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FACULTY OF BIOLOGY
Department of Vertebrate Zoology

Anna V. Klenova, Ilya A. Volodin

Practical guide for bioacoustics



Moscow University Press

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Translated from Russian by *E. V. Volodina*



Moscow University Press
2022

УДК [591.582:597/599](076.5)
ББК 28.671.32я73-5 + 28.693.3я73-5

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Practical guide for bioacoustics / A. V. Klenova, I. A. Volodin; transl. from Russian by E. V. Volodina. — Moscow: Moscow University Press, 2022. — 96 p.: ill. — (Electronic book for network distribution).

ISBN 978-5-19-011802-5 (e-book)

This is a practical guide for applying modern methods and approaches for measuring and analysis of the acoustic parameters in mammal and bird calls, along to principles of their recording in the field or under laboratory conditions. This guide contains a few practical tasks for call measuring and the comparative analysis of their acoustic variation. The practical tasks, containing detailed guidelines and comments, would help students to know more about the actual problems of bioacoustical research on particular examples during their individual work. The practical tasks are presented in the order as they should be fulfilled during the practical part of the «Bioacoustics of vertebrates» teaching course of the Department of Vertebrate Zoology, Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia.

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ISBN 978-5-19-011802-5 (e-book) © Moscow University Press, layout, 2022

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Introduction.

Essential terms and definitions of bioacoustics

What are animal calls and which main approaches can be used for their description and analysis

Everyday experience suggests that any sound can be described using three main parameters: duration (long or short), pitch (perceived by researcher call frequency characteristics) and loudness (perceived by researcher call intensity). For example, dog bark is short, low-frequency and loud, whereas dog whine is long, high-frequency and faint. These three groups of parameters are essential for describing the acoustic structure of any animal call.

Sounds are waves in their nature. Sound wave, as any oscillatory process, can be described via a combination of simple characteristics. Sound wave can be visualized with different ways by computer software.

How to measure call duration?

The simplest way to visualize sound is the *oscillogram* (= waveform), that graphically represents the relationship of call amplitude from time (Fig. 1). Oscillogram enables to measure the amplitude of sound wave and the oscillation period (T), the time space, during which the wave passes the entire cycle. Call fundamental frequency (= rate of oscillations) is the inverse value to the oscillation period $f=1/T$. The large oscillation period corresponds to the low values of call fundamental frequency, whereas the short oscillation period corresponds to its high values. By oscillogram, we can measure the entire call duration and sometimes also estimate call fundamental frequency (although it is difficult in most cases).

How to measure call fundamental frequency?

Any wave process can be presented as time-frequency spectrum using Fast Fourier Transform (FFT), the mathematical algorithm developed by Jean Baptiste Fourier at the beginning of 19th century. The FFT algorithm presents a time function as a sum of separate sinusoid and cosine waves with different amplitudes and frequencies, where spectrum of the summary function is equal to the sum of spectra of its' harmonic components. The FFT strongly simplifies the sound image, and enables to measure call frequency parameters.

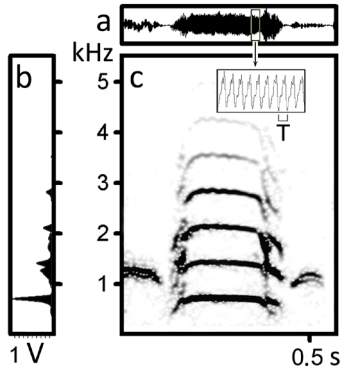


Figure 1. Three basic forms of sound visualizing — oscillogram (a), power spectrum (b) and spectrogram (c). T — oscillation period of sound wave.

Relationship between call frequency and time, created by FFT, is call *spectrogram* (Fig. 1). The unit for measuring call fundamental frequency is Hertz. One Hertz (1 Hz) is one sound wave oscillation per second; one kiloHertz (1000 Hz) is one thousand oscillations per second. Men's voice fundamental frequency is about 150–200 Hertz. In addition to the fundamental frequency, the spectrogram, as oscillogram, enables measuring the duration of entire calls or call parts (e.g., from the beginning of a call to its frequency maximum or from the maximum to the end of a call). Spectrogram is more informative than oscillogram.

How to measure call intensity?

Call intensity is determined by sound wave amplitude. On spectrogram, sound energy and, respectively, sound amplitude at particular frequency is as higher, as it is darker (blackier). Another way of sound visualization is *power spectrum*, displaying the dependence of intensity from call frequency, either at particular point of a call (single spectrum) or over the entire call duration (overall spectrum) (Fig. 1).

In contrast to call duration and frequency, which are absolute values, call energy is the relative value. As a rule, in call spectrum, the energy is distributed over a few frequency bands. The largest peak in the analysed time window is considered as zero, and the others are calculated relative to it to the negative side.

Call energy is commonly measured in decibels. Decibel means decimal logarithm of unsized ratio of two sound intensities. This is not an absolute value, as, e.g., seconds, Hertz or meters, but the relative value, as e.g., integer multiples («three-fold difference»), percent or propor-

tion, designed for measuring the ratios of two values of intensity, and to the obtained ratio, the logarithmic scale is applied. For subjective perception, increase of intensity for 10 dB approximately corresponds to increase the sound loudness twice.

Thus, there are three ways to visualize the sound wave: oscillogram, spectrogram and power spectrum (Fig. 1), which allow measuring three groups of sound parameters: time (in seconds), frequency (in Hz) and power (in decibels). Still recently, sound visualization demanded applying special devices. Now, progress of electronics and computer technique allows conducting the spectral analysis easily, fast and inexpensively. High-speed processor and sound card is all what is necessary for transforming a personal computer to powerful spectrograph.

Signals coming from microphone have analogous representation. For creating spectrograms on a computer, these signals should be digitized, that is, to receive the digital representation. This can be made using a special converter, which is a part of sound card or any digital audio recorder.

Analogous signals are continual by frequency and amplitude. This means that, at any time moment, sound amplitude can be measured with unlimited precision, in contrast to the digital signal representation, consisting of a number of separate measurements, which can only be setup using a limited number of different discrete values (11025, 22050, 44000, 48000 etc.). The number of sound wave measurements per second is termed sampling rate and measured in kHz. If sampling rate is high enough, the original analogous signal can be created again without any sufficient mistakes. Sampling rate should exceed the maximal fundamental frequency of the analogous sound signal at least

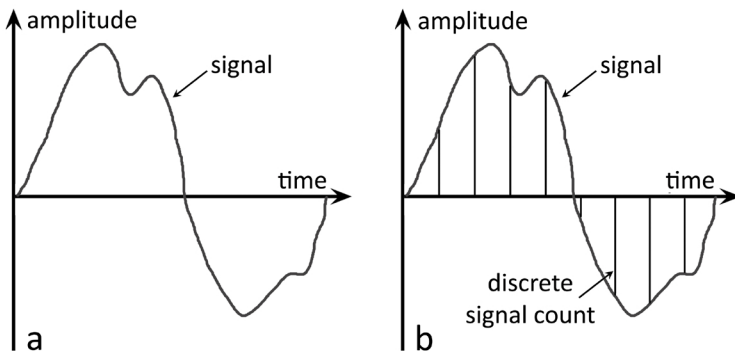


Figure 2. Analogous (a) and digital (b) ways of sound representation.

twice. The Fig. 2 illustrates the differences between the analogous (a) and digital (b) ways of sound representation.

Parameters for creating spectrogram

Spectrogram and information which can be extracted on its basis, are predetermined by parameters used for creating the spectrogram. These parameters determine resolution of call acoustic structure in time and frequency domains. Choice of parameters for creating spectrograms is always a trade-off, because, if you need the high resolution of frequency scale, you cannot receive respectively high resolution on time scale (keep in mind that $f=1/T$). So, you should decide in advance, whether you need the high resolution in frequency or high resolution in time, accordingly to the particular bioacoustical task which should be resolved.

When describing parameters for creating spectrogram, we will illustrate them by examples using the interface of the professional bioacoustical software Avisoft-SASLab Pro (www.avisoft.com). However, selection of parameters for creating spectrograms can be done similarly using any other professional software for sound analysis. Avisoft has the following options for creating spectrograms.

Sampling frequency

Sampling frequency sets the upper limit of call spectrum and thus determines the maximum frequency visible on spectrogram. Sampling frequency restricts the maximum frequency of the digitized signal to the half of its value. The higher is sampling frequency, the higher-quality digitizing will be achieved. Based on Kotelnikov theorem, sampling frequency for precise re-storing the original signal should exceed two times the maximum frequency of interest of the signal. Transformation of the analogous signal to the digital signal includes two stages: time discretization and amplitude quantization. Time discretization means that the signal is represented as a set of scores (samples), taken at equal time intervals. For example, when we say that sampling frequency is 44.1 kHz, this means that the signal was measured 44100 times per second.

FFT-length

The FFT (Fast Fourier Transform) length, together with frame size and window type, determines spectrogram frequency resolution. The FFT value reflects at which values two close-frequency components of the signal still can be separated. Larger FFT values smooth the structures of transitional signals, so that a few close-time tonal impulses cannot be separated. In other words, time resolution will be poor.

Frame size

The influence of frame size on the spectrogram image can be compared with the influence of FFT length. A smaller frame size results in the lower frequency resolution and the higher time resolution. Theoretically, FFT spectrum length of 512 points, combined with frame size 50% would result in the same spectral resolution as the spectrum with 256 points and frame size 100%. The only difference would be that, at larger FFT length, spectrogram will have a higher resolution for the account of the larger number of spectral lines.

Window type

The FFT algorithm provides precise spectra only if time section of audio recording overlaps the total number of periods in the periodic signal. This is a very special case, not reported in practical guidelines. If this demand was not met, the resulting spectrum will be thumb with false high frequencies (spectrum spread). For diminishing this effect, smoothing windows can be used, which decrease the amplitude near the edge of the recording section. There are different types of windows, influencing differently spectrum spread and frequency resolution. The Hamming Window has the best characteristics for most cases: good suppression of spectral spread and good frequency resolution. The FlatTop Window is distinctive by a larger frequency range with relatively flattened transmission characteristics. The FlatTop Window provides a high

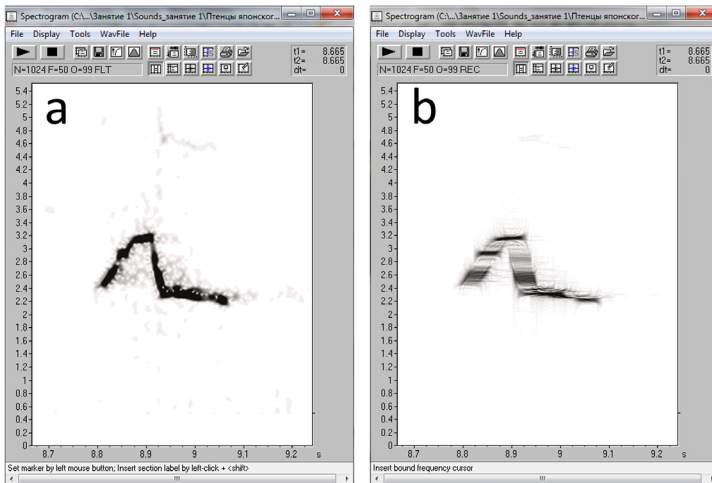


Figure 3. Influence of window type on the spectrogram image: a — FlatTop window and b — Rectangle window.

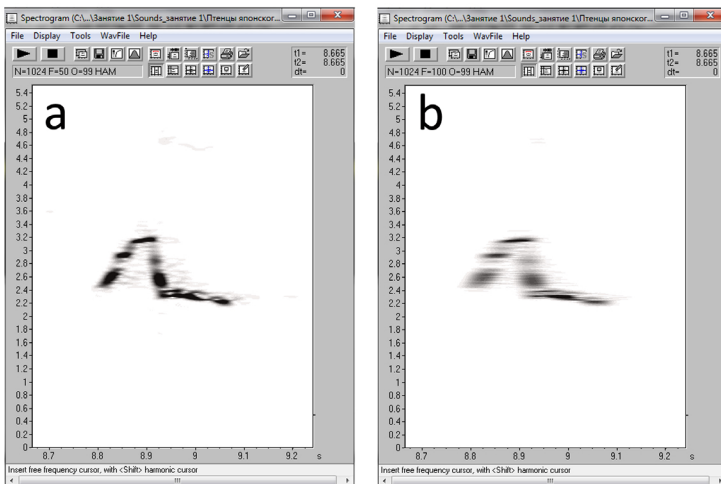


Figure 4. Influence of Overlap on the spectrogram image: a — overlap 50 % and b — overlap 100 % at the same Hamming type of window.

time resolution for the account of frequency resolution. This window allows creating spectrograms, similar with broadband spectrograms generated by dynamic spectrographs with wide filter. Please compare two figures: the first spectrogram was created with FlatTop Window, the second one was created with Rectangle Window (Fig. 3).

Overlap

Spectrogram image represents an ordered picture of single spectra. For each spectrum, a restricted part (time section) of the entire time signal is taken. Sequential time sections can be organised in overlapping mode, what means that the same part of time signal is used for a few sequential spectra. Such overlap provides a higher time resolution, as the number of spectra per second is increased. The value of overlap should be adapted to the type of window and frame size. Small frame sizes need a high overlap, because otherwise some parts of time signal will not be present in some spectra of the particular spectrogram. Please compare two spectrograms on the Figure 4: both of them are created with maximal window size, but the first one is created with overlap 50 %, and the second one with overlap 100 %.

Task 1.

Manual measurements of essential time-frequency parameters with Avisoft-SASLab Pro. Statistical analysis of acoustic variability of the calls

Study objects

Chicks of red-crowned crane, *Grus japonensis* (monogamous territorial species) and ancient murrelet, *Synthliboramphus antiquus* (monogamous colonial species) will be used as objects of this task.

Aims of the work

- 1) to measure time, frequency and power parameters of suggested calls of 8 individual chicks of the two bird species using the reticule cursor and the standard marker cursor for estimating variability of chick calls of each species;
- 2) to conduct a statistical analysis of the measured parameters for estimating the individual and sex-based variation of the calls.

Introduction. Why these two bird species were selected for comparison?

Red-crowned crane, as other Gruidae species, is strictly monogamous: a parental pair raises one or rarely two chicks per breeding season. For the first three months of chick life, parents stay on their family territory together with their offspring and defend the territory from other cranes. Members of own family cannot be therefore confused with alien cranes. The calls suggested for analysis were recorded from chicks of about one month of age, that is, during the season when crane family is strictly territorial. So, we can predict that individual differences of chick calls might not be well-expressed at this age. Red-crowned crane chicks produce calls of four structural classes: trills, whistles of two types, differing in the contour of frequency modulation, and intermediate calls between trills and chirps (Fig. 5).

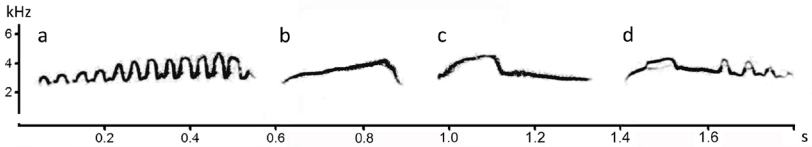


Figure 5. Spectrograms of four structural call classes of red-crowned crane chicks: trills (a), chirps C1 (b) and C2 (c), and intermediate calls between trills and chirps (d).

The suggested for analysis chirps C2 were emitted by the red-crowned crane chicks in various contexts, for instance during food begging, pre-flight activity or in other contexts including active movements. Chirps C2 are most widespread calls of red-crowned crane chicks, comprising 45–90% of all sound signals depending on the ontogenetic stage. During the first 8–12 months of chick life, time and frequency parameters of chirps C2 are kept practically unchanged in spite of the over 40-times body mass gain (these chirps retain their high juvenile fundamental frequency exceeding about three times the adult fundamental frequency). At 8.5–11 months of age, both sexes experience voice breaking with rapid (within a few weeks) changes of the high-frequency chick-like calls to the low-frequency adult-like calls.

Ancient murrelet is a colonial seabird from Alcidae family. In contrast to many other seabirds, ancient murrelets use colonies only for incubation. The hatched chicks depart their nest burrows in the second

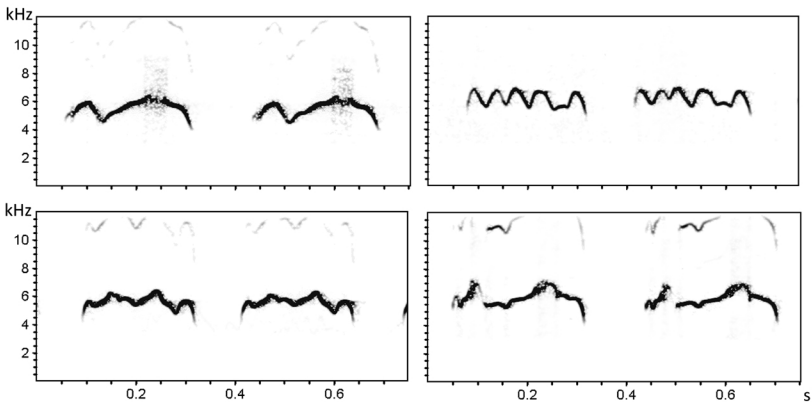


Figure 6. Spectrograms of the individualistic chirps of four different ancient murrelet chicks, two chirps per chick.

night and then are fed by their parents in the sea. Mass synchronous departure of thousands of chicks from a colony occurs at nights, to avoid predation from numerous diurnal raptors. Ancient murrelets have poor nocturnal vision, so, the acoustical channel is a single communicative channel between chicks and parents. Both parents and chicks have individually distinctive “signature calls”, differing by their unique frequency modulation, which enable unmistakable recognizing each other among thousands of conspecifics. Chicks of ancient murrelet, commonly emit their individualistic chirps in series of 2–8 calls, what probably allows to increase reliability of individual recognition. The suggested for analysis calls were recorded in a natural colony of ancient murrelets in the Talan Island (Okhotsk sea) during the mass chick departure (Fig. 6).

Important!!! *As example for illustrating acoustic variation, in this task, we will only talk about individual and sexual variation of calls. Other modalities of acoustic variation (age-related, context-related, population-specific etc.) can be studied with a similar way.*

Task for the practical work.

Part 1. Acoustics

The calls suggested for analysis were recorded at a distance 2–15 m to microphone from 8 individual 40–65-day-old red-crowned crane chicks and from 8 individual 2-day-old ancient murrelet chicks. Audio files of these calls are available at link http://bioacoustica.org/practicum/practicum_eng.html, the file names correspond to individual chicks; each file contains calls of only one individual chick (for the red-crowned crane, chicks 1–4 are females, 5–8 — are males; the ancient murrelet chicks are unsexed). The inter-call intervals were artificially shortened for analysis convenience; some recordings of ancient murrelet chicks contain also calls of conspecific adults.

For creating call samples, you should measure 10 calls per chick, 80 calls per species. Please select calls randomly from different parts of recordings avoiding to take calls following each other. For ancient murrelet chicks, preferably take one call per series selecting one of the last calls of a series, as the calls at the end of the series are commonly more intense and thus higher-quality. The random choice of calls for analysis decreases the potential effect of pseudoreplication because of the influence of the acoustic structure of a preceding call to the following call.

Step-by-step guide to the work with Avisoft-SASLab Pro

This section describes the measuring of the essential acoustic parameters and their export to external softs with a professional bioacoustical software Avisoft-SASLab Pro (www.avisoft.com). Below, you will find the instructions for selecting the settings for creating spectrograms of desirable image, for selecting call parameters for measuring, and for preparing the tables for saving the exported measurements.

1. Open Avisoft, then open one of suggested files, for example, file «g1». You will see 46 calls of one individual red-crowned crane chick. The calls are shown in two windows: as waveform (oscillogram) (Fig. 7a) and as spectrogram (Fig. 7b). Above, there are working buttons of Avisoft. The first three buttons enable audio recording (Fig. 7 (1)), playback of the existing recording (Fig. 7 (2)) and interruption of recording or playback (Fig. 7 (3)). In the current task, files for analysis are already prepared, so it is unnecessary to make an audio recording. You can listen the calls from this file by pressing the button «Playback of marked section» (Fig. 7 (2)). By placing the mouse to each button, you can read its function in the box below the spectrogram window. Using the mouse cursor, you can label some file fragment and then, with double press of the left key of the mouse on this fragment, to zoom it to all screen. For the same purposes, you can also use the 5 working buttons of the software (Fig. 7 (4)).

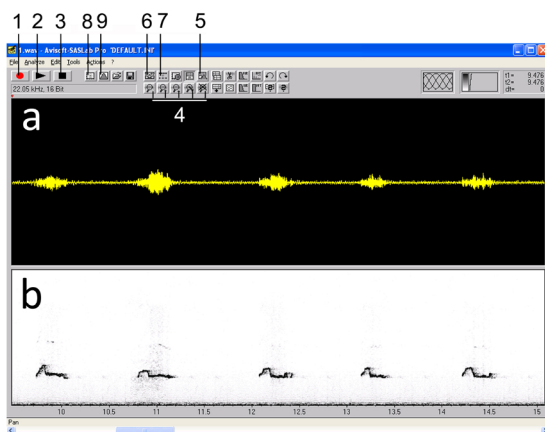


Figure 7. Main window of Avisoft-SASLab Pro: a — waveform (oscillogram), b — spectrogram. The mentioned in the description working buttons are marked with numbers.

2. Using the «Overview Display Parameters» button (Fig. 7 (5)), you can change the spectrogram settings in the main window for more convenient visual inspection of audio recording. Try to change each parameter of the settings separately and then observe, how this change modifies the spectrogram image.

