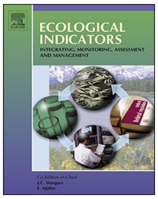
Ecological Indicators 103 (2019) 202–211

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind



Original Articles

Using acoustic indices to estimate wolf pack size

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ARTICLE INFO

Keywords:
Acoustic index
Bias
Conservation management
Pack size
Wolf howl

ABSTRACT

Acoustic indices were recently developed for biodiversity evaluation by measuring the acoustic heterogeneity generated by animals in natural environments. Some of these indices focus on the species diversity in a community by studying frequency and temporal variations in acoustic signals. We explored the possibility of using acoustic indices to estimate the population size of a specific species. More precisely, the objective was to estimate the size of grey wolf packs by testing six acoustic indices: *H*, *Hf*, *Ht*, *M*, *AR*, and *ACI*.

The relationship between the averaged values of the indices and the number of howling wolves was studied based on artificial solos and choruses created from howls extracted from wolf choruses recorded in captivity. Then, 16 real choruses were used to test the size predictions based on index values calculated previously and considered as references. Finally, we explored three biases that might influence the acoustic index values and thus the chorus size estimates.

All of the acoustic indices were positively correlated with chorus size, although large standard deviations were observed. Moreover, *H*, *Hf*, and *Ht* reached a plateau at 7–8 wolves. The size predictions based on real choruses were overestimated or underestimated. However, *ACI* was the most accurate with chorus size predictions close to the actual value. *M* and *AR* also had good predictive power, especially for choruses made by a relatively small number of howling wolves. The overestimates may be explained by several sources of bias related to the natural composition of real choruses. Indeed, the acoustic indices were influenced by the audio file duration, signal-to-noise ratio (SNR), and temporal overlap of the wolf howls, but not in the same manner for each index. In particular, *H*, *Ht*, and *M* were significantly influenced by the audio file duration and their values decreased as the duration increased. Excluding *AR*, all of the indices were affected by adding background noise. The *H* and *Hf* values decreased as the SNR decreased, but the opposite trend occurred for *ACI*. Only *Hf* and *AR* were not influenced by the temporal overlap of howls and the values of the four other indices decreased to a greater extent when more wolf howls overlapped.

The most promising indices were *ACI*, *AR*, and *Hf*, and they may provide an innovative census tool for estimating wolf pack size. Our results are encouraging although further research is needed to obtain a more effective and accurate tool. Several recommendations and directions for further studies are discussed.

1. Introduction

During the two last centuries, the extermination policies against the grey wolf (*Canis lupus*) led to the extinction of its populations throughout Europe and North America (Boitani, 2003). However, in Europe, it is now legally protected by the Bern Convention (1979) and the Habitats Directive (1992). Consequently, in recent decades, this elusive species has naturally recolonized its former areas (Fabbri et al., 2007; Valière et al., 2003). Indeed, this species comes into conflict with humans where its range overlaps with areas of human settlement and agriculture, mainly due to the predation of livestock (Mech, 2017; Rigg et al., 2011). In this context, understanding and monitoring the expansion of the grey wolf is important for preventing or mitigating intense conflicts. Documenting and updating the size of wolf packs is of great importance for the conservation and management of this protected species. However, wolf monitoring remains challenging in the field because it is a wide-ranging generalist species that lives at a low density and it is often secretive and elusive (Latham et al., 2014; Louvrier et al., 2017).

Howls are long-range vocal signals that are regularly used by wolves

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https://doi.org/10.1016/j.ecolind.2019.03.010
Received 2 October 2018; Received in revised form 29 January 2019; Accepted 8 March 2019

in solos or choruses for long distance communication (Harrington and Asa, 2003; Harrington and Mech, 1983, 1978; Joslin, 1967). This type of vocalization has been studied to explore different topics, such as identifying individual and pack vocal signatures (Harrington, 1989; Palacios et al., 2007; Passilongo et al., 2012, 2010; Root-Gutteridge et al., 2014; Tooze et al., 1990; Zaccaroni et al., 2012), the chorus structure (Harrington and Mech, 1982; Harrington, 1975; Theberge and Falls, 1967), the detection of individuals (Ausband et al., 2011; Bassi et al., 2015; Brennan et al., 2013; Duchamp et al., 2012; Fuller and Sampson, 1988; Llaneza et al., 2005; Suter et al., 2016) or reproduction events (Harrington, 1986; Llaneza et al., 2014; Longis et al., 2004; Nowak et al., 2008, 2007; Palacios et al., 2016; Sèbe et al., 2006), as well as for acoustic localization (Papin et al., 2018). In addition, different methods have been used to estimate wolf chorus size based on howls, including discriminating the fundamental and harmonic frequencies of howling wolves (Filibeck et al., 1982; Sèbe et al., 2004), image processing techniques based on spectrograms (Dugnol et al., 2008, 2007a, 2007b), and visual inspections of spectrograms obtained from chorus recordings (Passilongo et al., 2015).

These previously developed approaches are useful for estimating wolf chorus size based on howls but most of them are time consuming or subjective, and they include potential sources of errors. According to Passilongo et al. (2015), it is possible to count up to six wolves based on visual inspections of the spectrograms obtained from chorus recordings, but this method could include sources of error, especially when the howls made by many wolves are superimposed. Field experiments have also demonstrated that counting wolves directly by ear during simulated howling surveys could be difficult and limited to three wolves (Harrington, 1989; Harrington and Mech, 1982). Recently, Palacios et al. (2017) showed that estimating the number of wolves in choruses by the unaided human ear is inaccurate, regardless of the experience of the listener. Given the results of these previous studies, the development of a new method, based on the use of acoustic indices, may provide an alternative approach to overcome these inaccuracies, thereby providing a new tool for estimating wolf pack size.

Recently, the rapid acoustic survey (RAS) approach has been developed to quantify the global acoustic variability in the sounds produced by animal communities (Sueur et al., 2008a). Among the RAS tools, acoustic indices have been produced for rapid evaluations of biodiversity (Obrist et al., 2010; Sueur et al., 2008a). After the first tests performed on artificial animal choruses, this approach was successfully tested *in situ* (see Sueur et al., 2014 for a review). In particular, several studies found correlations between the acoustic heterogeneity of a short ambient sound recording (ranging from a few seconds to minutes) and the species richness (Depraetere et al., 2012; Sueur et al., 2008a; Towsey et al., 2014) or song types (Pieretti et al., 2011). Acoustic indices have many advantages such as permitting rapid analyses of large amounts of acoustic data recorded over large areas and long time periods using standardized or automatic processes without identifying species or individuals (Gasc et al., 2015).

