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1 **Title: Acoustic monitoring of rock ptarmigan: a multi-year comparison with point-**
2 **count protocol**

3 **Authors:** Thibaut Marin-Cudraz^{a,*}, Bertrand Muffat-Joly^b, Claude Novoa^c, Philippe Aubry^d,
4 Jean-François Desmet^e, Mathieu Mahamoud-Issa^{a,f}, Florence Nicolè^g, Mark H. Van Niekerk^a,
5 Nicolas Mathevon^a, Frédéric Sèbe^a

6 a: Equipe de Neuro-Ethologie Sensorielle ENES / Neuro-PSI, CNRS UMR 9197, University of Lyon /
7 Saint-Etienne, 23 rue Docteur Paul Michelon, Saint-Etienne Cedex 2, France.

8 Authors e-mail addresses: thibaut.marincudraz@gmail.com, markh.vn@gmail.com, mathevon@univ-
9 st-etienne.fr, frederic.sebe@univ-st-etienne.fr

10 b: Unité Faune de Montagne, Direction de la Recherche et de l'Expertise, Office National de la Chasse
11 et de la Faune Sauvage, 90, impasse les Daudes, 74320 Sevrier, France.

12 Authors e-mail addresses: bertrand.muffat-joly@oncfs.gouv.fr

13 c: Unité Faune de Montagne, Direction de la Recherche et de l'Expertise, Office National de la Chasse
14 et de la Faune Sauvage, Espace Alfred Sauvy, 66500 Prades, France.

15 Authors e-mail address: claudenovoa@oncfs.gouv.fr

16 d: Cellule d'appui méthodologique, Direction de la Recherche et de l'Expertise, Office National de la
17 Chasse et de la Faune Sauvage, Saint Benoist, BP 20, 78612 Le Perray-en-Yvelines, France.

18 Authors e-mail addresses: philippe.aubry@oncfs.gouv.fr

19 e: Groupe de Recherches et d'Information sur la Faune dans les Ecosystèmes de Montagne,
20 Samoëns, France.

21 Authors e-mail addresses: jfdesmet@orange.fr

22 f: Current adress: Department of Behavioral Ecology, Institute of Environmental Sciences, Faculty of
23 Biology, Adam Mickiewicz University, Umultowska 89, 61-614 Poznań, Poland.

24 Authors e-mail addresses: mathieumahamoudissa@hotmail.fr

25 g: Laboratoire de Biotechnologies Végétales appliquées aux Plantes Aromatiques et Médicinales,
26 FRE CNRS 3727 - EA 3061, Université de Lyon/Saint-Etienne, 23 rue Docteur Paul Michelon, Saint-
27 Etienne Cedex 2, France.

28 Authors e-mail addresses: florence.nicole@univ-st-etienne.fr

29 ***Corresponding author:** Equipe de Neuro-Ethologie Sensorielle ENES / Neuro-PSI, CNRS UMR
30 9197, University of Lyon / Saint-Etienne, 23 rue Docteur Paul Michelon, Saint-Etienne Cedex 2,
31 France.

32 *E-mail adress:* thibaut.marincudraz@gmail.com (T. Marin-Cudraz)

33

34 HIGHLIGHTS:

35 Calls of male rock ptarmigans show individual vocal signatures.

36 These signatures allow acoustic censusing of the number of males present in an area.

37 Acoustic sampling is more accurate than point-count protocol.

38

39 ABSTRACT:

40 The cost-effectiveness and reduced human effort employed in setting up acoustic monitoring
41 in the field makes bioacoustics an appealing option for wildlife monitoring. This is especially
42 true for secretive vocal species living in remote places. However, acoustic monitoring still
43 raises questions regarding its reliability when compared to other, human-driven methods. In
44 this study we compare different approaches to count rock ptarmigan males, an alpine bird
45 species which lives at high altitudes. The monitoring of rock ptarmigan populations is
46 traditionally conducted using a point-count protocol, with human observers counting singing
47 males from a set of different points. We assessed the (1) feasibility and (2) reliability of an
48 alternative counting method based on acoustic recordings followed by signal analysis and a
49 dedicated statistical approach to estimate the abundance of males. We then (3) compared
50 the results obtained with this bioacoustics monitoring method with those obtained through the
51 point-count protocol approach over three consecutive years. Acoustic analysis demonstrated
52 that rock ptarmigan vocalizations exhibit an individual stereotypy that can be used to
53 estimate the abundance of males. Simulations, using subsets of our recording dataset,
54 demonstrated that the clustering methods used to discriminate between males based on their
55 vocalizations are sensitive to both the number of recorded signals, as well as the number of
56 individuals to be discriminated. Despite these limitations, we highlight the reliability of the
57 bioacoustics approach, showing that it avoids both observer bias and double counting,
58 contrary to the point-count protocol where this may occur and impair the data reliability.
59 Overall, our study suggests that bioacoustics monitoring should be used in addition to
60 traditional counting methods to obtain a more accurate estimate of rock ptarmigan
61 abundance within Alpine environments.

62

63

64 *Keywords:*

65 acoustic monitoring; rock ptarmigan; point-count protocol; long-term study; individual
66 information; methodology comparison.

67

68 1. Introduction

69 Acoustic monitoring is becoming an effective means to assess wildlife diversity, resulting in
70 minimal impact to the environment (Towsey et al., 2014; Sueur and Farina, 2015).
71 Importantly, it enables to focus on species used as bioindicators as well as of patrimonial or
72 economic concern. The use of acoustic monitoring is usually motivated by the difficulty in
73 observing the species because of its secretive behavior or the difficulty in accessing its
74 habitat (Hoodless et al., 2008; Vögeli et al., 2008; Marques et al., 2009; Buxton and Jones,
75 2012; Dugan et al., 2013; Marques et al., 2013; Andreassen et al., 2014; Ulloa, 2016). Levels
76 of investigation range from the simple assessment of species' presence/absence to more
77 complex studies that aim at determining the number of individuals present on an area.
78 Depending on the species, these approaches may require the discrimination of individuals
79 (Terry et al., 2005; Pollard et al., 2010), which is only possible when vocalizations contain
80 individual vocal signatures based on morpho-physical, genetics and/or learning abilities
81 (Kroodsma, 1982; Tibbetts et Dale, 2007; Catchpole et al., 2008; Taylor and Reby, 2010;
82 Nowicki and Searcy, 2014; Tamura et al., 2018).

83 Although acoustic monitoring is promising, it still raises several potential issues: high cost of
84 monitoring material, design of monitoring protocols, analysis of long-duration recorded
85 signals, weather conditions impacting the quality of the data, needs of bioacoustics experts
86 for data analysis, and sensitivity to the density of the species (Budka et al., 2015; Linhart and
87 Šálek, 2017). To the best of our knowledge, acoustic monitoring has not yet replaced other
88 protocols. Point-counts protocols (Lancia et al., 2005) are still largely used to provide
89 estimates of the number of individuals. However, these conventional, human-operated
90 methods are exposed to biases due to inter-individual differences between observers,
91 unpredictability of field conditions, and biological parameters such as species abundance
92 (Tyre et al., 2003; Bart et al., 2004; Lotz and Allen, 2007; Elphick, 2008; Fitzpatrick, 2009).
93 Moreover, individuals of species emitting long-range signals can be counted by several
94 observers simultaneously, leading to double counting and abundance overestimation.
95 Besides, human presence can disturb birds' behavior making them stop singing and leading
96 to population underestimation.

97 While previous works have explored the technical feasibility of acoustic monitoring based on
98 vocal individual signature (Terry and McGregor, 2002; Hartwig, 2005; Grava et al., 2008;
99 Policht et al., 2009; Adi et al., 2010; Feng et al., 2014; Budka et al., 2015, 2018; Peri, 2018a)
100 most field applications were based on sounds recorded from already known individuals
101 (O'Farrell and Gannon, 1999; Peake and McGregor, 2001; Vögeli et al., 2008; Digby et al.,
102 2013; Peri, 2018b). To the best of our knowledge, there is no published study investigating

103 the generalization and reliability of an acoustic monitoring approach based on individual
104 vocal signatures aimed at estimating the number of individuals in real field conditions.
105 Focusing on the rock ptarmigan *Lagopus muta* in France, the present paper reports a multi-
106 year study aimed at assessing the potential interests, as well as the caveats, of acoustic
107 monitoring by comparing performances of this approach with a traditional point-count
108 methodology.

109 The rock ptarmigan is a bird species that inhabits the northern parts of Eurasia and North
110 America. In France, its range is restricted to sub-alpine and alpine habitats (altitude > 1800
111 m) of the Pyrenees and the Alps Mountain ranges (Sale and Potapov, 2013). This species is
112 secretive and difficult to access. It is highly adapted to its environment, has mimetic plumage
113 and vocalizes at dawn and dusk (MacDonald, 1970). Population abundances are decreasing
114 in both the Alps (Imperio et al., 2013; Furrer et al., 2016; Martinoli et al., 2017) and the
115 Pyrenees, where they are threatened by both climate change and habitat transformation
116 (Revermann et al., 2012; Bech et al., 2013). Due to these extreme environmental and
117 selective pressures, rock ptarmigan is often considered as a bioindicator of the ecosystem
118 health, a sentinel and umbrella species for biodiversity conservation of the alpine
119 environments (Sandercock et al., 2005; Hanser and Knick, 2011; Henden et al., 2017).