3. Press the button «Sound card settings» (Fig. 7 (6)). You will see, that Sampling frequency of the given file is 22050 Hz. This means that spectrogram allows analysing frequencies up to 11025 Hz. However, average call frequencies of red-crowned crane chicks are substantially lower, about 3000 Hz. So, for increasing the spectrogram frequency resolution, it is convenient to downsample the sampling frequency from 22050 Hz to 11025 Hz using the button «Sampling frequency conversion» (Fig. 7 (7)). Respectively downsample for analysis the calls of the ancient murrelet chicks from 48000 Hz to 22050 Hz (because in this species, chick calls are higher-frequency than in the red-crowned crane chicks).

Important!!! *Do not forget to change sampling frequency each time after a file opening. Calls of all individual chicks should be measured at the same frequency resolution.*

4. The opened main window of Avisoft (displaying waveform and spectrogram) is convenient for conducting the visual inspection of large call massive. However, conducting measurements is impossible in this window (for the exclusion of the overall call duration). For measuring other call parameters, you would need to use the spectrogram window. Press the button «Create spectrogram» to open the spectrogram window (Fig. 7 (8)). If you want to create spectrogram of the entire audio file, delete preliminary the labelling the file to fragments; otherwise, the spectrogram will only be created for the labelled fragment. By-default parameters for creating spectrogram can be inappropriate for the analysed calls. As a rule, for each particular case, the settings for creating spectrogram should be selected manually. To select parameters for creating spectrogram, close the already created spectrogram and press the button «Spectrogram parameters» (Fig. 7 (9)). Try to change the settings of the three parameters (FFT-length, Frame, Overlap) and then observe how this will change the spectrogram image. Select those spectrogram parameters, which, on your opinion, are most appropriate for analysis of the given call type. Two most convenient sets of parameters for analysis of red-crowned crane chick calls are shown on figure 8.

Important!!! *Write-down the parameters which you selected for creating the spectrogram, as well as the values of time and frequency resolution.*

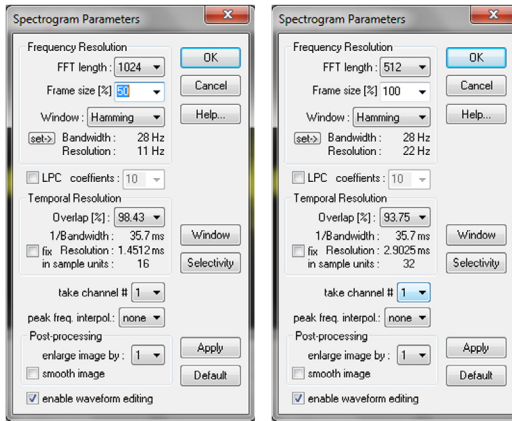


Figure 8. Example sets of parameters for creating spectrograms enabling the accurate measurements of red-crowned crane chick calls.

tions which these parameters allow to reach. This will allow to reproduce the measurements at any time with the same resolution and precision.

5. Open the spectrogram window to start measuring call parameters (Fig. 9). You can change the brightness and contrast of spectrogram by using the button «Display parameters» (Fig. 9 (1)). You can also obtain additional information about the spectrogram, by using the button «Additional Spectrogram Information» (Fig. 9 (2)). Please train yourself,

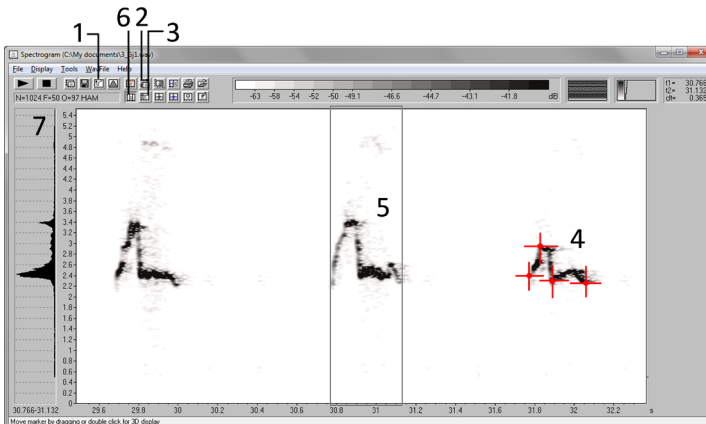


Figure 9. Spectrogram window in Avisoft-SASLab Pro. Working buttons mentioned in the description are marked with numbers.

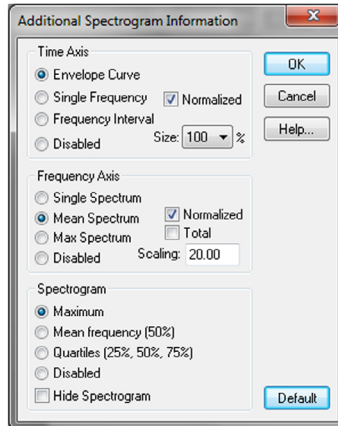


Figure 10. A possible set of additional parameters which can be used for creating spectrograms for accurate measuring of the acoustic parameters of red-crowned crane chick calls.

by changing each setting and observing how each change modifies the spectrogram image. Finally, select the combination of settings shown on figure 10. Checkmarking the «Maximum» checkbox of «Spectrogram» option (Fig. 10) will display a red line showing the positions of call frequency maxima at each time moment (Fig. 11). This option allows using the reticule cursor during manual measurements in cases when frequency contour is not enough clear, to make more precise measurements of frequency modulation on spectrogram.

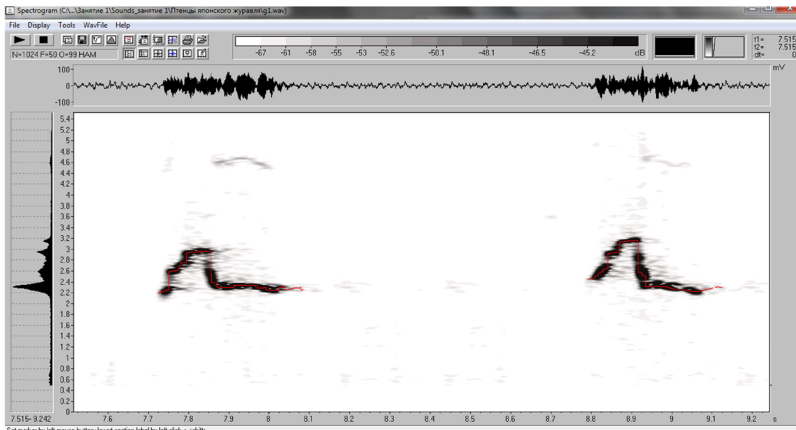


Figure 11. Spectrogram window in Avisoft-SASLab Pro, ready for analysis. Red line on the screen indicates the points of call maximal intensity at each moment of time.

6. Go to spectrogram window menu (Display → Lower Cut-Of Frequency). This option provides filters for cutting off the irrelevant to current analysis call frequencies, such as background noise higher or lower the frequency spectrum of interest. As chick calls of both species are higher than 1 kHz, the frequencies below 1 kHz are irrelevant to analysis, so you can apply the high-pass filtering to cut off the frequencies below 1 kHz.

Please also pay attention to another checkbox from the menu of the spectrogram window: Display → Absolute Time Scaling. If this checkbox is marked, time scale of the spectrogram window corresponds to the time scale of the main window of Avisoft. That is, if you created the spectrogram of a selected fragment within file (not spectrogram of the entire file), the time scale of the spectrogram window will not start from zero. If this checkbox is unmarked, time in the spectrogram window will start from zero. It is better to put this checkmark, and then you will easily determine from the transferred to Excel time scores, which calls were measured in each file.

7. Now you made the settings for the spectrogram window and all is ready for call measuring. Spectrograms of chick calls are created at good resolution in time and frequency, the alien noises are filtered out, all necessary information is put on the screen (Fig. 11). Your next step is to select the parameters for acoustic analysis. In this practical task, you will make the manual measurements of acoustic parameters using the «Free moveable reticule cursor» (Fig. 9 (3)). At clicking this cursor at any point of spectrogram image, three scores will be copied to the exchange buffer at the point of crossing the cursor lines: coordinates of time and frequency as well as call energy. By clicking the cursor on the beginning and then on the end of a call, you can calculate additional parameters, for example, the total call duration, the fundamental frequencies at the beginning and at the end of a call. Now, we should select the particular call points, whose coordinates we will measure in each call during this practical task.

Important!!! *For measuring the calls of the red-crowned crane chicks, you should select the following 4 points for measuring with the reticule cursor: the point of call beginning, the point of call fundamental frequency maximum, the point of the first extremum (the first inflection) of frequency contour after the call fundamental frequency maximum, and the point of call end (Fig. 9 (4)). For the ancient murrelet chicks, you should select the following 4 points for measuring for obtaining the comparable data with crane chicks: the point of call beginning, the point of call fundamental frequency maximum, the point of the following this maximum*

first extremum (first inflection after the maximum) and the point of call end. Selection of the same parameters sets (numbers and modalities) justifies conducting a comparison of the degree of acoustic variation between species.

8. You can also make another measurement for each study species: calculation of the dominant frequency of the entire call (the frequency of call maximum intensity). For doing this, please mark the entire call (Fig. 9 (5)) with the Standard marker cursor (Fig. 9 (6)), and then click twice with a mouse on the call power spectrum (Fig. 9 (7)). Another Avisoft window the Power spectrum window will be opened, where X axis indicates call frequency and Y axis indicates call amplitude (sound energy) at the given time moment (Fig. 12).

In the power spectrum window menu, put the checkmark in the checkbox Display → Special Characteristics. In the appeared window (Fig. 12), you can select parameters which Avisoft will measure from the labeled by you time section (it can be either the entire call or call fragment): dominant frequency («Maximum at Freq.»), quartiles («Quartiles»), the wide of the frequency peak («Bandwidth») and its lower and upper borders («min Frequency» and «max Frequency») at the arbitrary given distance from the frequency maximum («Threshold»). In this practical task, we will only need one power spectrum parameter, the dominant frequency. To solely copy the dominant frequency to the exchange buffer, please put the checkmark only in the checkbox «Peak

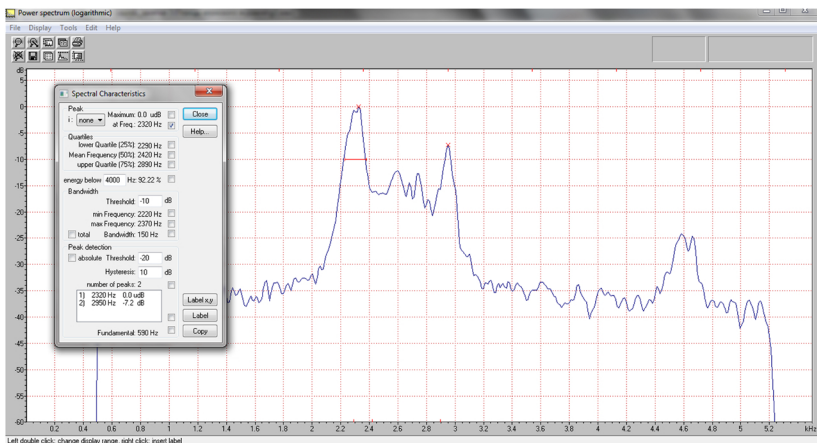


Figure 12. Power spectrum window in the Avisoft-SASLab Pro. The window for measuring power parameters is shown on the left.

at Frequency» (Fig. 12). Press «Copy» for copying the measured value of the selected parameters to the exchange buffer. If you will fill out the window «Special Characteristics» as it is shown on figure 12 and then press the button «Copy», only one value will be copied to the exchange buffer (2320 Hz in this particular case).

9. The next step is to prepare an Excel file to which Avisoft-SASLab Pro will automatically copy your measurements. Please open a new Excel table and save it (file name should be in not more than 7 Latin letters). For convenient analysis of the obtained measurements, please prepare the table with the following way: all measurements taken from a call should be in one line of table, all measurements from the next call should be in the next line of table etc. The Fig. 13 displays an example table, which format is convenient for the purposes of the given practical task.

First three columns of this table should be filled manually. The first column contains chick individual numbers (IDs ##1–8), the second column contains chick sex (males are indicated with digit 1, females with digit 2, so, female red-crowned crane chicks IDs ##1–4 should be marked with digit 2, whereas male red-crowned crane chicks IDs ##5–8 should be marked with digit 1). The third column contains the sequential number of a call from each chick (you should measure at minimum 10 calls per chick). Next 13 columns of the table will be automatically filled by Avisoft-SASLab Pro. First 12 of them will contain the values copied to the exchange buffer when you press the reticule cursor on each of the described above call points (indicating fundamental frequencies at the beginning, maximum, inflection point and end of the call). A click of the reticule cursor on any point of spectrogram will always copy to the exchange buffer the all three measurements in one time. The measurements will be always recorded in the same order: first the coordinates on the *X* axis (time axis); second the coordinates

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	Chick N	Sex	Sound N	T_beg	F_beg	E_beg	T_max	F_max	E_max	T_ext	F_ext	E_ext	T_end	F_end	E_end	F_peak
2																
3																
4																
5																
6																
7																
8																
9																

Figure 13. Example of table ready for the automatic data export from Avisoft-SASLab Pro.

the Y axis (frequency axis); and third the coordinates on the power axis (reflected on spectrogram by gradations of grey color). Finally, in the last (13th) column, the measurements of the dominant frequency of the entire call will be written (the parameter which you measured separately in the power spectrum window).

Important!!! *Be careful: you can only conduct the measurements in the strict order (for example, you can at first conduct the measurement with the reticule cursor and then measure the dominant frequency; the illustrative table on Fig. 13 just indicates this order). The selected order of measurements cannot be changed during the work; all call parameters should be measured in the same order! Otherwise, your table with measurements will turn to be a senseless set of digits.*

10. Finally, you should connect the Avisoft and Excel and indicate, to which Excel file and to which lines of the Excel table the Avisoft should copy your measurements. To make this, go back to the spectrogram window and select from menu: File → Data export → DDE-Parameters/Log-File. If your Excel table is looking in the same way as the table on Fig. 13, the appeared window should be filled with the following way (Fig. 14):

- 1) fill the field Topic (name and extension of your Excel file);
- 2) fill the field Item — as it is necessary to indicate the coordinates of the cell of your table, to which Avisoft should copy the first measure-

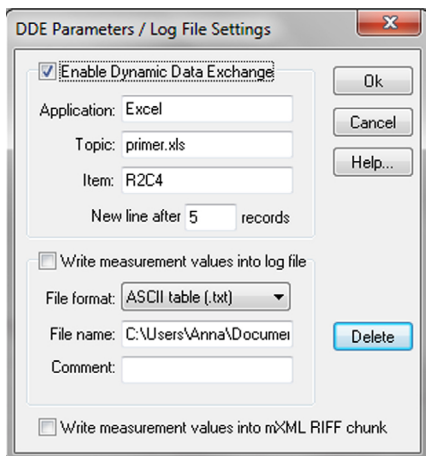


Figure 14. Avisoft-SASLab Pro window enabling external export of measurements. The way how this window has to be filled corresponds to the learning purposes of practical Task 1.

ment (R — line, C — column); the further measurements Avisoft will copy to the same line, one after another;

3) indicate after how many clicks (= measurements), Avisoft should hop to another line (that is, how many measurements of each call you are going to do); in our example table it is after five measurements (of the five measurements, four we conduct with the reticule cursor and one in the power spectrum window).

11. Now, after making all necessary settings, you can start the measurements. Measurements of calls would be better to make in a separate table for each species or on separate pages of one table.

Task for the practical work.

Part 2. Statistics

After measuring a sufficient number of calls per chick, come to the next part of work, to evaluate the degree of individual and sexual variation in the two study species. Let's consider a few most widespread statistical methods for estimating acoustic variation (including the individual variation of animal calls).

One simple method is calculating coefficients of variation (CV), measured in percent by formula $CV = (SD/Mean) * 100\%$, where *SD* is standard deviation and Mean is the average value of the measured parameter values. You can also calculate the ratio of CV between individuals to CV within individual, to estimate how strongly interindividual variation exceeds the intraindividual variation. However, coefficient of variation only enables comparing the intra- and interindividual variation for each call parameter and for each individual separately, but CV does not allow to evaluate individual variability on the basis of the entire set of the measured parameters.

More precise statistical methods for estimating acoustic variation are ANOVA, MANOVA, DFA (Discriminant Function Analysis), cluster analyses, classification tree methods etc. ANOVA and MANOVA enable estimating the effects of one or a few factors (in our example, the factors are chick individual identity and sex) on dispersion of the normally distributed random value, observed at different factor values (in our example study, the random value is represented by the values of one of the measured call parameters). ANOVA and MANOVA enable to compare variation of each of the measured parameters for calls of each individual animal with variation of the random value in calls of all individuals. ANOVA and MANOVA calculate Fisher's criterion (*F*-ratio) for each of the measured call parameters and allow to estimate, which

call parameters are most individual-specific (that is, possess by weak intra-individual and high inter-individual variability). However, ANOVA or MANOVA-based methods have the same problem as CV-based method, as they deal with each parameter separately and cannot consider a few call parameters in combination.

As the number of call parameters measured during a study is often large, so, for estimating the individual differences would be convenient to use the methods of multiparametric statistics, which estimate the effect of the whole set of parameters. Cluster analysis is one such method. Cluster analysis is designed to search a similarity between the analysed objects (calls in the given case). Objects are considered the more similar, the less are the differences between the matched parameters (measured call parameters in this case). For determining the spatial closeness of a pair of points in multidimensional space, most used option is the Euclidean distance, which is equal to square root of the sum of squares of differences in values of matched parameters, taken for the given pair of objects. Among deficiencies of cluster analysis is a substantial limitation on the number of analysed objects (on the number of calls in this case).

One of the most common and powerful statistical methods used for estimating the individual and sexual variations is discriminant analysis. For applying this analysis, a researcher should select the analysed objects (all massive of calls in this case), parameters, characterizing these objects (all acoustic parameters in this case), and number of groups, to which it is necessary to subdivide the analysed object (number of individuals in this case). The aim of discriminant analysis is to calculate $(n-1)$ of linear functions, which maximize the probability of correct assigning the objects to n groups (to maximize the probability of correct assigning the analysed calls from each individual to a particular group, in this case). Due to the general estimating all parameters of analysed objects, discriminant analysis currently is mostly used statistical method, applied in studies of vocal individuality. However, the results of discriminant analysis (percentage of correctly assigned calls) strongly depend on the number of groups (individuals), calls, included in analysis, and selected by researcher parameters, included in analysis, so, they should be estimated (by using an especial randomization procedure) for the degree of significance. Limitations of discriminant analysis and the ways of their estimation and testing the results will be considered below, in the practical Task 2.

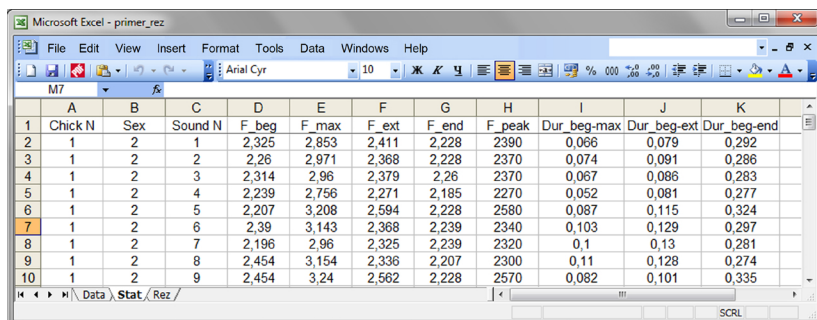
Step-by-step guide to the work with STATISTICA and The R software

In this section, we describe the way of preparing data tables, based on the results of a preceding task, on measuring of time-frequency parameters in calls of chicks of two species, for further statistical analysis with STATISTICA (<http://statsoft.ru/>) or The R (<https://www.r-project.org/>). Instructions for conducting discriminant analysis with each of these software and calculating the average percent of correct assignment of calls to individuals (for red-crowned crane chicks to both individual and sex) are given below.

STATISTICA

1. Prepare your Excel table with measurements for the statistical analysis. Let's start with the values of time parameters (as the reticule cursor copies to the exchange buffer only coordinates of respective points, the durations of different call parts might be calculated on the basis of the obtained measurements manually). Using simple formulas available in Excel, you can calculate, for example, Dur_beg-max (duration from the beginning of a call to the point of call maximum fundamental frequency) = $T_{max} - T_{beg}$; Dur_beg-ext (duration from the beginning of a call to the inflection point of call contour) = $T_{ext} - T_{beg}$; Dur_beg-end (entire call duration) = $T_{end} - T_{beg}$.

After calculating these time parameters, you can delete the already unnecessary columns with time and power coordinates (before deleting them, please obligatory save the Excel table under a new name, in order to return to the initial file in case of mistake, and change the introduced formulas to the values). Example of table prepared for the statistical analysis is present on Figure 15.



	A	B	C	D	E	F	G	H	I	J	K
1	Chick N	Sex	Sound N	F beg	F max	F ext	F end	F peak	Dur_beg-max	Dur_beg-ext	Dur_beg-end
2	1	2	1	2,325	2,853	2,411	2,228	2390	0,066	0,079	0,292
3	1	2	2	2,26	2,971	2,368	2,228	2370	0,074	0,091	0,286
4	1	2	3	2,314	2,96	2,379	2,26	2370	0,067	0,086	0,283
5	1	2	4	2,239	2,756	2,271	2,185	2270	0,052	0,081	0,277
6	1	2	5	2,207	3,208	2,594	2,228	2580	0,087	0,115	0,324
7	1	2	6	2,39	3,143	2,368	2,239	2340	0,103	0,129	0,297
8	1	2	7	2,196	2,96	2,325	2,239	2320	0,1	0,13	0,281
9	1	2	8	2,454	3,154	2,336	2,207	2300	0,11	0,128	0,274
10	1	2	9	2,454	3,24	2,562	2,228	2570	0,082	0,101	0,335

Figure 15. Example of table prepared for statistical analysis with STATISTICA.

