor et al. 2018), removing

440   any opportunities for learning vocal behavior from parents. Of known call types, alarm calls are

441   more likely to be preserved since they potentially share some innate characteristics that make them

442   harder for predators to locate (Maynard et al. 2003). Additionally, animals, including corvids, will

443   often make alarm calls to elicit assistance from conspecifics in investigating or confronting a

444   predator (Curio 1978; Coomes et al. 2019). In the case of 'alalā, we found that birds were more

445   likely to approach alarm call playbacks than other auditory stimuli. However, they were not more

446   likely to make alarm calls in response to alarm playbacks. This lack of vocal response is consistent

447   with what was reported in some pilot work on 'alalā anti-predator behavior (Greggor et al. 2021),

448   suggesting that alarm calls may function to alert birds to the need to gather additional information

449   or that birds may not respond to alarm calls with their own calls in order to avoid drawing the

450   attention of the threat to themselves. It is also possible that because we presented alarm calls

451   without other stimuli that could indicate danger, they investigated but did not respond with their

452   own alarm calls when they saw no clear threat. Further testing of alarm calls with and without

453   presenting a dangerous context would be necessary to tease this apart. However, 'alalā's approach

454   of the source of the playback alone may still have had a social function. The fact that birds

455   approached the alarm calls (and not just conspecific playbacks in general) suggests they may still

456   function to elicit social assistance: approach of the caller may precede mobbing or other group

457   antipredator defense. Although mobbing-like behavior has been anecdotally reported in 'alalā

458   (Greggor et al. 2021, this study provides some empirical support that the birds responded

459   appropriately to alarm calls in the absence of other signs of danger and have not become

460   completely desensitized to them.

461   Territorial calls are also an important part of vocal communication, including in the

462   historical vocal repertoire of 'alalā (Banko et al. 2002; Tanimoto et al. 2017a). We played two

463   types of territorial calls, a neutral territorial maintenance call and an aggressive territorial intrusion

464   call. 'Alalā did not significantly differ in their response to the territorial maintenance call from

465   other call types. However, we found that 'alalā responded to a simulated territorial intrusion with

466   territorial intrusion calls significantly more than they did to any other playback stimuli. A vocal

467   territorial response is the natural reaction we would expect to a territorial challenge (Maynard et

468   al. 2003; Bradbury and Vehrencamp 2011), considering we presented the birds with this stimulus

469   in their home aviaries. We did not test any of the birds in a new location outside of their artificial

470   "territory" so we do not know if their responses are flexible and adaptive to context, i.e. defending

471   only their occupied territory. However, given that 'alalā exhibit fewer territorial calls than their

472   wild counterparts (Tanimoto et al. 2017b), we find it encouraging that 'alalā displayed this

473   behavioral response suggesting some normal signaler-receiver exchange. Additionally, we found

474   that males were more likely to make territorial intrusion calls, which mirrors patterns noted

475   previously in the wild (Banko et al. 2002). As a sexually dimorphic species with respect to size,

476   males are the larger and more aggressive sex, and thus their greater role in territorial defense is

477   expected (Archer 1988).

478   While we found encouraging signs that some birds exhibited natural responses to our

479   playbacks in the absence of any other context or stimuli, many of the birds did not respond vocally

480   when faced with any type of conspecific call. Given that the birds are living in social densities that

481   are far higher than observed in the wild (Flanagan et al. 2020), the high levels of individual

482   variation we found suggest that some 'alalā may have become desensitized to the calls of

483   conspecifics vocalizations, perhaps a result of repeated exposure without consequence or that

484   salient context was missing from our stimulus presentations. While 'alalā in the conservation

485   breeding facilities may not necessarily be less vocal than wild birds (Tanimoto et al. 2017b), the

486   lack of vocal behavior we saw in many individuals in response to social cues suggests a potential

487   decoupling of the meaning or relevance of conspecific calls. Alternatively, perhaps there is

488   additional context we did not adequately capture in our recordings (e.g. individual caller ID, social

489   status, etc.), that could have differentially influenced some individuals more than others. Corvids

490   are known to respond to individual qualities of callers (Boeckle et al. 2012), such as their

491   dominance status (Massen et al. 2014), and membership in the breeding colony (Woods et al.

492   2018), with call signatures that may also help with distinguishing sex (Mates et al., 2015).

493   Additionally, since calls were recorded opportunistically, there may be subtle differences to the

494   different calls (for example, the particular stimulus causing the alarm calls) that we are unaware

495   of. Therefore, we may have inadvertently broadcast information beyond the content of the calls.

496   However, we saw no differences in the number of calls birds made in response to the three different

497   call sets, suggesting that the identity of the caller was not a major cause of variation. A final

498   explanation for the variation could be that by using recordings of birds in the conservation breeding

499   centers, which was necessary for controlling context and individual factors such as sex and

500   identity, the calls themselves may no longer retain the same information as wild calls would have.

501   Examinations of historical recordings show that similar call types to the ones we broadcast were

502   used by the last wild birds (Tanimoto et al. 2017a). Although some differences likely exist

503   (Tanimoto et al. 2017b), the call types we chose were similar to those produced by wild birds, and

504   the fact that responses were largely consistent with our predictions suggest that the calls used have

505   retained their function. Regardless of the cause of the low responses of many individuals, there

506   were still other birds that clearly demonstrated survival-relevant responses.

507   Although we found no evidence of widespread erosion of vocal communication behavior

508   under human care, the individual differences that we saw in how birds responded to the playbacks

509   could indicate that not all individuals are equally as well equipped for release into the wild if these

510   call responses would indeed result in reduced predation risk or increased territory defense in the

511   wild. Many factors go into determining fitness for release, and these results suggest that we may

512   need to consider whether birds demonstrate adequate responses to conspecific calls as a criterion

513   for release. Additionally, future research could investigate how likely birds are to regain survival-

514   relevant responses to vocalizations if they are exposed to training that encourages associations

515   between conspecific calls and relevant responses. Given the critical conservation status of the

516   species, any technique that could limit the impact of the conservation breeding environment on

517   survival-relevant behavior is warranted.

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