Until now, acoustic indices have mainly been applied to quantifying the diversity of multispecies assemblages. However, they could also be applied in the same manner to monospecies assemblages (i.e. several individuals from the same species), e.g. as a proxy for the abundance based on a chorus made by a species. In particular, the grey wolf is a good candidate for testing this type of approach with monospecies assemblages. Indeed, howling wolves exhibit complex vocal signatures (Harrington, 1989; Palacios et al., 2007; Passilongo et al., 2012; Root-Gutteridge et al., 2014; Tooze et al., 1990), so this individuality could induce acoustic variability in choruses allowing to quantify the number of howling wolves using acoustic indices. Furthermore, this kind of approach is interesting because estimating wolf pack size is challenging.

In the present study, we hypothesized that quantifying the global acoustic variability in wolf choruses could be used for estimating chorus size. We examined the relationship between measures of acoustic variability and the number of howling individuals in choruses expecting that individuality would induce a correlation between acoustic index values and the number of howling wolves in choruses. Therefore, we tested six acoustic indices for estimating wolf chorus size. First, we studied the relationship between the averaged values of the indices and the number of howling wolves based on artificial solos and choruses created from wolf howls recorded in captivity. Next, we used a generalized linear model fitted to previously calculated index values for artificial solos and choruses in order to predict the size of 16 real choruses. Finally, we explored three biases that might influence the acoustic index values and thus the chorus size estimates.

2. Materials and methods

2.1. Acoustic recordings

The howls made by 12 adult grey wolves from three packs living in captivity were recorded from February to May 2014. Ten wolves (six females and four males) from two different packs were recorded in the Parc Animalier de Sainte Croix (Moselle, France) and two wolves (two females) from a pack living in the Zoo of Pescheray (Sarthe, France).

The recordings were acquired close to the packs (15–25 m) during optimal meteorological conditions (without rainfall or wind) in order to optimize the quality of the sounds recorded. The recordings were made with a digital recorder (Zoom H4n Handheld Audio Recorder; Zoom Corporation, Tokyo, Japan) at a sampling rate of 44.1 kHz with a 16-bit accuracy and a Rode NTG-3 directional microphone (super-cardioid, frequency response: 40 Hz to 20 kHz; Rode Microphones Company, Sydney, Australia; see Supplementary material A). In addition, wolves making solos and choruses were filmed with a camcorder (model: JVC HD Everio GZ-HD3; JVC Kenwood Corporation, Yokohama, Japan). The audio recordings and videos were synchronized in order to assign each howl to an individual. Finally, the howls in ".wav" format assigned to each wolf were extracted using Sony SpectraLayers Pro™ software (Sony Creative Software, 2013; version 2.0).

2.2. Creation of the data set of artificial solos and choruses

Artificial solos and choruses were created in order to obtain samples that included a known number of howling wolves, only howl type vocalizations, and no background noise. The recorded and extracted howls (see Section 2.1) were employed to create howling sequences with a duration of 30 s using Audacity software (Audacity Team, 2014; version 2.0.6).

First, a set of artificial solos was created for each of the 12 wolves recorded. Artificial solos consisted in a combination of several extracted howls made by single individual. This set of artificial solos was then used to create 10 series comprising one solo and 11 choruses from two to 12 individuals. Each series was prepared according to two consecutive random selection steps: selecting a solo for each individual and then selecting the order of the individual solos in the choruses. From one series to the next series, the previously selected solos were removed from the sample (random sampling without replacement). Finally, a total of 10 artificial solos (10*1 wolf) and 110 artificial choruses (10*2, 10*3, 10*4, …, 10*12 wolves: 11 chorus size from 2 to 12 wolves) were obtained (see Supplementary material B).

2.3. Acoustic indices

The use of six acoustic indices was explored to estimate the number of howling wolves in choruses: the spectral entropy *Hf* (Sueur et al., 2008a), the temporal entropy *Ht* (Sueur et al., 2008a), the acoustic entropy *H* (Sueur et al., 2008a), the median of the amplitude envelope *M* (Depraetere et al., 2012), the acoustic richness *AR* (Depraetere et al., 2012), and the acoustic complexity index *ACI* (Pieretti et al., 2011).

The spectral entropy *Hf* (spectral index) was obtained from a mean and normalized spectrum, which was the average of a short-term Fourier transform (STFT) with a non-overlapping Hanning window of 512 samples (Sueur et al., 2008a). The mean spectrum was scaled by its maximum to obtain index values between 0 and 1 (Gasc et al., 2015). The two temporal indices comprising the temporal entropy *Ht* (Sueur et al., 2008a) and median of the amplitude envelope *M* (Depraetere et al., 2012) were computed based on the Hilbert amplitude envelope, which was scaled by its maximum to obtain index values between 0 and 1. The acoustic entropy *H* is the product of *Hf* and *Ht* (Sueur et al., 2008a), and the acoustic richness *AR* is based on the two temporal indices, i.e. *Ht* and *M* (Depraetere et al., 2012). The last acoustic index used in this study was the acoustic complexity index *ACI* (Pieretti et al., 2011), which was computed based on a non-scaled STFT with a non-overlapping Hanning window of 512 samples. *ACI* can be calculated over the total duration of the audio file or based on multiple parts of the file with the same duration, where the sum of the *ACI* values gives the final value for this index. The number of cutouts was adapted to the total duration of the audio files considered in order to obtain windows with a duration of 0.128 s. The final *ACI* was then scaled by its maximum to also obtain index values between 0 and 1 (Gasc et al., 2015).

The six acoustic indices comprising *Hf*, *Ht*, *H*, *M*, *AR*, and *ACI* were calculated for the 10 artificial solos and 110 artificial choruses (see Section 2.2) using the Seewave package (Sueur et al., 2008b) with R software (R Development Core Team, 2014; version 3.1.2). The values were averaged based on the number of individuals. For the following investigations (see Section 2.4), these averaged values were considered as references values (Treatment T0, T: treatment).

2.4. *Effects of three biases on the reference values for the acoustic indices*

Different sources of bias may be encountered when calculating acoustic indices based on field recordings (Gasc et al., 2015; Zhang et al., 2016) and they could influence the chorus size estimates. Thus, in this study, we explored the effects of three biases by using reference values for the acoustic indices obtained from artificial solos and choruses with durations of 30 s.