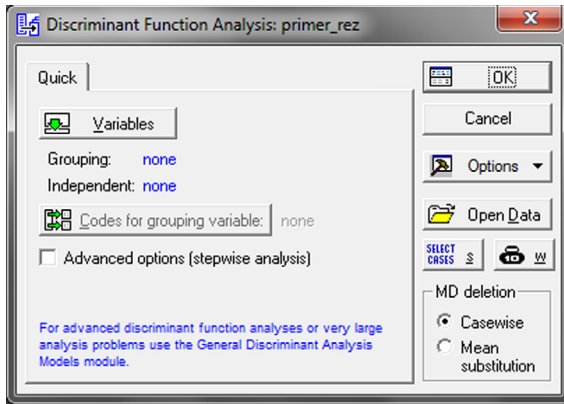


Figure 16. Window in STATISTICA providing options for selecting the grouping variable and call parameters for conducting discriminant analysis.

2. Open the prepared table with STATISTICA software. To do this, please checkmark File → Open in the main menu; checkmark «All Files» in checkbox «File type» and open the table. In the pop-up window, checkmark «Import selected sheet to a Spreadsheet» and label the page where there are data for the statistical analysis. In the next opened window, put a checkmark in the «Get variable names from first row» checkbox and press «Ok». Your table will be opened in the main window of STATISTICA.

3. To run the discriminant analysis procedure for analysis of individual-based acoustic variation, please select from the main menu Statistics → Multivariate Exploratory Techniques → Discriminant Analysis. A window will appear, in which you will need to indicate to how many groups STATISTICA should subdivide the analysed objects (calls) and also indicate the variables (call parameters), which will serve for classifying the calls to the predicted number of groups (Fig. 16).

Press the button «Variables». Select a column encoding individual identity of the calls in the box «Grouping variable». Select a set of columns with measured parameters in the field «Independent variable list» (Fig. 17). Press «Ok».

Press the button «Codes for grouping variables», select «All» and press «Ok». The button «Select Cases» in the right part of window (Fig. 16) can be used for selecting the groups to which you are going to apply the discriminant analysis procedure. For example, if you would like to include in analysis only the calls of males or females, you can, with appropriate choice of these settings, calculate the values of correct assignment of the calls to each sex separately.

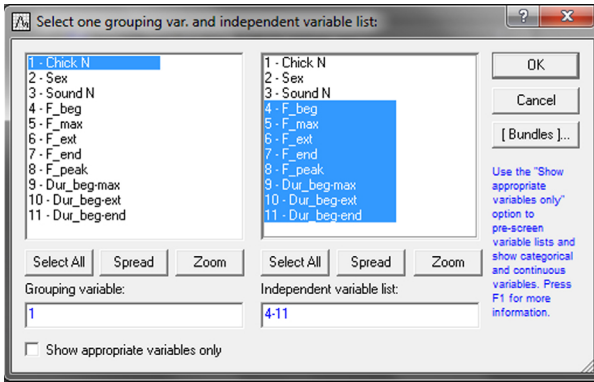


Figure 17. Window in STATISTICA displaying how to select the grouping variable and measured parameters for conducting discriminant analysis suggested in this task.

Checkmark the «Advanced options» checkbox (Fig. 16), press «Ok». In the checkbox «Method» select «Forward stepwise». Press «Ok» again. You have run the discriminant analysis procedure.

4. Let's consider the obtained results of the discriminant analysis. If you have run discriminant analysis successfully, you will see the window with its results. The simplest way of presenting the results is calculating percentages of correct assignment of calls to particular individuals. Select the box «Classification», and then press the button «Classification Matrix». STATISTICA will show you the table with the results (Fig. 18).

Classification Matrix (primer_rez)

Rows: Observed classifications

Columns: Predicted classifications

Group	Percent	G. 1.1	G. 2.2	G. 3.3	G. 4.4	G. 5.5	G. 6.6	G. 7.7	G. 8.8
Correct	p=,12500	p=,12500	p=,12500	p=,12500	p=,12500	p=,12500	p=,12500	p=,12500	p=,12500
G. 1.1	100.0000	10	0	0	0	0	0	0	0
G. 2.2	100.0000	0	10	0	0	0	0	0	0
G. 3.3	100.0000	0	0	10	0	0	0	0	0
G. 4.4	100.0000	0	0	0	10	0	0	0	0
G. 5.5	80.0000	0	0	0	2	8	0	0	0
G. 6.6	100.0000	0	0	0	0	0	10	0	0
G. 7.7	100.0000	0	0	0	0	0	0	10	0
G. 8.8	100.0000	0	0	0	0	0	0	0	10
Total	97.5000	10	10	10	12	8	10	10	10

Figure 18. Window in STATISTICA displaying classification matrix of discriminant analysis, obtained for 80 calls of 8 individual red-crowned crane chicks calculated on the basis of the 8 time-frequency parameters of the calls.

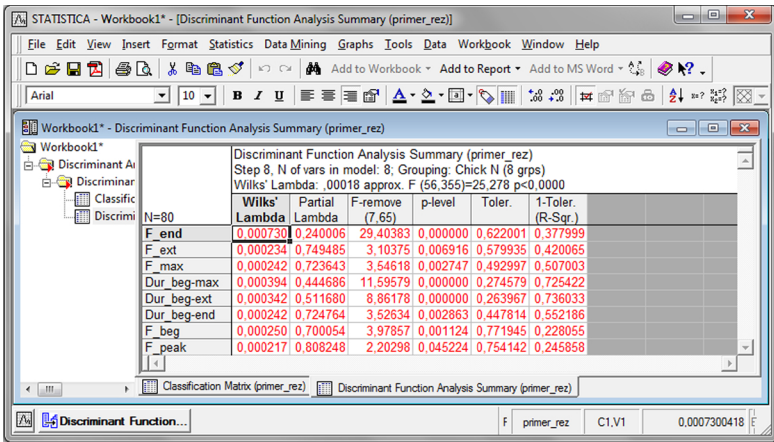


Figure 19. Window in STATISTICA displaying parameters included in discriminant analysis and percentages of their contribution to classification.

For instance, in the example given on Fig. 18, the average percent of correct assignment of calls to individual is 97.5%. Among individuals, the percent varies from 80 (in Chick #5) to 100% (in all remaining chicks). You can see that 2 calls of Chick #5 were classified inaccurately (being assigned to a group of calls of Chick #4).

Additionally to calculating percentage of correct assignment, discriminant analysis shows the contribution of each parameter to accurate classification (that is, it shows which parameters are more individual-specific and which are less). In order to see this, please return to the start window with results of discriminant analysis (by pressing the button «Discriminant Function...» in the bottom of the main window of STATISTICA), select the box «Advanced» and then press the button «Summary: variables in the model».

You will see the list of parameters, included to analysis by STATISTICA. In the case presented on Figure 19, the entire set of all variables was included in analysis, although, in principle, the software can discard some parameters non-contributing to classification (in order to see the list of parameters discarded from analysis, please select the button «Summary: variables not in the model»).

Stepwise discriminant analysis put parameters in the order of their decreasing importance for classifying success. In the case illustrated on Figure 19, the parameter mostly introducing in discrimination of calls of different individuals is the end fundamental frequency (F_end), whereas parameter least introducing in discrimination was dominant frequency (F_peak).

You also can create the graph of distribution of the analysed objects in two-dimensional space (Fig. 20). For doing this, select box «Advanced», then button «Perform canonical analysis», then «Scatterplot of canonical scores» and select any two axes of the multidimensional space; press «Ok». Unfortunately, in STATISTICA 8.0, objects from different groups are only color-labeled if the number of groups (here the chicks) does not exceed 6; in other cases, the objects of different groups do not differ by color and form. Axes («Roots») which discriminant analysis calculates, are functions of a few correlated parameters, so their number is not equal to the number of parameters included in analysis.

You can look which calls were classified incorrectly. Press the box «Classification», then the button «Classification of cases». In the appeared table, you will see the results of classification for each call; the incorrectly classified calls are marked with asterisk.

5. For sexual (or any other) variation, discriminant analysis can be done in a similar way. Please return to the start of discriminant analysis and select «Sex» in the «Grouping variable» box instead of «Chick N» (in the current task, this possibility is only available for red-crowned crane chicks). Then repeat all the described above procedures and try answering the questions: What differences (individual or sexual) are expressed clearer in the chicks? What can be reasoning for this?

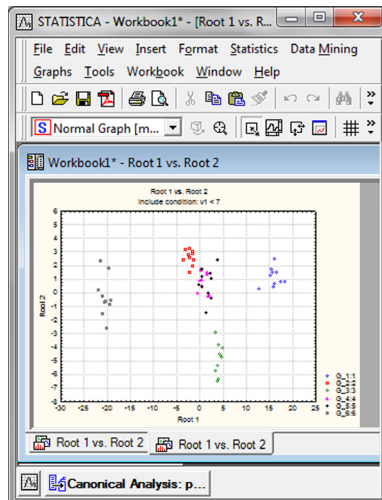


Figure 20. Window in STATISTICA displaying the graph of distribution of the analysed objects in two-dimensional space using two first functions, calculated on the basis of discriminant analysis. In this case, only first 6 chicks were included in analysis.

The R

1. For conducting discriminant analysis in The R software, modify slightly the initial data table prepared for analysis with STATISTICA (Fig. 15). First, avoid the intervals between words in names of the columns (for example, the name of column 1 from our example «Chick N», should be replaced with «Chick_N»); second, for simplifying the procedure, delete all extra columns from the table, keeping only those which will be included in analysis (for example, you should delete the column «Sound N» and column «Sex» or «Chick_N» depending on what analysis you are conducting in the given moment (for individual or for sexual variation of the calls; Fig. 15). Then, Excel file should be saved in txt format with separating tabulation.

2. Upload the obtained table to The R. To do this, enter the following code to the working field of R:

```
library(MASS)
setwd(«C:/My documents/R/»)
crane <- read.table(file = «primer.txt», dec = «,», header = TRUE)
```

where the field «C:/My documents/R/» indicates the place where your table is saved; «primer.txt» is the name of your table. The field «crane» is the name of database of the prepared for analysis table; you can select any different coding name for subsequent actions with the uploaded database. For this task, example coding can be downloaded at link http://bioacoustica.org/practicum/practicum_eng.html.

3. Please check whether your database was uploaded successfully. To do this, use the following commands in the working space of R:

```
str(crane)
names (crane)
```

If the database was uploaded correctly, The R will show the list of values of the variables and the list of names of the variables which it sees in the database:

```
> str(crane)
'data.frame': 80 obs. of 9 variables:
 $ Chick_N   : int  1 1 1 1 1 1 1 1 1 1 1 ...
 $ F_beg     : num  2.33 2.26 2.31 2.24 2.21 ...
 $ F_max     : num  2.85 2.97 2.96 2.76 3.21 ...
 $ F_ext     : num  2.41 2.37 2.38 2.27 2.59 ...
 $ F_end     : num  2.23 2.23 2.26 2.18 2.23 ...
 $ F_peak    : int  2390 2370 2370 2270 2580 2340 2320 2300 2570
2400 ...
 $ Dur_beg-max: num  0.066 0.074 0.067 0.052 0.087 0.103 0.1 0.11
0.082 0.11 ...
```

```

$ Dur_beg-ext: num 0.079 0.091 0.086 0.081 0.115 0.129 0.13 0.128
0.101 0.142 ...
$ Dur_beg-end: num 0.292 0.286 0.283 0.277 0.324 0.297 0.281
0.274 0.335 0.355 ...
> names (crane)
[1] «Chick_N» «F_beg» «F_max» «F_ext» «F_end»
[6] «F_peak» «Dur_beg-max» «Dur_beg-ext» «Dur_beg-end»

```

In our example the R reports us that it uploaded the database, in which 80 observations are present, differing by 8 parameters.

4. To start discriminant analysis, use the following commands in the working space of the R:

```

M3 <- lda(crane$Chick_N~.,crane)
misclass <- function(pred, obs) {
tbl <- table(pred, obs)
sum <- colSums(tbl)
dia <- diag(tbl)
msc <- dia/sum * 100
m.m <- mean(msc)
cat(«Classification table:», «\n»)
print(tbl)
cat(«percent of correct classification:», «\n»)
print(round(msc, 1))
cat(«Mean percent of correct classification:», round(m.m, 1), «\n»)
}
misclass(predict(M3)$class, crane$Chick_N)

```

where «**crane**» is the given by you name for the uploaded to the R database and a «**Chick_N**» is the grouping variable for conducting discriminant analysis.

The R will show you the following results:

Classification table:

```

obs
pred 1 2 3 4 5 6 7 8
1 10 0 0 0 0 0 0 0
2 0 10 0 0 0 0 0 0
3 0 0 10 0 0 0 0 0
4 0 0 0 10 2 0 0 0
5 0 0 0 0 8 0 0 0
6 0 0 0 0 0 10 0 0
7 0 0 0 0 0 0 10 0
8 0 0 0 0 0 0 0 10

```

percent of correct classification:

1 2 3 4 5 6 7 8
100 100 100 100 80 100 100 100
Mean percent of correct classification: 97.5

We obtained the classification matrix, where the average percent of correct assignment of calls to individual is 97.5%, that is, the same percent as was obtained with STATISTICA. Among individual chicks, percent of correct assignment of calls to individual varies from 80 (Chick #5) to 100% (all other chicks). Two calls of Chick #5 were incorrectly classified (and were assigned to a group of calls of Chick #4).

5. In the same way, you can conduct discriminant analysis for sexual (or any other) variation. Please return to the initial database table and use the column «Sex» instead of the column «Chick_N» (in this task, this possibility is only available for red-crowned crane chicks). Then repeat all the above-described procedures and compare the obtained results with those from STATISTICA.

Task 2.

Visual inspection of acoustic variation, definition of «signature» calls. Comparison of results of quantitative (measuring) and qualitative (visual) analyses

Study objects

Again, in the practical Task 2, our objects are the chicks of the red-crowned crane *Grus japonensis* (monogamous territorial species) and of the ancient murrelet *Synthliboramphus antiquus* (monogamous colonial species).

Aims of the work

- 1) to visually compare call contours of 8 individual chicks per species for estimating the acoustic variation of chick calls of each species;
- 2) to compare the two ways of calls classifying, the quantitative (conducting using discriminant analysis in the practical Task 1) and qualitative (conducted in the current practical Task 2), applied to the same samples of calls and matched samples of individuals;
- 3) to evaluate, by using randomization procedure, the results of discriminant analysis of acoustic variation in call parameters obtained in the practical Task 1.

Introduction. Methods for estimating the degree of acoustic variation of the calls

There are different methods for estimating the degree of acoustic variation of the calls. These methods include call playbacks followed with analysis of behaviour of the focal recipient individual, as well as analysis of vocalizations themselves. Analysis of vocalization can be qualitative and quantitative. The quantitative analysis is based on measuring call parameters followed by some statistical procedures (just this work was done in the preceding Task 1). The qualitative analysis is based on the visual classifying the calls by human observers, relying their classification on provided spectrograms or power spectra of referential calls or acting without the referential calls.

Classic example of applying visual inspection for classifying calls is the describing the signature whistles produced by toothed whales (*Odontoceti*). In this example, visual classifying of the whistles even when made by unexperienced humans, was more successful than classification based on the quantitative measurements of the acoustic parameters of the same whistles, because toothed whales often compress or expand their calls by keeping their characteristic pattern. Humans remember this pattern, whereas the statistical methods fail to resolve this problem. However, human classifying can be biased or depend on how much the person is informed about the tasks and what instruction were given to him/her before the start of the visual classifying task. All these things can strongly affect the obtained results and the conclusions made on their basis.

A lot of various mathematical models are currently available for visual classification, which allow a comparison of contours of particular calls, for example, by using the methods of cross-correlation or dynamic time warping algorithm (Fig. 21). However, the practical work with such models is out of frames of this teaching course.

In any research, you can use both the visual and quantitative classifications together for obtaining more convincing results. But most studies use only one method for estimating the individual acoustic variation, as a rule, without reasoning for method selection. Only some studies compare the data obtained by different methods applied to the same call sample. Whereas selection of the method can substantially affect the results of the study, the knowledge of their powers and limitations is important for planning and conducting research.

In this practical Task 2, we will compare the two methods of call classifying: the quantitative (using the results of discriminant analysis from

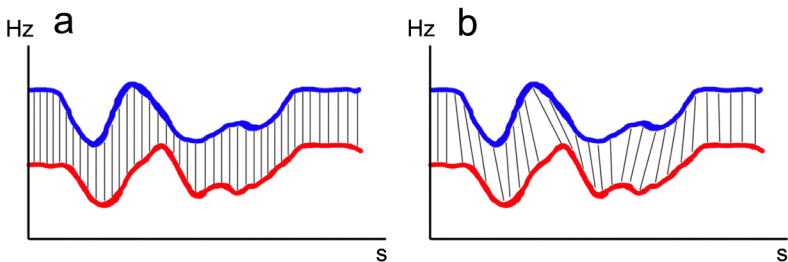


Figure 21. Variants of comparison of call contours by using the cross-correlation (a) and dynamic time warping algorithms (b).

the preceding Task 1) and the qualitative (classification made manually in frames of the current Task 2 using visual inspection of the calls). We will apply both analyses to the same call samples: of the ancient murrelet chicks and of the red-crowned crane chicks. Matched samples of call and equal number of individuals justify the statistical comparison of classifying success between the methods.

Task for the practical work.

Part 1. Acoustics

For visual inspection of spectrograms of ancient murrelet and red-crowned crane chick calls, you will receive the cards with spectrograms, with one call per card. A total of 72 calls per species should be subdivided to 8 groups (corresponding to the 8 chicks) based on their image. For doing this task, you can use a ruler. The number of calls in each group can be different, but the number of groups should be obligatory eight. The calls can be inspected a few times; time for doing the task is unlimited. After classifying the calls to the eight groups, please turn the cards with face side down and then list the numbers of calls (written on the back side) to a separate table. You can find the cards with calls and the referential table at link http://bioacoustica.org/practicum/practicum_eng.html.

Task for the practical work.

Part 2. Statistics

In the preceding task, we learned the opportunities of discriminant analysis for classifying the objects to a predicted number of groups. Now we should learn the limitations of discriminant analysis, which should be met to avoid obtaining the incorrect results. There are a few limitations on applying discriminant analysis:

1. Objects (observations) should belong to one of two (or more) classes (groups). Objects are the main units of analysis. Objects can be represented by humans, animals, countries etc.; here the objects are calls. In each class, should be at least two objects.

On practice, you will often meet the objects which cannot be attributed to any group (for example, if calls of individually unidentified unsexed chick were recorded). Such objects cannot be immediately included in discriminant analysis, however, they can be classified later, on the basis of mathematical functions, obtained from analysis of objects with «known» individual and/or sexual identity. Discriminant

analysis calculates one or a few such functions (so-called discriminant functions) based on object characteristics and assign each object to one of the groups. After classifying the objects with known individual identities (as calls from individually identified chicks), cross-validation analysis can be applied, using the already calculated discriminant functions for classifying the objects with unknown individual identity (as, for example, calls of an individually unidentified chick).

2. Characteristics of objects applied for separating the classes are named discriminant variables. Discriminant analysis does not work with qualitative variables. For applying discriminant analysis, the variables should be measured on either the interval scale, or on the ratios scale. Then, mathematical expectancies and dispersions can be calculated so that using discriminant equations is becoming legitimate. Generally, the number of discriminant variables is unlimited, but in sum, the number of objects should always exceed the number of variables at least by two.

3. Parameters used in discriminant analysis should not be the linear combinations of other variables (it is impossible to use the sum of variables of their arithmetic averages together with the variables themselves). Parameters whose correlation coefficient is equal to 1 are also inappropriate.

4. Distribution of each parameter should be normal.

These four limitations are essential for discriminant analysis. If the experimental data do not meet these assumptions, the obtained results cannot be considered as reflecting precisely the reality.

However, even if all the assumptions are met, the choice of parameters has an important effect on the results of discriminant analysis. If the parameters are not linear combinations of each other but there is some degree of correlation between them, the final result (percent of classifying success) may be significantly overestimated. Nevertheless, the real sets of call parameters practically always, at least weakly, correlate to each other (for example, the higher is the call minimum fundamental frequency, the higher, automatically, will be the call maximum fundamental frequency). The number of parameters used in analysis can also affect the final result. For controlling the effects of all possible factors and estimating the significance of the obtained percentages of correct assignment of the calls (to individuals, sexes, etc.) with discriminant analysis, we should apply the so-called randomization procedure. The randomization procedure calculates by-chance percent of correct assignment (random value), which can be then compared with the real percent of correct assignment.

For calculating the random value, it is necessary to randomly mix the objects among groups (here, to mix the calls among the chicks), to conduct the discriminant analysis procedure and to repeated this ac-

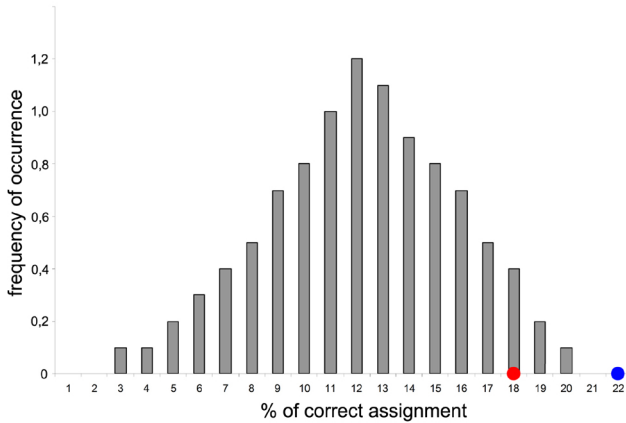


Figure 22. Histogram displaying the distribution of 1000 percentages of random assignment, calculated using randomization procedure. If the actual percent of correct assignment is 22% (marked with a blue point), it differs from the random distribution with probability $p < 0.001$. If the actual percentage of correct assignment is 18% (marked with a red point), it does not differ from the random distribution with probability $p > 0.05$.

tion 1000 times. In the result, we will obtain the normal distribution of the random assignment value (Fig. 22), which can be used for comparison with the real percentage of correct assignment. For example, if distribution of the correct assignment value matches those presented on Figure 22, whereas the real percent of correct assignment is 22% or more (on the figure, it is marked with a blue point), this would mean that it differs from the random distribution with probability $p < 0.001$. In case if the real percent of correct assignment is 18% (on the figure, it is marked with a red point), this would mean that it does not differ from the random distribution with probability $p > 0.05$.