120 During the mating season (May-June), males display courtship rituals, which often includes
121 simple, pulsatile vocalizations as well as a peculiar “singing in flight” behavior before dawn
122 (MacDonald, 1970). The flight is hyperbolic, and vocalization starts when the bird reaches
123 the point of highest altitude. Point-count protocols rely on this acoustic behavior to evaluate
124 the number of males after they have established their breeding territories (Bossert, 1977).
125 Although vocalizations are loud and easily heard by an observer, low visibility, birds’ mobility,
126 frequent harsh weather conditions and other constraints associated with the alpine
127 environment are likely to increase the possibility of double counting and overestimation of the
128 monitored population. Conversely, this may also impair song perception by observers
129 (Andreev, 1971). The use of an acoustic monitoring technique could provide a feasible
130 alternative as a response to these difficulties and potential biases. Despite these constraints,
131 rock ptarmigan presents several advantages in terms of monitoring. Vocalizations are easily
132 recognizable, population densities are generally low (around 5 males/km²), and males are
133 mostly located in stable territories which facilitates their localization and recording.

134 The present study proposes (1) to assess the individual vocal signature embedded in rock
135 ptarmigan males’ calls, (2) to test the feasibility and reliability of a bioacoustic monitoring
136 approach over several years, and (3) to compare the results obtained using this approach to
137 those obtained with a traditional point-count protocol and long-term field observations.

138

139 **2. Material and Methods**

140 We performed this study in 2015, 2016 and 2017, at the ski resort of Flaine (French Alps,
141 Haute-Savoie, 45°59'32.8"N 6°43'44.2"E; altitude: 1600-2500 m).

142

143 *2.1 Counting of singing males*

144 We used three different methods to evaluate males' abundance: a point-count protocol, long-
145 term observations and acoustic monitoring. The latter was followed by signal processing and
146 statistical analysis.

147

148 *Point-count protocol*

149 The counting area was delimited empirically to cover roughly 100 hectares (Fig.1). Previous
150 field observations and literature reviews had suggested that male ptarmigans could be heard
151 at distances of 500-1000 m (Watson, 1965; Bossert, 1977; Marty and Mossoll-Torres, 2012).
152 We selected three counting points, approximately 500 m apart from one another to maximize
153 the listening area (Fig.1). These counting points were located at precisely the same positions
154 over the three-year period of study. The counting procedure followed that proposed by
155 Léonard (1995), which was based on the work of Bossert (1977). Three experienced
156 observers, with previous knowledge of the field (TMC, FS, BMJ; 1 per counting point),
157 accompanied by volunteers, were placed at each point. As ptarmigan males mainly display
158 their acoustic signal early in the morning, the observation periods started at 4.30 a.m. and
159 ended at 5.30 a.m. The observers were positioned 15 min before the beginning of the
160 observation period. Throughout the observation period, each observer noted on an
161 observation form (with a drawn map of the area) the timing, the number and the approximate
162 estimated locations of the vocalizing ptarmigans. At the end of the observation period, BMJ
163 collected all observers' forms.

164 To estimate the total number of males in the area, we counted 1 male for each group of
165 neighboring vocalizations indicated on the maps (each group had to be clearly separated
166 from the others; see Fig. 1). Each male was confirmed by cross-checking the observers'
167 data. To avoid double-counting by two different observers, vocalizations localized nearby and
168 heard at approximately the same time (interval < 20 sec.), were considered identical. We
169 considered the total number of males unambiguously localized as the minimum total number
170 of males. To take into account ambiguous localizations (e.g. when one observer noted two
171 birds on the same location while another observer noted a single one) we defined a

172 maximum total number of birds by adding these ambiguous males to the minimum total
173 number. We thus defined an estimated interval (min-max) of the total number of males. This
174 procedure was repeated on several consecutive days namely: June 3rd and 4th in 2015 and
175 2016; June 6th, 7th, 9th in 2017.

176

177 *Long-term observations and acoustic recordings*

178 After the point-counting days, two bioacousticians (TMC and FS) remained in the field for a
179 period of one month to observe and record all the birds present in the area (recording
180 material: Sennheiser MKH70 shotgun microphones connected to Marantz PMD 660
181 recorders; sampling rate: 48000 Hz). This was an important long-term observation effort
182 aimed at ensuring a thorough knowledge and identification of each bird present within the
183 area, which may have been acoustically recorded during the point-count protocol. The
184 resulting number of males spotted by this technique therefore represented the expected
185 maximum value that could be deduced using the other methods.

186 Despite this effort however, we were only able to record birds during 7, 9, and 15 days in
187 2015, 2016 and 2017 respectively, primarily due to the harsh weather conditions. We used
188 two different strategies in order to build up our bank of recordings:

189 1) Recording of non-identified birds before sunrise (4.30 a.m. - 6 a.m.), i.e. during the time
190 slot corresponding to the spontaneous singing activity peak. Due to the low ambient
191 luminosity, the recorded males could not be visually observed and identified. The recording
192 data sets were thus named: “unknown datasets”. Each day, both bioacousticians recorded
193 from different locations within the study area to sample a maximum number of males.

194 2) Recording of identified birds (6 a.m. – around 10 a.m.). In 2017, we equipped 5 males
195 present on the area with GPS solar tags (e-obs GmbH, Grünwald, Germany), and used
196 these tags to pinpoint the males with their individual UHF (Ultra High Frequency)
197 radiofrequencies. One male had already been equipped with a VHF (Very High Frequency)
198 radio-emitter collar since 2015. Two additional males were identified using visual cues only.
199 Both had mated with females and remained within stable and well-defined territories. The
200 combination of direct observations and GPS localizations greatly minimized the risk of mis-
201 identifying or confounding males during recordings. A ninth male had its territory on a
202 neighboring summit (3 km away). As this male was alone on this site and easy to identify, it
203 was thus added to the recording database (total = 9 birds in 2017).

204 The recording strategy was as follows: after sunrise, when the peak of males' vocal activity
205 ended, we played back calls from an individual recorded in another area to elicit the focus

206 male's territorial response. This allowed us to record each focus male while double-checking
207 for its individual identity.

208 In the preceding years (2015 and 2016), we used the same approach although the results of
209 the field effort were weaker:

210 - June 2016: 5 males recorded. Two of the males were equipped with GPS tags and 3 other
211 males were identified using visual observations only.

212 - June 2015: 7 males recorded. One male was equipped with a VHF radiotransmitter
213 necklace; 6 males were identified using visual observations only.

214 The 2015, 2016 and 2017 recording data sets obtained with this method were named "known
215 datasets".

216

217 2.2 Acoustic analysis of recorded signals

218 *Data bank of calls*

219 Rock ptarmigan vocalizations are sequences of pulse trains, with energy spread over a
220 frequency spectrum ranging from 900 to 3700 Hz. There are two major types of calls, namely
221 short and long calls. These differ by the number of successive pulse trains, namely 3 and 4
222 respectively (MacDonald, 1970; Watson, 1965). For the present study, we focused on the
223 short calls, which are the most frequently recorded (Fig. 2). Our annual data bank of calls
224 consisted of the following:

225 - 2015: 183 short calls, of which 100 were of sufficient quality (in terms of signal-to-
226 noise ratio) to be analyzed ("unknown" dataset: 75 calls; "known" dataset: 25 calls,
227 3.6 ± 2.4 calls/male, min = 1 call, max = 8 calls).

228 - 2016: 249 short calls, with 98 of sufficient quality ("unknown" dataset: 66; "known"
229 dataset: 32 calls, 6.4 ± 2.3 calls/male, min = 4 calls, max = 10 calls).

230 - 2017: 180 short calls, with 133 of sufficient quality ("unknown" dataset: 52 calls;
231 "known" dataset: 81 calls, 7.1 ± 3.9 calls/male, min = 3 calls, max = 24 calls).

232

233

234 *Automatic detection of group of pulses*

235 Due to harsh weather conditions (wind and rain), recorded signals were frequently corrupted
236 by noise. Before performing the automatic detection of pulses, we first filtered the signals
237 with a 950-2700 Hz bandpass filter, and then processed a *wavelet continuous transform*

238 (WaveleComp R package, Roesch and Schmidbauer, 2018) to optimize the signal-to-noise
239 ratio (see Supplementary Material for details).

240 After denoising, amplitude pulses were detected using a customized script (Seewave R
241 package, Sueur et al., 2008). The absolute amplitude of the signal was first smoothed using
242 a Daniell kernel (length = 100). The time position of the pulses was then identified using an
243 amplitude threshold fixed at 3 % of the maximum amplitude of the considered signal.

244 Pulses were gathered in “groups” (G1, G2, G3, see Fig. 2) by automatically measuring
245 intervals between pulses (Fig. 2c) and computing the ratios between two successive intervals
246 (Fig. 2d). Ratios superior to 1.7 characterized boundaries between groups of pulses (Sil1 and
247 Sil2; see Fig. 2b).