2.4.1. *Bias due to the audio file duration*

Both the chorus and audio file durations could vary in the recordings, which might influence the acoustic index values (Gasc et al., 2015) and thus the chorus size estimates. Two treatments were applied to the 10 artificial solos and 110 artificial choruses in order to explore the effect of the sound file duration on the reference values for the acoustic indices (see Supplementary material C).

The 10 artificial solos and 110 artificial choruses (described in Section 2.2) were used as a control data set (T0, 120 audio files of 30 s). The first treatment involved adding 30 s of silence to each T0 audio file in order to increase the total duration of the file to 60 s (T1, 120 audio files of 60 s). The second treatment involved adding 60 s of silence to each T0 audio file in order to increase the total duration of the file to 90 s (T2, 120 audio files of 90 s). The six acoustic indices were calculated for these 360 audio files.

2.4.2. *Bias due to the signal-to-noise ratio (SNR)*

Field recordings are often characterized by a low SNR because of the distance separating the target sound source from the recording equipment, the background noise level, and the intensity of the signal investigated (Araya-Salas et al., 2017; Wiley and Richards, 1982, 1978). The effect of the SNR on reference values for the acoustic indices was tested by applying three treatments to the 10 artificial solos and 110 artificial choruses (see Supplementary material D).

The 10 artificial solos and 110 artificial choruses (see Section 2.2) were used as a control data set (T0, 120 audio files without any background noise). The treatments involved mixing T0 with a sequence comprising 30 s of natural background noise recorded during 2012 in the Vosges Mountains. Three different amplitude levels of added background noise were applied to vary the SNR threshold: high SNR with the original amplitude level of the background noise (T1), medium SNR where the amplitude level was multiplied by 2.5 (T2), and low SNR where the amplitude level was multiplied by 5 (T3). The six acoustic indices were calculated based on these 480 audio files.

2.4.3. *Bias due to the temporal overlap of howls in choruses*

Variations in the temporal overlap between individual songs or vocalizations within choruses may influence the size estimates obtained with acoustic indices (Gasc et al., 2015). To test this bias, we combined artificial solos from six wolves to obtain new artificial choruses of six individuals for three different treatments (see Supplementary material E).

For each of the six wolves, 100 artificial solos with durations of 30 s (see Section 2.2) were randomly selected (with replacement) to obtain artificial choruses by six wolves (180 s) with three different time lags. The solos were alternated in the first treatment (T1, no temporal overlap). The second treatment comprised a half overlap between individual solos (T2). The third treatment comprised the maximum overlap (superposition) between the six individual solos (T3). The six acoustic indices were calculated based on these 300 new choruses with durations of 180 s.

2.5. *Statistical analysis*

2.5.1. *Relationship between the acoustic indices and numbers of howling wolves*

Spearman's rank correlation coefficients were calculated to investigate: (i) the possible relationship between the averaged values of the indices and numbers of howling wolves (artificial solos and choruses); and (ii) the relationship between the averaged values of the indices including bias (audio file duration and SNR biases; see Sections 2.4.1 and 2.4.2) and the number of howling wolves. For both investigations (with or without biases), pairwise comparisons were conducted with Mood's median test (pairwise.mood.medtest function in RVAideMemoire package; Hervé, 2017) to explore the potential differences in the index values with different wolf chorus sizes.

2.5.2. *Wolf chorus size prediction*

The wolf chorus size was predicted using the reference values for the acoustic indices obtained from artificial solos and choruses (see Section 2.3). The six acoustic indices were calculated based on sequences with a duration of 30 s selected from 16 real wolf choruses (with background noise, potentially different vocalization types, etc.), which were recorded in the Parc Animalier de Sainte Croix (see Section 2.1 and Supplementary material A; eight choruses made by three howling wolves and eight choruses made by six wolves). To predict the number of howling individuals in these real choruses, Poisson regression fitted to the reference values for the acoustic indices obtained from artificial solos and choruses (see Section 2.3) was performed using a generalized linear model (glm function in stats package). Before making predictions, the assumptions required to use a generalized linear model (i.e. homogeneity, normality and independence of the residuals) have been verified thanks to graphical analysis (qqnorm function in stats package and plotresid function in RVAideMemoire package; Hervé, 2017). The ratio of the residual deviance over residual degrees of freedom was calculated to evaluate the deviance goodness of fit (DGOF) for Poisson regressions. The model was considered as correctly fitted in the case where the ratio approached 1.

2.5.3. *Differences between treatments in the bias tests*

The following tests were applied to the data obtained from the three bias tests (see Section 2.4). In the case of homogeneity of variances, permutational analysis of the variance table was first used to determine whether significant differences existed between treatments. Next, pairwise comparisons were conducted using permutational *t*-tests to

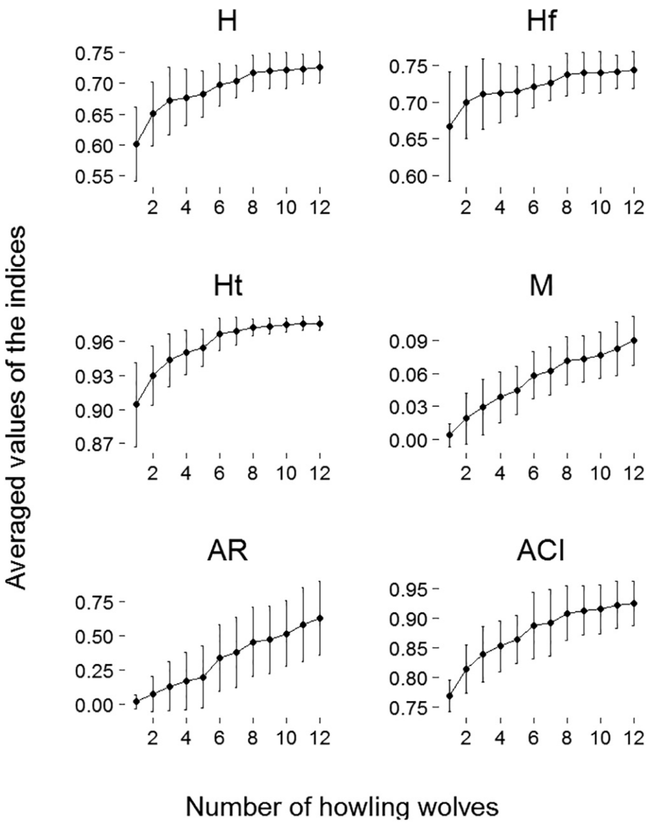


Fig. 1. Relationship between the averaged values of the indices (± SD) and the number of howling wolves in artificial solos and choruses. All indices were significantly and positively correlated with the number of howling wolves (Spearman's rank correlation coefficient, rho = 1, $P < 0.01$).

identify differences more precisely. Alternatively, a Friedman rank sum test was conducted followed by pairwise comparisons using the Wilcoxon signed rank test with the same objective.

