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From the Columbus Zoo to the Borror Laboratory of Bioacoustics: Animal Vocalizations III

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This full-day workshop was devoted to learning about animal vocalizations and what characteristics students can measure. This was the third workshop on animal vocalizations held at ABLE conferences. We visited the Columbus Zoo to record animals such as birds and primates using professional recording equipment (Marantz PMD660 and PMD670 recorders and Audio Technica and Sennheiser microphones). We analyzed vocalizations recorded at the Borror Laboratory of Bioacoustics, located at the Ohio State University (OSU) Museum of Biological Diversity. We examined our own recordings with the free-downloadable software Raven Lite, in order to learn about sounds displayed as spectrograms and as waveforms and the characteristics that scientists measure from these. Sample data included an Excel spreadsheet with the number of vocalizations, duration of each, and frequencies of the various tones or formants seen in the spectrograms. We then formulated hypotheses as to the meaning or function of the various vocalizations. This exercise is transferable to any zoo or park where animal vocalizations can be heard and recorded.

Keywords: animal sound recording and analysis, bioacoustics, animal vocalization

Introduction

Objectives

The objectives of this lab were: to record animal vocalizations at the Columbus Zoo using professional recording equipment provided by the Borror Laboratory of Bioacoustics, analyze these recordings using freely available sound analysis software, investigate features of the vocalizations and, where possible, see how these correlate with the context in which they were given.

Prior to this lab, participants should familiarize themselves with how animals use sound to communicate by exploring, for example, the webpages on this topic by the Howard Hughes Medical Institute (http://www.hhmi.org/biointeractive/how-animals-use-sound-communicate). This tutorial explores three animals that are communicating in different ways. Elephants can detect low frequency sounds through surface vibrations of the earth through their limbs, which helps them communicate with each other. Birds make different patterns of vocalization, and the second exercise in the tutorial challenges the students to differentiate between two species of birds by listening to or viewing their songs. In a third exercise, students listen to and watch a recording of bat sonar. Of course, the frequencies of the bats are too high for the human ear to detect, but one can hear and see the spectra of the insects (in addition to seeing the spectra of the bats' sonar). The tutorial allows the student to slow down the recording, which drops the frequency to the human hearing range, which the students find very intriguing.

Animals vocalize for various reasons and may alter their vocalizations depending on context. In a zoo environment, we can expect animals to give food-related calls (e.g. to get the attention of a keeper), alarm calls, and possibly also contact and social calls if the animals are housed in groups; less likely are territorial or mating calls (although male sea lions at the Queens Zoo in New York barked incessantly during mating season, according to zookeepers (Nolan personal communication)). Birds are particularly vocal animals and may be best suited for this exercise, but early in the morning, mammals may also be vocalizing, especially in the context of feeding.

At the Columbus Zoo where this workshop took place we could have expected the following animals to vocalize: African grey parrot *Psittacus erithacus*, Asian small-clawed otter *Amblonyx cinereus*, bettong

Bettongia penicillata, black and white colobus *Colobus angolensis*, black swan *Cygnus atratus*, Caribbean flamingo *Phoenicopterus ruber*, East African grey-crowned crane *Phacochoerus aethiopicus*, Guinea fowl Numididae, Humboldt penguin *Spheniscus humboldti*, kiwi *Apteryx* sp., langur *Trachypithecus cristatus*, mandrill *Mandrillus sphinx*, red crowned crane *Grus japonensis*, saddle billed stork *Ephippiorhynchus senegalensis*, vervet monkey *Cercopithecus aethiops*, Western lowland gorilla *Gorilla gorilla*, and white handed gibbon *Hylobates lar*.

Given the exotic nature of many of these animals (as might be the case with animals in a zoo near you), we suggest that participants (and students) familiarize themselves with one or two focal animal species ahead of time before a field trip. A good resource is the Animal Diversity Web http://animaldiversity.org/, which also provides information about some of the species' vocalizations.

Participants in this workshop (and future students) will need time to locate an animal of interest and set up for their observation period, i.e., find a spot where they can observe the animal without disturbing its behavior. They will need to connect microphone and recorder and can start recording as soon as they are settled. The recordist should begin the session by speaking the date, time, location, and the species of the focal animal. The recordist can then add any behavior he/she observes as well as record the animal's vocalizations.

