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**Mixed-species flock size affects contact call frequencies
of the crested tit, *Lophophanes cristatus***

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TIIVISTELMÄ

Ään telemällä tapahtuva kommunikointi voi altistaa linnut saalistukselle, koska pedot voivat paikantaa saaliseläimet ääntelyn perusteella. Yksi mahdollinen tapa suojautua saalistajilta on liittyminen parviin. Parveutuminen voi vaikuttaa ääntelyfrekvensseihin lisääntyneen parvensisäisen kommunikointitarpeen kautta, mutta myös koska ääntely suuremmissa parvissa voi olla saalistusriskin suhteen turvallisempaa. Tässä tutkimuksessa tutkin lajienvälisen sekaparven koon vaikutusta töyhtötiaisen pitkän etäisyyden kommunikointiin yleensä, parven sisäiseen kommunikointiin ja parvien väliseen revii rin puolustus kommunikointiin. Tutkimusmenetelmänä oli töyhtötiäisparvien tarkkailu luonnollisissa olosuhteissa. Pitkän etäisyyden kommunikointi kokonaisuudessaan sekä parven sisäisen kommunikoinnin ja revii rin puolustus kommunikoinnin frekvenssit olivat positiivisesti riippuvaisia sekaparven koosta. Tulokset osoittavat, että parvensisäinen kommunikointi on positiivisesti riippuvainen parvikoosta, todennäköisesti koska parven sisäinen kommunikointitarve kasvaa parvikoon myötä. Tulokset myös viittaavat siihen, että parvien välinen revii rin puolustus kommunikointi on riippuvainen sekaparven koosta. Esitän, että tämä on seurausta suurempien parvien pienemmästä saalistusriskistä.

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ABSTRACT

Vocal communication can subject birds to increased predation risk, due to predators homing in on conspicuous vocalizations. One potential way to decrease predation risk is flocking. Flocking may affect vocalization frequencies due to increased intra-flock communication needs, but also because vocalizations in large flocks may be less costly in terms of predation risk. In this study, I examined the relationship between mixed-species flock size and long range call rates generally and the effect on intra-flock and territorial call rates of the crested tit. The general methodology of the study was direct observation of crested tit flocks in natural conditions. Call rates generally and both intra-flock and territorial call rates were affected by mixed-species flock size. The results show that intra-flock communication frequency is positively dependent on mixed-species flock size, most likely due to increased needs for communication in large flocks. The results also suggest that crested tit territorial communication rates are dependent on mixed-species flock size. I suggest that this is due to the decreased predation risk in larger flocks.

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1. INTRODUCTION

Mixed-species social groups have been documented in fish (Lukoschek & McCormick 2002), mammals (Stensland et al. 2003) and birds (Sridhar et al. 2009). Mixed-species bird flocks occur in terrestrial ecosystems over the world, mostly in forested areas and particularly in the tropics (Powell 1985, Thiollay 1999). One of the main hypotheses for the adaptive significance of flocking is decreased predation risk (Sridhar et al. 2009).

Flocking with hetero-specifics is generally supposed to infer advantages by increases in either protection from predation or feeding efficiency (reviewed in Morse 1977). There are several ways how flocking can affect predation risk. First, dilution effect refers to the reduced probability of a prey-individual being attacked in larger flocks. Dilution effect requires, however, that increased attack frequencies on larger flocks do not negate the effect (Foster & Treherne 1981). Second, increased vigilance refers to the better predator detection abilities of a flock, compared to solitary individuals (Pulliam 1973 sit. Elgar 1989). Third, the confusion effect, which takes place when the movements of dense prey items make capturing less successful (Neill & Cullen 1974). Fourth, a phenomenon that can apply especially to dominant species in mixed-species flocks, is the “selfish herd”-effect under which some individuals or species may be safer than others due to safe positions in the middle of the flock or more protected microhabitats (Hamilton 1971, Ekman 1986, Suhonen et al. 1993). Finally, flock size can affect the prey-individuals' chance to physically disturb predators (Morse 1977). If individuals warn other flock members of approaching predators with calls, or individuals can deduce the presence or approach of predators from the movements of other individuals (Lima 1994), at least the increased vigilance effect can be assumed to be positively related to flock size, and therefore individual predation risk in large flocks is likely to be lower.

Vocal communication is an essential part of a typical passerine's life. Birds use vocalizations for many functions, including contact signals, territory defense, mate attraction, alarm and mobbing. Birds derive benefits by communicating vocally, such as keeping neighbors from trespassing into their territory (Krams 2000) and communicating their whereabouts or actions to mates or other flock members. Some calls are used more frequently than others, and even within a call type there is much context-specific variation in frequency. Therefore the benefits of calling can be assumed to be in some way dependent on the calling frequency. Flock size and composition can presumably affect the benefits from vocal communication. These changes could result from changes in, for example, flock movement speed (Hutto 1988), distances between conspecifics or aggressive activity related to changes in the amount of competition.

However, conspicuous calling may also incur costs in the form of predation risk if predators use vocal cues when searching for prey (Klump & Shalter 1984, Leech & Leonard 1997, Krams 2001, Hale 2004). If predation related costs for vocal communication are high, it may be advantageous for birds to alter the amount of vocal communication depending on flock size, or, to put it simply, avoid making noise when flocks are small, despite the benefits of vocal communication. If, specifically, predation risk related changes in the flock size–calling frequency relationship are to be studied, the effect must be discernible from other possible flock size related changes in intra-flock communication benefits. An obvious choice for the study is territory defense calling, which, by definition, is not directed at flock mates. Territorial calling must also be loud in order to be heard to neighbors, and therefore can be assumed to be conspicuous to predators as well.