All of the statistical analyses were conducted with R software (R Development Core Team, 2014; version 3.1.2) and type 1 error threshold was set at 5% for all statistical tests.

3. Results

3.1. Relationship between the acoustic indices and numbers of howling wolves

As expected, all of the averaged values of the indices calculated based on artificial solos and choruses had significant and positive correlation with the number of howling wolves (Spearman's rank correlation coefficient, rho = 1, $P < 0.01$; Fig. 1). However, the standard deviations associated with the averaged values of the indices were large for each chorus size. Moreover, *H*, *Hf*, and *Ht* reached a plateau at 7–8 wolves. Pairwise comparisons using Mood's median tests detected more significant differences between the chorus sizes based on the values of *Ht*, *AR*, and *ACI* ($P < 0.05$), especially between one and five wolves (Fig. 2).

3.2. Size prediction based on real choruses

The values of the six acoustic indices calculated from the 16 real choruses were subjected to Poisson regression fitted based on the reference values obtained from artificial solos and choruses (Fig. 3). The *ACI* index was the most accurate and the chorus size predictions were closest to the actual values. The five other indices overestimated the chorus size but *M* and *AR* obtained interesting predictions, especially for choruses made by a relatively small number of howling individuals (three wolves in this case).

3.3. Effects of the biases on the acoustic indices

3.3.1. Effect of the audio file duration

H, *Ht*, and *M* were significantly influenced by the addition of silence, and thus by the audio file duration (permutational analysis of variance table, $P < 0.05$; Fig. 4), whereas *Hf*, *AR*, and *ACI* did not differ significantly between treatments (permutational analysis of variance table, $P > 0.05$). In particular, the *H*, *Ht*, and *M* values decreased as the duration of the audio file increased.

Under each treatment, all of the indices remained significantly and positively correlated with the number of howling wolves (Spearman's rank correlation coefficient, $P < 0.01$; Fig. 5).

3.3.2. Effect of the SNR

All of the indices were affected by the addition of background noise (Friedman's rank sum test, $P < 0.05$), except for *AR* (permutational analysis of variance table, $P > 0.05$; Fig. 6). The *H* and *Hf* values decreased as the SNR decreased. By contrast, the *ACI* values increased as the SNR decreased.

Under each treatment, all of the indices remained significantly and positively correlated with the number of howling wolves (Spearman's rank correlation coefficient, $P < 0.01$; Fig. 7).

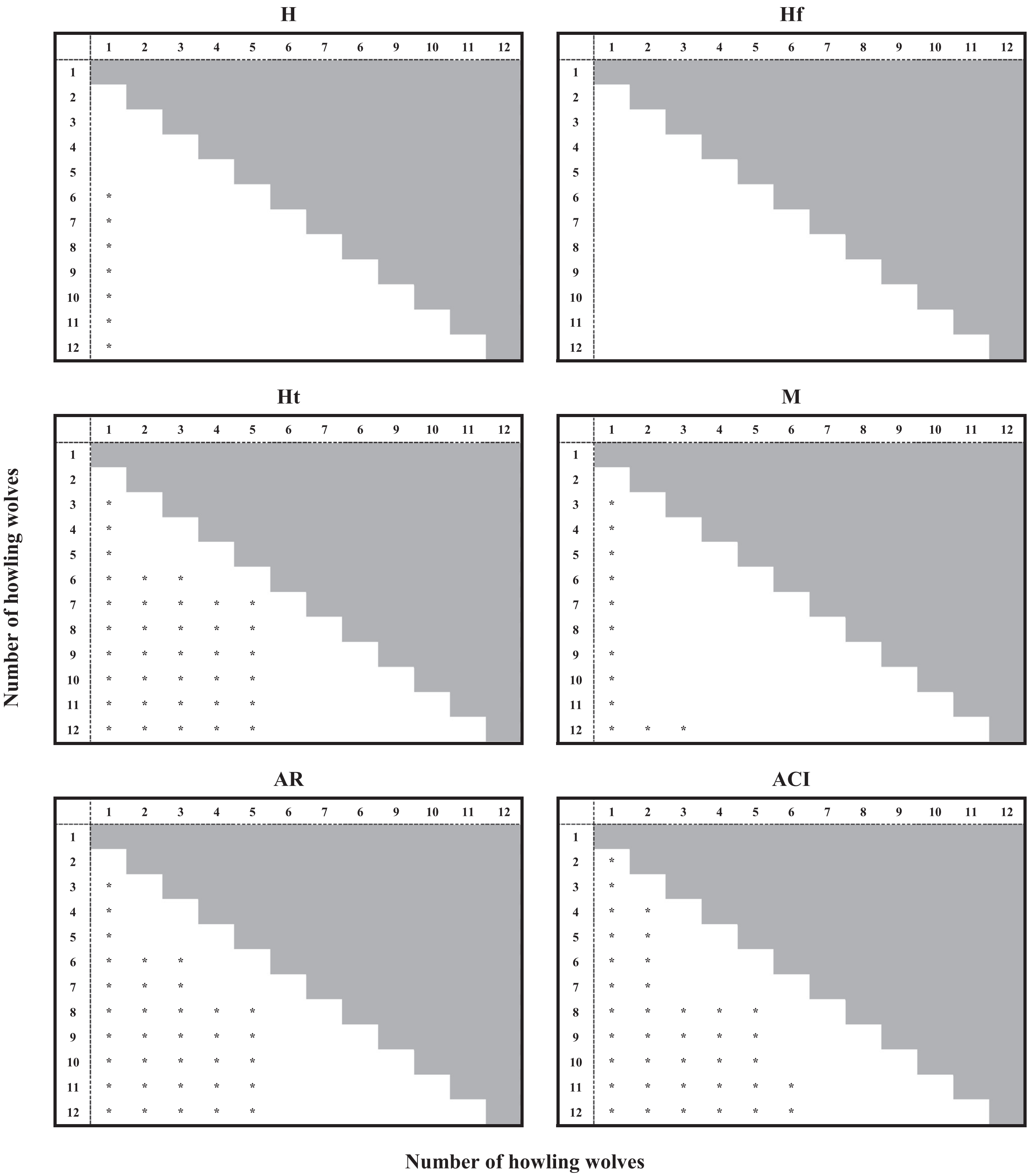
3.3.3. Effect of the temporal overlap of howls in choruses

Only *Hf* and *AR* were not influenced by the different temporal overlap of howls in choruses in the test of this bias (permutational analysis of variance table, $P > 0.05$; Fig. 8). The values of *H*, *Ht*, *M*, and *ACI* decreased when the howls of six wolves overlapped to a greater extent.