Given the opportunistic nature of most vocalizations, participants may have to spend considerable time at the animal's enclosure to obtain a good recording. We suggest that participants focus on one species/animal and follow it for a set amount of time (e.g. 30 minutes), observing its behavior while recording its vocalizations. The behavior can be spoken onto the sound recording, but this should be done only intermittently, taking care to not overlap the animal's sounds. We know from experience that continuous narration makes animal sounds difficult to isolate and analyze in the lab.

The amount of time then spent analyzing the recordings is flexible. Prior to the start of the workshop, participants (and students) should download the free software Raven Lite from the Cornell Lab of Ornithology (http://www.birds.cornell.edu/brp/raven/RavenOverview.html) onto their laptop. With advance preparation, summary measurements of the number, durations, and peak frequencies of vocalizations can be done quickly. With additional time, these measurements can be used in various calculations and different vocalizations can be compared.

To illustrate how such comparisons might be useful in hypothesis testing, Figure 1 shows a visual representation, including both a waveform and a spectrogram, of two different calls recorded from male and female northern white-cheeked gibbon *Hylobates leucogenys,* by Ted Spellmire at the Columbus Zoo in 1996. These are the typical morning calls of this gibbon pair while brachiating and displaying. The recording is archived in the Borror Laboratory of Bioacoustics at The Ohio State University (cut number BLB21327, http://portal.vertnet.org/o/blb/recordings?id=urn-lsid-biosci-ohio-state-edu-osuc-occurrences-blb21327).

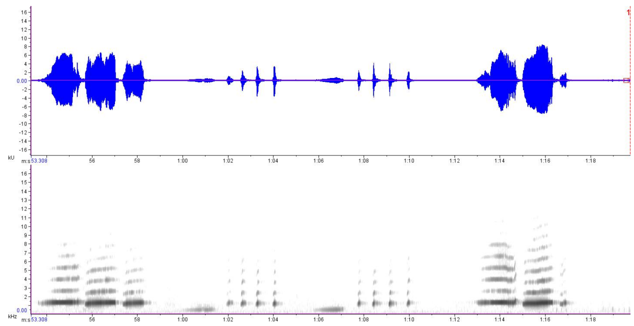

Figure 1. Spectrogram of morning calls of a pair of male and female white-cheeked gibbon *Hylobates leucogenys* (BLB21327). Total duration is about 26 seconds (horizontal axis).

The top part of Figure 1 shows the waveform of the sound (amplitude over time) and the bottom part shows the spectrogram (frequency over time). Multiple horizontal lines in the spectrogram depict harmonic overtones of the fundamental frequency (darkest line at the bottom).

For information on similar previous workshops, see Nolan et al. (2015), Nolan et al. (2016) and Nolan and Callahan (2018).

History of the Borror Laboratory of Bioacoustics

The analysis of the recordings can be performed in any lab with computers and Internet access. For this specific workshop, participants visited the Borror Laboratory of Bioacoustics (BLB), one of the largest sound archives in North America, housed at the Museum of Biological Diversity at The Ohio State University, Columbus OH.

The BLB's collection of recorded animal sounds began in 1948 as the research tool of Dr. Donald J. Borror, professor of entomology at The Ohio State University, and his students. It is one of the oldest and most extensive in the world, exceeded in size and in taxonomic diversity in the United States only by the Macaulay Library at Cornell University's Laboratory of Ornithology, and in taxonomic diversity by the sound collection of the Florida Museum of Natural History. Today the BLB houses over 49,000 recordings of birds, insects, amphibians, and mammals (https://osuc.biosci.ohio-state.edu/blb/).

In a project funded by the National Science Foundation, the BLB copied Don Borror's entire collection of recorded sounds from 1/4" analog tape to digital .WAV files. Since then, work in the lab has focused on digitizing recordings by other researchers (e.g. Louis Baptista at the California Academy of Sciences, San Francisco CA) and other collections (e.g. Florida Museum of Natural History, Gainesville FL). The BLB employs undergraduate students for this task and teaches them valuable skills in maintaining research collections.

Literature Search and Examples of Types of Information Gained from Recording Animal Vocalizations

The literature carries a tremendous number of examples of animal vocalizations and their analysis; here we cite just a few examples to give students ideas for hypothesis testing or future research projects. There appear to be three environments for acquiring animal sound information.

1. Following and recording animals in the wild---this can involve observations, setting up cameras and recorders and/or data loggers, or using radio collars.
2. Recording captive animals in a zoo---these animals may have been in captivity for a time---also, studying behavior and vocalizations of babies, juveniles, and adolescents appears to be popular---scientists are studying both parent-offspring interaction, and a change in vocalization with age, called vocal ontogeny.
3. Capturing animals from the wild and studying them for a short period of time in a laboratory to record their vocalizations and then releasing them back into the wild.