248

249 *Measurement of acoustic parameters*

250 We measured 12 acoustic parameters from groups of pulses G1 and G2 (see list in Table 1).
251 We chose to ignore the G3 group, as the signal-to-noise ratio of this part of the call was
252 usually very weak (this was mostly due to males flying away from the recorder while singing).

253

254

255 The mean acceleration (Acc.G1) was calculated as follows:

$$Acc = \frac{1}{n-2} \sum_{k=1}^{n-2} \frac{1}{Pr_{k+1}} - \frac{1}{Pr_k}$$

256 Where n is number of pulses in G1; Pr is the pulse rate and t is the time of occurrence of the
257 pulse’s maximum amplitude.

258 The normalized Pairwise Variability Index ($nPVI$) is an index commonly used in phonetic
259 studies (Grabe and Low 2002) to illustrate the variability between consecutive pairs of
260 intervals:

$$261 \quad nPVI = 100 \times \sum_{k=1}^{n-1} \left| \frac{Pr_k - Pr_{k+1}}{\frac{(Pr_k + Pr_{k+1})}{2}} \right|.$$

262 We used continuous wavelet transformation to calculate the peak frequency parameters
263 (Fq1.G1 and Fq2.G1). Since pulse locations in the signal had already been calculated, each
264 pulse was isolated from the original sound, filtered with an 800 Hz -3000 Hz passband filter.
265 For each pulse, wavelet power spectrum was then calculated. The two scales with the
266 maximum power were then selected. The scales were further multiplied by the Fourier factor

267 $6 / (2\pi)$ to obtain the classical Fourier periods (Aguiar-Conraria and Soares, 2011) with the
268 corresponding frequencies. The median of each peak frequency was then calculated across
269 the entire pulse train. Overall, medians were preferred to means since they are more
270 conservative and less influenced by possible outliers resulting from analytical errors (e.g. due
271 to rain drops occurring within a pulse group).

272

273 2.3. Acoustic space and individual vocal signatures

274 In order to build a functional tool allowing the acoustic discrimination between rock ptarmigan
275 males, we proceeded as follows: 1) we built a 4-D acoustic space with a dedicated
276 discriminant analysis which optimized separation between males using the 2017 “known”
277 dataset (reference dataset); 2) we used this acoustic space to perform an unsupervised
278 clustering analysis using the reference dataset for tuning the clustering hyperparameters; 3)
279 we applied the workflow (centering, projection in the acoustic space and then tuned
280 clustering) on the 2015, 2016 and 2017 complete data sets to further estimate the number of
281 males present each year.

282

283 *Acoustic space definition*

284 We analyzed the differences between calls from the individuals of the 2017 “known” dataset
285 (9 identified males) using powered partial least squares discriminant analysis (PPLS-DA,
286 Liland and Indahl, 2009; “pls” R package, Mevik et al., 2016). PPLS-DA enables more
287 accurate analysis of a small sample size with a high number of acoustic parameters, better
288 than the linear discriminant analysis signatures (Hervé et al., 2018), commonly used in
289 studies on animal vocalizations. PPLS-DA calculates new variables as combinations of all
290 centered acoustic variables, leading to a new acoustic space optimizing the discrimination
291 between individuals. The number of dimensions was chosen by model cross validation
292 (Szymańska et al., 2012). The mean classification error rate was established after 100 model
293 cross validations for each number of dimensions (varying between 2 to 11, Fig. 3). We
294 followed an analog method of the elbow method (Cattell, 1966) to assess the optimal number
295 of dimensions and we considered the first four PPLS-DA variables, which define a 4-D
296 acoustic space and explained 36.52 % of the variability (see Results and Fig. 4).

297

298

299 We tested the statistical significance of our PPLS-DA model with a procedure implemented in
300 the RVAideMemoire package (Westerhuis et al. 2008; Hervé, 2018). The PPLS-DA

301 significance validation is composed of two steps. Firstly, a set of discriminant functions is
302 obtained from a training data set and secondly, these functions are used to test the
303 classification on a validation set. The measure of standard error is obtained by analyzing the
304 correct assignment percentage of 999 random selections of the original data set, which have
305 been divided into a fitting and testing set.

306

307 *Clustering analysis and bootstrap reliability testing*

308 We used an unsupervised classification method (high dimensional data clustering, HDDC,
309 Bouveyron et al., 2007) to estimate the number of individual males present within the
310 datasets. HDDC has already been applied on acoustic data with some success (Ulloa, 2018).
311 HDDA is known to be consistent and reliable with unbalanced datasets because it is based
312 on gaussian mixture models (Fraley and Raftery, 2002). It is more parsimonious and flexible
313 than gaussian mixture modelling by adding a noise term within the model covariance
314 parametrization. The mixture model aims at identifying the meaningful variables for each
315 cluster and is fitted with the E-M algorithm. The number of mixture components of the model
316 maximizing the Bayesian information Criterion (BIC, Schwarz, 1978) is set as the number of
317 clusters. The E-M algorithm is sensible to the selected random points during its initialization.
318 Thus, we ran the clustering algorithm several times in order to obtain a reliable value for the
319 number of clusters.

320 The 2017 'known' reference dataset was used for tuning the HDDC hyperparameters ($K = 9$
321 clusters in 2017; the covariance model M ; the threshold t used to parametrize the dimension
322 of each cluster; see R package HDclassif, Bergé et al., 2012 for details). Each call was
323 represented by its 4 acoustic dimensions previously calculated through PPLS-DA. We tested
324 10 values of t namely: 0.000001, 0.00001, 0.0001, 0.01, 0.03, 0.05, 0.07, 0.1, 0.15, 0.2
325 (adapted from Ulloa, 2018) and the 14 possible models of covariance parametrization. Each
326 association of t and M value were tested.

327 The clustering algorithm was run 100 times for each association. For each run, we measured
328 the similarity between the clustering output and the clustering membership with the adjusted
329 Rand Index (ARI, Hubert and Arabie, 1985; package mclust, Scrucca et al., 2016). The ARI
330 ranges from -1 to 1 and is an indicator of the concordance of two classifications for the same
331 dataset: when $ARI = -1$, the classifications are totally opposed, or different. When $ARI = 0$,
332 the classifications are considered random; when $ARI = 1$, they are identical. The mean ARI
333 was then calculated for the 100 values and the tuning parameters associated with the
334 highest mean were selected. The maximum mean adjusted Rand Index ($ARI = 0.91$) was

335 found for the simplest covariance model (“abqd”) and a threshold value of 0.1 was assigned.
336 We thus used these tuning parameters.

337 Once M and t fitted with our data, the reliability of the clustering process was further tested
338 using sub-sets of the 2017 reference ‘known’ dataset. Subsets were built by randomly
339 sampling several various males (random sampling of 1 to 9 males; 900 subsets; 100
340 trials/subset) or a various total number of calls (random sampling of 20 to 81 calls; 6200
341 subsets; 100 trials/subset). We tested models with K values ranging from 1 to 20.

342

343 2.4 Comparison between counting methods

344 To assess the number of males through the acoustic analysis of calls, we performed the
345 clustering analysis on each year separately, using the entire datasets obtained by pooling
346 “known” and “unknown” calls (100 trials/year). The male of 2017 that was geographically
347 isolated was however excluded because it was located outside the point-counting area. We
348 calculated the 4 dimensions of each call using the PPLS-DA functions previously defined
349 with the 2017 “known” dataset for each year separately. Each acoustic dataset was centered
350 before its projection in the 2017 acoustic space by subtracting the means of each acoustic
351 variable calculated on the 2017 “known” dataset.

352 The number of males (i.e. the number of acoustic clusters) estimated for each year through
353 the clustering analysis was then compared with the number of males estimated through the
354 two other counting methods, i.e. 1) the point-count protocol and 2) the long-term observation.

355 All the acoustic and statistical analysis was performed on R version 3.5.0 (R core team,
356 2018).

357

358 **3. Results**

359 3.1 Acoustic discrimination between males and definition of the acoustic space

360 The PPLS-DA identified significant acoustic differences between ptarmigan males, with 4
361 significant functions that allowed maximizing individual separation (Fig. 4, mean classification
362 rate = 79 %; min-max classification rate per individual = 0 – 100 %; $p = 0.001$). Table 2
363 shows the correlation between each of the 4 components, the acoustic variables and shows
364 that all parameter types (pulse number, pulse rate, durations, frequency parameters)
365 contribute towards separating the males.

366

367

368

369

370 3.2 Reliability of High Dimensional Data Clustering

371 To test the reliability of HDDC, we compared the median number of males obtained through
372 resampled HDDC with the actual number of males of each sub-data set. As displayed in Fig.
373 5, HDDC gives a reliable estimate of the number of recorded males if this number does not
374 exceed 5 individuals. HDDC underestimates the number of recorded males when 6 or more
375 individuals were included in the sub-dataset. It also underestimates the number of recorded
376 males, when the number of vocalizations in the sub-datasets are sampled, and consistently
377 predicts 7 clusters (i.e. 7 individuals) for sub-datasets composed of a minimum of 33
378 vocalizations (i.e. 41 % of the total number of calls) (Fig. 5b).