Step-by-step guide to the work with The R software

Please conduct the randomization procedure and check the result of discriminant analysis obtained in Task 1. For doing this, please repeat in R the actions described in Task 1 (conduct discriminant analysis of your own data). The sample code for the randomization procedure you can download also at link http://bioacoustica.org/practicum/practicum_eng.html.

1. After obtaining the results of discriminant analysis, please run the randomization procedure:

```
misclass <- function(pred, obs) {  
tbl <- table(pred, obs)  
sum <- colSums(tbl)  
dia <- diag(tbl)  
msc <- dia/sum * 100  
m.m <- mean(msc)  
m.m  
}
```

```
output<-data.frame(attempt=NA, mean.perc=NA)  
for (i in 1:1000){  
rand1 <-sample(crane$Chick_N)  
M7 <- lda(rand1~.,crane)  
m.m<-misclass(predict(M7)$class, rand1)  
output[i,1] <-i  
output[i,2] <-m.m  
}
```

To print out the results of the randomization procedure, use the command:

```
print(output)
```

2. Calculate the descriptive statistics for the obtained distribution of 1000 results of discriminant analysis:

```
average<-mean(output[,2])  
cat(«Average m.m is »,average, «\n» )  
sd<-sd(output[,2])  
min<-min(output[,2])  
max<-max(output[,2])  
cat(«SD m.m is »,sd, «\n» )  
cat(«Min m.m is »,min, «\n» )  
cat(«Max m.m is »,max, «\n» )  
sd<-sd(output[,2])
```

Now you can see the calculated average, maximal and minimal values for the by-chance percentage of correct assignment, along to standard deviation. Using this distribution, please compare it with the real percent of correct assignment (in our example case, it was 97.5 %):

```
output[output$mean.perc>A,]
```

where **A** should be replaced with the real percent of correct assign-

ment. As you can see, in our case there is no any single overlap between distribution of the by-chance value of correct assignment and the real percent of correct assignment, that is, the real result differs from the by-chance value with probability $p < 0.001$.

3. As an additional task we can create 3D graph of distribution of calls of our study chicks in the space of the three most important for classifying discriminant functions. To do this, please indicate again the parameters which should be used for calculating the discriminant functions:

```
lda.fit <- lda(formula = Chick_N ~ F_beg + F_max + F_ext + F_end + F_peak + Dur_beg-max + Dur_beg-ext + Dur_beg-end, data=crane)
```

Using the following function, you will calculate the position of each object (call) in the three-dimensional space of the three discriminant functions:

```
plda <- predict(object = lda.fit,
newdata = crane)
```

Please declare arbitrary the axes x, y and z as discriminant functions 1, 2 and 3:

```
x <- plda$x[, 1]
y <- plda$x[, 2]
z <- plda$x[, 3]
```

Please declare arbitrary the colors for each individual:

```
mapdf <- data.frame(id=c(1,2,3,4,5,6,7,8),
paint=c(«white»,«black»,«blue»,«red»,«green»,«yellow»,«purple»,«coral»))
colors <- mapdf$paint[match(crane$Chick_N,mapdf$id)]
```

Then please install the graphical packages of R (if they are not already installed on your computer):

```
install.packages(«rgl»)
install.packages(«magick»)
library(rgl)
library(magick)
```

Please set the size of labels on the figure:

```
viewport_size <- 512
par3d(windowRect=c(50, 50, 50+viewport_size, 50+viewport_size))
par3d(zoom=1.1)
```

Then display the graph to the screen (Fig. 23):

```

plot3d(
x, y, z,
col = colors,
type = 's',
radius = .3,
xlab=»Function 1«, ylab=»Function 2«, zlab=»Function 3»)
play3d( spin3d( axis = c(0, 0, 1), rpm = 4), duration = 5 )

```

Additional information of how to start discriminant analysis of your data and what other results can be obtained is available at links <https://jcoliver.github.io/learn-r/003-intro-multivariate.html> and <https://rpubs.com/ifn1411/LDA>

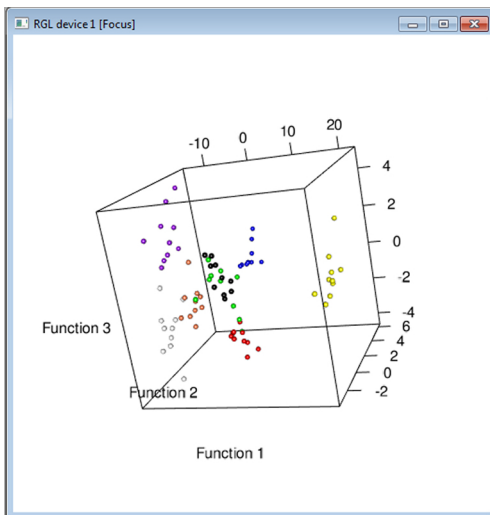


Figure 23. Graph of distribution of analysed calls in the three-dimensional space using tree first discriminant functions. In this case, all the 8 individual red-crowned crane chicks are included in analysis.

Task 3.

Nonlinear phenomena in calls of mammals and birds. Measuring the fundamental frequency in calls containing nonlinear phenomena

Study objects

Mammals (adults)

Carnivora: red fox *Vulpes vulpes*, polar fox *Vulpes lagopus*, domestic dog *Canis lupus familiaris*, dhole *Cuon alpinus*; Cetacea: killer whale *Orcinus orca*.

Birds (adults)

Passeriformes: domestic sparrow *Passer domesticus*; Charadriiformes: puffin *Fratercula cirrhata*, crested auklet *Aethia cristatella*.

Birds (chicks)

Charadriiformes: puffin *Fratercula cirrhata*, crested auklet *Aethia cristatella*, horned puffin *Fratercula corniculata*, Gruiformes: Siberian crane *Leucogeranus leucogeranus*, red-crowned crane *Grus japonensis*; Strigiformes: Great grey owl *Strix nebulosa*; Sphenisciformes: Humboldt penguin *Spheniscus humboldti*.

Aims of the work

- 1) to recognize different kinds of nonlinear phenomena in calls of mammals and birds;
- 2) to determine the values of fundamental frequency in calls complicated with presence of nonlinear phenomena.

Introduction. Kinds of nonlinear phenomena and why it is important to recognize them

There are many calls of complex structure (Fig. 24, 25), whose analysis is difficult even for specialists. Nonlinear phenomena affect substantially the acoustic structure of calls, by masking or entirely hiding the fundamental frequency band. This would result in mistaken classifying calls to the incorrect type and problems with measuring the fundamen-

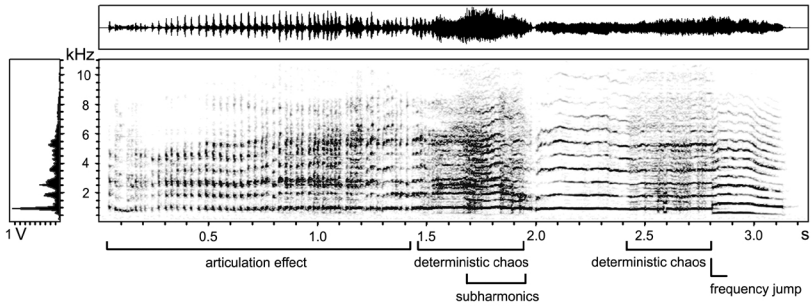


Figure 24. Diversity of nonlinear phenomena in whines of red fox *Vulpes vulpes*. This call contains an articulation effect, deterministic chaos, subharmonics and frequency jump.

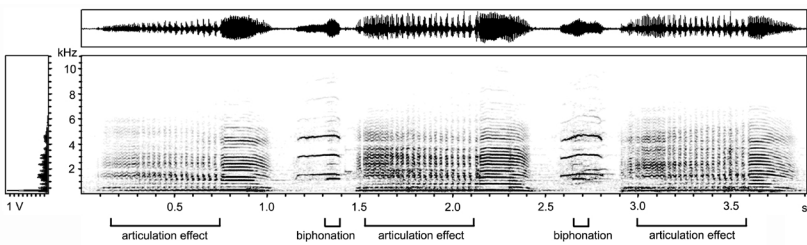


Figure 25. Diversity of nonlinear phenomena in calls of Humboldt penguin *Spheniscus humboldti*. This call contains an articulation effect and biphonation.

tal frequency or with selecting parameters for analysis of structural variation. So, you should learn how to find the fundamental frequency band, even if it is masked with different nonlinear phenomena.

Determining fundamental frequency band in calls produced by phonation

Mechanism for producing calls by vibration of the vocal folds (in mammals) or syringeal membranes (in birds) is termed the phonation mode of vocal production. Call fundamental frequency (f_0) corresponds to the vibration rate of vocal folds, visible as pulse rate at oscillogram. Call, produced by phonation mechanism, has a harmonic structure, that is, its spectrum contains power peaks not only of f_0 , but also of its integer multiple bands (harmonics: f_1, f_2 etc.). On spectrogram, harmonics represent frequency bands, lying above the f_0 band, and their values are re-

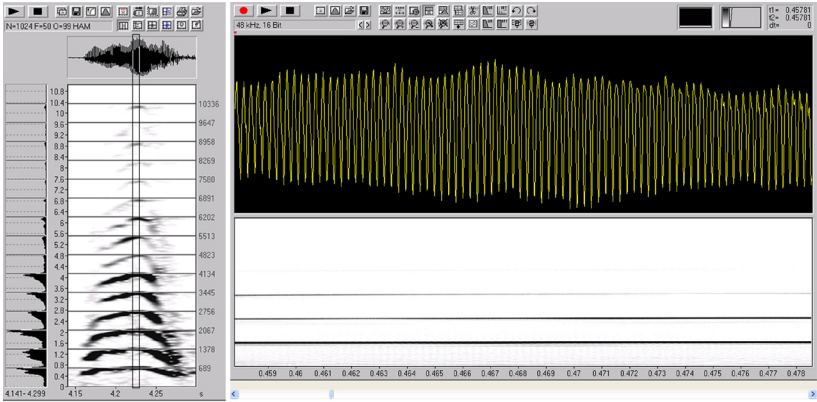


Figure 26. Spectrogram and oscillogram of a bark call of a crested auklet *Aethia cristatella* emitted in the mode of normal phonation. On spectrogram (left), the harmonic cursor indicates a position of the fundamental frequency band and its integer multiple harmonic bands. In the right window, a call fragment is shown indicated on spectrogram with a rectangular.

lated to the f_0 band values as whole numbers (2:1, 3:1 etc.). Oscillogram of normal phonation (that is, without nonlinear phenomena) represents a row of vibrations with constant period and amplitude (Fig. 26).

For measuring f_0 at normal phonation, it is sufficient to measure the values of the lowest band of call frequency spectrum on spectrogram or to measure the duration of one vibrational period on oscillogram (the fundamental frequency can be further calculated by formula $f_0 = 1/T$). As a rule, the f_0 band is the most accented band, bearing most energy and looking on spectrogram as the brightest frequency bands. In ideal case, $E(f_0) = 2E(f_1) = 4E(f_2)$ etc., that is, the first harmonic band bears twice less energy than the f_0 band, the second harmonic bears twice less energy than the first harmonic etc.. However, in reality, such «ideal» energy ratio does not occur, as sound of the vocal source (larynx, syrinx) is filtered by passing the vocal tract to output from the nose or from the mouth and then degrades at propagation through the environment (Fig. 26).

Determining the fundamental frequency in calls with nonlinear phenomena

Subharmonics

Sound source (vocal folds) represents a system of two coupled oscillators, which synchronize their vibrations during each cycle, ensuring the

periodical regular phonation. In case when vibratory modes of the vocal folds are substantially different (for example, when tension of one vocal fold is slightly stronger), coupling between them can result in synchronizing of vibrations, but at different rate for each of the folds. So, for example, one vocal fold will pass two periods of vibrations during those time, when the second fold will pass only one period. On spectrogram (in this case) the intermediate frequency bands of 1/2, 1/3 or 1/4 steps between harmonics, termed *subharmonics*, will appear.

Subharmonics, as a rule, appear not for the duration of the entire calls uninterruptedly, what facilitates their recognition. To find the fundamental frequency in a call with subharmonics, it is necessary to find the frequency band, which would be the integer multiple to harmonics as 1:2, 1:3 etc., or would be integer multiple to subharmonics as 1:0.5, 1:1.5 etc. or as 1:0.33, 1:0.66 etc.. Again, the fundamental frequency band is commonly the most accented band of a call, but there are exclusions. Subharmonics can be discriminated from frequency jumps by oscillogram: the oscillogram of the subharmonic mode clearly displays a regular alternation of the high and low-amplitude peaks (Fig. 27).

Deterministic chaos

This nonlinear phenomenon results from aperiodic, irregular vibrations of the vocal folds. However, in spite of the chaotic character of the vibrations, the vocal folds still behave as coupled oscillators, retaining the mechanic and aerodynamic relationship to each other. As

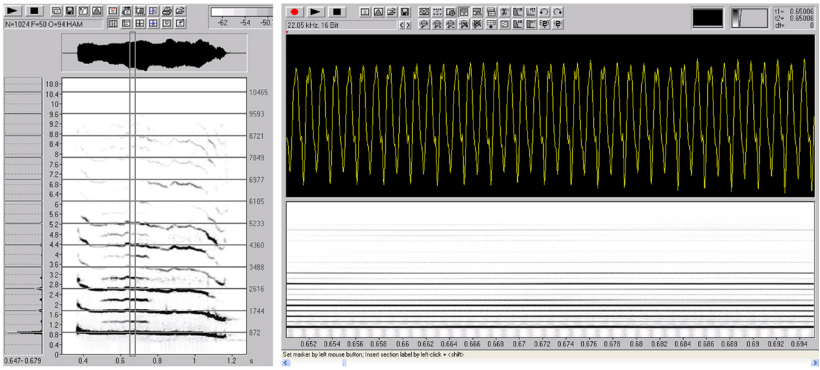


Figure 27. Spectrogram and oscillogram of a red fox *Vulpes vulpes* whine call produced in the subharmonic mode. On spectrogram (left), the harmonic cursor indicates the position of the fundamental frequency band and its integer multiple harmonic bands. In the right window, a call fragment is shown indicated on spectrogram with a rectangular.

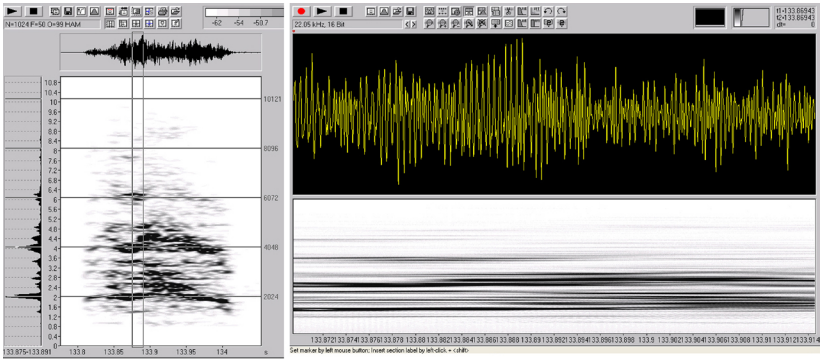


Figure 28. Spectrogram and oscillogram of Humboldt penguin *Spheniscus humboldti* chick call produced in the deterministic chaos mode. On spectrogram (left), the harmonic cursor indicates a position of the fundamental frequency band and its integer multiple harmonics. An approximate position of the fundamental frequency band is indicated by power peak at the area of 2 kHz. In the right window, a call fragment is shown indicated on spectrogram with a rectangular.

the result, the aperiodic vibrations are arranged around some average frequency which commonly corresponds to the call fundamental frequency. On spectrogram, *deterministic chaos* looks like rather uniform darkness of the entire spectrum, with residual energy in the area of the fundamental frequency and its harmonics (Fig. 28). This allows to reliably distinguish this variant of vibratory mode of the vocal folds from the *turbulent noise*, which also looks as uniform darkness of the entire spectrum, but at which the vocal folds do not work, and the air is blowing under press through the glottic or the narrowing of the vocal tract.

Measuring the fundamental frequency in a call with deterministic chaos is often difficult. Commonly, the fundamental frequency run can be predicted by power peaks which correspond to the fundamental frequency and its harmonics, but only in cases when the degree of desynchronization of the work of the vocal folds is not too strong (Fig. 28). In addition, the calls with deterministic chaos can contain the «frequency windows» with fragments of normal phonation, when the vocal folds return for some time to the work in the coupled mode (Fig. 29a). These «windows» commonly facilitate the search of the fundamental frequency in a call. However, in cases when the degree of desynchronization in the work of the vocal folds is high, determining the fundamental frequency of a call can be impossible (Fig. 29b).

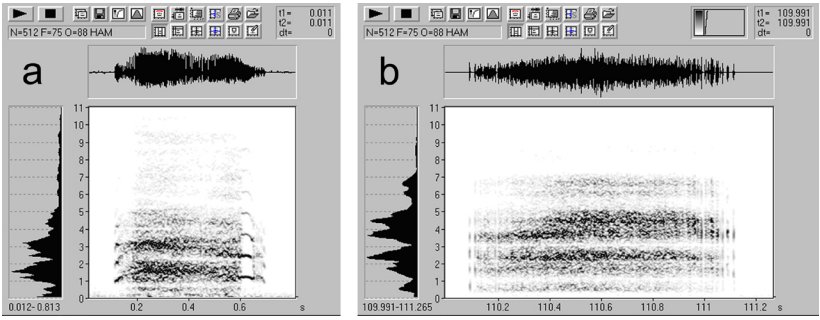


Figure 29. Spectrogram of parakeet auklet *Aethia psittacula* chick calls in the deterministic chaos mode. The first call (a) contains «windows» with normal phonation at the beginning and at the end, where the position of the fundamental frequency and its integer multiple harmonics is visible; the second call (b) does not contain the «windows» with normal phonation, so determining the fundamental frequency is difficult.

Frequency jumps

The nonlinear phenomenon *frequency jump* represents an abrupt change of fundamental frequency within one cycle of vibration. Frequency jumps may occur within one sound source (vocal folds) or between two different sound sources (Fig. 30). Sometimes it is difficult to

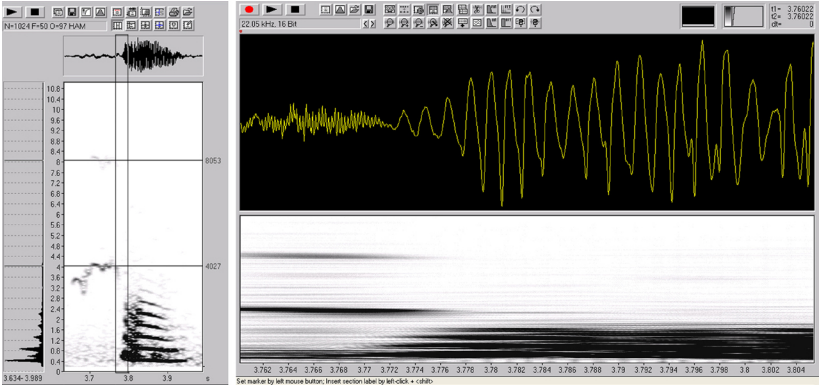


Figure 30. Spectrogram and oscillogram of red-crowned crane *Grus japonensis* fledging call with frequency jump. On spectrogram (left), the harmonic cursor indicates the position of the fundamental frequency and its integer multiple harmonics at the beginning of the call. In the right window, a call fragment is shown indicated on spectrogram with a rectangular.

distinguish between frequency jumps and subharmonics (if the change of fundamental frequency in the frequency jump occurs by integer multiple ways relative to preceding fundamental frequency). In such case, it is convenient to use again the oscillogram. In case of frequency jump, oscillogram always clearly displays an abrupt change of vibration rate from one mode to another (Fig. 30), whereas the oscillogram of the subharmonic mode displays a regular alternation of the high and low-amplitude peaks (Fig. 27).

Two independent frequencies in the call (biphonation in a broad sense)

Biphonation appears when two independent fundamental frequencies are present within a call. There are three patterns of biphonations, which cannot be discriminated physically (because in all the cases, there are two independent fundamental frequencies within call, interacting to each other), but they differ in the pattern of displaying on spectrograms. Even by spectrogram, these patterns are not always well distinguishable from each other, and the intermediate forms between them are relatively widespread. In such cases, it is better to speak about biphonations in a broad sense, without concentrating attention on the particular pattern of biphonation.