4. Discussion

All of the selected acoustic indices are potentially useful for obtaining chorus size estimates. Indeed, the averaged values of the indices calculated based on artificial solos and choruses were positively correlated with the number of howling wolves. We found that the standard deviations were large due to high intra- and inter-individual variability. The identification of a threshold above a chorus size of 7–8 wolves when using *H*, *Hf*, and *Ht* indicated a limit on the estimation of the number of howling wolves, especially in large packs. This could be problematic even though the size of European packs rarely exceeds 10 wolves (see Duchamp et al., 2017). However, *M*, *AR*, and *ACI* may be the most promising acoustic indices because they had the advantage of not reaching this threshold.

In terms of the predictions of the number of howling wolves in choruses based on the reference values of the acoustic indices calculated from artificial solos and choruses, *ACI* was the only index that yielded good predictions (the chorus size was predicted correctly or it was very close to the actual size). Indeed, *ACI* has been developed in the objective to be less sensitive to constant background noise (i.e. constant human-generated-noise such as car passing or airplane transit – Pieretti et al., 2011) compared to the other acoustic indices. In our case, *ACI* seemed not to be influenced by the constant human-generated-noise contained in the real choruses tested (see Supplementary material A). For all of the other indices, the values obtained from real choruses often corresponded to a higher number of wolves than the actual chorus size. These overestimates can be explained by the presence of background noise such as bird songs and constant human-generated-noise. In addition to the background noise, the values of the indices could be influenced by the different vocalization types (e.g. barks, squeaks, and growls; Harrington and Asa, 2003; Harrington and Mech, 1978; Joslin, 1967), and howl modulations (amplitude and frequency). Indeed, according to the Beau Geste hypothesis (Krebs, 1977), wolves can



H

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4												
5												
6	*											
7	*											
8	*											
9	*											
10	*											
11	*											
12	*											

Hf

	1	2	3	4	5	6	7	8	9	10	11	12
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Ht

	1	2	3	4	5	6	7	6	9	10	11	12
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2												
3	*											
4	*											
5	*											
6	*	*	*									
7	*	*	*	*	*							
8	*	*	*	*	*							
9	*	*	*	*	*							
10	*	*	*	*	*							
11	*	*	*	*	*							
12	*	*	*	*	*							

M

	1	2	3	4	5	6	7	8	9	10	11	12
1												
2												
3	*											
4	*											
5	*											
6	*											
7	*											
8	*											
9	*											
10	*											
11	*											
12	*	*	*									

AR

	1	2	3	4	5	6	7	6	9	10	11	12
1												
2												
3	*											
4	*											
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7	*	*	*									
8	*	*	*	*	*							
9	*	*	*	*	*							
10	*	*	*	*	*							
11	*	*	*	*	*							
12	*	*	*	*	*							

ACI

	1	2	3	4	5	6	7	8	9	10	11	12
1												
2	*											
3	*											
4	*	*										
5	*	*										
6	*	*										
7	*	*										
8	*	*	*	*	*							
9	*	*	*	*	*							
10	*	*	*	*	*							
11	*	*	*	*	*	*						
12	*	*	*	*	*	*						

Fig. 2. Summary tables of pairwise comparisons using Mood's median test between index values obtained from artificial solos and choruses. Asterisks "*" indicate significant differences between chorus sizes ($P < 0.05$).

modulate their howls to convince a neighboring pack that they are more numerous (Harrington, 1989). Therefore, modulations could be responsible for the increases in the index values, thereby leading to overestimates of the chorus size estimates based on the reference values for the acoustic indices. All of these factors combined with the other biases were sources of acoustic heterogeneity that could explain the higher index values obtained and the possibility of incorrect wolf chorus size estimates.

In our study, the reference values for each of the acoustic indices were obtained from artificial solos and choruses with a duration of 30 s, whereas a wolf chorus may last for approximately 1 min in the field (Harrington, 1989; Holt, 1998). Our investigations of how the values of the acoustic indices were influenced by the duration of the audio file showed that *Hf*, *AR*, and *ACI* were not sensitive to this bias. These results are consistent with previous research (e.g. Gasc et al., 2015), except for *ACI* because of an error in the function relative to the *ACI* values calculated during silence, which has now been corrected (see version 2.1.0 of the Seewave package). Moreover, all of the averaged

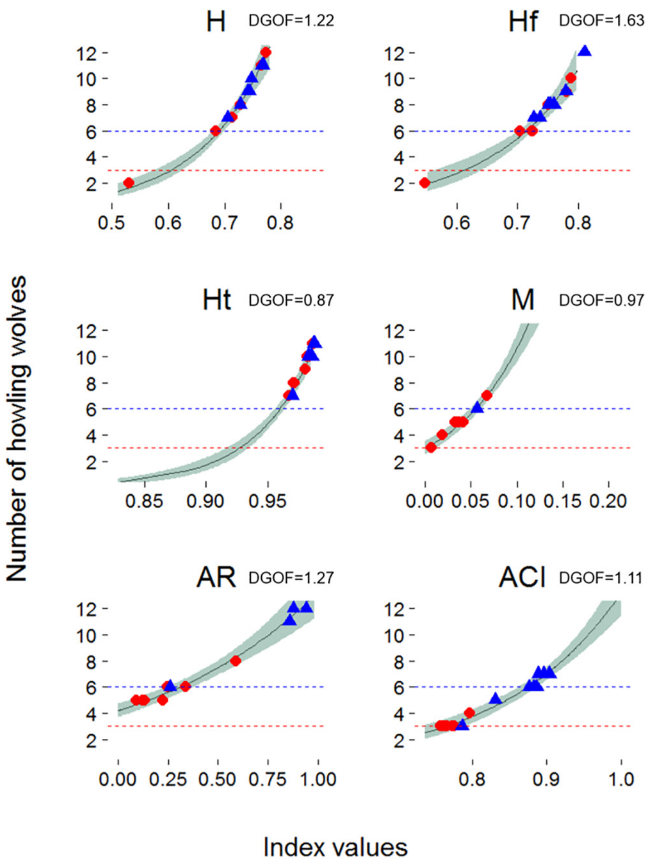

Fig. 3. Prediction of the chorus size in real choruses using Poisson regression. Black lines correspond to the Poisson regression fitted with index values obtained from the artificial solos and choruses, with the confidence interval in grey. DGOF values correspond to the deviance goodness of fit for Poisson regression. Red and blue dashed lines correspond to the number of wolves expected for the tested choruses, i.e. three and six wolves, respectively. Red circles and blue triangles correspond to the predicted number of howling wolves in the test choruses.