379

380 3.3 Comparison of counting methods' reliability

381 In 2017 and 2016, the most congruent results were given by the acoustic monitoring and
382 long-term observation. In both years, the point-count protocol resulted in a lower estimation
383 than the two other counting methods. Still, the long-term results were reached by the point-
384 count intervals for at least one day per year. Estimation through the point-count protocol
385 appears to be highly dependent on the day of observation (this is particularly obvious in
386 2017, with an estimate of 5-8 males on the first day *versus* 4-5 males on the second day).

387 The 2015 results differed significantly from those of 2016 and 2017, with an apparent under-
388 estimation of the number of males through the acoustic monitoring method compared to
389 long-term observations. However, the distribution is widespread and looks bimodal, with the
390 second mode (6 clusters) being close to the actual number of males (7 individuals). This can
391 be clearly seen in Fig. 6 which displays the number of males estimated by each counting
392 method (point-count protocol, long-term observation, acoustic monitoring).

393

394 4. Discussion

395 4.1 Does the acoustic space built from recordings encompass the vocal variability of rock 396 ptarmigan males?

397 The relative inconsistency of individual males' vocal signature might limit the bioacoustics
398 approach. Although the mean PPLS-DA classification rate of recorded calls was around
399 80%, individual rates differed greatly among males (from 0% to 100%). Moreover, the
400 discriminant functions used to build the acoustic space explained only 36% of the total

401 acoustic variability of calls. A significant proportion of the variability remains out of reach,
402 suggesting that individual identity is not the only factor driving the calls' structure. Rock
403 ptarmigan' vocalizations are sequences of stereotyped pulses with few frequency
404 modulations. The acoustic variation between individuals may thus be reduced when
405 compared to other bird species with more complex signals. Ptarmigan are non-oscine birds
406 (Kroodsma et al., 1982; Slater, 1989), and their vocalizations thus lack then the individual
407 variability that could have been induced by song learning. Inter-individual differences in
408 ptarmigan acoustic signals mostly result from differences in their genetic background and
409 their physiological conditions. It is known that ptarmigan males are highly philopatric and
410 closely related genetically in the Alps at large scale (Caizergues et al., 2003). In addition to
411 this, a genetic study of a closely related species, the red grouse (*Lagopus lagopus scoticus*),
412 showed that males were highly related at local scales (Piertney et al., 1998). The genetic
413 variability between rock ptarmigan males is thus rather low. Moreover, the highly variable
414 alpine weather conditions should promote great annual variations in food availability,
415 especially due to snow cover and the timing of snow melt (Körner, 2003; Edwards, 2007;
416 Jonas, 2008). Thus, males' physiological state might be different both between individuals
417 (e.g. depending on the individual food intake in each territory) and from year to year within
418 individuals (depending on the availability of resources). The variability from year to year is of
419 special concern as vocalizations from the same male could be very different each year, thus
420 impairing recapturing males over consecutive years using acoustics only. For this reason, we
421 considered each year independently within the present study.

422 Our analysis demonstrates that, despite these potential limitations and thanks to their low
423 densities in France (few males present on the same area), it is possible to rely on individual
424 vocal signatures to identify local rock ptarmigan males (Linhart and Šálek, 2017) and,
425 ultimately, to count them. The first requirement is to include only high-quality recordings in
426 the analysis step (recordings are regularly corrupted by background noise, mostly induced by
427 wind). Besides, analysis should be mainly performed on the temporal acoustics parameters,
428 since these are less influenced by noise than the frequency cues. These conservative
429 choices and the fact that, by design, PPLS-DA optimizes the separation between males and
430 not the explained variability, can partly explain the low percentage found. Nevertheless, we
431 were still able to separate the males well enough for our purpose.

432

433 4.2 High dimensional data clustering

434 A second potential limitation of the bioacoustics method may arise in cases where some
435 males are represented by only a few recordings, resulting in unbalanced recording datasets.

436 HDDC is a model-based clustering, fitted by maximizing log-likelihood estimation (MLE),
437 itself based on the probabilities of clusters membership. The likelihood will tend to favour
438 clusters forming large and homogenous groups of points (Fraley and Raftery, 1998). When
439 the recording dataset is strongly unbalanced between males, individuals with few
440 vocalizations can be confounded (i.e. included in the same cluster) with other males that
441 show close vocalization characteristics. One of the males was overrepresented in the 2017
442 “known” dataset and its calls formed a cluster that incorporated vocalizations from some
443 other males. This resulted in an underestimated total number of individuals. After removing
444 this male from the dataset (for comparing between methods), the number of estimated
445 clusters was higher and more reliable. Unbalanced situations are more likely to arise when
446 the recording effort is mitigated, as was the case in 2015. This caused HDDC to under-
447 perform, resulting in an underestimation of the number of males present in the observation
448 area. Such a difference can be explained when looking at the call’s clusters obtained through
449 the HDDC method for each year, using both ‘known’ and ‘unknown’ data sets (Fig. 7). In
450 2015, the vocalizations were mostly from unknown emitters. As a result, clusters strongly
451 overlapped each other. Conversely, 2016 and 2017 vocalizations are more clearly separated
452 into homogenous groups. The recording sampling effort must then be homogeneous over the
453 whole study area and cautiously planned to optimize the representativity of recording banks
454 (Heupel et al., 2006).

455

456

457 4.3 Sampling effort and balance

458 An adequate acoustic dataset needs a major field effort, due to frequent harsh weather
459 conditions and difficulties associated with approaching males’ territories. Despite these
460 constraints, comparable results were found between bioacoustics and long-term methods.
461 Long-term observations seem the most reliable approach, although not feasible on a regular
462 schedule given the required workforce. However, most of the vocalizations were obtained
463 using playbacks and males were recorded directly within their territories (“known” datasets).
464 Marginal males were included (Fig. 1) as we assumed that they could be heard and could fly
465 inside the study area. The area of interest was therefore slightly larger than the area covered
466 by the point-count protocol. This increased the probability of male detection during the
467 acoustic monitoring compared to the point-count protocol. Conversely, point-count monitoring
468 appears to be less accurate, with a greater variability of males’ abundance estimations
469 between counting days.

470 The reduced reliability of the point-count census is not surprising, given that observers focus
471 generally on only one observation day. We extended the counting period for a few days to
472 show the variability of this method in this study. Weather conditions (e.g. wind speed) and
473 variation in males' motivation to vocalize may impair the detectability of males. Moreover, the
474 number of males present in the area fluctuates through the season and between observation
475 days. In contrary to northern latitudes (Unander and Steen, 1985; Cotter, 1999), ptarmigan
476 males differ in their arrival dates depending on their mating status. Mated males return to
477 their territory in late summer / autumn, while single males arrive only in spring of the
478 subsequent year. Snow cover plays an important role in the availability of territories and
479 reproductive success of the species (Novoa et al., 2008) by delaying the males' arrival (one
480 of the 2015 males equipped with VHF was not present in 2016 –a year during which snow
481 covered its territory- but arrived in late June in 2017, when the snow cover of its territory
482 started to dissipate).

483 Point-count census is a “one-shot” process: it estimates the number of males at a given day
484 whereas bioacoustics and long-term observation estimations are obtained over larger time
485 periods. The point-count protocol is therefore not able to capture changes throughout the
486 mating season. In practice, only long-term monitoring using direct observations or acoustic
487 recordings could overcome this variability.

488 In addition, our study was able to test the reliability of the census method based on the
489 number of individuals and the number of signals taken into consideration. We showed that a
490 minimum number of sampled vocalizations were necessary (approximately 33 vocalizations
491 for 9 individuals, Fig. 5B) to ensure a consistent assessment of males' number. Although the
492 efficiency of the clustering method is density dependent, this is on a smaller scale than those
493 of classical counting methods (Budka et al., 2015; Linhart and Šálek, 2017). To apply our
494 analysis methodology across populations in different locations, we would probably need to
495 consider a range of higher signal diversities. The generalization of our study would thus need
496 to train our statistical model (PPLS-DA) with recordings from identified males, from other rock
497 ptarmigan populations, to encompass greater signal variability and to avoid staying at local
498 scale variability.

499

500 4.4 Is bioacoustics monitoring a good solution for rock ptarmigan population monitoring?

501 The choice of a monitoring method is the result of a balance between the scale of the study
502 and the expected results. At a fixed cost, the same number of automatic recorders allow to
503 gather precise information regarding males' abundance and locations within a restricted area
504 or could provide simple presence/absence survey of a wider region. Besides, the

505 bioacoustics approach could enable the censusing of more areas without requiring an
506 increase of the number of observers and/or days of observation. This may be particularly
507 advantageous in large and remote mountain massifs where rock ptarmigan habitats can be
508 situated in remote areas.