Many studies regarding mixed-species flocks and the use of alarm calls have been done. For example, Goodale & Kotagama (2005) showed that several species in mixed-species flocks contribute to alarm calling, albeit with inter-specific differences in alarm

calling frequency. It has also been shown that birds can react to alarm calls made by hetero-specifics (Magrath et al. 2007), and do so in mixed-species flocks (Goodale & Kotagama 2008). Furthermore, Hetrick & Sieving recently (2012) showed that chickadee species can correctly interpret predator related information coded in hetero-specific alarm calls. Studies have also shown that vocal mimicry (Goodale & Kotagama 2006) and long distance contact calls (Suzuki 2011) can facilitate the formation of mixed-species flocks. Krams (2000) showed that dominance and sex affected the frequency of loud contact call utterance in crested tit flocks. However, to my knowledge, no studies have been made on the role of mixed-species flocking on contact calling or territorial calling frequencies, from a predation risk perspective or otherwise.

The purpose of this study is to determine whether the frequency of conspicuous calling is related to mixed-species flock size. I address the questions, if 1) the call rate of crested tit trill calls generally (total call rate of trill calls), 2) the call rate of crested tit intra-flock contact calls (short call series and sparsely used individual trill calls, i.e. calls of low calling intensity) and 3) the rate of territory defense calling (the frequency of periods when trill calls are made in long series or repeated frequently, i.e. high calling intensity), is related to mixed-species flock size. Due to possible increases in flock movement speed (Hutto 1988), distances between conspecifics, inter-specific communication and protection from predators, I predict that intra-flock contact call rate is higher in larger mixed-species flocks. Due to the increased predation risk of conspicuous calling and the presumably increased safety of larger flocks, I predict the territorial defense calling rate to be positively dependent on mixed-species flock size. From the combination of positive dependency of both territorial calling and intra-flock calling on mixed-species flock size, I predict total call rate to be also positively dependent on mixed-species flock size.

2. MATERIAL AND METHODS

2.1. Study area

The study was conducted between September 2011 and February 2012 in Nuuksio National Park (66,87°N 36,11°E) and the surrounding forested areas in southern Finland. Most of the study area is dominated by Scots pine (*Pinus sylvestris*) with some areas dominated by Norway spruce (*Picea abies*). To maximize the lengths of observation periods, the area was selected to be continuously forested, with no clear-cuts or sapling stands to induce long distance flights, which often lead to contact loss between the observer and bird flocks (personal observation). The locations for the observations were selected to be at least 500m from the nearest settlement, to avoid possible complications associated with bird feeders, such as, long distance flights to and from feeders and possible increased vocal activity associated with either territory defense or within-flock aggressive behavior.

2.2. Study species

The crested tit is a sedentary species that forms stable territorial flocks outside the breeding season. In Northern Europe these flocks consist of 2-4 individuals (Ekman 1979, Lens & Dhont 1992, Krams 2000). Flocks usually consist of an adult mated pair and 0-2 first year birds (Ekman 1979). Crested tits hoard food in caches inside the territory, especially during the autumn months (Haftorn 1954). The flocks form a stable linear hierarchy, with males dominating females and, within sexes, old individuals dominating young (Krams 1996, 2000). On warm days, the flocks tend to split into sub flocks within the same territory for

part of the day, with the dominant pair forming one sub flock, and the subordinate pair the other, but stay together more consistently during cold days (Lens & Dhont 1992).

In Finland, crested tits frequently form mixed-species flocks with other species outside the breeding season. In my study area in southern Finland, these flocks can consist of typical coniferous forest tit guild members, i.e. willow tits (*Poecile montanus*), coal tits (*Periparus ater*), goldcrests (*Regulus regulus*) and Eurasian treecreepers (*Certhia familiaris*) and the primarily deciduous great tits (*Parus major*) and blue tits (*Cyanistes caeruleus*). These mixed-species flocks are not stable, with birds joining and leaving the flock from time to time.

The tit guild of coniferous forests is well studied (see e.g. Alatalo 1982a for a review). The members of the mixed-species flock segregate regarding tree species and vertical and horizontal positions in the trees. Great tits and blue tits mostly prefer deciduous trees while crested tits, willow tits, coal tits and goldcrests prefer conifers (Alatalo 1982b). Crested tits tend to forage in the middle part of the vertical plane of the tree foliage, in the lower parts of the tree, whereas willow tits forage in the innermost parts, treecreepers forage mostly on the trunk and coal tits and goldcrests forage in the outer and upper parts of trees (Alatalo 1982b, Alatalo et al. 1987, Suhonen et al. 1993).

A dominance hierarchy also exists between species, the heavier species usually dominating lighter ones (Morse 1978, but see Haftorn 1993). Crested tits are subordinate to great tits and the blue tits, but dominant regarding willow tits, goldcrests and possibly coal tits (Hogstad 1978, Haftorn 1993, Mönkkönen & Orell 1997). This dominance regarding willow tits, the species with the greatest niche overlap with the crested tit (Alatalo 1982b), allows crested tits to occupy the supposedly safer parts of trees, forcing the willow tits to more vulnerable places (Krams 1996). During winter, pygmy owls (*Glaucidium passerinum*) are known to take proportionately larger amounts of coal tits and goldcrests, the species which forage in the outer parts of trees (Ekman 1986, Suhonen et al. 1993). This could be because predators tend to select the more vulnerable individuals, i.e. the individuals in the outer parts of the foliage, when attacking mixed-species flocks. If this is the case, and not a direct effect of less successful attacks towards birds that forage in the inner parts of trees, it would mean that crested tits gain significant increases to safety by joining mixed-species flocks with goldcrests, coal tits and, possibly, willow tits.