Below we give examples of information about animal vocalization gleaned from each environment listed. Change of vocalizations with geography, categorization of vocalizations, sexual differences of vocalizations, change in vocalizations during aging, and correlation with types of behavior such as mating or food handling were explored.

Students also might want to read about the concept of soundscape ecology, in which sound could serve as a signature of an ecosystem (e.g. Pijanowski et al. 2011). Students should be encouraged to categorize sounds and to label them themselves. An example by Řeháóvá-Petrů et al. (2012) includes five long-distance calls between or among adult tarsiers (radio-collared) ("loud call, smack-whistle, and whistle - then a soft locust-like chirp and a bird-like trill"). These authors called an additional two calls between mother and offspring "twitter and cheep".

Following and Recording Animals in the Wild

Ng et al. (2016) found only a weak correlation between geography and species of cuckoo doves in Pacific islands. However, using a set criterion for bioacoustics data, they decided that several cryptic species existed in their sampling. Thus, bioacoustics data might be able to resolve differences among cryptic species groups.

The use of bioacoustics could be expanded when students read about the work of Frommolt (2017), who conducted long-term recordings in a remote restored bog area in Germany. He detected 61 species of birds, including rare nocturnal ones. He is using these recordings to study breeding calls, migration, and perhaps even the influence of climate change on birds. He points out that these recordings are good for determining species richness, but that it is more difficult to determine abundance. Luef et al. (2016) depicted two very different spectrograms that were produced from two different types of calls of wild gorillas - singing and humming - while handling food.

Zubakin et al. (2010) studied the spectra produced by trumpeting, babbling, and cackling of auklets during their mating. Ellis et al. (2015) studied sexual selection in koalas and noted that the amplitude of male bellows during breeding season was correlated to size and thus perhaps both attraction to females as well as avoidance of older, aggressive males. Volodina et al. (2018) analyzed the spectrograms of hisses and snorts (considered vigilance calls) of wild giraffes.

Recording Captive Animals in a Zoo

It might be useful for students to attempt to characterize categories of behavior and make an ethogram. An example is given in Smith and Wassmer (2016), in which behavior of subspecies of an endangered bird on a Pacific Island chain was recorded. The behaviors of animals they studied in captivity included: sexual, parental, antagonistic, social, maintenance, ingestion/egestion, movement, vocalization, and resting behavior and behavior based on the captivity itself. When students begin to learn to categorize behavior, a next step might be investigating whether the vocalization correlates with certain types of behavior.

Vocalizations may be used as a survival mechanism to help mothers locate their offspring and vice versa. This is a powerful addition to sight, because it can be used nocturnally as well as diurnally. One example has been shown in farmed red deer, in which oral and nasal vocalizations can both be used to discriminate among individual hinds and by calves to identify their mother (Sibiryakova et al. 2015). Seiler et al. (2015) were able to identify at least six types of loud calls in certain sportive lemurs on Madagascar. Through playback of known calls, they believe that loud calls function as advertisement and territory marking, but more work needs to be done. Whines, snorts, threats, and pants, were the names given to vocalizations of juvenile white rhinoceroses by Linn et al. (2018). Their study was a preliminary study to investigate how the vocalizations changed with age. They found that, of these four vocalizations, only the whines decreased with increasing age of the animal.

Herler and Stoeger (2012) recorded and examined vocalizations of six Asian elephant calves in two European zoos. They studied both characteristics of the spectra of four types of vocalizations - roar, rumble, chirp, and trumpet - and their associations with various types of behavior. For example, they found that chirps and trumpets were more common when the calves were playing, and that they would roar in a stressful situation, such as being separated from their mothers. This points out the importance of long-term studies of vocalizations if one is seeking to use them to identify specific individuals.

Hradec et al. (2017) studied vocal ontogeny (development) of juvenile and adolescent male gibbons in Czech zoos. Adult calls are sex-specific. A fascinating hypothesis that these authors developed was that the more "female" juvenile vocalizations might protect them from adult aggressive males.

Silver foxes raised on experimental farms in Russia exhibited a variety of vocalizations (whine, moo, cackle, growl, bark, pant, snort, and cough) in their responses to humans (Gogoleva et al. 2013). The vocalizations of 14 adult cheetahs were studied in four Russian zoos (Smirnova et al. 2016). Chirr, growls, howls, and hisses were related to courting or aggressive behavior. Chirps, purrs, and meows did not appear to be context-specific. One interesting conclusion they made was that vocal individuality was unstable over the years.