509

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720 **Acknowledgements**

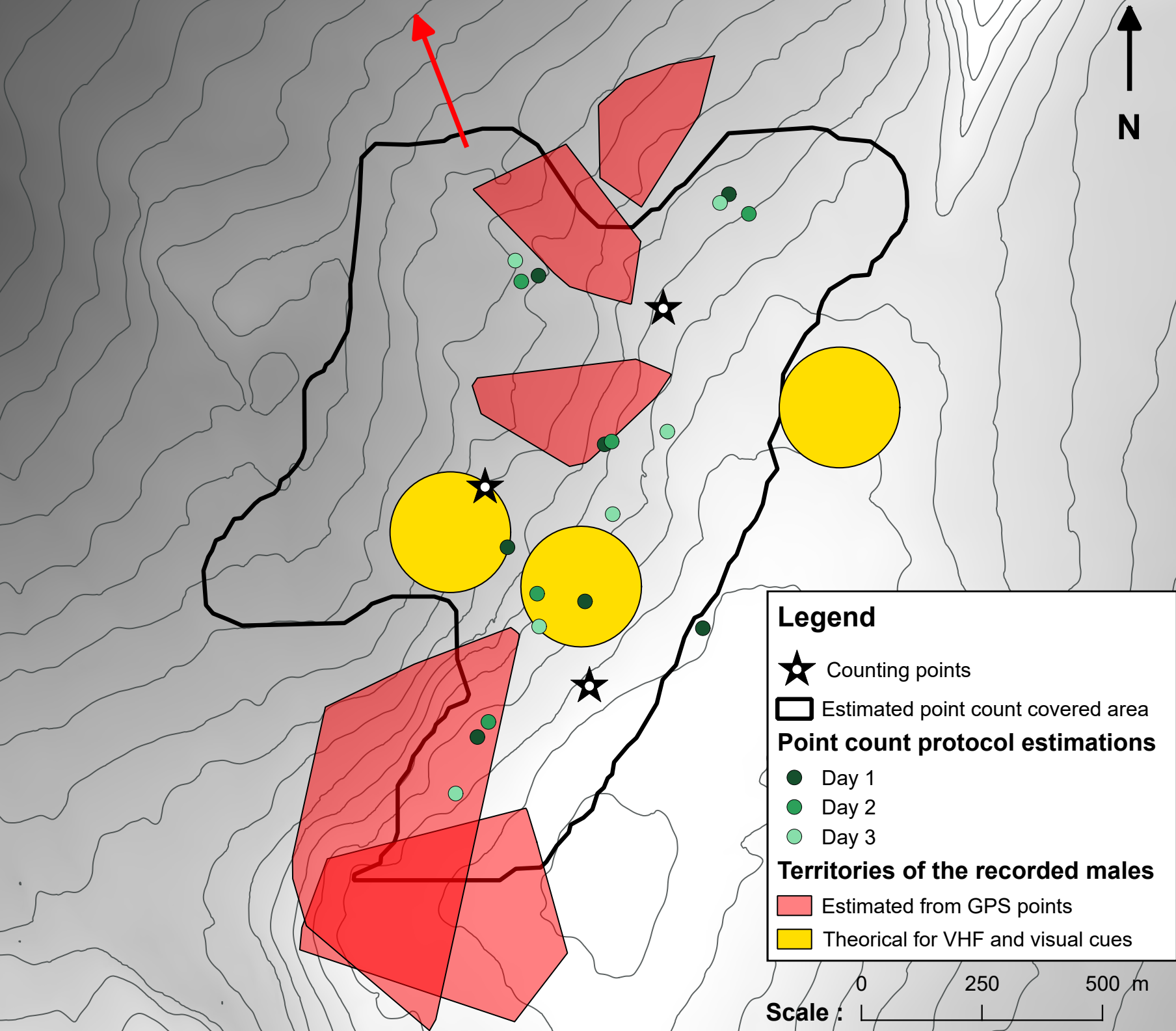
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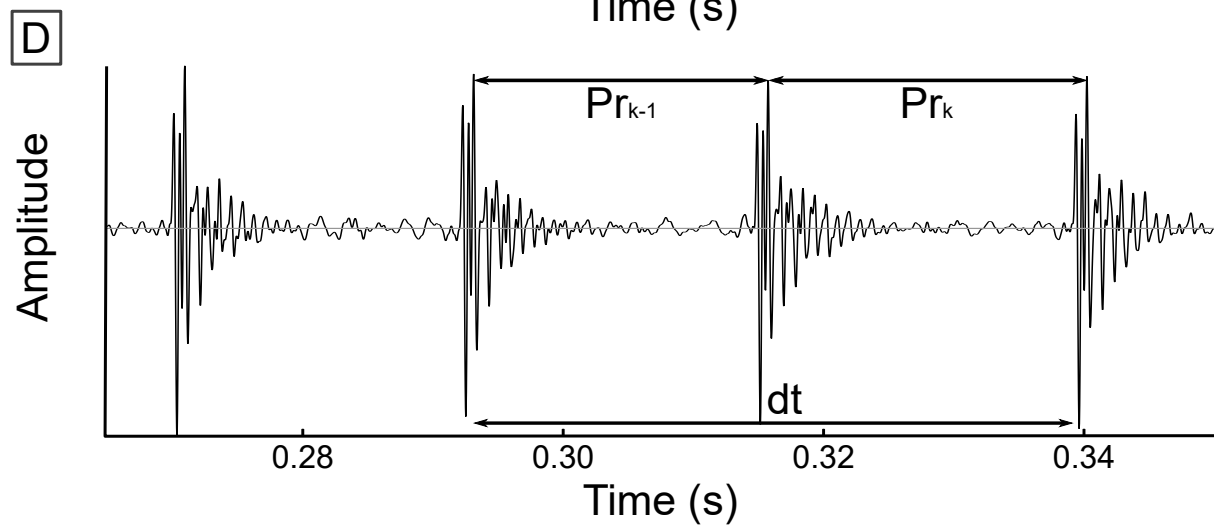
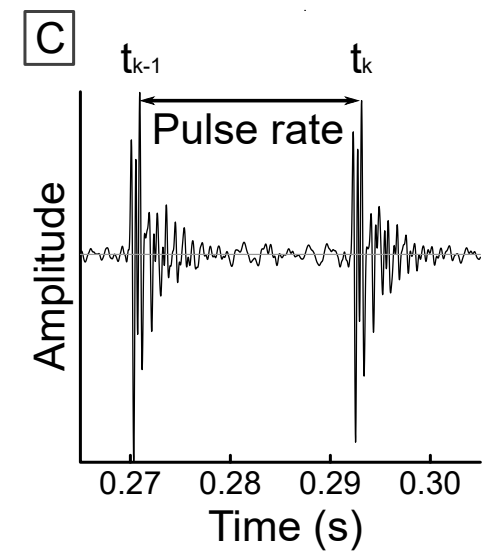
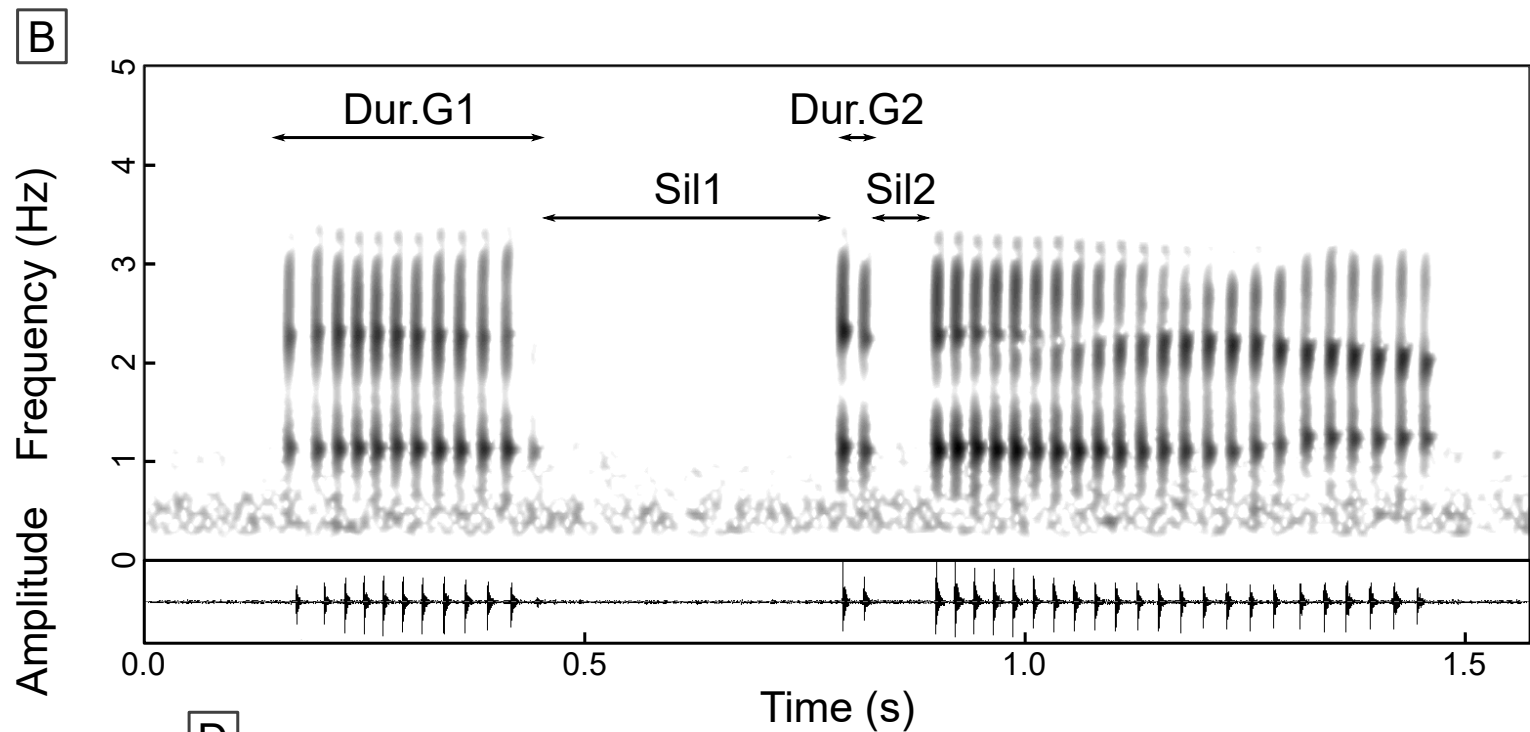