1. *Sidebands.* In case when the difference between two independent fundamental frequencies is large (over 1 kHz), one of the frequencies is rather low, and the interaction between these two frequencies results in the appearance of an additional amplitude modulation, we observe the pattern termed sidebands. In calls with sidebands, the low fundamental frequency can be invisible in call spectrum, being filtered out by the vocal tract. Thus, we observe on spectrogram only the upper fundamental frequency and the combinatory frequency bands (resulting from interaction between the two fundamental frequencies of a call), looking as bands spreading up and down from the fundamental frequency band and the bands of harmonics, with step between the combinatory bands equal to the frequency of amplitude modulation. The sidebands which are nearest to the fundamental frequency band and harmonics are most accented energetically and fade more and more with increasing distance from them. As amplitude modulation can change for the duration of a call, the step between sidebands will increase or decrease respectively, what allows to distinguish them from subharmonics, which are integer multiples of fundamental frequency throughout a call. Sidebands can also be easily distinguished from the classical biphonations and subharmonics by call oscillogram, displaying clearly both the fundamental frequency period and modulating frequency period (Fig. 31). Commonly, calculating fundamental frequency in a call with sidebands is not difficult (most often, this is

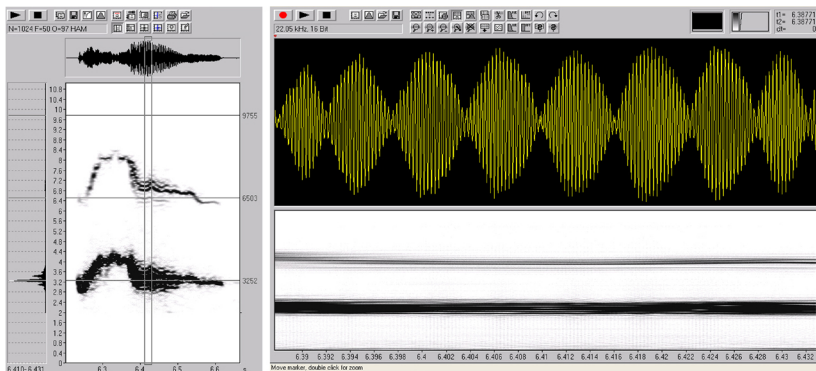


Figure 31. Spectrogram and oscillogram of red-crowned crane *Grus japonensis* fledging call with sidebands arising due to the additional amplitude modulation of fundamental frequency. On spectrogram (left), the harmonic cursor indicates the position of fundamental frequency and its integer multiple harmonics. In the right window, a call fragment is shown indicated on spectrogram with a rectangular.

the brightest band in call spectrum), but sometimes, sidebands practically entirely mask the fundamental frequency. Then, it is possible to calculate it by oscillogram by the formula $f=1/T$.

2. *Biphonation (in narrow sense)*. At biphonation, two independent fundamental frequencies and the results of their interaction are visible in call spectrum (Fig. 32). The difference between two independent fundamental frequencies is rather large (not less than 1 kHz). Commonly, you can see, on spectrogram, two non-integers to each other frequency bands (f_0 и g_0) with independent contours of frequency modulations, and each frequency has its own harmonics ($f_1=2f_0, f_2=3f_0$ etc., $g_1=2g_0, g_2=2g_0$ etc.). Moreover, these frequencies interact to each other with creating the linear combinatory frequencies (g_0-f_0, g_0-2f_0) (Fig. 33). By presence in the spectrum of the independent frequencies with their harmonics and combinatory frequency bands, biphonation can be easily distinguished from other nonlinear phenomena. On oscillogram of the biphonation in narrow sense, the amplitude modulation is commonly invisible.

3. *Beating*. This nonlinear vocal phenomenon results from interaction of two independent fundamental frequencies with similar values (with differences less than 1 kHz). Call oscillogram at beating arises by arithmetic summation of values of the two sinusoids corresponding to each of the fundamental frequencies in each point, so, as in the case of side-

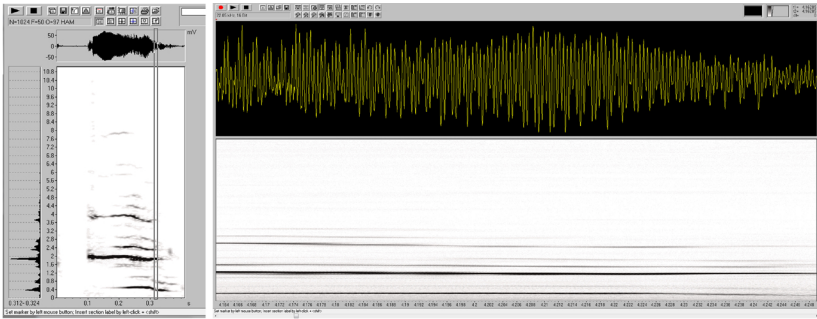


Figure 32. Spectrogram and oscillogram of Siberian crane *Leucogeranus leucogeranus* fledging call with biphonation. In the right window, a call fragment is shown indicated on spectrogram with a rectangular.

bands, on the oscillogram, we observe amplitude modulation (Fig. 34). Theoretically, at beating, call spectrum should lack the additional frequency bands, but in reality, we see, rather often, not only two fundamental frequencies, but also their steadily fading combinatory frequencies. The beating can be easily distinguished from subharmonics, as two frequencies commonly have independent run from each other, whereas subharmonics are always parallel. The beating can be easily confused

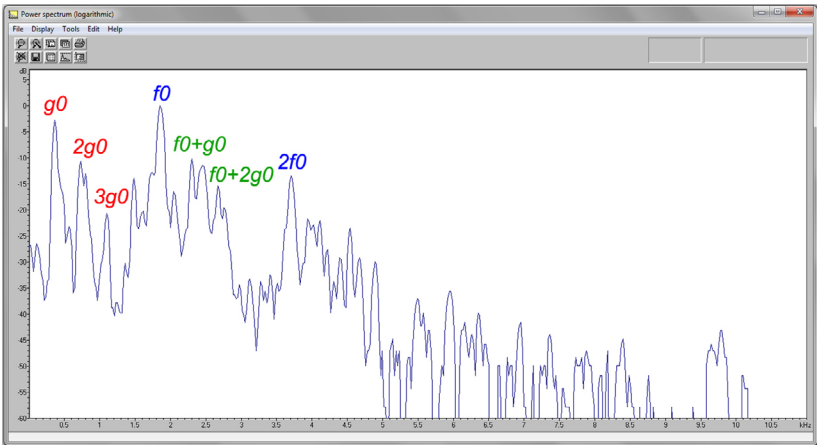


Figure 33. Power spectrum of call fragment containing biphonation, indicated on spectrogram with a rectangular on the figure 32; f_0 — the upper fundamental frequency, g_0 — the low fundamental frequency, $2f_0$, $2g_0$, $3g_0$ — harmonics of the upper and low fundamental frequencies; f_0+g_0 , f_0+2g_0 — combinatory frequencies.

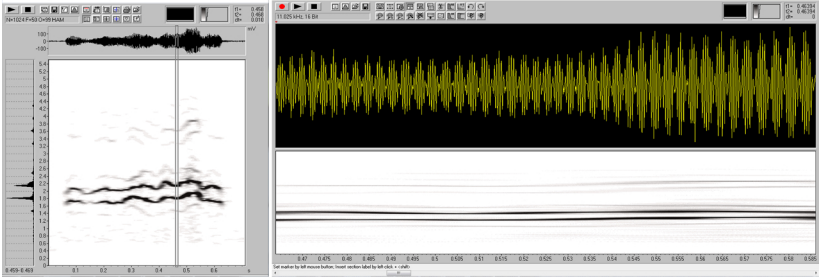


Figure 34. Spectrogram and oscillogram of Siberian crane *Leucogeranus leucogeranus* fledging call with beating. In the right window, a call fragment is shown, indicated on spectrogram with a rectangular.

with sidebands by call oscillogram, on which also commonly well visible both the fundamental frequency period and the modulating frequency period (Fig. 34), but in a call with sidebands, as a rule, one most bright band is visible, with coming up and down steadily fading additional frequency bands, whereas in the call with beating, two close fundamental frequencies are always well-visible, whereas the additional frequency bands are expressed weaker or even can be missing (Fig. 34).

Thus, biphonations in narrow sense, beatings and sidebands represent different poles of the same physical phenomenon; differences between them can be summarized by the following scheme (Fig. 35).

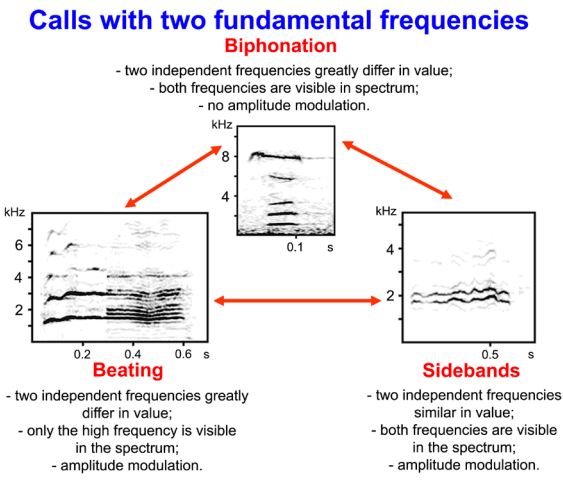


Figure 35. Differences between biphonations, beating and sidebands.

Task for the practical work

The calls intended for analysis are recorded from young and adult individual mammals and birds. The 17 files with recordings can be download at link http://bioacoustica.org/practicum/practicum_eng.html; file names correspond to the task numbers. Each file contains 2–4 calls from one individual of a given species, intervals between calls were cut off for more convenient analysis. In the task description you will find a precise location of the time fragment of the call which should be used in the work.

Inspect visually the 17 files with recordings. Please determine, what acoustic mode is attributive for each of these proposed calls (normal phonation, subharmonics, deterministic chaos, frequency jump, sidebands, biphonation, beating). Please determine the values of call maximum fundamental frequency on the marked time fragments (for biphonations and beatings, please determine the maximal values for each of the fundamental frequencies):

File name	Call number within file	Time fragment for analysis (s)	Species
1	second	1.32–1.52	red fox <i>Vulpes vulpes</i>
2	first	0.13–0.26	red fox <i>Vulpes vulpes</i>
3	third	0.75–0.77	domestic sparrow <i>Passer domesticus</i>
4	second	1.00–1.20	domestic dog <i>Canis lupus familiaris</i>
5	first	0.15–0.20	crested auklet chick <i>Aethia cristatella</i>
6	first	0.83–1.17	tufted puffin chick <i>Fratercula cirrhata</i>
7	second	2.64–3.00	Siberian crane chick <i>Leucogeranus leucogeranus</i>
8	first	0.21–0.51	horned puffin chick <i>Fratercula corniculata</i>
9	first	0.40–0.45	Humboldt penguin chick <i>Spheniscus humboldti</i>
10	first	0.16–1.00	tufted puffin <i>Fratercula cirrhata</i>
11	forth	1.65–1.85	crested auklet <i>Aethia cristatella</i>
12	first	0.65–0.90	horned puffin chick <i>Fratercula corniculata</i>
13	first	0.24–0.25	dhole <i>Cuon alpinus</i>
14	first	0.5–0.6	killer whale <i>Orcinus orca</i>
15	second	0.45–0.59	great grey owl chick <i>Strix nebulosa</i>
16	first	0.50–0.60	polar fox <i>Vulpes lagopus</i>
17	first	0.32–0.35	red-crowned crane chick <i>Grus japonensis</i>

Important!!! *At measuring frequency in the labeled call fragment, do not zoom the call so strongly to have in the window only the labeled fragment. At such extending, the sound will be visible very unclearly. It is better to select the degree of extension at which the call will be visible on the screen entirely, but the measurements should only be done for the labeled fragment, because the fundamental frequency is changing strongly for the duration of the call, so the obtained values can differ strongly depending on the selected point of measuring.*

Guidelines to the work with Avisoft-SASLab Pro

For making this task, use all the three windows of Avisoft: oscillogram, spectrogram and power spectrum. For creating spectrogram, select in the spectrogram window Analyze → Create Spectrogram. For creating spectrogram, use each time the most appropriate parameters for creating spectrogram for the calls of the given pattern. In order to change them, please select Analyze → Spectrogram parameters; from the proposed parameters, try to change only «Overlap» and «Frame Size», ensuring resolution in time and frequency. For creating the detailed frequency spectrum, please click with mouse two times on the panel of frequency spectrum in the spectrogram window (preliminary, select with the standard cursor in the spectrogram window only the necessary call fragment, otherwise the spectrum will be created for the entire call). For finding the harmonics, integer multiples of fundamental frequency, it is convenient to use the special harmonic cursor (spectrogram window: Tools → Cursors → Insert harmonic frequency cursor; the power spectrum window: the harmonic cursor is «pulling» with mouse from-under the y axis). In the spectrogram window, you can use only one harmonic cursor, whereas in the window of power spectrum there are two cursors, what can be convenient at analysis of biphonations.

Task 4.

Vocal repertoires and methods of call classifying. Advanced options of Avisoft-SASLab Pro (automatic call parameter measuring, call labeling, filtering etc.) and the essential options of Syrinx and Audacity software (visual inspection and call labeling)

Study objects

Mammals (adults)

Rodentia: yellow steppe lemming *Eolagurus luteus*.

Birds (adults)

Passeriformes: Siberian blue robin *Luscinia cyane*.

Birds (chicks)

Gruiformes: common crane *Grus grus*.

Aims of the work

- 1) to describe the vocal repertoire of three animal species based on provided audio recordings of calls sequences;
- 2) to conduct the automatic measuring of the selected for analysis calls from the three species using three different kinds of software for bioacoustical analysis.

Introduction. Vocal repertoires and why their describing is important?

Any study of acoustic behaviour of animal species starts with describing the vocal repertoire. If the vocal repertoire was not already described in literature, you should start with creating a simple and convenient scheme describing variation of calls of the given species. Based on this scheme, you can subdivide the vocal repertoire to call types. For

describing the vocal repertoire, it is necessary to collect a representative sample of calls from several individuals of the given species (and, desirably, at different age classes) in different contexts (e.g., during social contacts with conspecifics, during courtship and aggressive interactions, during communication with offspring/parents, in situation of food deficit, cooling, etc., depending on species biology). Then, the recorded calls should be classified to call types.

For describing the vocal repertoires of mammals and birds, two approaches of classifying can be applied: by structure and by functions. Avoid mixing these two approaches, but when applied one after another, they complete each other very well.

At classifying calls by function, the main attention is focused on relationship of calls with certain behaviour, e.g., calls can be subdivided to categories attending comfort, aggression, appeasing, sexual or maternal behaviour.

At classifying calls by the acoustic structure, the main attention is only focused on physical parameters of the calls. In this case, calls are subdivided to structural classes, for example, tonal or noisy, with rhythmic or without rhythmic etc., and further divided accordingly to greater detail within each structural class. Fragmentation of classifying is arbitrary given by researcher depending on the particular research task, so the number of call types to which the repertoire is subdivided can vary broadly, from a few to a few dozen call types.

At classifying by structure, sometimes it is difficult to strictly discriminate call types from each other. There are intermediate forms, so that call types, from one side, represent discrete units, but from another side, all vocalizations, due to intermediate forms, may represent one or a few structural continua.

Call classifying to types primarily based on the following criteria:

1. Source of sound production. Calls can be subdivided to groups on the basis of sound production. For example, the voice calls produced by phonation with self-sustained vibration of the vocal folds, the hiss calls, produced by aerodynamic turbulence, the whistle calls, produced by aerodynamic jet or edge-tone mechanism and purring calls, produced by muscle activity applied to the vocal folds (e.g., cat purring at both phases of breathing).
2. Presence or absence of rhythmicity.
3. Duration of vocalization.
4. Contour of frequency modulation.
5. Presence or absence of some nonlinear phenomena.

Variation of duration or frequency modulation and presence of nonlinear phenomena can be different for different call types. The degree

of possible variation is determined by researchers themselves basing on the aims of the study and peculiarities of certain species. However, to make the classification reproducible by other researcher, it is important to precisely describe in the methods, which borders were arbitrary selected between call types. Verifying the own classification is possible by using the statistical procedure of discriminant analysis. Discriminant analysis clearly demonstrates how realistic the established borders between call types are.

Suggestions for practical work.

Part 1. Avisoft-SASLab Pro

Advantages of Avisoft-SASLab Pro software

In the last years, many researchers throughout the world conduct bioacoustical studies, so the number of bioacoustical programs increases constantly. Today, you can find many bioacoustical programs with different capacities at different prices (from available for free to very expensive). Choice of most appropriate and convenient soft can be difficult. With Avisoft-SASLab Pro, we made the Tasks 1 and 3; in current task 4, we will evaluate some special options of Avisoft and will additionally consider two other bioacoustical programs, Syrinx and Audacity.

First versions of Avisoft-SASLab Pro were developed by German specialist Raimund Specht in 1990th. Now Avisoft is one of most used programs for bioacoustical studies. New versions of Avisoft appear regularly. The program is convenient and user-friendly, allowing to conduct various measurements manually and automatically, create high-resolution spectrograms and oscillograms, label and filter the calls etc. However, Avisoft has some limitations: it can only be installed on computers with Windows, works with only files of particular formats (.WAV .BWF .AIF, .SND, .AU) and is not available for free.

In the current Task 4, we will learn the abilities of Avisoft for labeling and selection of calls for analysis, including the automatic call parameter measuring.

Guidelines for individual work

Inspect visually the file Common crane.wav in Avisoft; make the visual classification of call types occurring in this file; describe them shortly in the attending file with comments or provide their spectrograms in a separate textual file. Using markers of Avisoft, label 10–15 calls per call type in the audio file. Export the labeled calls to separate files, one file per call type. Save the prepared files. Conduct the measurements

of 10 calls per call type using the panel for the automatic call measurements in Avisoft; check whether data export is functioning normally and provides the adequate values of call measurements; save the obtained Excel table.

Step-by-step guidelines to the work with Avisoft-SASLab Pro

1. Please run Avisoft and open the audio file; inspect visually the recording. If necessary, make call downsampling to 11025 Hz (Edit → Format → Sampling frequency conversion), filter out the alien low-frequency noises (Edit → Filter → High pass IIR filter → select the low-frequency zone which should be filtered out from the file, for example 1 kHz). Tune out, with most convenient for you way, the parameters for visual inspection of spectrograms (Analyze → Overview parameters), for example, try to change the settings of FFT-length, Frame and Threshold and observe how this will affect the quality of spectrogram in the main window of Avisoft.

2. Make the visual classification of call types occurring within the file. Describe shortly the distinctive traits and basic characteristics of each call type. Using the markers of Avisoft (Tool → Labels → Insert section label from marker), label 10–15 calls of each type. At labeling, for each call type, use different layers (layer 1, layer 2 etc.). For convenient work, you can set the «hot key» on the keyboard, in order to add the marker by pressing the key. Do it using Actions → Keyboard Shortcuts and Popup Menu. In the opened window, select the convenient for you key on the keyboard by putting the checkmark against Tool ... Labels ... Insert section label from marker, for example, «2». Please export the labeled calls to separate files depending on call type (Tool → Labels → Save labeled sections of the same class into single wav file). Save the obtained files.

3. Open one of the obtained files and create the spectrogram using the most convenient for your parameters for creating spectrogram (Analyze → Spectrogram parameters; for example, FFT-length 1024, Frame size 25, Overlap 98.43).

4. In the spectrogram window, establish the set of parameters for the automatic measurements. Select from the main menu of the spectrogram window Tools → Automatic parameter measurements → Automatic parameter measurements setup. This will open the window, in which you can select the acoustic parameters for measurements. Select the acoustic parameters for measurements, for example, as it is indicated on Figure 36.

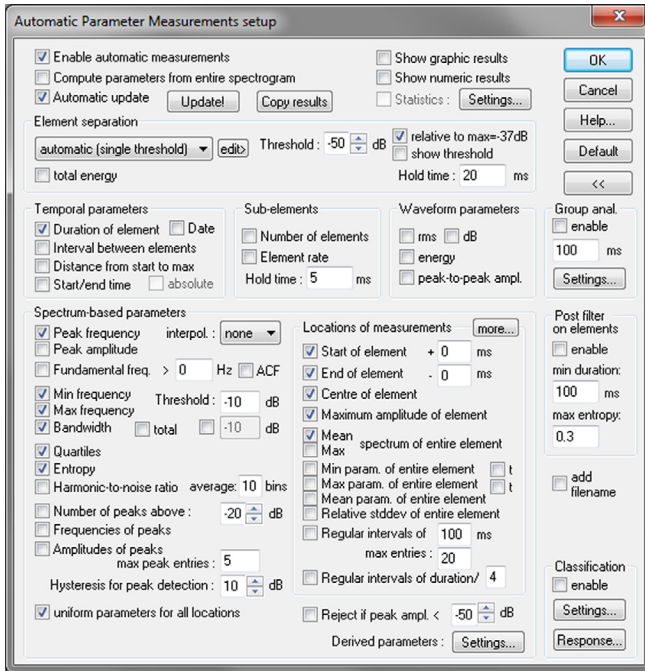


Figure 36. Window in Avisoft-SASLab Pro for selecting parameters for the automatic measurements. The way of filling this window is in accordance to the aims of the practical task 4.

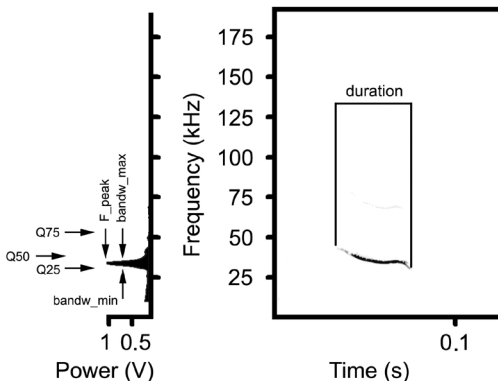


Figure 37. Spectrogram (right) and power spectrum (left) of a call of a yellow steppe lemming *Eolagurus luteus*, illustrating some measured parameters that mentioned in the text.

When you selected the settings as indicated on Figure 36, please label the call with the standard cursor and select the command «Copy parameter measurement values» (in spectrogram menu, select Tools → Automatic parameter measurements → Copy parameter measurement values). The following parameter values will be exported to the external file:

- 1) duration of the labeled fragment (duration, Fig. 37);
- 2) peak frequency of the labeled fragment (F_{peak} , Fig. 37);
- 3) minimum and maximum frequencies of a power peak at the level -10dB from the point of a call power maximum (bandw_min, bandw_max, Fig. 37);
- 4) the wide of power peak at level -10dB from the point of a call power maximum (bandwidth);
- 5) three power quartiles of a call, displaying the frequencies, lower which are disposed 25, 50 and 75% of call energy respectively (Q25, Q50 and Q75, Fig. 37);
- 6) call entropy (Entropy), reflecting the degree of call noisiness (this value varies from 0 to 1, where the values around 0 characterize practically ideal tonal call, whereas the values around 1 characterize, otherwise, practically white noise.