Therrien et al. (2012) were able to discern different patterns in whistle types of dolphins which they recorded using underwater hydrophones. This workshop recording airborne sounds could be extended to recording animals that vocalize underwater, such as whales and dolphins with hydrophones.

In a study of captive diurnal frogs, Quiguango-Ubillu and Coloma (2008) were able to match the animals' vocalization to the behavior. For example, males made long-range advertisement calls when seeking a mate, different kinds of short-range courtship calls, and aggression calls and other sounds before clasping a female, and encounter calls upon body contact. They produced a table in which they listed all the various types of vocalizations: call length (seconds), calls per minute, notes per call, note length (seconds), note interval (seconds), call interval (seconds), fundamental frequency (Hz), dominant frequency (Hz), and sample size (Hz). These types of behavior can even be verified by vocalizations, and both could be used to tease out intra- versus interspecific variation and in the construction of phylogenetic trees.

Capturing Animals from the Wild and Studying Them for a Short Period of Time in a Laboratory

A few studies of animal sounds are done in the laboratory. This is necessary when controlling the acoustic environment of the animals is crucial, such as in studies of song development in songbirds (Soha et al. 2009) or mating call preferences in female frogs (Gerhardt et al. 2000). However, to work with vertebrates in the lab, highly regulated permits and animal protocols are necessary. More amenable for laboratory courses is to investigate the non-vocal sounds of invertebrates, such as the calls of crickets, produced by stridulation. For example, laboratory studies have shown that the rate of these calls depends on ambient temperature (Walker and Collins 2010).

Regardless of the original environment in which a recording is made, animal sound archives are a treasure trove for comparative studies. Students could peruse the literature and recordings from archives such as the BLB or the Macaulay Library at the Cornell University to investigate, for example, geographic variation in animal sounds, as has been done for the white-crowned sparrows of the Pacific coast (Nelson and Soha 2004).

Laboratory Sound Analysis

Once everyone finished recording animal sounds at the zoo, we returned to the Museum of Biological Diversity, specifically the Borror Laboratory of Bioacoustics (BLB), one of the largest sound archives in North America.

Student Outline

Student Learning Objectives

- Students will learn how to record and analyze animal vocalizations using recording equipment and free-downloadable software Audacity and/or Raven Lite.
- Students will compare and contrast various animal vocalizations and see if they can interpret the type of behavior involved.
- Students will enter various parameters of vocalizations into an Excel spreadsheet such as number of vocalizations over a specific time period, range of frequencies, dominant frequencies and others, and will look for and write about patterns and differences in these patterns.
- Students will write a cogent lab report

Introduction

Your professor might ask you why animals vocalize. Pause for a moment and come up with a list of reasons why they might do so. In this lab you will record animal vocalizations, observe the behavior of the animal during or shortly before and after the vocalization, and analyze the vocalizations with the behavioral context in mind using sound analysis software on your computer.

Procedure

Background

To obtain background information about how animals use sound to communicate, complete the Howard Hughes Medical Institute Biointeractive online lab "How Animals Use Sound to Communicate" (http://www.hhmi.org/biointeractive/how-animals-use-sound-communicate) before class. (Alternatively, the instructor may decide to include this with the class as part of the lab period.) This activity will introduce some important concepts such as sound waves, amplitude, frequency, and their units. You can find this information also in the Raven Lite Manual, which you can download at http://www.birds.cornell.edu/brp/RavenLite/RavenLite10UsersGuide.pdf.

Field Recording

If you will be making your own recordings, learn how to use the recording equipment, such as the Marantz PMD670 or PMD660 recorder, before going to the zoo or other field site. Know how to start a recording, pause and stop it, and monitor the recording level throughout.

At the recording site, choose an animal to focus on and find a good place to observe the animal without disturbing its natural behavior patterns. Birds are generally good recording subjects because they tend to be active and vocal during the day.

Once you arrive at the site and are settled, stay for 20-30 minutes to record the animal's behavior and (hopefully) vocalizations. Start your recording when you are ready; do not wait for the animal to vocalize before you start. Point the microphone at the vocalizing animal and monitor the recording level as to not overload the recording. At the beginning of the recording, say your name, date and start time, your location, and the animal species you are observing. You may want to tilt the microphone slightly towards you when you speak, but please note that sensitive microphones will overload if you speak directly into the front unless you turn down the gain on the recorder substantially. Continue to state occasionally any observations of the animal's behavior throughout the observation period.