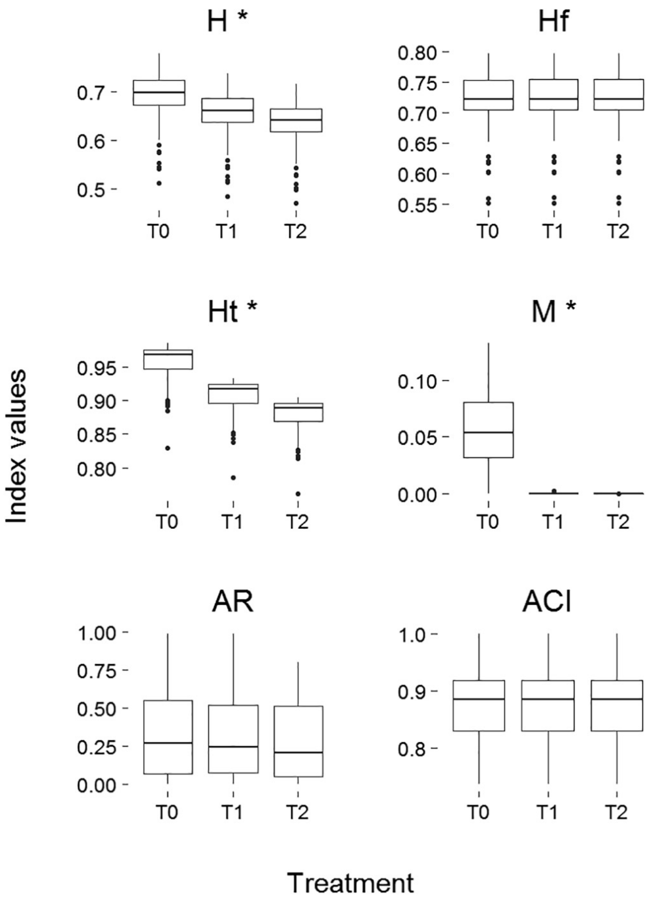

Fig. 4. Effect of the audio file duration on the distributions of the index values. T0: control situation with artificial solos and choruses with a duration of 30 s. T1: Addition of 30 s of silence to T0 audio files. T2: Addition of 60 s of silence to T0 audio files. *H*, *Ht*, and *M* (*) were significantly influenced by the addition of silence, and thus by the audio file duration (permutational analysis of variance table, $P < 0.05$).

values of the indices remained correlated with the number of howling wolves even with a longer audio file duration. Thus, if a field recording with a duration of less than 30 s containing chorus is analyzed, it would be possible to estimate the wolf chorus size by using the reference values for *Hf*, *AR*, and *ACI*. If the recording exceeds 30 s, it would be preferable to select only the 30 s interval where the acoustic diversity appears to be the most important (based on a visual inspection of the spectrogram) in order to increase the likelihood of detecting the maximum number of wolves. In future research, it would be useful to define a standardized file duration for calculating these indices, as suggested by Gasc et al. (2015).

Field recordings are inevitably affected by bias due to the presence of background noise. Thus, in order to estimate the number of wolves, it is important to determine the extent to which the reference values for the acoustic indices are affected by a decrease in the SNR. As shown by Gasc et al. (2015) for bird choruses, we found that all of the indices were influenced by the added background noise (i.e. decreased SNR), except for *AR*. Regardless of the treatment, all of the averaged values of the indices remained positively correlated with the number of howling wolves. Very narrow value ranges were determined for *M* and *Ht*, thereby demonstrating the difficulty of distinguishing choruses made by variable numbers of wolves in the presence of background noise. Thus, the reference values for *AR* could be used to estimate the number of howling wolves in real choruses containing background noise. However, in order to obtain choruses with good acoustic quality (i.e. with a high SNR), recommendations are required in terms of the recording period (e.g. selecting the optimal meteorological conditions; Wiley and Richards, 1978), acoustic recording equipment employed (microphone sensitivity, microphone type, etc.), and the optimal and maximal distance between the howling wolves and the recording equipment. Acoustic filters could also be employed to reduce the background noise. However, using filters would be difficult when the noise and wolf howls share common frequencies (e.g. Fairbrass et al., 2017).

Finally, the temporal overlap of howls in choruses affected the acoustic index values. Regardless of the treatment, only *Hf* and *AR* were not influenced by the temporal overlap of howls, which suggests that their reference values could be used to estimate the number of wolves in real choruses, irrespective of whether the howls overlap. However, this bias was only tested for choruses of six wolves, so it would be interesting to test the responses of these indices to choruses comprising more or less wolves.

Ideally, as suggested by Gasc et al., 2017 for assemblages of bird species, a chorus database should be produced based on the field recordings containing a known number of wolves and used to obtain new reference values for the acoustic indices. These values would integrate different SNRs, variable background noise compositions, and various wolf vocalization types, and thus the estimates of the number of howling wolves in real choruses could be more accurate. Additionally, a SNR measurement of the files containing a known number of wolves could be made in order to applying a correction to the estimation of howling wolves related to these SNR values.