In forested habitats of the boreal zone, the predation related risks of loud vocalizations may be pronounced during late autumn and winter after most birds have migrated, when loud vocalizations in the otherwise relatively silent landscape can make the wintering birds particularly conspicuous to predators, such as the resident pygmy owl and the sparrow hawk (*Accipiter nisus*). Both species occur in my study area and use the vocalizations of prey-species to locate them (Kullberg 1995, Krams 2001). The predation risk generally is probably also dependent on season, as the proportions of predators and prey will change according to migration habits and mortality rates.

Crested tits have a variety of calls for different purposes (Cramp & Perrins 1993). The focus of this study is the long range contact calling of the crested tit. These calls are known as trills (Krams 2001). Trills are used for keeping contact with flock members, but also have an important role in territory defense, by keeping neighboring crested tit flocks from entering the territory (Krams 2000). Crested tits also use trills for other functions, such predator mobbing and territorial skirmishes (Hailman 1989, Cramp & Perrins 1993). The dominant male does most of the calling, the subordinate females calling least (Krams 2000). While foraging, crested tits most often use singular or several consecutive trills (personal observations), for instance when the flock moves constantly while foraging or suddenly changes direction. Most of these trills are obviously used as contact calls within the flock. At other times, trills are used in longer series, even if all flock mates are within

close proximity and in no obvious need for contact keeping. These long series of calls often elicit no obvious behavioral response from flock mates but sometimes induce other flock members to call as well. In these instances territorial advertisement seems the only obvious explanation. Crested tits are also known to use long series of calls between series of flights along territory borders, which also suggests that long call series are used for territory defense purposes (Krams 1996). Calling may be dependent on season for a multitude of reasons, e.g. changes in territorial activity levels, differences in the amount of social interactions during the initial phases of intra-specific flocking or changes in foraging movement rates due to differences in food availability.

2.3. Observation of call rates

The general methodology of the study was direct observation of crested tit flocks in natural conditions. A total of 39 crested tit flocks were observed in the study. The flocks consisted of 1-4 crested tits (mean 2.19). Several sub-flocks were observed in this study. Members of the same territorial flock temporarily split up into mated pairs to forage in different parts of the mutual territory and joined either momentarily or permanently for the subsequent duration of the observation period. It is likely that most, if not all, of the observed sub-flocks consisted of the dominant pair and not the sub-dominant individuals. This was due to the significantly higher territorial calling frequencies of the dominant pair (Krams 2000), which makes the finding of dominant pairs easier.

A new flock was observed on each day. A playback of a willow tit mobbing call was used to locate crested tit flocks. This playback was always played for two minutes before flock observation, even when crested tits were located by chance encounter before playback calls were played. During observation call rates, flock composition was recorded.

I randomly selected one crested tit individual as a focal individual. Due to the fact that eye contact was frequently lost, and the following of the crested tit flock was often easiest by listening to their trill calls, the focal individual tended to change into the most vocally conspicuous individual, most likely the dominant male, which calls the most (Krams 2000). Because individual recognition was not possible and the “trill” calls of the crested tit are audible over sufficient distances, call rates were recorded both for the focal individual and collectively for the rest of the flock.

Calls were not recorded during the first 5 minutes of observation to avoid bias associated with either the encounter or playback-call. Disturbances, such as predator attacks and periods of competitive calling between neighboring flocks, were recorded. Before analysis, the disturbances and the following five minutes were removed from the data. The flock was followed for as long as possible, which often meant that the same crested tit flock was observed in different mixed-species flock compositions. When contact was finally lost, a new flock was searched for.

Observations were not made during rain or on days with strong wind. The variables I recorded were: crested tit flock number, focus individual trill call rate, flock trill call rate (not including calls of the focal individual), length of the observation period, crested tit flock size, interspecific flock size and date. Trill call rates and changes in flock size, when evident, were recorded at one minute intervals, with the help of a stopwatch.

The observation date can cause variation in call rates (e.g. if territorial activity is more pronounced during the onset of intra-specific flocking) but in addition could cause bias due to effects on both call rate and flock composition. The dates of observations formed two distinct clusters, one between 09.09.2010–12.10.2010 and the second between 18.11.2011–09.02.2012. Therefore, the date variable was substituted by season, the earlier date cluster defined as autumn and the later defined as winter.

The observation periods were determined by splitting the data into periods of

constant flock composition, so that the same crested tit individuals could be represented in different sized inter-specific flocks. As this caused the different observations to be dependent of each other, the flock number was also recorded as a variable (see statistical analysis).

I classified call rate data in five ways. First, I calculated total call rate as the sum of trill calls for the whole crested tit flock, including the focal individual, over the observation period. I further aimed at distinguishing within-flock contact calls from territory defense calls. Because there are no detailed studies on crested tit territorial calling behavior, separating territorial calls from within-flock contact calls must be done subjectively.

Due to the uncertainty that follows from a subjective classification, I classified the call rate for contact calls in two ways. First, I summed up the trill calls of the whole flock, again including the focal individual, only for minutes with less than four calls over the observation period, removing all minutes with four or more calls from the data. I assume this variable (short intra-flock call rate) strictly represents intra-flock contact call rate. Secondly, I summed up the whole flock calls for all minutes with less than six calls, removing all minutes with six or more calls from the original data. I assume this variable (long intra-flock call rate) to also represent intra-flock communication, but it includes more data to improve the quality of statistical analysis, however, with a larger risk of including some instances of territorial calling than the short intra-flock call rate.