According to the settings shown on Figure 36, all parameters aside the duration of the labeled element will be measured by a program for the following locations:

- 1) for the beginning and end of the element;
- 2) for the middle of the element;
- 3) in the point of the maximum call intensity (or in the point of maximum amplitude);
- 4) and, at last, for the entire call spectrum.

As you can see on Figure 36, the listed parameters can also be taken from the entire call contour, for example, by «cutting» it to the given number of equal sections or with the given time interval. This is useful, for example, for those tasks, where the entire contours of calls are compared.

5. Prepare the Excel file, to which Avisoft will automatically export the measured parameters (for example, use the table Prim2, which can be downloaded together with audio files for the given Task 4 at link http://bioacoustica.org/practicum/practicum_eng.html). In the spectrogram window of Avisoft, establish the settings for export of measured parameters (see also Fig. 14). For this, select File → Data export → DDE-Parameters/Log-File.

In the appeared window, you need:

- 1) first, fill the field «Topic» (the name of your Excel file);

- 2) then, in the field «Item», indicate the cell of your Excel table, to which the program should copy the first measurement (R — line, C — column); next measurements of the same call Avisoft will copy to the same line, one after another;
- 3) at last, indicate, after how many clicks (measurements) Avisoft should hop to a new line (that is, how many measurements of each call you are going to make); in our case, the measurement will be only one (one click on the labeled with the standard marked cursor call).

6. Copy the names of measured parameters to the prepared Excel table. For doing this, select in the spectrogram window Tools → Automatic parameter measurements → Copy parameter measurement headings. If export parameters are tuned correctly, Avisoft should copy the names of the proposed for measuring parameters to the first line of Excel table. Now you can start the measurements.

7. For measuring, label the call with the standard marker cursor from the beginning to end in the spectrogram window and select Tools → Automatic parameter measurements → Copy parameter measurement values. For convenience of measuring, you can set the «hot key» to this command, then the measurement will be made at pressing the particular key on the keyboard. For setting the «hot key», select in the spec-

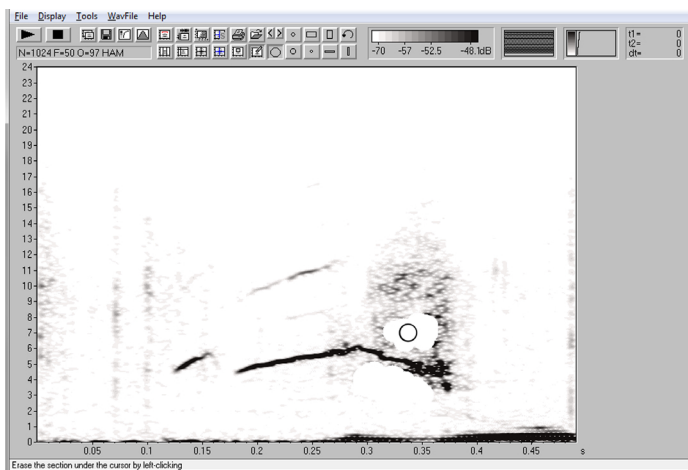


Figure 38. Spectrogram window in Avisoft-SASLab Pro illustrating the using the manual filter «Eraser» for removal the alien noise complicating call analysis.

rogram window Tools → Keyboard Shortcuts and Popup Menu. In the opened window, checkmark the convenient for you key of the keyboard against Tools → Automatic parameter measurements → Copy parameter measurement values, for example, 1. Then, for measuring the call, it is sufficient to label it with the standard marker cursor and press the key «1» on the keyboard.

8. Make the measurements on 10 calls of two main call types; check whether the program is working normally and displays the adequate values. If the call is strongly polluted with noise, clean it manually of the alien noise, because the selected power parameters are very sensitive to the pollution. For the cleaning, you can select the manual filter «Eraser» from the buttons in the spectrogram window (Eraser cursor style, Fig. 38).

Suggestions for practical work.

Part 2. Syrinx

Advantages of Syrinx software

Distinctive to Avisoft, the Syrinx program is free available, but the author of Syrinx (John Burt) asked to refer on him in papers. The program was created in 1995. Syrinx was actively applied in 90-th for analysis of bird songs, however, in the last years, it was not upgraded. In spite of the prolonged absence of upgrades, Syrinx works with all versions of Windows and with files of different types, including the formats wav and mp3. Syrinx allows creating call spectrograms

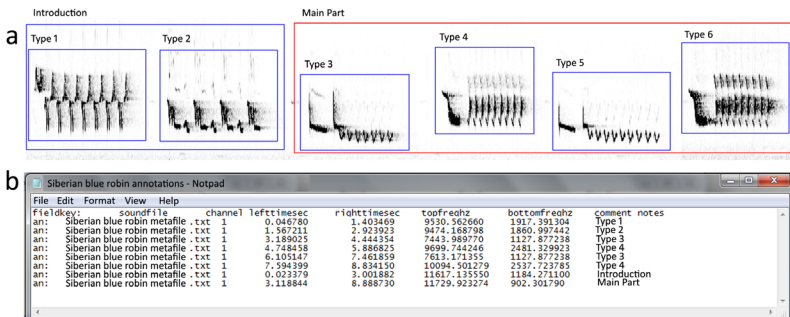


Figure 39. Spectrogram of Siberian blue robin *Luscinia cyane* songs, with labeling at different levels using Syrinx (a) and the textual file with automatically exported parameters (b).

and conducting express-analyses, including the visual inspection of recordings, catalogizing the calls using a convenient labeling option, measuring the essential call parameters (call duration and the maximum and minimum fundamental frequencies). The measured values are exported to the textual file. However, power parameters cannot be measured with Syrinx.

One important advantage of Syrinx is a possibility to easy and conveniently label the recordings at different levels. The measured parameters of the labeled calls are automatically exported to the textual file (Avisoft does not allow to conduct the labeling and measuring parameters simultaneously; Fig. 39). Measuring parameters with Syrinx can also be conducted without the labeling. In this mode, the values will also be automatically exported to a textual file. In frames of this Task 4, we will know about the labeling calls with Syrinx for further analysis and with automatic measuring call parameters.

Suggestions for practical work

Inspect visually the file Siberian blue robin.wav in Syrinx program. Classify visually the song types present in the file. Describe shortly the distinctive and basic characteristics of each song type or provide their spectrograms in a separate textual file. By using the markers of Syrinx, label the songs in the file minding their type; then save the obtained file-annotation with measurements of the song types. Export the obtained measurements of the song types to Excel. Calculate with Excel the Mean \pm SD for each of the measured parameters in each song type.

Step-by-step guidelines to the work with Syrinx

1. Start the Syrinx and open the audio file Siberian blue robin.wav. By selecting from menu View \rightarrow Make Time Scale Fit Window you can zoom a small fragment of recording to the entire screen; if you need the higher or less resolution, use the commands View \rightarrow Zoom In / Zoom Out. For increasing / decreasing the brightness of the image, use View \rightarrow Make Spectrogram darker / Make Spectrogram lighter. Combination of the keys «Shift + C» will allow to reproduce the selected fragment of recording. Command Edit \rightarrow Copy Bitmap of Current Window will allow to copy the spectrogram to the external sources.

2. Inspect visually the recording. Create a visual classification of song types occurring in the file. Briefly describe the distinctive and basic characteristics of each of the song types. Using the markers of Syrinx, label the songs in the file (Tool \rightarrow Annotation \rightarrow Add Annotation Link \rightarrow indicate the place of storage and the name of the forming meta-

file and we obtain a separate metafile with labeling and comments). At labeling, contour accurately each call type so that the borders of the rectangular would pass through the minimal and maximal values of song frequencies, as well as the beginning and end of the vocalization. In such case, the created metafile will contain the actual values that can be used for the statistical analyses of parameters of vocalizations (the minimum and maximum values of sound frequencies, time of the beginning and end of sound). After the contouring, press the key «Enter» for exporting the measurements to metafile. Please label (by catalogizing simultaneously song types and measuring their parameters) all songs in the file, save the obtained file-annotation with measurements of all present song types. Export the obtained measurements of song types to Excel.

For convenient visual inspection of long spectrograms, select, in the section Options: Window Display Settings and then select the Number of display rows equal to 4. Then, on monitor, the spectrogram will be shown in a few lines, that can be convenient for comparative analysis of call structure. In the same section, it is possible to zoom the spectrogram image on the entire screen (the values of frequency should be indicated in Hz).

Suggestions for practical work.

Part 3. Audacity

Advantages of Audacity program

As Syrinx, the Audacity is free available software, but it is much more modern program (developed by the group Foos.Hub). As Syrinx, the Audacity allows creating call spectrograms and conducting express-analyses, including visual inspection of recordings, catalogizing calls using a convenient option of labeling and measuring the temporal parameters. The measured values are exported as a text file. However, the frequency and power parameters are unavailable for measuring. The Audacity is compatible with platforms Windows, Mac and Linux as well as with files of different types, including the wav and mp3 formats. In frames of this Task 4, we will familiarize with possibilities of Audacity for labeling calls for further analysis and with automatic measuring their parameters.

Suggestions for practical work

Inspect visually the file Yellow steppe lemming.wav using the program Audacity. Make the visual classification of call types occurring in the file. Describe shortly the distinctive and basic characteristics of

each of the call types or provide their spectrograms in a separate textual file. Using markers of Audacity, label 10–15 calls of each type, save the markers in the textual file-annotation. Export the obtained measurements of calls types to Excel. Calculate in Excel Mean \pm SD for each of the measured parameters in each call type.

Step-by-step guide to the work with Audacity software

1. Start the Audacity program (available for free downloading at link <https://www.audacityteam.org/>) and open the audio file (for example, Yellow steppe lemming.wav) from Menu: File \rightarrow Open. In the opened file, the main window is looking as illustrated on Figure 40 (at the initial stage, you will only see the oscillogram of the audio file).

2. In the left window (marked with red arrow on Fig. 40), press the panel with file name and arrow down; select «Spectrogram». The program will create the spectrogram of the entire file. Click with mouse on the lower border of spectrogram and extend it down to the borders of the screen. Again, in the left window (arrow-marked), press the box with the file name and, with pressing the arrow down, select «Spectrogram settings». For choosing most convenient parameters for visual

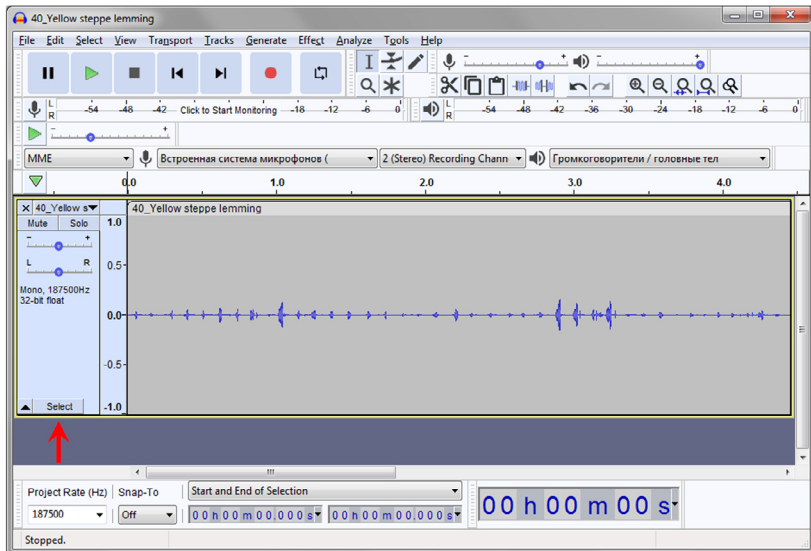


Figure 40. The main working window of Audacity. The working field described above in the text, is marked with red arrow.

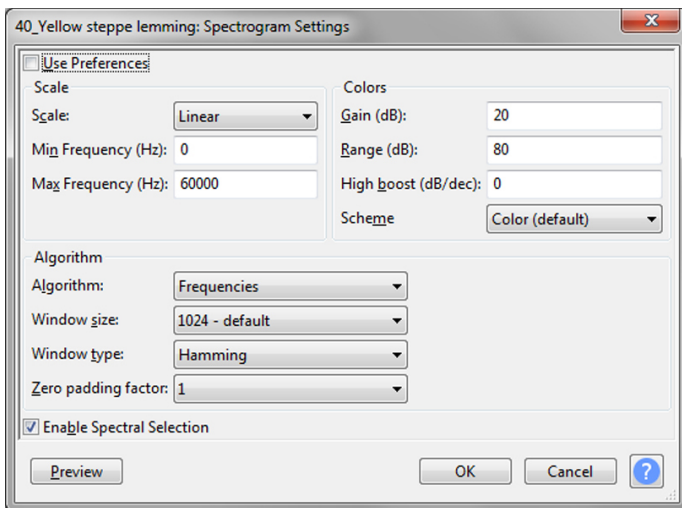


Figure 41. Example set of parameters for creating spectrogram of a yellow steppe lemming *Eolagurus luteus* ultrasonic call.

inspection of the calls, try to manually change the parameter values, e.g., «Scale», «Window size» or «Scheme». Example set of parameters appropriate for ultrasounds of yellow steppe lemmings is shown on Figure 41.

Important!!! Example settings indicated on Figure 41 are specially selected for the ultrasonic calls of yellow steppe lemmings. You can try to change them and to look for the result (for training). For other species with different characteristics of the calls, the settings should be selected separately.

3. Now you can train to scale the file on time axis using the keys with loupe images (Fig. 40), to playback some fragments of recording. You can try to edit the file (select from Menu Edit → Copy / Paste etc.). The program allows to open many windows (files) in one time, what is convenient for the visual confronting of calls of different animals.

A zoomed fragment of the file Yellow steppe lemming.wav with indicated spectrogram settings and scaled in time can look as it is shown on Figure 42.

4. As you can see, Audacity creates spectrograms of sufficient quality for visual inspection and catalogizing of recordings, and for describing the vocal repertoires of particular species. In addition, Audacity allows

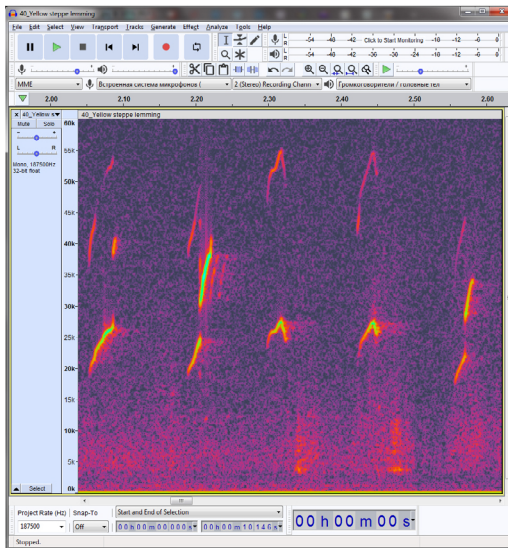


Figure 42. Main working window of Audacity, displaying spectrogram of yellow steppe lemming *Eolagurus luteus* calls.

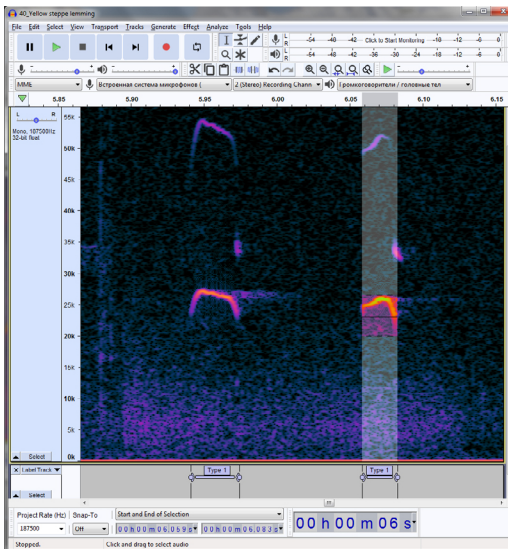


Figure 43. Main working window of Audacity, illustrating two labeled calls of yellow steppe lemming *Eolagurus luteus*.

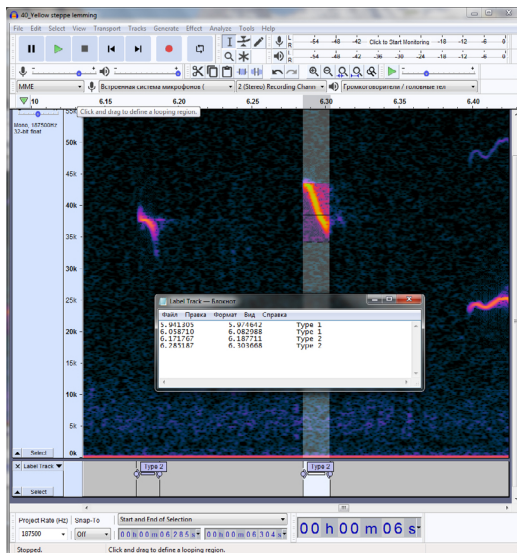


Figure 44. Textual file of Audacity program with automatically exported call parameters at conducting the marking procedure.

labelling the selected calls and conducting measurements of call duration.

With mouse cursor, label the selected call with maximum accuracy from the beginning to end. Then select from Menu: Edit → Labels → Add Label at Selection. In the window that appeared under spectrogram, write the name of call type which you assign to calls of such structure (for example, «Type 1»; Fig. 43).

5. After adding all labels, you can save time marks for each call to the textual file. For this, select File → Export → Export Labels, and write the name for the textual file. Time marks of the beginning and end of the selected sections and the names of the call types will appear in the list (Fig. 44). The file can then be opened with Excel, by converting it to the respective Excel format, and then you can calculate, basing on the obtained time marks, the mean durations of all call types.

Task 5.

Tracking and measuring call fundamental frequency and formants with Praat

Study objects

Mammals (adults)

Artiodactyla: three subspecies of red deer (*Cervus elaphus hippelaphus*, *C. e. hispanicus*, *C. e. sibiricus*), goitred gazelle (*Gazella subgutturosa*), saiga (*Saiga tatarica*).

Aims of the work

- 1) to track and measure call fundamental frequency;
- 2) to track and measure the frequencies of the first four (lowest) formants in calls of three ungulate species.

Introduction. The vocal systems of terrestrial vertebrates

In all terrestrial vertebrates, the vocal systems are arranged according to the general scheme and consist of three parts: the lungs, the sound source and the vocal tract. The lungs create the airflow which provides energy for sound production. Sound source (the larynx in mammals and the syrinx in birds) transforms the energy of the airflow to the acoustic energy, and creates the sound wave. The rate of vibration of the vocal folds (in mammals) or of the syringeal membranes (in birds) corresponds to call fundamental frequency.

Sound wave created by sound source passes through the volumes of the vocal tract: throat, mouth and/or nose, and, in birds, additionally via the trachea (because bird syrinx is positioned lower than the mammalian larynx, namely in the place of the trachea branching to the two bronchi). The vocal tract modifies the sound, working as the acoustical filter re-distributing sound energy, with accenting it in the area of the resonance frequencies (formants) for the account of the weakening the energy in the area of anti-resonances, thus changing the structure and sounding of calls created with sound source. As sound source of all terrestrial vertebrates is positioned inside the body, the output sound signal is always the result of work of both the source and the vocal tract.

Along to call duration and fundamental frequency, the formant frequencies represent the essential acoustic parameters which encode information about the caller: physical size, age, sex, physiological status (aggressivity, fear, motivation), emotional state (degree of discomfort, degree of arousal, emotional valence), individuality and species-specific traits. So, acoustic parameters related to the fundamental frequency and formants are measured in many bioacoustical studies. Modern methods of call analysis allow measuring the acoustic parameters related to both sound source (fundamental frequency) and vocal tract (formant frequencies).

All programs for call analysis we used so far (Avisoft, Syrinx, Audacity), only allow measuring parameters of fundamental frequency and duration. For transforming the sound wave to call spectrum, these programs use Fast Fourier Transform (FFT). However, for calculating the formant frequencies, another algorithm of sound wave transforming to call spectrum is necessary, the Linear predictive coding (LPC). The LPC algorithm, along to FFT, well-realized only in one program for sound analysis, the Praat, which was initially created for analysis of human speech.

Task for the practical work

Inspect visually the suggested files with audio recordings of the three species of ungulates using Praat; conduct the tracking and measuring the fundamental frequency and then track the first four formant frequencies in the indicated calls. The results of the conducted work (measurements of the fundamental frequency in 10 calls and measurements of formant frequencies in 7 calls) should be presented in the Excel table.

Step-by-step guide to the work with Praat

The Praat software is free. The latest version of Praat can be downloaded at link <http://www.fon.hum.uva.nl/praat/>. Unpack and start the program; the special installing is unnecessary. Below, the work with files will be described for Praat version 6.1.12.

Creating spectrogram

Start Praat. You will see two windows: Praat Objects (this is the main window of the program) and Praat Picture (which can be immediately closed, as it will not be used in frames of the current Task 5).