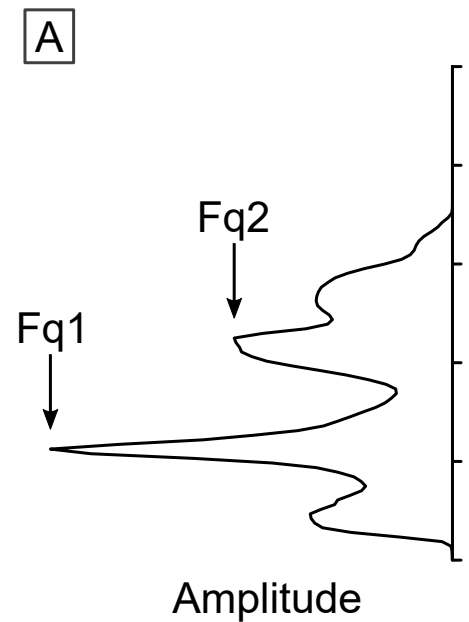
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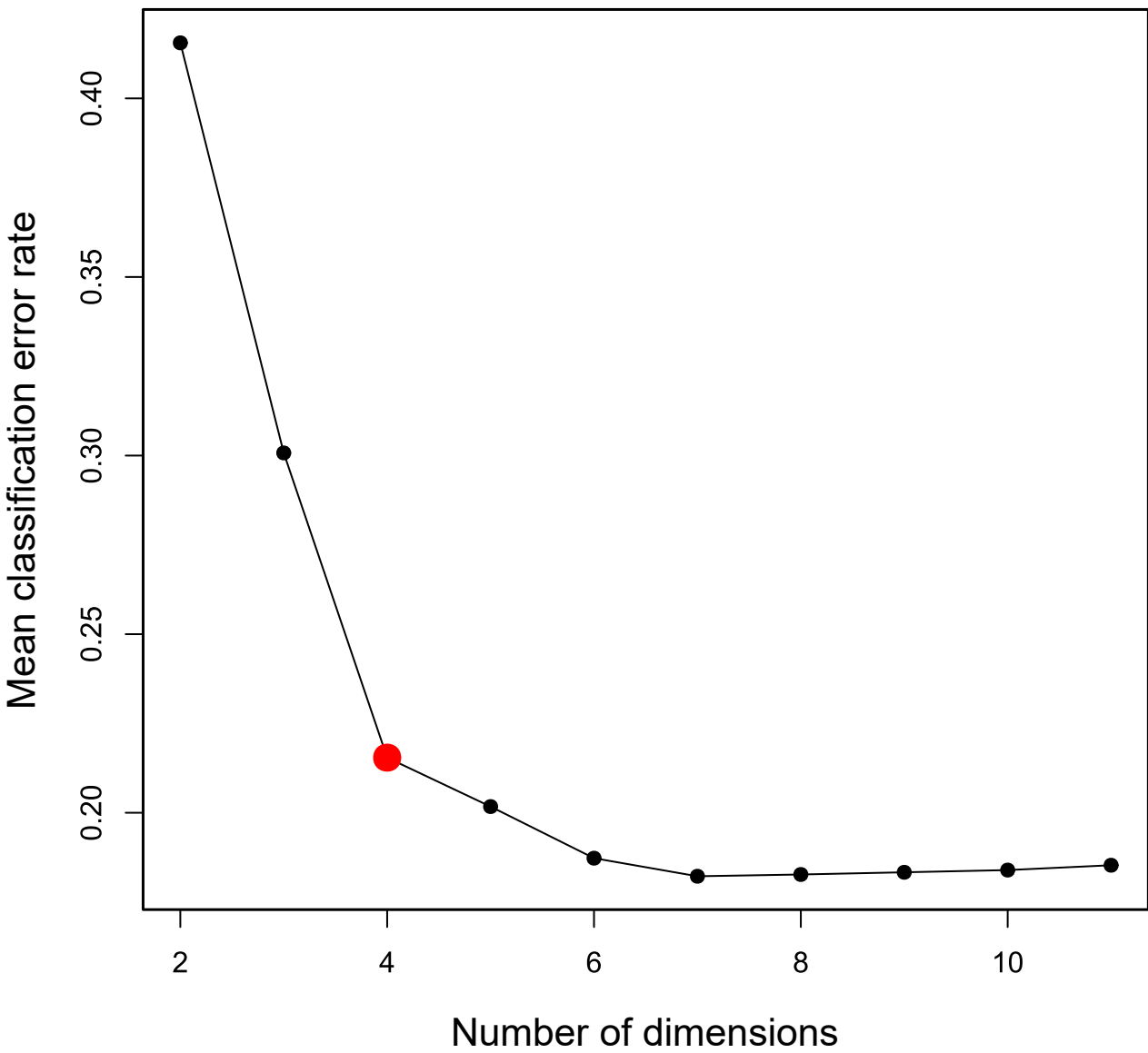
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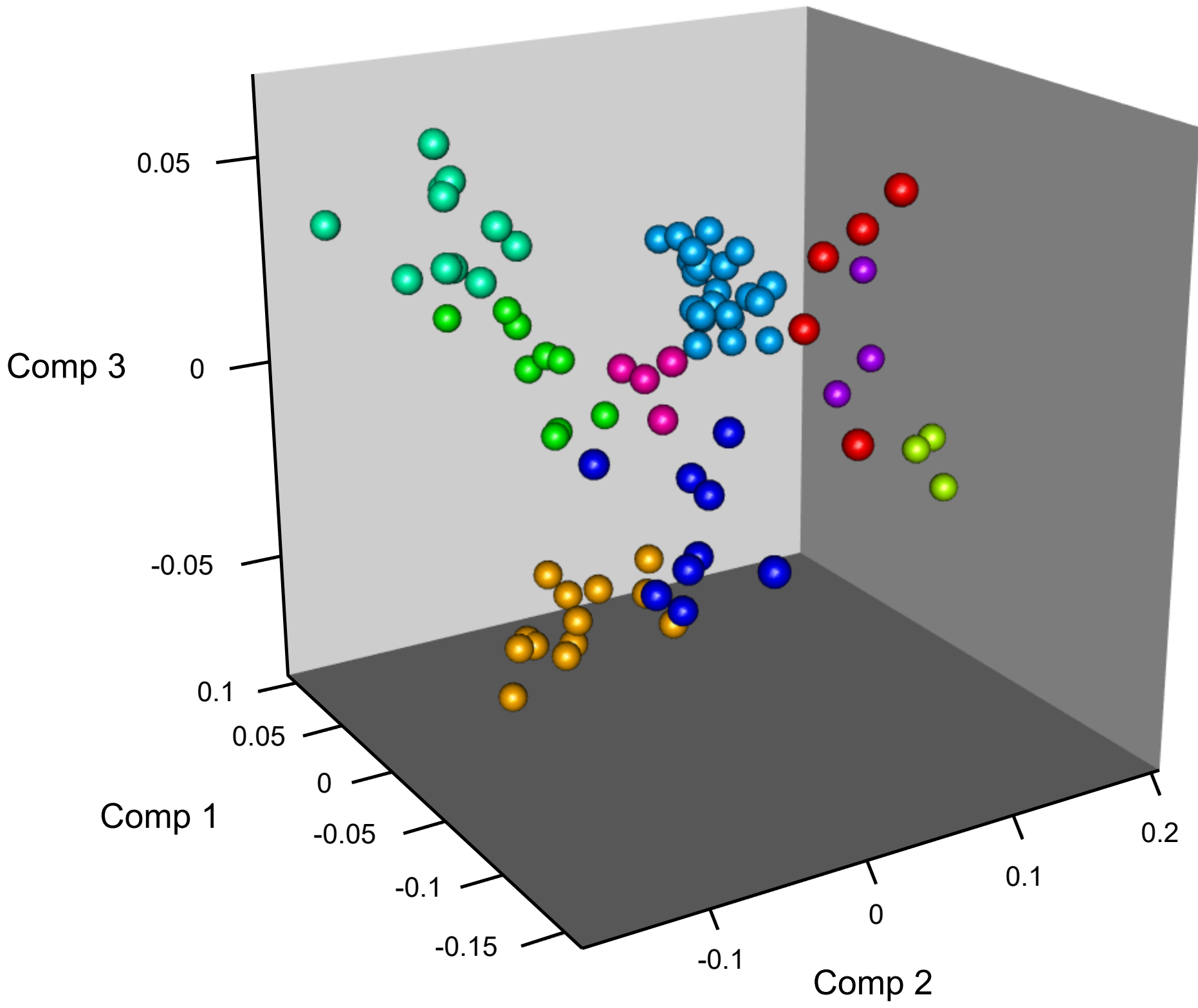
731 **Author contribution statement**