The amount of trills per minute varied widely with longer calling frequencies, which I assume to be territorial in nature (ranging from 10 to 61 trills per minute in this study). This means that territorial calling behavior can be affected by changes in calling frequency (the frequency of periods of territorial communication) and through calling intensity (the amount of calls during one instance of territorial communication). The predation risk that follows from calling may be more strongly related to territorial calling frequency than intensity (e.g. two separate periods of territorial calling may subject an individual to greater predation risk than one period which contains twice as many calls). Therefore I first calculated the call rate for territorial calls by summing the frequency of minutes with one individual of the flock producing more than nine calls over the observation period, i.e. if the call rates of the focal individual or average call rate of the other flock members exceeded nine. Thus this variable (territorial call rate) attempts to measure the frequency, not the intensity, of instances of territorial communication. However, due to the tendency of crested tits to make calls synchronously, the territorial call rate may not show effects of intra-specific flock size on calling behavior (if larger crested tit flocks call more, but simultaneously). Therefore, I finally calculated the average amount of trill calls made by the whole crested tit flock during minutes of territorial calling (when at least one individual made over 9 trill calls per minute) (territorial calling intensity).

The classification of territorial call rate is supported by the fact that, in this study, the average call rate when neighboring flocks were in audible contact, when most calls can be presumed to be territory defense related, was 11.7 trills per minute. Other causes that can elicit long series of calls are predator mobbing (Hailman 1989, Cramp & Perrins 1993) and some social interactions, such as the joining of sub flocks, but these are readily identifiable and were removed before analysis. Using subjective call classifications means that the call categories probably do not consist only of the appropriate calls, and/or do not contain all of the appropriate calls. By excluding intermediate call rates (over three or five flock-made calls per minute for intra-flock contact calls and less than ten individual-made calls per minute for territorial calls) I attempted to direct the error in call classification to a conservative direction, i.e. call categories likely consist only of the appropriate calls even if part of the appropriate calls are lost.

2.4. Statistical analysis

I used linear mixed models with Maximum Likelihood approach to analyze the data. I constructed five separate models in which the dependent variables were total call rate, short intra-flock call rate, long intra-flock call rate, territorial call rate and territorial calling intensity. The dependent variables were $\ln(\text{call rate} + 1)$ -transformed to normalize model residuals. Because of the substantial variation in call rates within short observation periods, as shown in Figure 1 for total call rate, \ln -transformation was not enough to normalize model residuals. I therefore reduced the amount of (random) variation in the data by only including into analyses the observation periods exceeding 19 minutes. The minimum limit of 20 minutes was determined by searching for a minimum value after which the residuals of all five models had normal distributions (see below for model structure). This resulted in the final data in the analysis originating from 25 different crested tit flocks. These 25 flocks yielded 53 observation periods.

In all models, except the model of territorial calling intensity, independent variables were observation length, mixed-species flock size (number of individuals in the flock other than crested tits), intra-specific flock size (number of crested tits in the flock) and season as fixed factors, and flock number as a random factor. Observation length was entered to control for the obvious dependence of the frequency of calls on the time the flocks were observed. This way I avoided transforming the data into call frequencies per minute (i.e. call density). As average calling intensity is not dependent on observation length, the model for territorial calling intensity did not contain observation length. Otherwise the models contained the same independent variables. Because some observations were not independent of each other but originated from the same crested tit flock, I used nested design where flock size and observation length were nested within the crested tit flock number. This effectively controls for the dependencies among observations within a flock but allows analyzing all observation periods. Season was entered to control for potential overall difference in call rates in autumn vs. winter as one may expect birds to be less vocal in winter than in autumn. Because the same flocks were not observed in autumn and winter I could not test for season*flock size interaction with nested design mixed models. All analyses were conducted with Pasw v.18 statistical package for windows (SPSS inc., Chicago, IL, USA).

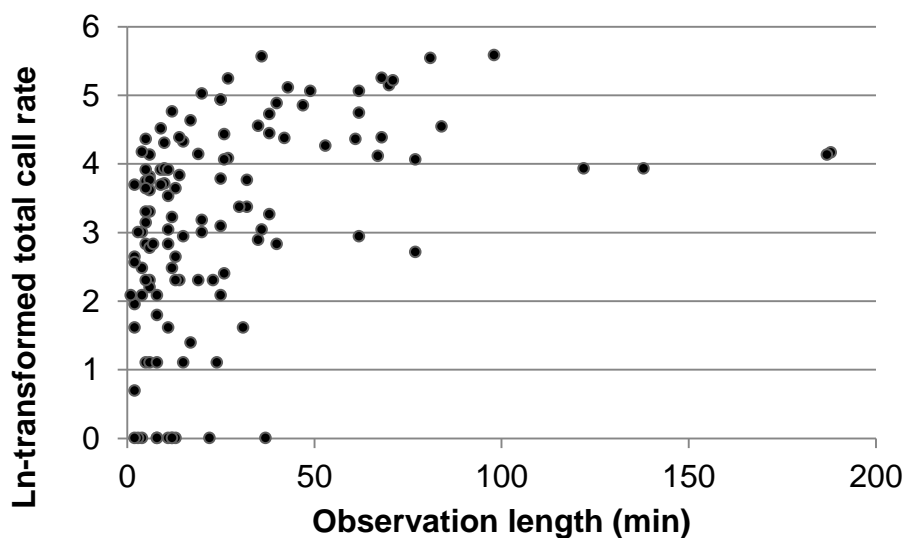


Figure 1. The relationship between \ln -transformed total call rate and observation length.