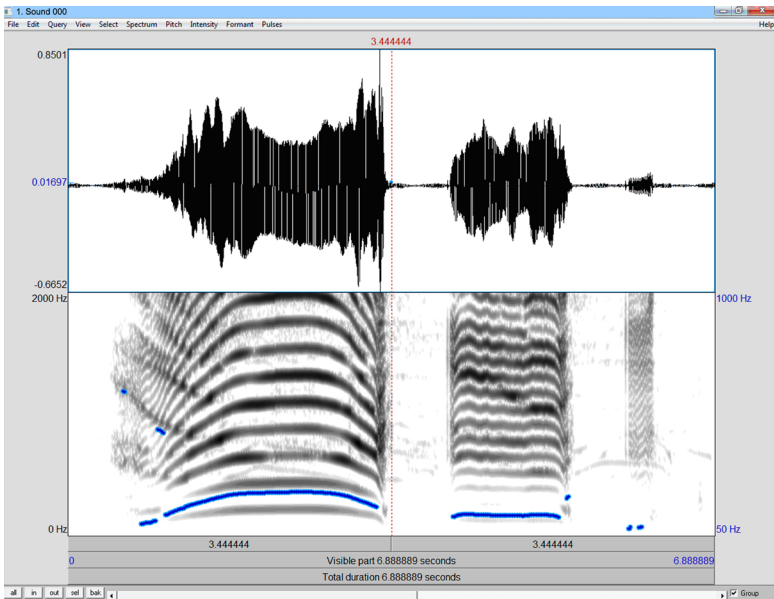


Figure 45. Spectrogram and oscillogram of male red deer *Cervus elaphus hispanicus* calls in the working window Sound of Praat.

For downloading and visual inspection of the audio file, select from menu Read → Read from file. The downloaded file will appear as the line in the section «Praat Objects». In the right column, select «View & Edit», and you will see the spectrogram of the file in a separate window Sound (Fig. 45).

Spectrogram zooming can be made using the keys «All», «Sel», «In», «Out», located in the left low angle of the window (sometimes they are not visible immediately, then you should extend the window to the entire screen of the monitor):

In — zooming;

Out — deleting;

Sel — extending the window up to the borders of the cursor (marked with red dotted vertical lines);

All — show the entire file on the screen;

Bak — cancel the last change (step back);

Under the spectrogram, there are three grey bands for playing back the audio file. Pressing the lowest band allows playing back the entire file, pressing the middle plays back would play back the visible part of the file and pressing the upper one would play back the part of file between the cursors.

Settings for tracking the fundamental frequency

At file opening, the program applies by default the settings used in the last time. However, if the current analysis demands different settings, they should be changed. For doing this, select from menu of the spectrogram window «Sound»: View → Show analyses → 50 s. Put a checkmark in the checkbox «Spectrogram, pitch». In Praat, the term «pitch» encodes call fundamental frequency. Now you will see call spectrogram and track its fundamental frequency (drawn in blue color), as on Figure 45.

Further, select from menu of the spectrogram window «Sound» View → Time step settings → Fixed, select «Fixed time step» equal to 0.01 s.

Then select Spectrum → Spectrogram settings from menu of the spectrogram window «Sound», select the following parameters:

View range — 0–2000 Hz;

Windows — 0.04 s;

Dynamic range — 60 dB.

Scale of values of the fundamental frequency is shown to the left of spectrogram (it varies from 0 to 2000 Hz on Figure 45).

Further, from menu of the spectrogram window «Sound», select Pitch → Pitch settings and specify the following settings:

Pitch range — 50–1000 Hz (these values should be set for each file separately);

Analysis method — cross-correlation;

Drawing – speckles.

Finally, select Pitch → Advanced Pitch settings from menu of the spectrogram window «Sound»: checkmark the box «Very accurate».

Scale of values for the fundamental frequency for «pitch» is shown with blue color to the right of spectrogram (50–1000 Hz on Figure 45).

Tracking the fundamental frequency — action steps

1. In the window «Sound», select, with cursor bordering, a fragment of sound file, then press «Sel» to zoom this call fragment to the entire window.

2. From menu of the spectrogram window «Sound», select Pitch → Extract visible Pitch contour (Fig. 46). In the window «Praat objects», the window «Pitch untitled» will appear.

3. Press «View & Edit» (in the right column of the window «Praat objects»). You will see the window «Pitch untitled» with track of the fundamental frequency band (Fig. 47).

4. In the window «Pitch untitled», set manually the missing points and delete the incorrect points. Inspect visually by spectrogram, which points are correct.

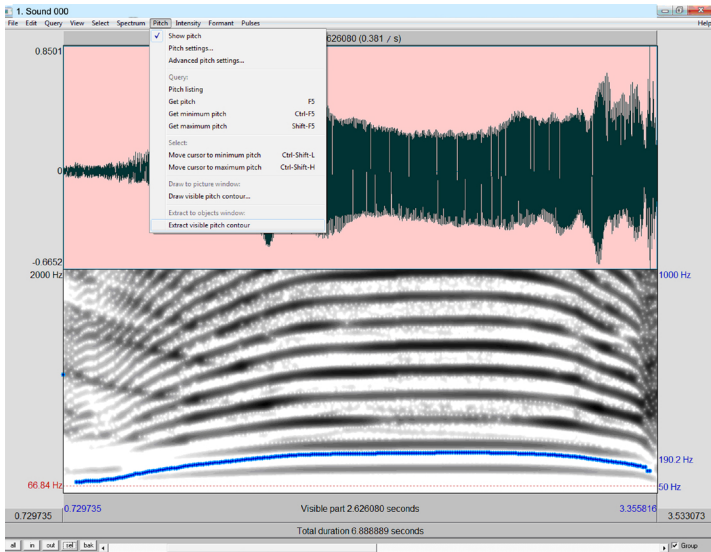


Figure 46. Spectrogram window prepared for tracking the fundamental frequency of a male red deer *Cervus elaphus hispanicus* call in Praat.

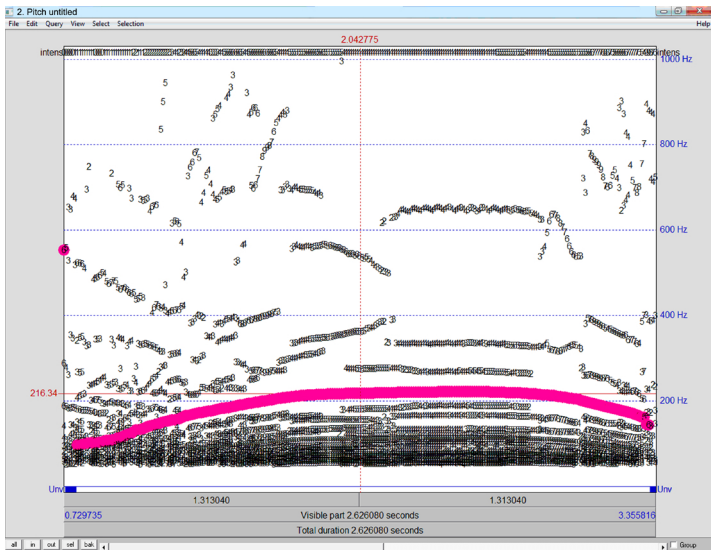


Figure 47. Window «Pitch untitled» with track of the fundamental frequency band of a male red deer *Cervus elaphus hispanicus* call in Praat.

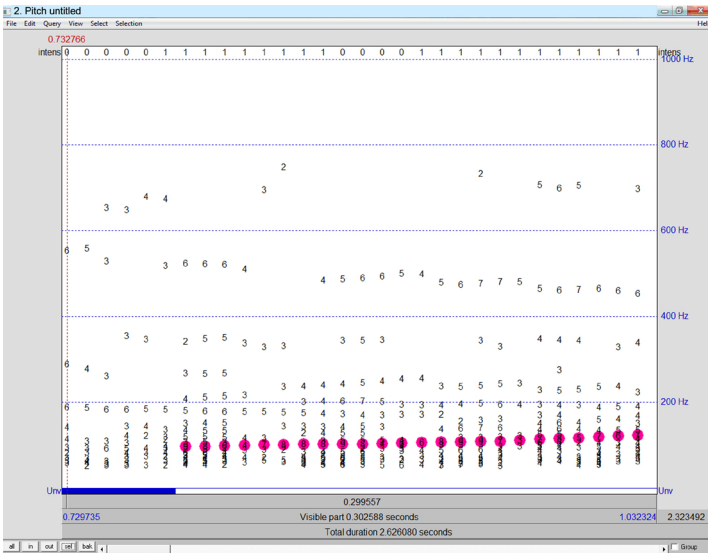


Figure 48. Window «Pitch untitled» with a zoomed track of the fundamental frequency band of a male red deer *Cervus elaphus hispanicus* call in Praat. Active values of the fundamental frequency used for the track calculating, are shown with pink points.

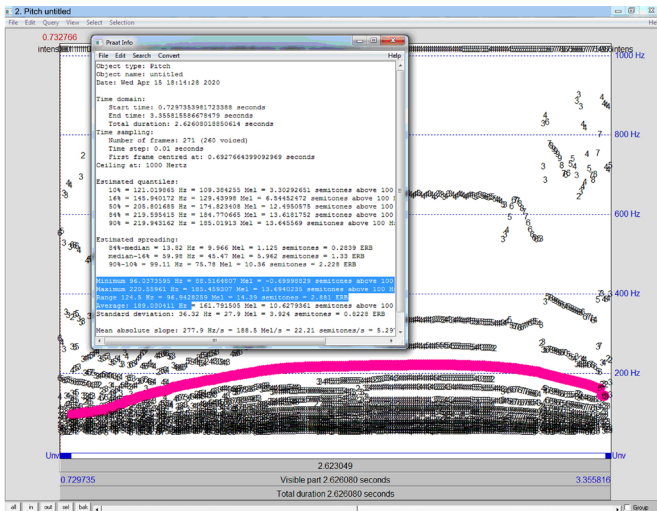


Figure 49. Window «Praat Info» with calculated values of the obtained track of the fundamental frequency band of a male red deer *Cervus elaphus hispanicus* call in Praat.

To add a new point, press the respective digit; it will become pink-colored at activation. For deleting the incorrect point, press the band «Unv» below the window, whereas the pink pouring of the digit will disappear and the point will become inactive. On the band «Unv», you will see the blue color of the inactive time frame.

Zooming the window can be done with keys «Sel», «In», «Out», located on the left under the window with track (Fig. 48).

5. For obtaining data from menu of the window «Pitch untitled», select Query → Pitch info. From the opened window (Fig. 49), copy (using «Ctrl+C») and paste to the Excel file (you can use the preliminary prepared file Praat.xls) the following values: «Minimum», «Maximum», «Range», «Average».

The Praat provides descriptive statistics only in the textual format. So, it is necessary to manually copy the values and re-distribute them to the respective cells of the table (Praat.xls, page «Fundamental frequency»).

Practical work on tracking the fundamental frequency

Please, measure the four values of the fundamental frequency (the minimum fundamental frequency — «Minimum», the maximum fundamental frequency — «Maximum», the depth of frequency modulation — «Range», the average fundamental frequency — «Average») for 10 calls of the three species (each call is in a separate file, the list of files is provided below) in Praat, and introduce the data of measurements in the file Praat.xls on the page «Fundamental frequency». Files with calls for analysis and the Excel table are available at link http://bioacoustica.org/practicum/practicum_eng.html. On the page «Fundamental frequency» the necessary actions (column «Additional Actions») on correcting (editing) the track of fundamental frequency in the window «Pitch untitled» are indicated.

The list of audio files for tracking and measuring fundamental frequency and additional settings «Pitch range» (which should be set before the beginning the work with each file separately in menu of the spectrogram window «Sound» using Pitch → Pitch settings → Pitch range) for each file:

File name	Pitch range (Hz)
C_elaphus_hippelaphus_1.wav	50–500
C_elaphus_hippelaphus_2.wav	50–500
C_elaphus_hispanicus_1.wav	50–500
C_elaphus_hispanicus_2.wav	50–500
C_elaphus_sibiricus_1.wav	100–2000

File name	Pitch range (Hz)
C_elaphus_sibiricus_2.wav	100–2000
C_elaphus_sibiricus_3.wav	100–2000
Gazella_subgutturosa_1.wav	20–200
Saiga_tatarica_1.wav	20–200
Saiga_tatarica_2.wav	20–200

Program settings for tracking the formant frequencies

For preparing the program for tracking the formant frequencies of the calls, delete the fundamental frequency track from the spectrogram window: in menu of the spectrogram window «Sound» select «Pitch» and delete the checkmark against «Show pitch». Now select from menu «Formant» and put the checkmark against «Show formant». The track of fundamental frequency will disappear and the red formant tracks will appear.

Select from menu of the spectrogram window «Sound» Spectrum → Spectrogram settings and set the working range for creating the tracks of the formant frequencies (now you can set the range 0–5000 Hz, but this range should be set for each file separately).

Then select from menu of the spectrogram window «Sound» Formant → Formant settings and set the following parameters for calculating the formant tracks:

Maximum formant — 4200 (will be set for each file separately);

Number of formants — 4 (for all files of the given task this parameter remains unchanged, but in reality, it can substantially differ between species depending on their specific morphological traits);

Window length — 0.04 s;

Dynamic range — 30 dB;

Dot size — 2 mm (Fig. 50).

What you should know for the correct selecting the settings for calculating the formant frequencies?

What are the values which we established in the fields «Maximum formant» and «Number of formants» and why they should be tuned for each species and call type separately for the correct tracking and measuring the formant frequencies? The parameter named as «Maximum formant» represents the upper (maximum) border of frequency range, in which the calculation of formant frequencies will be done. «Number of formants» means the number of formants (in units), which can be within limits of this frequency range. That is, if «Maximum formant» = 4200 Hz, and «Number of formants» = 4, this means, that you want

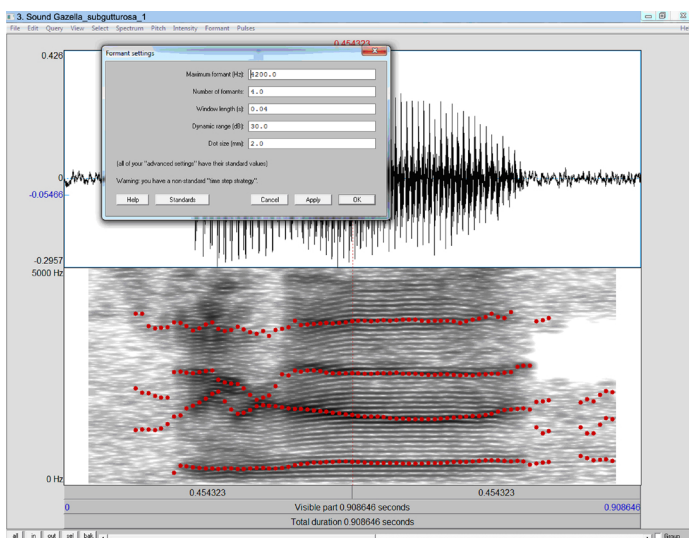


Figure 50. Spectrogram window prepared for tracking formant frequencies in goitred gazelle *Gazella subgutturosa* call in Praat.

to calculate the values of the four formants within range from 0 to 4200 Hz. Why we selected here these settings and why they are so important for conducting the correct analysis?

The values of formant frequencies depend, in the first order, on the length of the vocal tract and (for the one-end closed tube) can be roughly calculated by formula $F_n = (2n-1) * C / 4L$, where n — the formant number, C — sound speed (in moist warm air, approximately 350 m/s), L — the vocal tract length. That is, the longer is the vocal tract, the lower are the values of the formant frequencies. So, before the start of measuring formants in the calls, you should know (at least approximately) the length of the vocal tract of individuals of the given species, sex and age, in order to understand at which frequencies should be posed the formants in animal subjects of your study and to select correctly the settings for «Maximum formant» and «Number of formants» at calculating the tracks of the formant frequencies.

However, determining vocal tract length is not so easy. In particular, you should to take into account, that mammals can vocalize through the mouth and through the nose (in mammals, the nasal vocal tract length is always longer than the oral). In addition, transits from the nasal to oral emission or back can occur within a call. Moreover, some mammals can elongate the vocal tract length during the calls, by retracting the larynx down or by extending the nasal trunk. So, the anatomical-based

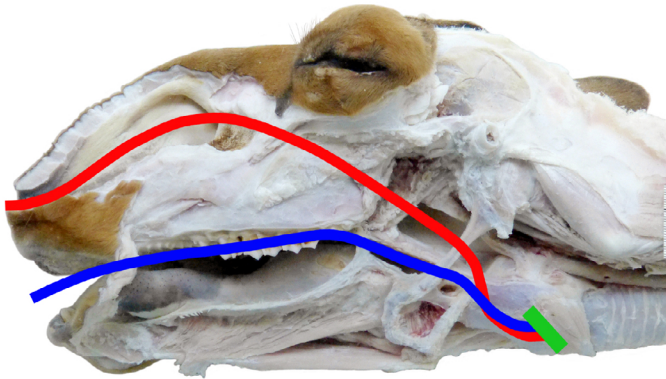


Figure 51. The lengths of the oral vocal tract (blue line) and of the nasal vocal tract (red line) of female saiga *Saiga tatarica*, obtained at conducting the anatomical dissection. A position of the vocal folds is marked in green. Scale bar is 10 mm. Taken from Volodin et al. 2014.

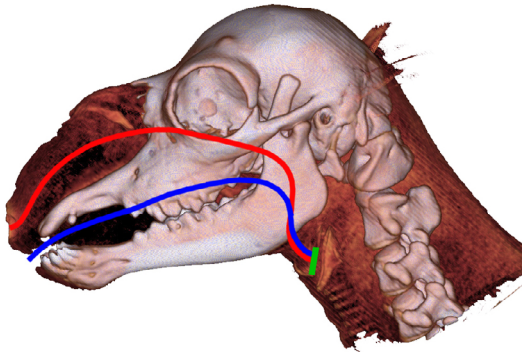


Figure 52. The lengths of the oral vocal tract length (blue line) and the nasal vocal tract length (red line) of saiga newborn calf *Saiga tatarica*, obtained using computer tomography. Position of the vocal folds is marked in green. Scale bar is 10 mm. Taken from Volodin et al. 2014.

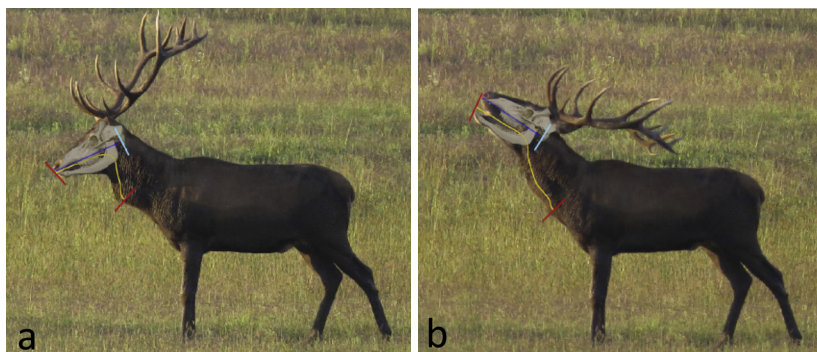


Figure 53. Calculated basal skull length (blue line) and the oral vocal tract length (yellow line) at rest (a) and during vocalization (b) of a male red deer *Cervus elaphus hippelaphus*, obtained at conducting video frame-by-frame analysis, as well as skull measurements and reconstruction of skull position. Taken from Volodin et al. 2019.

measurements of the vocal tract length can be insufficient and therefore the visual observations and even video recording of vocalizing animals can be necessary for understanding whether the calls are produced through the nose or through the mouth and how the vocal tract length changes during a call.

Below, we provide some examples of how the measurements of the vocal tract lengths of different species were obtained.

1. Saiga *Saiga tatarica*, adult female vocal tracts were measured at conducting the anatomical dissection. The oral vocal tract length (Volodin et al. 2014) is 240 mm, the nasal vocal tract length 320 mm (Fig. 51).

2. Saiga *Saiga tatarica* newborn. Vocal tracts were measured using computer tomography (Volodin et al. 2014). The vocal tract length is 116 mm, the nasal vocal tract length is 142 mm (Fig. 52).

3. Adult male red deer of the Central European subspecies *Cervus elaphus hippelaphus*. Vocal tracts were measured at conducting frame-by-frame video analysis along to skull measurements and reconstructions of skull positions (Volodin et al. 2019). The average skull basal length is 372 mm, the oral vocal tract length in resting position is 562 mm, the oral vocal tract length during vocalization is 800–810 mm (Fig. 53).

Tracking formant frequencies: sequence of actions

Download the audio file and create its spectrogram. In the opened window «Sound» select the audio file fragment which you want to analyse, then press the key «Sel» to expand the spectrogram to the entire

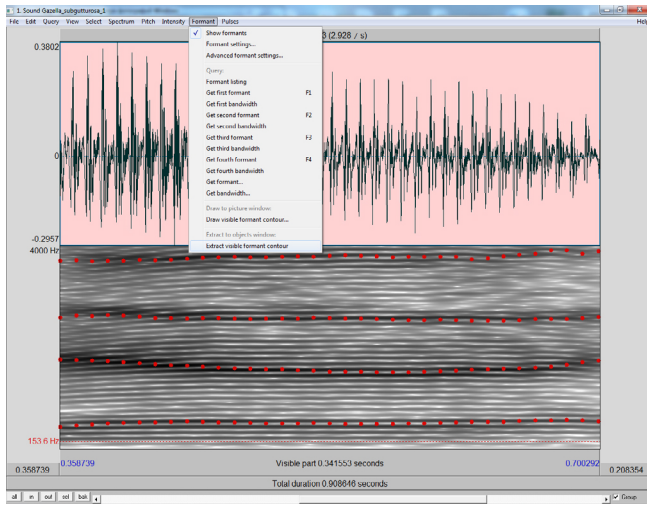


Figure 54. Spectrogram of a goitred gazelle *Gazella subgutturosa* call with tracks of the four formant frequencies and with opened panel for setting the parameters of formant frequencies in Praat.