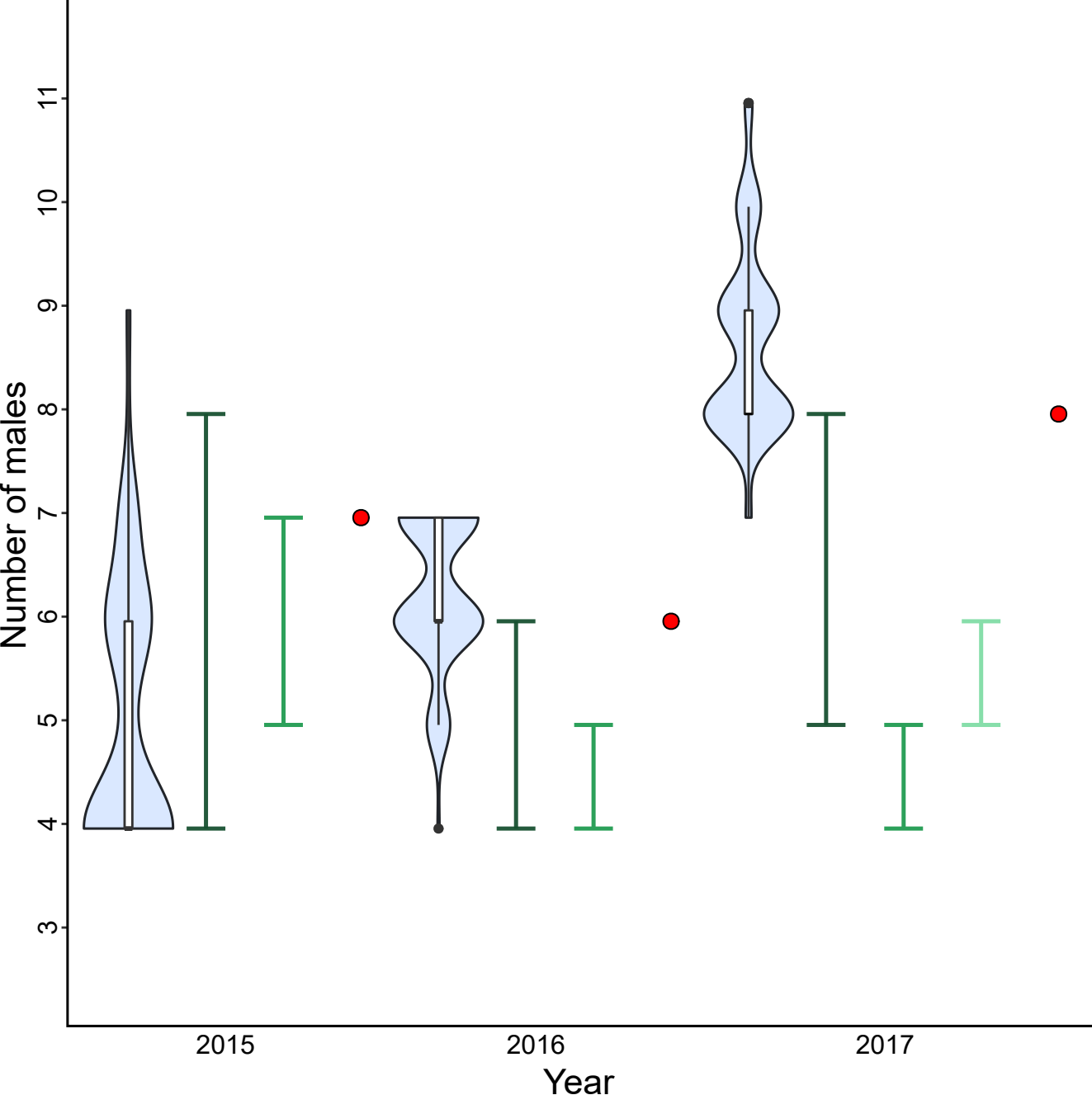
732 TMC, FS, BMJ, JFD, MMI, FN, PA, CN and NM conceived the ideas and designed the
733 methodology; BMJ captured the males, equipped the GPS collar and organized the point-
734 count protocols. TMC and FS collected the acoustic data; TMC, MMI, FN analyzed the data;
735 TMC, FS and NM wrote the manuscript. All authors contributed critically to the drafts and
736 gave final approval for publication.