3. RESULTS

After controlling for the observation length, total call rate was affected by mixed-species flock size and intra-specific flock size, but I found no effect of season on total call rate (Table 1). Increase in the number of individuals to a mixed-species flock increased the ln-transformed call rate. When transformed back to the number of calls (converted with the formula $100 * (\exp(\ln(\text{call rate})) - 1)$) the model parameters suggest that an increment of one hetero-specific individual was associated with an 7.1% (95% confidence interval (CI) [3.1%, 11.4%]) increase in the call rate of crested tits (Figure 2a). Similarly, one additional crested tit in the flock was associated with an 83.9% (95% CI [27.6%, 164.9%]) increase in the total call rate of the flock.

Table 1. Results from a repeated measures mixed model analysis, with ln (total call rate + 1) as the dependent; season, mixed-species flock size, observation length and intra-specific flock size as fixed variables and flock number as the subject of repeated measures.

Estimates of Fixed Effects							
Parameter	Estimate	Std. Error	df	t	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Intercept	,611998	,616459	52,347	,993	,325	-,624822	1,848817
Season = autumn	,460129	,442708	53,000	1,039	,303	-,427830	1,348089
Mixed-species flock size	,068973	,019348	52,825	3,565	,001	,030162	,107783
Observation length	,013934	,004270	7,560	3,264	,012	,003988	,023880
Intra-specific flock size	,608920	,181709	50,335	3,351	,002	,244008	,973833

Dependent Variable: Ln (total call rate +1)

After controlling for observation length, both short (Table 2) and long (Table 3) intra-flock call rates were affected by mixed-species flock size, intra-specific flock size and season. Both call rates increased with increases in mixed-species flock size. Back-transformed model parameters suggest that an increase of one hetero-specific individual to a mixed-species flock increases short intra-flock call rate by 4,2% (95% CI [1.7%, 6.6%]) and long intra-flock call rate by 5.3% (95% CI [2.9%, 7.7%]) (Figures 2 b, c). Both call rates also increased with increases in intra-specific flock size. An increase of one crested tit to the flock increased short intra-flock call rate 46.1% (95% CI [17.7%, 81.5%]) and long intra-flock call rate 59.2% (95% CI [28.7%, 96.8%]). Short intra-flock call rates were 2.8 times (95% CI [1.7, 4.9]) more frequent and long intra-flock call rates 2 times (95% CI [1.2, 3.3]) more frequent in autumn than in winter.

Table 2. Results from a repeated measures mixed model analysis, with \ln (short intraspecific call rate + 1) as the dependent; season, mixed-species flock size, observation length and intra-specific flock size as fixed variables and flock number as the subject of repeated measures.

Estimates of Fixed Effects							
Parameter	Estimate	Std. Error	df	t	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Intercept	-,530521	,368771	52,707	-1,439	,156	-1,270278	,209236
Season = autumn	1,048555	,266685	52,965	3,932	,000	,513645	1,583465
Mixed-species flock size	,040617	,011600	52,812	3,502	,001	,017349	,063885
Observation length	,016658	,002850	22,646	5,845	,000	,010757	,022560
Intra-specific flock size	,379421	,108006	51,604	3,513	,001	,162652	,596189

Dependent Variable: \ln (short intra-flock call rate + 1)

Table 3. Results from a repeated measures mixed model analysis, with \ln (long intraspecific call rate + 1) as the dependent; season, mixed-species flock size, observation length and intra-specific flock size as fixed variables and flock number as the subject of repeated measures.

Estimates of Fixed Effects							
Parameter	Estimate	Std. Error	df	t	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Intercept	-,202442	,362143	52,684	-,559	,579	-,928910	,524026
Season = autumn	,673826	,262608	52,945	2,566	,013	,147089	1,200563
Mixed-species flock size	,051411	,011394	52,687	4,512	,000	,028555	,074267
Observation length	,016712	,002890	22,612	5,782	,000	,010727	,022697
Intra-specific flock size	,464507	,105797	51,297	4,391	,000	,252141	,676873

Dependent Variable: \ln (long intra-flock call rate + 1)

After controlling for observation length, territorial call rate was affected by mixed-species flock size. I found no effect of season or intra-specific flock size on territorial call rate (Table 4). Territorial call rate increased with increasing mixed-species flock size. Back-transformed model parameters suggest that territorial call rate increases 3.4% (95% CI [0.6%, 6.2%]) for an increment of one hetero-specific individual. (Figure 2 d).

Table 4. Results from a repeated measures mixed model analysis, with $\ln(\text{territorial call rate} + 1)$ as the dependent; season, mixed-species flock size, observation length and intra-specific flock size as fixed variables and flock number as the subject of repeated measures.

Estimates of Fixed Effects							
Parameter	Estimate	Std. Error	df	t	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Intercept	,221993	,425664	53	,522	,604	-,631781	1,075766
Season = Autumn	-,241990	,305245	53	-,793	,431	-,854234	,370255
Mixed-species flock size	,033178	,013349	53	2,485	,016	,006404	,059952
Observation length	,002170	,002816	53	,770	,444	-,003479	,007818
Intra-specific flock size	,089709	,125711	53	,714	,479	-,162434	,341853

Dependent Variable: $\ln(\text{territorial call rate} + 1)$

I found no effect of season on territorial calling intensity but it was tentatively affected by mixed-species flock size and intra-specific flock size, (Table 5). Back-transformed model variables suggest that territorial calling intensity increases 1.3% (95% CI [-0.1%, 2.7%]) for an increment of one hetero-specific individual to the mixed-species flock. Similarly, the model results suggest that an increase of one crested tit to the flock increases territorial calling intensity 12.4% (95% CI [-0.6%, 27.0%]).

Table 5. Results from a repeated measures mixed model analysis, with $\ln(\text{territorial calling intensity})$ as the dependent; season, mixed-species flock size and intra-specific flock size as fixed variables and flock number as the subject of repeated measures.