Finally, not all pack members participate in choruses (Harrington,

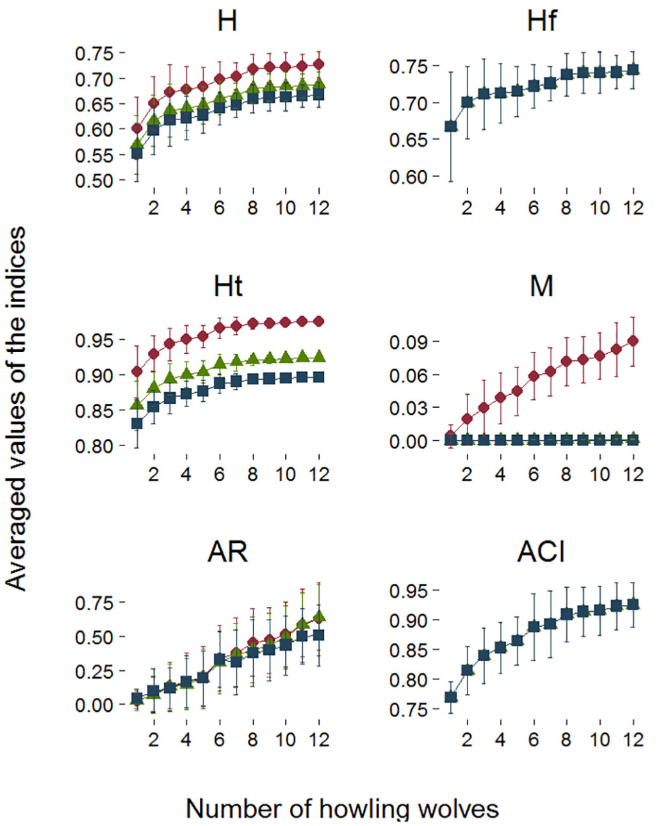

Fig. 5. Relationship between the averaged values of the indices (± SD) and the number of howling wolves in artificial solos and choruses according to different audio file durations. Red circles: control situation (T0) with artificial solos and choruses with a duration of 30 s. Green triangles: treatment T1 with 90 s audio file duration (T0 with 30 s of silence added). Blue squares: treatment T1 with 120 s audio file duration (T0 with 60 s of silence added). All indices for all treatments were significantly positively correlated with the number of howling wolves (Spearman's rank correlation coefficient, $P < 0.01$).

1987) and they could be temporarily and/or spatially separated from each other (Holt, 1998), so it is important to emphasize that the chorus size estimated using acoustic indices is not an exact estimate of the wolf pack size. In order to maximize the likelihood of recording choruses containing all pack members, summer is the best period for obtaining field recordings because of the high and frequent howling activity (Gazzola et al., 2002; Harrington and Mech, 1979) in restricted areas called "rendezvous sites" during pup rearing (Harrington and Mech, 1978).

5. Conclusion

To the best of our knowledge, this is the first study to explore the possibility of using acoustic indices for estimating wolf pack size. The most promising indices are the acoustic richness index *AR*, acoustic complexity index *ACI*, and spectral entropy index *Hf*, although they were sensitive to at least one of the three bias investigated in this study. As reported by Gasc et al. (2015), no index based on field recordings can provide a perfect estimate of the species richness, and this also applies to estimating the wolf pack size. Further investigations of acoustic indices could contribute to the development of an index or a set of indices for estimating the number of wolves in a pack and for reducing and/or correcting bias. We are convinced that a larger chorus database (real choruses with a known number of howling wolves) would provide more reliable predictions of the number of howling wolves in chorus tested. Indeed, the more the number of real choruses will be important, the more the recording conditions will be varied (e.g. distance, SNR, background noise, chorus structure, etc.) and the more the statistical model can take into account these variabilities to obtain a better fitted model. In addition, it would be possible to use a combination of acoustic indices rather than a single index (multimetric approach). Also, pack size could be estimated in relative values (e.g. class estimates) and not in absolute values.

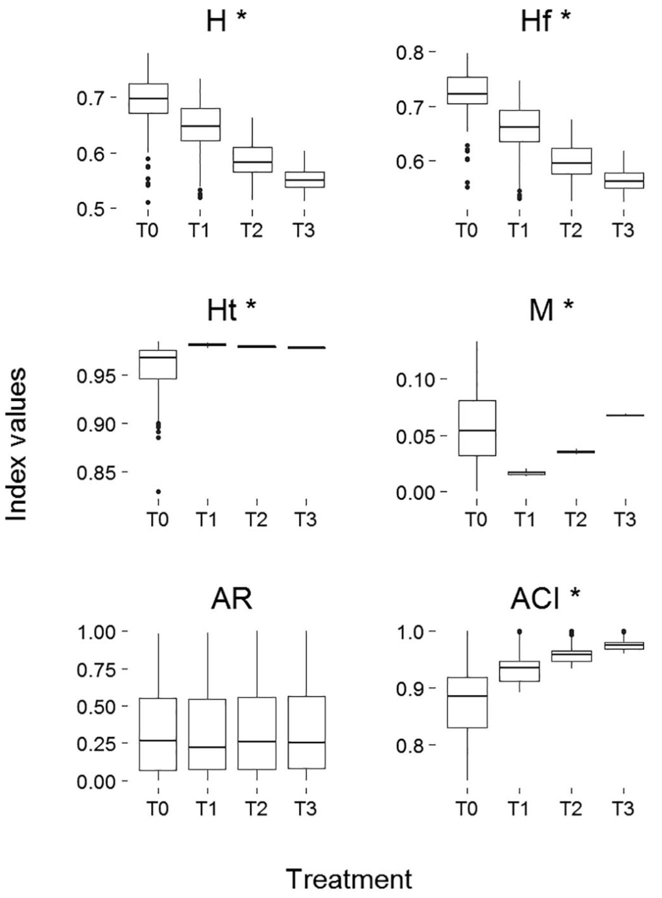

Fig. 6. Effect of the signal-to-noise ratio (SNR) on the distributions of the index values. T0: Control with artificial solos and choruses without any background noise. T1: Artificial solos and choruses mixed with background noise at the amplitude level recorded in the field (high SNR). T2: Artificial solos and choruses mixed with background noise at the amplitude level multiplied by 2.5 (medium SNR). T3: Artificial solos and choruses mixed with background noise at the amplitude level multiplied by 5 (low SNR). Indices (*) were affected by adding background noise (Friedman's rank sum test, $P < 0.05$), and thus by decreases in the SNR, except for *AR* (permutational analysis of variance table, $P > 0.05$).

Grey wolf monitoring remains a major challenge and conservation and management efforts require pack size and population size estimates that are as accurate as possible. Our findings concerning the use of acoustic indices for wolf monitoring are encouraging and promising even if further refinements are required. Future research is needed, as collaboration between bioacousticians and ecoacousticians is necessary, to develop effective tools for grey wolf monitoring that could be applied easily in the field, thereby enhancing current survey methods.