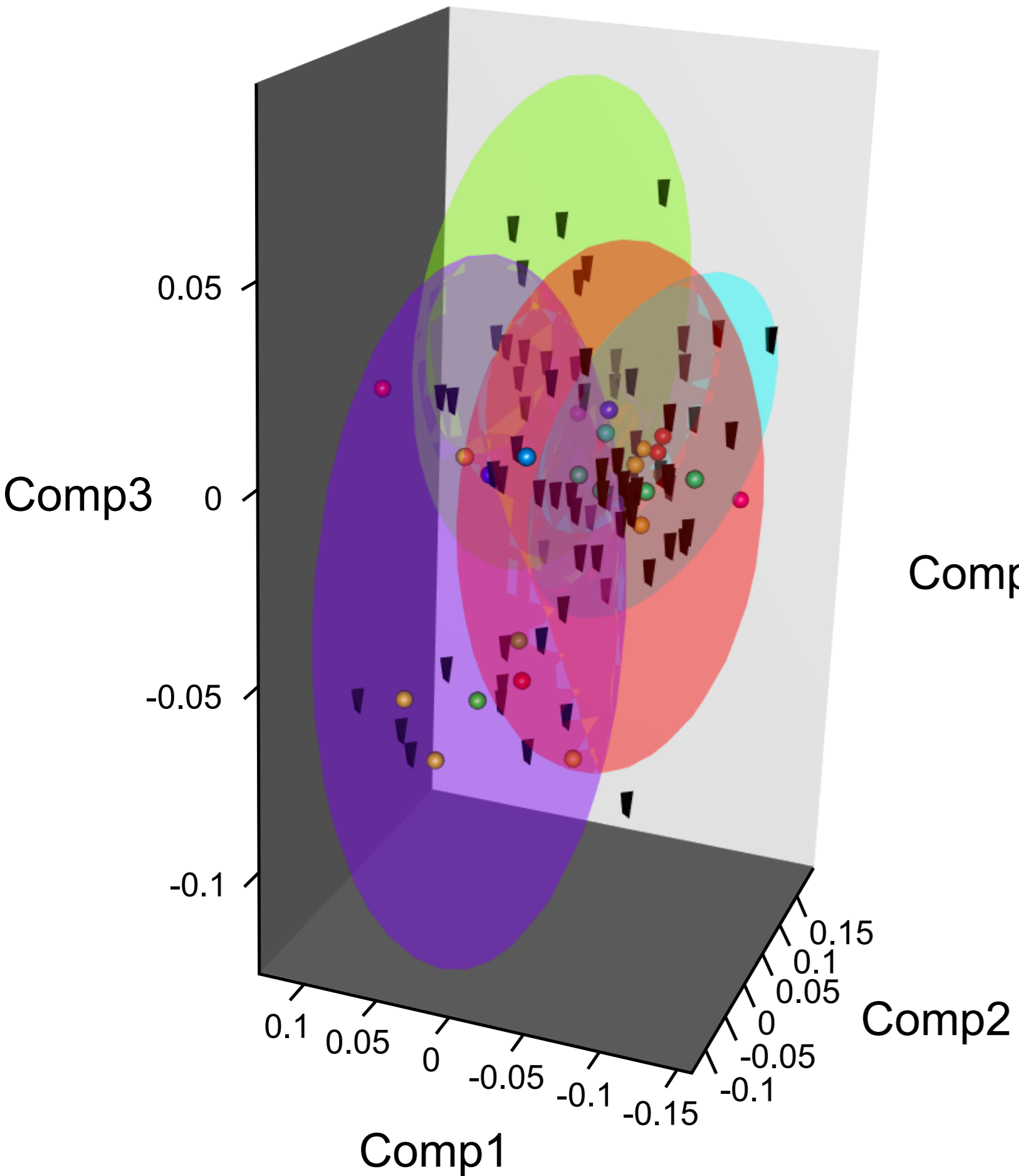




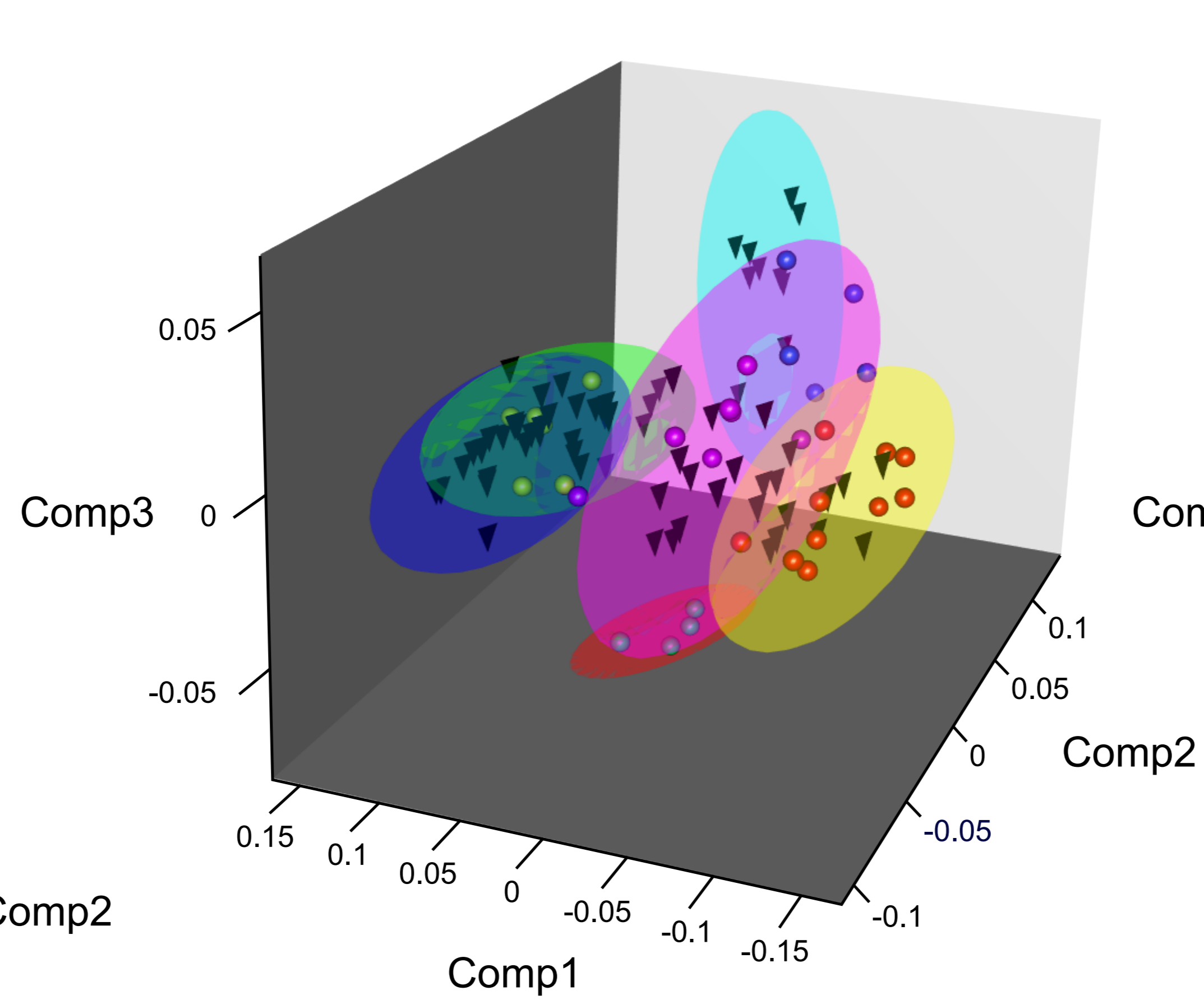




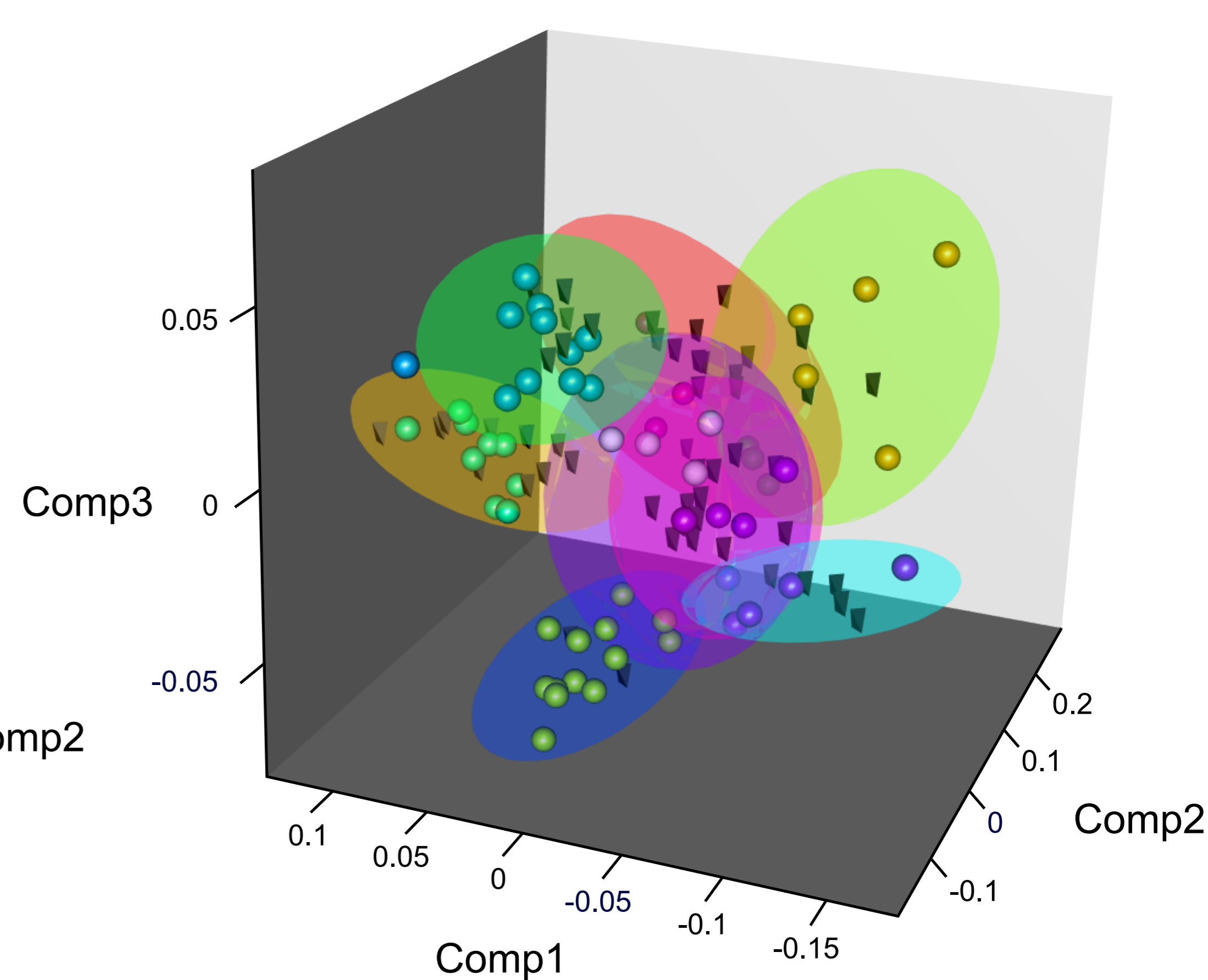
2015



2016



2017



Tables

Category	Acoustic parameter		Mean \pm sd	Min	Max
Pulse number	Number of pulses in G1	Pln.G1	15.88 \pm 3.51	8.0	25.0
	Number of pulses in G2	Pln.G2	2.47 \pm 0.55	2.0	4
Temporal	G1 duration (sec)	Dur.G1	0.31 \pm 0.06	0.18	0.47
	G2 duration (sec)	Dur.G2	0.031 \pm 0.009	0.016	0.054
	Duration between G1 and G2 (sec)	Sil1	0.38 \pm 0.03	0.33	0.48
	Duration between G2 and G3 (sec)	Sil2	0.09 \pm 0.02	0.048	0.14
	Pulse rate Median in G1 (sec)	Plr.G1	0.021 \pm 0.0031	0.0145	0.028
	Pulse rate median in G2 (sec)	Plr.G2	0.022 \pm 0.0034	0.0147	0.031
Pulse rate	Mean acceleration in G1	Acc.G1	22.51 \pm 24.32	-31.22	130.59
	nPVI in G1	nPVI.G1	5.42 \pm 3.98	1.56	28.76
	nPVI in G2	nPVI.G2	4.16 \pm 6.34	0	20.84
	Median of the first peak frequency in G1 (Hz)	Fq1.G1	1.12 \pm 0.06	0.97	1.30
Frequency	Median of the second peak frequency G1 (Hz)	Fq2.G1	2.25 \pm 0.11	1.93	2.50

Table 1. Acoustic parameters describing the acoustic structure of the male ptarmigan call.

Acoustic variables	Comp 1	Comp 2	Comp 3	Comp 4
Pln.G1	0.86	0.16	0.30	0.061
Pln.G2	0.69	0.24	0.40	0.051
Dur.G1	0.80	0.44	0.15	0.11

Dur.G2	0.58	0.17	0.18	0.013
Sil1	-0.45	0.42	0.53	0.22
Sil2	0.22	0.36	0.71	0.25
Plr.G1	0.48	0.29	0.63	0.22
Plr.G2	0.53	0.26	0.43	0.097
nPVI.G1	0.058	0.48	0.023	0.030
nPVI.G2	0.46	0.26	-0.19	0.49
Acc.G1	0.079	0.13	0.35	-0.45
Fq1.G1	0.34	0.69	0.47	0.020
Fq2. G1	0.76	-0.43	0.049	0.21

Table 2. Correlation between acoustic variable and PLS-DA components