Estimates of Fixed Effects							
Parameter	Estimate	Std. Error	df	t	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Intercept	2,478834	,189637	34,000	13,071	,000	2,093445	2,864222
Season = Autumn	-,163373	,141643	34,000	-1,153	,257	-,451226	,124480
Mixed-species flock size	,013111	,006915	34,000	1,896	,066	-,000943	,027165
Intra-specific flock size	,116612	,060135	34,000	1,939	,061	-,005597	,238821

Dependent Variable: $\ln(\text{territorial call intensity})$

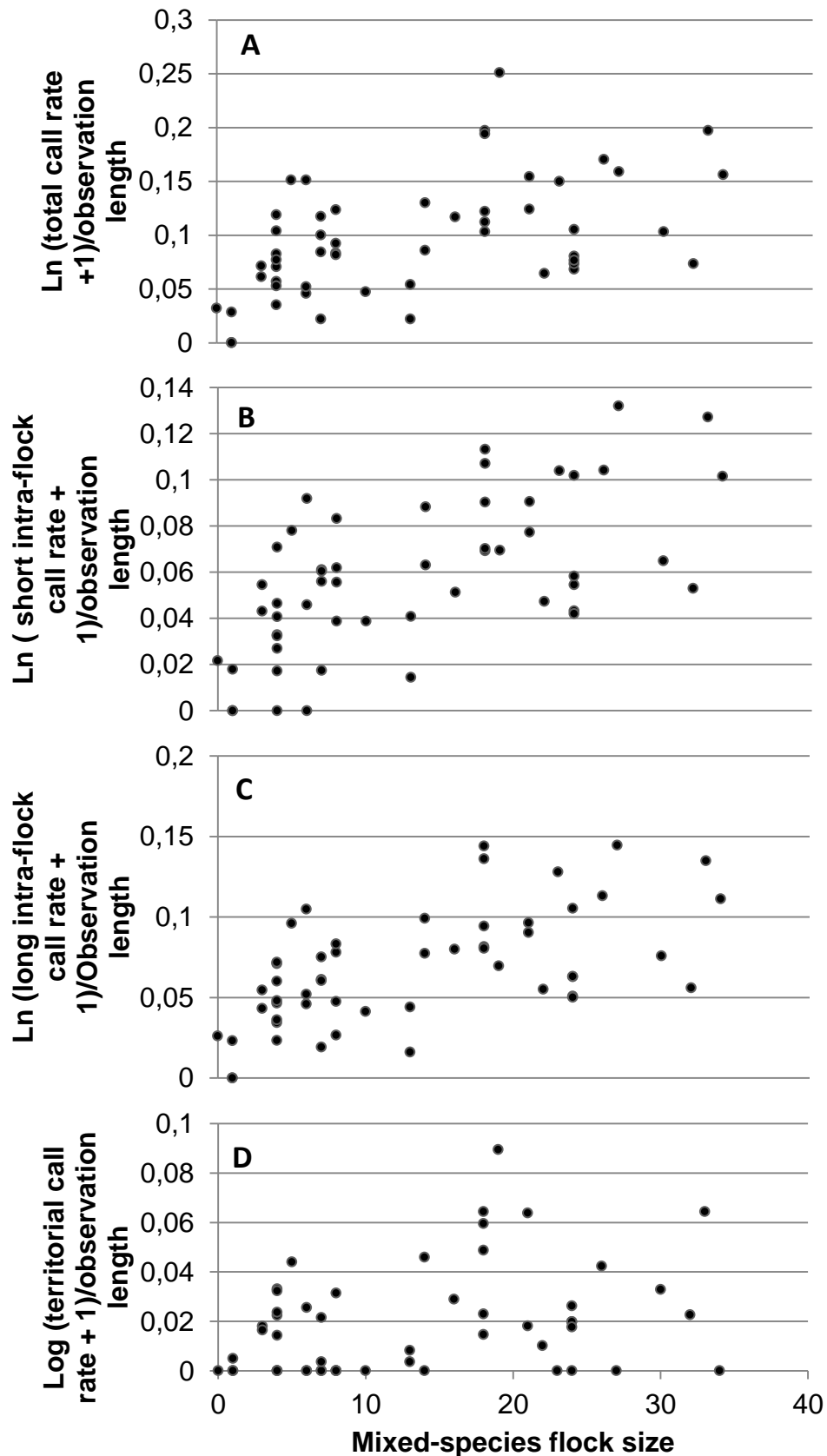


Figure 2. The relationship between mixed-species flock size and average ln-transformed values (calls per minute, or instances of calling per minute in the case of territorial call rate) of (A) total call rate, (B) short intra-flock call rate, (C) long intra-flock call rate and (D) territorial call rate. Call rates are divided by observation length here only for visual representation, i.e. to clarify the relationship between call rates and flock size after controlling for the observation length.

4. DISCUSSION

In this study, total call rate, both classifications of intra-flock contact call rates and territorial call rate were, as predicted, all positively associated with mixed-species flock size. This provides clear evidence that mixed-species flocking has a significant effect on the calling behavior of the crested tit. This is also the first time that mixed-species flock size is shown to be associated with the frequency of contact and territorial calls.