Acknowledgments

We are grateful to Dr A. Gasc and Dr J. Sueur for their advice and constructive discussions about the ACI function in the Seewave package. We would like to thank the main financial partners of this study: the European Union within the framework of the Operational Program FEDER-FSE "Lorraine et Massif des Vosges 2014-2020", the Commissariat à l'Aménagement du Massif des Vosges for the FNADT

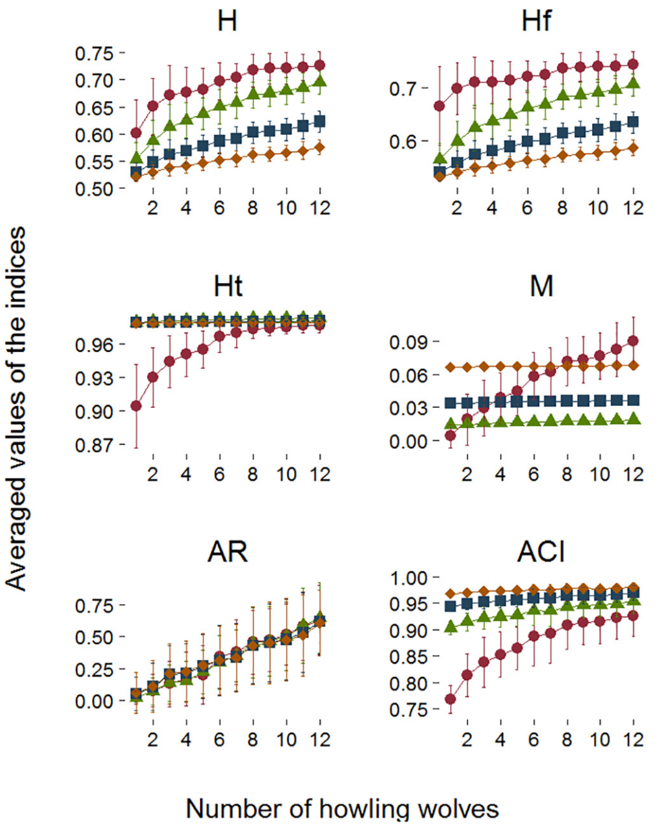

Fig. 7. Relationship between the averaged values of the indices (± SD) and the number of howling wolves in artificial solos and choruses according to different signal-to-noise ratios (SNRs). Red circles: Control (T0) with artificial solos and choruses without any background noise. Green triangles: Treatment T1 (T0 mixed with background noise with a high SNR). Blue squares: treatment T2 (T0 mixed with background noise with a medium SNR). Orange diamonds: treatment T3 (T0 mixed with background noise with a low SNR). All indices for all treatments remained significantly positively correlated with the number of howling wolves (Spearman's rank correlation coefficient, $P < 0.01$).

(Fonds National d'Aménagement et de Développement du Territoire), the DREAL Grand Est (Direction Régionale pour l'Environnement, l'Aménagement et le Logement), the Région Grand Est, the ANRT (Agence Nationale de la Recherche et de la Technologie, CIFRE award), the Zoo d'Amnéville, and the Parc Animalier de Sainte Croix. We would also like to thank the Fondation Le Pal Nature for its complementary financial support. This study would not have been conducted without authorizations and agreements from animal parks (Parc Animalier de Sainte Croix and Zoo de Pescheray) to record wolf choruses. We also thank the two anonymous reviewers for their careful reading and their perceptive comments and suggestions.

Author contributions statement

Conceived the ideas and designed methodology: MP MA EG JP. Collected the data: MP JP. Analyzed the data: MP MA JP. Led the writing of the manuscript: MP MA EG FG JP. All authors contributed critically to the drafts and gave final approval for publication.

Role of the funding source

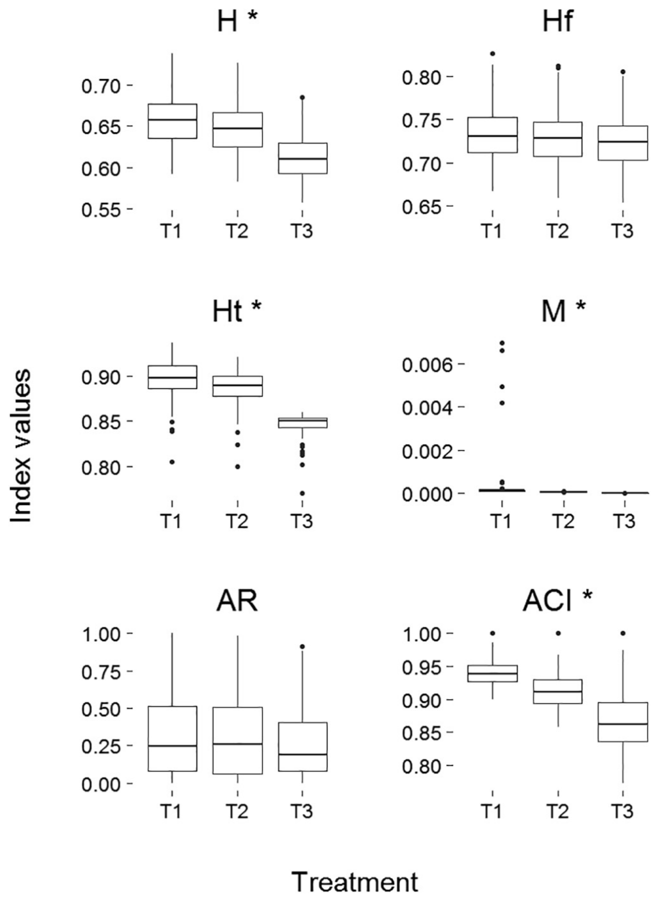

Fig. 8. Effect of the temporal overlap of howls on the distributions of the index values. T1: Alternation of the six solos. T2: Half overlapping between the six solos. T3: Superposition of the six solos. Except for *Hf* and *AR*, all of the other indices (*) were influenced by the different test treatments, and thus they were affected by the temporal overlap of wolf howls (permutational analysis of variance table, $P < 0.05$).

This study was supported by the European Union within the framework of the Operational Program FEDER-FSE "Lorraine et Massif des Vosges 2014–2020" [DPR-NT N2016-102], the Commissariat à l'Aménagement du Massif des Vosges for the FNADT (Fonds National d'Aménagement et de Développement du Territoire) [06/11/2014; 17/11/2015; 05/12/2016], the DREAL Grand Est (Direction Régionale pour l'Environnement, l'Aménagement et le Logement) [Lorraine N°EJ-2101550346; Alsace Arrêté 16/03/2], the Région Grand Est [Lorraine: DPR-NT N2015-11332; Alsace: D1502014], the ANRT (Agence Nationale de la Recherche et de la Technologie) [CIFRE award N° 2014/1220], the Zoo d'Amnéville, and the Parc Animalier de Sainte Croix. It was also supported by the Fondation Le Pal Nature. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Data accessibility

Data are archived in the CROC research center. The data sets used and/or analyzed in the current study are available from the corresponding author on reasonable request.

Declaration of interest

The authors declare that they have no competing interests.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2019.03.010.

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