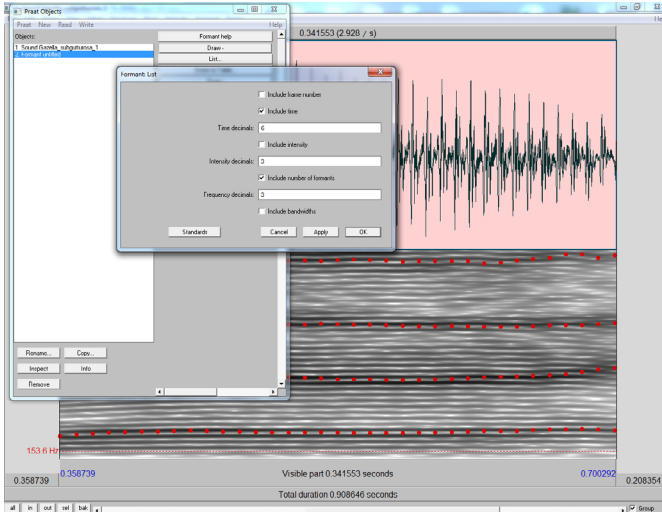


Figure 55. Spectrogram window displaying a goitred gazelle *Gazella subgutturosa* call with tracks of the four formant frequencies, with opened windows «Praat objects» and «Formant List» in Praat. Parameters for tracking the formants correspond to their description in the text.

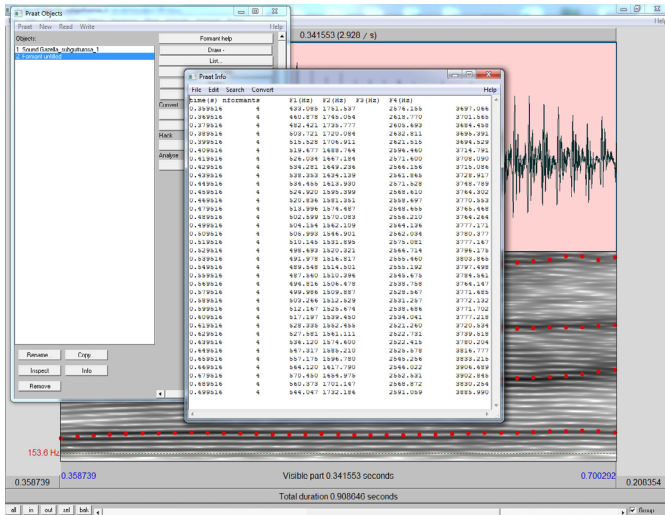


Figure 56. Spectrogram window displaying a goitred gazelle *Gazella subgutturosa* call with tracks of the four formants and with opened windows «Praat objects» and «Praat info» in Praat. Parameters for tracking the formants correspond to their description in the text.

window (for each file, rely on time marks «time1» and «time2» on the page «Formants» in the file Praat.xls).

Select from menu: Formant → Extract visible formant contour (Fig. 54).

In the window «Praat objects», the line «Formant untitled» will appear. Then select «Tabulate», then select «List» (in the right column of the window «Praat objects»). The window «Formant List» will open. Put the checkmark only against «Include time» and «Include number of formants», leave other fields unmarked (Fig. 55). Press «Ok».

The window «Praat info», with the necessary information on the calculated tracks of formant frequencies will open (Fig. 56).

Mark all data with cursor, copy them and insert in the file Praat.xls, page «Temp». At this page, two data volumes are generated, for the small (yellow) and large (brown) number of values. Insert the copied from «Praat info» data volume to the cell «time(s)» and mark it with cursor.

In the copied values, replace the decimal point with comma («Praat info» provides the values in which the decimal sign is the point). For doing this, select in Excel Edit → Change, and introduce the respective symbols; press «Change all». As the result, we receive the time marks indicating the real time of this mark in the audio file and the values of each formant in each time window. Besides this, below the volume,

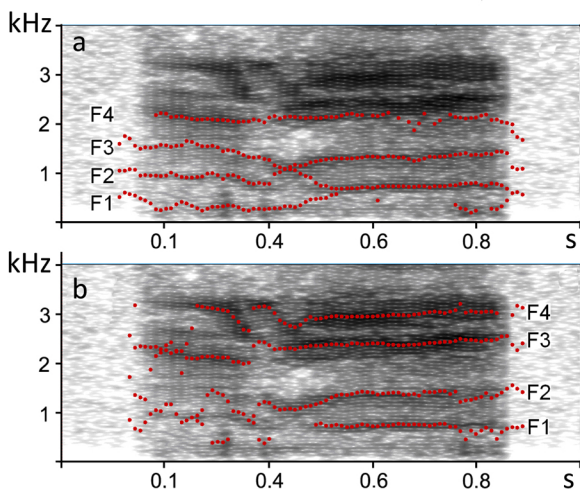


Figure 57. Positions of tracks of the first four formants of saiga *Saiga tatarica* calls, calculated for the nasal part (a) and the oral part (b) of the same call.

the average value for each column will be automatically calculated, that is, we will obtain the average values of the four formant frequencies (F1-F4) all over the track on the analysed call section.

Then, copy the average values of the formant tracks and insert them by selecting Special insert → Values) to the page «Formants» of the file Praat.xls.

Tracking formant frequencies — practical work

In frames of this task, you should measure (with Praat by tracks), the values of the first four formants for each of the 7 calls (each call is in a separate file, the list of the files is provided below) and introduce data of the measurements to the file Praat.xls on the page «Formants».

On the page «Formants», the borders for each call are indicated (in seconds, «time1» and «time2»), in which you should conduct the measurements of the formant frequencies (the borders for the measurements should be set with cursor in the spectrogram window «Sound», then press «Sel» and further follow to the above given description). Files with calls for analysis and the Excel table are available at link http://bioacoustica.org/practicum/practicum_eng.html. Examples of the correct positions of the tracks of the nasal and oral formant frequencies on spectrograms of saiga calls *Saiga_tatarica_3.wav* are shown on Figure 57.

The list of the audio files for tracking and measuring formant frequencies and additional settings «Spectrogram settings» and «Formant settings» (which should be established before the start of the work with each file manually via «Spectrogram → Spectrogram settings» and «Formant → Formant settings») for each file:

File name	Spectrogram settings	Formant settings
Gazella_subgutturosa_1.wav	0–5000 Hz	Maximum formant 4200 Number of formant 4
Gazella_subgutturosa_2.wav	0–5000 Hz	Maximum formant 4600 Number of formant 4
Gazella_subgutturosa_3.wav	0–6000 Hz	Maximum formant 5200 Number of formant 4
Saiga_tatarica_1.wav	0–8000 Hz	Maximum formant 6200 Number of formant 4
Saiga_tatarica_2.wav	0–5000 Hz	Maximum formant 3100 Number of formant 4
Saiga_tatarica_3.wav nasal part	0–5000 Hz	Maximum formant 2300 Number of formant 4
Saiga_tatarica_3.wav oral part	0–5000 Hz	Maximum formant 3400 Number of formant 4

Task 6.

Recording animal vocalizations for bioacoustical studies: choice of equipment and main rules for call collection under the field conditions

Study objects

Study objects can be any highly vocal species of mammals and birds (as many as possible), producing calls within human hearing range. The best place for conducting the practical training is a zoo.

Aims of the work

- 1) to collect audio recordings of as much as possible number of mammal and bird species, with detailed comments and descriptions in a paper diary followed with copying to electronic table;
- 2) to conduct a visual inspection of the obtained audio recordings and to make a short description of each recorded audio file.

Introduction. Choice of equipment for audio recording

Applying the bioacoustical approach in zoology

In the last decades, the applied aspects of bioacoustical studies develop actively, both in the field and in captivity. In captivity, bioacoustics approaches are applied for estimating animal reproductive state (e.g., in zoo felids for determining time for pairing, in farmed cows, for determining the moment of artificial insemination); for determining and correcting the degrees of discomfort in farmed animals (including the automatic acoustic detection), for sexing bird species without external sexual dimorphism and for developing the methods of individual acoustic monitoring. In the field, the bioacoustical approaches are used for searching the individual (group) markers in voice and monitoring “known” individuals or groups during the annual life cycle or from season to season; for playing back the recordings for luring and censuses of animals and determining their individual tags. Automated audio recording can be used for determining animal species composition and its changes throughout many years, as a

rule, by applying the automatic systems for call recognition, as well as for estimating biodiversity, estimating landscape quality and estimating the degree of habitat degradation (ecoacoustical approach).

Recording calls for conducting bioacoustical studies can be done manually or automatically. In the first case, a researcher can observe, in which situations the animals produce these or those calls and see which individual is calling (and can, therefore, to identify individuality, sex, age and other parameters of the focal animal), regulate the level of recording, actively change the distance to the focal animal. However, researcher's presence potentially affects animal behaviour, so, in some cases, animals (even tolerate to humans farmed animals) can change or even stop the normal behavioural activity. Moreover, animals can vocalize only time to time (for example, at night or irregularly, with large intervals), what makes the manual recording difficult. In such cases, the automated recording is preferable, using different automated audio recorders (SongMeter, Audiomoth, Audiolog and others). With automated recording, it is possible to obtain a large massive of high-qualitative calls, because animals, in this case, do not afraid of human presence and vocalize in vicinity of recording equipment; however, identifying individuals from the automated recordings is impossible. Automated recorders allow conducting audio recording by predetermined schedule for periods up to a few months in the autonomous mode. Such recorders are very solid and water-proof. With help of automated recorders, it is possible to track species' vocal activity in dynamics for prolonged time periods (for example, to track the dynamics of rutting vocal activity). However, using such devices, a researcher should keep in mind, that then a lot of time should be spent for visual inspection of the audio files and searching the recorded calls of the focal species.

Equipment for audio recording

Equipment for audio recording includes a portable recorder and microphone. In the last years, in relation to progress of digital audio recording, the classical tape and cassette recorders became completely out of use. Currently, audio recording is primarily made using portable digital recorders with memory cards. Digital recorders immediately transform the calls to audio files and store them on the memory cards, emancipating the researcher from the need to digitize the collected material. Furthermore, digital recorders have not any movable parts, what makes them more reliable.

Digital recorders for field recording should be portable and well-defended from environmental impact, because they should work at various temperatures and moisture as well as at the wind, and periodically resist against quite strong mechanical shocks. Control must be carried

out by mechanical buttons; sensory panels are inappropriate, as they are capricious and poorly visible in the sun. Recorders should be controlled with one hand; the number of control buttons should be minimal (ideally, only three — Record, Pause, Stop); they should be positioned comfortably under the fingers of a hand. It is better to use batteries or rechargeable batteries, as built-in storage batteries cannot be charged during long trips (because during the day you work, whereas at nights, there is no sun for charging the devices).

Field recorders should obligatorily have a possibility of manual regulation of recording level, which allows to take into account the intensity of caller vocalizations. Automated regulation of recording level, occurring on non-professional recorders and smartphones is inappropriate, as, in this mode, a recorder tunes itself for the loudest calls (as the wind, transport noise, loud calls of other animals), smoothies the differences between different call parts, and aligns the entire recording by intensity.

At selection of recorder, pay attention on the very convenient function of prerecording, which enables to record calls emitted a few seconds before the real start of recording. This function is especially convenient for recording animals, producing calls rather rarely, for example one time per 5–15 minutes. Using prerecording, you can be «on pause» during intervals between calls, turning on recording only at the moment of calling activity. In this case, the prerecording function, from one side, will not give you to miss the first seconds of vocalization, and from another side, you should not waste the memory and power for recording the long silent spaces between calls.

It is important to pay attention on the range of frequencies covered by recorder. All modern digital recorders qualitatively record calls in a broad range of frequencies, at least up to 24 kHz. But if you plan to work with animals using the ultrasonic range of frequencies (for example, with bats or rodents), this range is insufficient. Anyway, mind that the working frequency range of recorder corresponds to the half of the maximal value of sampling range, that is, at sampling rate 48 kHz, recorder will collect the calls at frequency range up to 24 kHz, at sampling rate 96 kHz recorder will collect the calls at frequency range up to 48 kHz etc. However, doubling sampling rate will increase twice the volume of the audio file. So, before the start of recording, it is necessary to select the working range of frequencies of recorder (sampling rate) correspondingly to the maximally possible frequencies of calls of the study animal species.

The recorder should have the option for recording calls in non-compressed format (to form the files with extension *.wav). This allows further conduct a detailed analysis of call acoustic structure, because the professional spectrographic programs, as a rule, do not work with compressed audio files (with extensions *.mp3 and similar).

It is recommended to use the recorders with professional acoustic ports for connecting microphones (XLR), as they provide the reliable fixation and low level of noise. The more simple ports (JACK) are commonly destroyed fast and can damage the recording by adding the unpleasant acoustic background (noise).

Mechanical reliability of buttons on the working panels is also important. The buttons should tolerate the permanent changes of working modes, weight of recorder and amount of used energy.

For outdoor recording, the following recorders are currently available: Zoom (H1, H4, H5, H6); Marantz (PMD-660, PMD-661, PMD-671); Tascam HD-P2; Fostex FR-2. For recording the ultrasonic calls, there are portable recorders Pettersson D1000X, Echo Meter Touch2 Pro. The Echo Meter Touch2 Pro allows conducting visualization of audio recording in live mode (showing the spectrograms of ultrasonic calls on the smartphone display), what is very convenient for the researchers, as ultrasonic calls are inaudible and therefore researchers cannot control the audio recording process by ear. Important, that new models of recorders appear each year, so, it is better to buy them not «for the future», but for the current particular task.

Unlike recorders, microphones update much more rarely and can serve 10 years or more. Microphones, by their construction, can be subdivided to the dynamic and condenser microphones. The dynamic microphones are simpler constructively and therefore cheaper and more reliable during the work; whereas the condenser microphones allow recording fainter calls and higher-frequency calls. The upper border of the nominal frequency range of microphone (that is, frequency range, in which the microphone minimally distorts the recorded calls) should be, as a rule, not below 15–20 kHz.

For different tasks, microphones with different «sensitivity» and directionality should be used. For recording intense calls at close distance (dog barking), weakly-directional (cardioid) microphones with sensitivity of about 2 mV/Pa are appropriate, whereas for recording faint calls at a remote distance (e.g., contact calls from the red deer herd), directional, highly sensitive microphones should be used, with sensitivity at least 30–40 mV/Pa. Below, you can find the list of relatively inexpensive microphones in the order of increasing their sensitivity and directionality: Sennheiser-e845 (dynamic); AKG-C1000S; Audiotechnica AT-835B; Sennheiser K6-ME-66 and Sennheiser K6-ME-67 (condenser).

Conducting audio recording

In some aspects, audio recording of animal calls is similar with photography: whereas many people have good photo cameras, much effort is necessary in order to make a good photo. The general principle

is simple: the closer is the animal, the higher-qualitative calls you can record. But during recording, you should minimally affect the animal behaviour. If it is impossible to approach secretively (e.g., in the open steppe, desert, tundra), you can use the automated recorders as remote microphones. In places of animal concentration, researcher can establish the automated recorders for the continuous recording and observe from a few hundred meters or depart if the animals continue to react to a human. During recording in captivity, the distance should not be too close, as at very close distance (e.g., less than 0.5 m for a not large animal of fox size or less than 0.3 m for a rodent of a ground squirrel size) animal breathing can also be recorded and substantially distorts the audio file.

Before starting the recording, select preliminary, in the pause mode, the approximate level of recording, corresponding to the intensity of calls of the focal animal. Indication on the panel of recording level should display that target calls will be recorded without distortion (overloading), with a high, but not too high, level of recording. That is, the indication of recording level should be within the green field and do not come to the red field. The recording made with overloading is scrap, such recordings have a destroyed structure and can only be used very restrictively.

If it is impossible to qualitatively record the series consisting of calls strongly differing in intensity, you should make two recordings one after another. During the recording, you can initially ignore the quality of faint calls, by using for recording the low level of recording; then, you can record qualitatively the faint calls at high level of recording, with the exceeding level of recording for the intense calls. In the first recording, faint calls will be practically inaudible, whereas in the second recording, faint calls will be recorded normally, whereas intense calls will be recorded with overloading.

During recording, researcher should observe behaviour of a caller, and, when necessary, comment it, trying to put the comments during the intervals between calls. For this, in some cases, you can use the stereo recording, where the animal calls are recorded to one channel, whereas the researcher' comments are recorded to another channel. For recording the voice comments, in this case you can use loop microphone, fixed on clothes, whereas the main microphone for recording would be better to locate distantly on the long cord, closer to the focal individual.

The comments should also include information about all changes of the mode of recording (level of recording, distance to the animal, its orientation relative to microphone, location of researcher). In cases when a few animals vocalize simultaneously or the recording is too noisy,

each call of the focal animal caller should be commented by voice (for example, at call-over or duet of two animals, using the words «first – second» / «male — female» / «parent — offspring / «call» etc.). After ending the recording, it is necessary to make the expanded commentary («label»), which should include the place, date and time of recording, animal species, individual, distance, orientation of the focal individual to microphone, situation and behaviour of the focal animal, presence of other individuals etc.

Introducing some noisiness during the recording is unavoidable, however, it should be kept at minimum. As a microphone records well even very weak mechanical oscillations, for excluding the interferences, you should avoid the touches by the microphone to any objects (wire mesh, branches, grass). Fingers keeping the microphone should be immobile. The microphone cord should not be dragging on the ground or touch any objects. Always use the windproof (especial foam head, which is in complete of any qualitative microphone). However, in windy weather, would be better do not conduct the recordings at all, without the extreme need (distortions are unavoidable), if it is necessary, the microphone should be shield-proofed against wind, at least by own body, and the recording should be longer, with hope that at least some calls will be recorded between wind gusts.

Batteries should be kept separately from the recorder or condenser microphone and attached to the equipment immediately before the work. This will allow to avoid damaging the equipment by the electrolyte occasionally leaking from the strongly used batteries. During recording at a negative temperature, the power cells can discharge very fast, so you will need two complects of batteries used alternately, one in the recorder, another one is heating inside clothes. Otherwise, you can use the external power cell kept inside clothes. If the recorder is small in size, it can be kept inside clothes, by hanging around your neck, so that only the external microphone on the cord was outside.

Researcher should avoid the rustling synthetical clothes, because they produce intense sounds with spectrum up to 40–50 kHz at arm and leg movements. These noisy clouds can overlap entirely the target calls. In addition, many animals hear in the ultrasonic range (for example, domestic dogs hear up to 41–47 kHz, Heffner, 1983) and therefore can behave unnaturally.

When you go to collect the calls, take with you an additional complect of fresh batteries or accuses and the additional memory cards. There is a probability that batteries will be discharged at the most interesting moment of recording, or a well-familiar animal will start vocalizing somehow unusually, or just something other interesting thing will occur.

Same-day processing and storage of audio recordings

Audio files with recordings of animal calls should be copied from the memory cards to computer. During the multi-day call collecting in the field, the collected recordings should be visually inspected using a spectrographic program in the evening of the same day. This provides researcher's feedback, allowing to adjust the distance to the animal callers and the settings for recording their calls in the next day. Moreover, you will immediately obtain the impression regarding the real number of well-recorded calls: you can think that you recorded many calls, whereas in reality you may see, for example, that 90% of the calls are broken with the wind.

Ideally, the audio files should be re-named and sorted by the folders correspondingly to animal species. Detailed data regarding the mode and conditions of recording with all comments should be copied from the diary to the electronic tables. As a rule, the full passport of recording contains the name of the audio file; date and time of recording; caller species, sex; age, maybe name; call type and context of emission; level of recording and the distance to the animal; place of recording; weather; call collector (researcher) name. For better storage of the audio files, their copies should be stored also on other computers and other external digital storages.

Task for the practical work

Please, conduct the audio recording of the calls of the vocally active species of mammals and birds. During the recording, keep in mind the following things:

1. For each situation, please select the particular level of recording (desirably, it should be done when the recorder in «pause» mode).
2. Do not forget about the «label» for each file (comment with voice the place, date, species, individual, situation, behaviour and other additional information when necessary), it is convenient to do this at the end of the file, when recording of target calls is ended.
3. Comment for the duration of the recording, labelling the calls with voice (this way, it will be easier to find them and to distinguish them from the alien noise and calls of other individuals).
4. Do not forget about the defence of the equipment against rain and wind.
5. After conducting each recording, put on a comment to the field diary, with indicating the file (files) name, their duration, animal species, individual, distance, orientation of the individual caller to microphone, other conditions of recording, situation and behaviour, presence of conspecifics and other interesting things if necessary.

6. Do not forget to take with you the extra batteries and memory cards.

After conducting the recording, please copy the collected audio files to your own computer. Inspect visually the collected recordings, estimate the quality of the recorded calls, make up a catalogue of the recordings (fill the Excel table with indicating the date, time, author or the recording, used equipment, ordering numbers of recordings, duration of recording, vocalizing species during the recording, approximate numbers of recorded calls, situations and comments for each file). Please conduct the work over the mistakes, by discussing the made mistakes at conducting the recordings and think how they can be avoided in the future.

Acknowledgments

Authors thank Elena Volodina, Vladimir Ivanitsky, Irina Marova, Irina Beme, Eugenia Bragina, Olga Filatova, Maria Goncharova and Vera Matrosova for help with preparing particular parts of this guide and for providing the teaching materials for the practicum on bioacoustics. Authors thank the reviewers Marina Rutovskaya and Leonid Korzun, for their valuable comments and discussing the text and content of this guide book.

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Учебное издание

Klenova Anna Viktorovna
Volodin Ilya Aleksandrovich

PRACTICAL GUIDE FOR BIOACOUSTICS

Translated from Russian by *E. V. Volodina*

Electronic book for network distribution

Художественное оформление, верстка, обработка иллюстраций *К. В. Саутенков*

Макет утвержден 18.11.2022. Формат 60×90/16. Усл. печ. л. 6,0. Изд. № 12230



ИЗДАТЕЛЬСТВО
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УНИВЕРСИТЕТА

119991, Москва, ГСП-1, Ленинские горы, д. 1, стр. 15
(ул. Академика Хохлова, 11).
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ISBN 978-5-19-011802-5



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