4.1. Total call rate

Krams (2000) showed that all individuals of a crested tit flock do not contribute equally to long distance (trill) calling but dominant individuals call more than subdominant individuals. Despite individual differences in calling rates, the assumption that intra-specific flock size affects calling frequencies over the whole flock is rather obvious, as one may expect more calls per flock even if individual communication rates remain constant when flock size changes. For example, if the flock size increases from 2 to 3 individuals (in this study, average intraspecific flock size was 2.2 individuals) one would expect approximately 1.5 times higher call rate due to additional individuals only. I observed that one additional crested tit in the flock was associated with on average 1.8 times more calls (95% CI 1.3 – 2.6) which roughly correspond with the expected increase. Thus, larger intraspecific flock size was associated with higher total call rate of the whole flock but not necessarily with higher call rate of an individual crested tit.

The situation is simpler when related to mixed-species flock size, where differences in total call rates of a species must be a direct consequence of increases in individual communication rates. In this study, the total call rate was positively related to mixed-species flock size. The increment of one hetero-specific individual to a mixed-species flock increased total call rate of the crested tit flock by 7.1%, meaning that average individual crested tit calling rates also increased the same amount. I predicted that both contact call rates and territorial call rates would be positively dependent on mixed-species flock size. As the total call rate is the sum of contact and territorial calling rates (other cases of trill call use were removed before analysis), this is in accordance with the study hypotheses. Contact call rates and territorial call rates were both positively dependent on inter-specific flock size. Therefore, the increase in total call rate is due to the combined increases in territorial and contact calling rates.

4.2. Contact calls

The strong relationship between flock size and intra-flock contact call rate, whether defined as call rates of less than either 6 or 4 calls per minute, provides evidence that intra-flock communication rates of crested tits are higher in larger mixed-species flocks, supporting my hypothesis that contact call rates are positively dependent on mixed-species flock size. Using the more strict classification (containing less data but with less risk of containing territorial calls) of short intra-flock call rate, the rate of contact calls increased 4.2% with an addition of one hetero-specific individual to the mixed-species flock. The less strict (more data with a larger risk of including some territorial calls) long intra-flock call rate provided a similar increase, 5.3% for an increment of one hetero-specific to the flock. The small differences between the two contact call variables indicate that they do not significantly differ in the type of communication they represent. Probably they are both composed almost completely of intra-flock contact calls, and either of them is suitable for the task of studying intra-flock communication in the crested tit.

Several hypotheses suggest that mixed-species flock size could influence the need for intra-flock communication, i.e. contact call rates. Firstly, Hutto (1988) demonstrated that

different members of mixed-species flocks have different foraging movement patterns and rates of progression, forcing some members of the flock to make adjustments to movement rates, or be forced to leave the mixed-species flock. An increase in movement rates could force an increase in contact call rates, or conspecifics risk being separated from each other. Secondly, Krams (2000) found that subordinate crested tits gave contact calls more frequently, when social cohesion decreased. If intra-specific flock cohesion is not maintained within the more widely dispersed mixed-species flock, mixed-species flock size could be directly related to flock contact call rates. Thirdly, Suzuki (2011) showed that willow tit contact calls facilitate the formation of mixed-species flocks. If crested tits function as “flock leaders” (Sridhar 2009), and actively try to manipulate mixed-species flock movements and cohesion, a larger flock could result in higher calling rates. Finally, the predation risk that follows from conspicuous calls could cause the costs of making contact calls to be higher in smaller flock sizes. (Klump & Shalter 1984, Leech & Leonard 1997, Krams 2001, Hale 2004, Krama et al. 2007). Determining the underlying reason for the relationship between contact call rates and mixed-species flock size is beyond the scope of this study. Any further attempt to investigate the causality between mixed-species flock size and contact call rates must make measurements of flock movement speed and intra-specific flock cohesion. Separating the possible effects of predation risk and inter-specific communication (manipulation of mixed-species flock movement and cohesion) on contact call rates is more complicated, but may be solved by manipulating perceived predation risk. Also, playback experiments using contact calls can be used to determine if contact calls affect mixed-species flock cohesion and movement, and if more frequent calling is necessary in larger mixed-species flocks.

Contact call rates were also affected by intra-specific flock size. However, the estimates for the effect of an increment of one individual to the intra-specific flock size were only 46.1% for short contact call rate and 59.2% for long contact call rate. Even if using the larger estimate of the long contact call rate, and accepting the perhaps unrealistic assumption of an exponential relationship between the back-transformed call rate and intra-specific flock size (which would tend to overestimate calling rates with large intra-specific flock sizes), the model estimates that the maximum intra-specific flock size of 4 individuals recorded in this study has 4.0 times the contact calling frequency of a solitary individual, exactly the amount expected if individual contact call rates are constant regarding intra-specific flock size. Therefore, one cannot conclude that an increase in intra-specific flock size was associated with an increase in the contact call rate of an individual.

Both short and long intra-flock contact call rates were affected by season. The model estimated that crested tits called short intra-flock contact calls 2.8 times and long intra-flock contact calls 2 times more often during autumn when compared to winter, when observation length and intra- and mixed-species flock sizes were held constant at average values. The result that season affects contact calling is somewhat unexpected, as there is no obvious reason why contact calling specifically might be more pronounced during autumn. Nonetheless, the results imply that crested tits have less need, or perhaps increased costs, for contact calls during winter. There are many possible explanations why this could be the case. For example, increased energy demands following from decreased food supply, colder temperatures and decreased day length during winter could make the energetic costs of vocalizations more significant. Also, during winter flock members can be expected to be more familiar with territory food supplies, which in addition to the hoarding behavior of crested tits during autumn (Haftorn 1954) may result in decreased flock movement speeds during winter, which in turn could decrease call rates. Crested tits could also have become so accustomed to movement patterns of other flock members that cohesion can be maintained with less calling.

4.3. Territorial calls

In this study, the amount of crested tits in a flock varied and the effect of intra-specific flock size could be estimated for the different call variables. Intra-specific flock size increased total call rate and both of the intra-flock communication rates and suggested an increase in territorial calling intensity, but I found no effect on territorial call rate. Furthermore, the suggestive increase in territorial calling intensity was much smaller than the increase in contact call rates (12.4% increase per additional crested tit for territorial calling intensity versus 46.1% for short and 59.2% for long intra-flock call rate). This supports Krams' (2000) suggestion that the addition of subdominant individuals does not add significantly to the territorial call rate of the flock. Krams (2000) found that in crested tit flocks consisting of 4 individuals the dominant male made the most trill calls, their mates (the dominant female) second most and the subdominant males and females called the least. Furthermore, he discovered that the dominant male and his mate made close to 1.5 times more trill calls when closer to territory borders, while the calling rates of subdominant individuals remained the same irrespective of location. This implies that the calling behavior of subdominant individuals is mostly directed towards members of the same flock, and territorial calling is mostly done by the dominant male and, to a lesser extent, his mate. My results, that the total call rate and both classes of contact call rates were strongly dependent on intra-specific flock size while the territorial call rate was independent, and territorial calling intensity was only weakly (suggestively) dependent on intra-specific flock size, suggest that the call classification in this study was successful in separating territorial calling from intra-flock contact calls.

The results of this study suggest that crested tits modify their territorial call rates according to mixed-species flock size. Back-transformed model parameters suggested that territorial call rate (the frequency of minutes of territorial calling) increased 3.4% with an increment of one hetero-specific flock member. Although less strong than the effect on contact calls, the large range of mixed-species flock sizes (0-34 individuals, see Fig 3) in this study still makes the relationship highly biologically significant.

Conspicuous calling is known to attract acoustically oriented predators (Klump & Shalter 1984, Leech & Leonard 1997, Hale 2004). For example, Krams (2001) showed that life-like stuffed crested tit models were attacked significantly more often when associated with conspicuous contact calls, than control individuals or models associated with short range contact calls, convincingly proving that the use of trill calls increases predation risk. Krama et al. (2007) further showed that crested tits used less trill calls when visiting exposed feeders, when compared to feeders in safe sites, suggesting that perceived predation risk affects crested tit trill call use.

Many of the hypotheses for increased predator protection in flocks have also received support (reviewed in Sridhar et al. 2009), which leads to the assumption that predation risk in mixed-species flocks is generally inversely related to flock size. This is especially likely to be the case with the crested tit, as the close similarities in foraging niche that exists between tit species (Alatalo 1982b) is likely to increase competition rather than foraging efficiency, making predator protection the most likely benefit from flocking with hetero-specifics. However, foraging efficiency could also increase in mixed-species flocks through the decreased predation risk, if decreased vigilance times allow more time to be allocated for foraging.

Territorial call rate (frequency of minutes of territorial calling) was not affected by the variation in the number of conspecific flock members but increased significantly with the number of hetero-specifics (mixed-species flock size). I found only a suggestive weak increase between territorial calling intensity (average amount of calls during territorial calling minutes) and both the amount of conspecifics and hetero-specifics. This indicates

that territorial calling was mostly done by the dominant crested tits in the flock, and that they adjusted their call rate according to mixed-species flock size by increasing the frequency of periods of territorial calling in larger mixed-species flocks. I suggest that this adjustment is due to a decreased perceived predation risk in larger flocks.

4.4. Assumptions, potential sources of error and challenges for future studies

Observational studies are generally hampered by substantial random variation, which leads to problems in defining the precise nature of the relationship between the original calling frequencies and model factors and necessitates large data sets. Unfortunately, a controlled experiment, for example in an aviary, where random variation could be limited by controlling many of the factors that influence calling behavior, can also lead to unhelpful changes in calling behavior by influencing territorial behavior and limiting movement, so that the experiment does not reflect natural conditions. Therefore, future studies will either have to rely on large-scale observation studies, which include measurements for variables such as flock cohesion and movement speed, or else be limited to studying calling behavior that is not strongly affected by experimental conditions.

The assumption of an exponential relationship between the model factors and dependents, that must necessarily be made when log-transforming the dependent variable for statistical reasons, is not probably valid for the relationship between flock sizes (mixed species and intra-specific) and calling rates. If the relationship between untransformed dependents and factors is linear, the estimates derived from the models will overestimate effect sizes at high dependent values, and underestimate effect sizes at small values. In this study, the log-transformations were made for the dependents because dependent variation increased with larger factor values. The relationship between the untransformed call rates and mixed-species flock size seemed to be linear. Therefore, call rates probably increase closer to a direct proportion with mixed-species flock size, rather than a percentage increase. Adding to the uncertainty are the considerably wide confidence intervals for all the model parameter estimates. Therefore the back-transformed estimates derived from the model parameters must be used with caution when estimating the strength of the relationship between model factors and dependents.

The classification of calls into contact and territorial calling used in this study was subjective and has not been verified to be correct. Therefore, territorial calling rate, for example, could contain intra-flock contact calls and result in the observed increase in the call rate for reasons related to within flock communication. However, territorial trill calling rate was not related to the number of conspecifics and territorial calling intensity was only suggestively and weakly related to the number of conspecifics. In contrast, contact call rates were strongly related to the amount of conspecifics in the flock. This suggests that my classification has succeeded in separating contact calls from territorial calls. However, it is obvious that further studies would benefit from better knowledge of calling behavior, so that the ecological context of calling can be accurately identified. Despite this, the fact that mixed-species flock sizes ranged widely from 0 to 34 hetero-specifics in the study, the estimates derived from the model parameters suggest a biologically highly significant effect of mixed-species flocking on both intra-flock and territorial communication behavior, and consequently, trill calling behavior generally.

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