You may want to mention how many animals are in the vicinity of your focal animal, what sex they are, how far apart they are from each other, whether they have yet been fed (if in a zoo or other captive facility), and anything else that you notice. It is important to avoid talking over the animal's sounds once it has started to vocalize, however; wait until it has paused before speaking.

If using your own recording device, such as your cell phone, it is best to use software that allows you to save the file in uncompressed WAV format, such as the Awesome Voice Recorder or Voice Recorder Lite. MP3 compression alters the nature of the sound and cuts out frequencies beyond human hearing. However, these frequencies might still be in the range of the animal's hearing and part of the message. These frequencies can be made visible with sound analysis software.

Sound Analysis

To analyze the recorded sounds, we will use the software Raven Lite: http://www.birds.cornell.edu/brp/raven/RavenOverview.html. Please follow detailed instructions provided in the manual: http://www.birds.cornell.edu/brp/RavenLite/RavenLite10UsersGuide.pdf. Briefly:

- **Opening Raven Lite**: Double click on the icon on the desktop to start the application.
- **Opening files**: Locate one of your recordings, drag and drop it into the application window. Click OK on the next dialog window to open the entire sound.
- **Default views**: You will now see two panels open with representations of the sound. The top view is of the Waveform indicating the loudness of the sound over time, the bottom view is of the spectrogram indicating the frequency (perceived as pitch) of the sound over time. Only one of these views is active at a time; the active view is highlighted in the views window and has a blue bar on its left edge.
- **Playing the sound**: You can play the sound by pressing one of the triangular buttons on the right top of the window. Try changing the rate at which the song is played. How does a rate of 0.5 affect the sound playback? How about a rate of 2?
- **Position marker**: Click anywhere in the wave or spectrogram view and a marker will appear. Move these position markers by clicking and dragging and watch what happens to the numbers on the bottom left of the window.
- **Selecting and expanding**: Select a section of the sound by dragging with the cursor or clicking once then shift-clicking again at a second spot. You can expand the selection by clicking on the "+" button on the horizontal axis in the bottom right corner. You can expand the frequency range by clicking the "+" button on the vertical axis in the bottom right corner.
- **Simple selection measurements**: Below the spectrogram window you will find a selection table which shows simple measurements, such as begin and end time, low and high frequency of the position of the cursor in the sound. You can calculate duration of the sound between the cursors as well as frequency range. From the spectrogram you can count the number of notes, usually defined as a continuous trace on the spectrogram. The goal of this analysis section is to generate an Excel spreadsheet with each vocalization listed, duration of each vocalization, number of "substructure" vocalizations (for example, we once counted 13 "pecks" in a woodpecker's 1-second peck!). Choose a type of vocalization that is meaningful for your focal animal, e.g. song or trills of songbirds, barks of sea lions, or grunts of betton. Enter the total number of vocalizations per time period and give the average duration of each vocalization, and, if relevant, the average number of sub-vocalizations. You may want to list the average number of formants, which are distinct frequencies that you will notice in your spectrograms. For example, in Figure 1 (calls of white-cheeked gibbon), there are at least five distinct formants in the first call.

Lab Report

Your lab report should include an Introduction that will include finishing the exercises in How Animals Use Sound to Communicate. Then, pick a reference from the animals recorded in the wild and one from animals recorded in captivity and compare and contrast them. Then compare and contrast your own results with those of the two articles you chose. Next, describe your Materials and Methods, and include your Excel table in the Results section. Also write about your results in prose. Then, in your Discussion section, write about the implications of your work.

Materials

For this exercise, students will need sound recorders with microphones and computers with the analysis software Raven Lite installed. For the recording equipment, see specific suggestions and pricing in Table 1. The Macaulay library at Cornell University provides some details about audio recording equipment and techniques online: https://www.macaulaylibrary.org/contribute/recording-techniques/.

The choice of digital sound recorder depends on several factors such as cost, the recorder's microphone preamplifier performance, size and weight of the recorder, to name a few. Marantz recorders have proven to be very durable and easy to use. Discontinued models can be purchased used on eBay or at retailers such as B&H.

Two types of microphones are commonly used to make animal sound recordings: parabolic reflector systems and shotgun microphones. Both systems are directional, i.e. they help focus on the target animal's sound. The parabolic reflector in addition amplifies the target sound by collecting sound waves over the surface of the reflector and centering them into the microphone. A parabolic reflector system tends to be more expensive than a shotgun microphone.

High-end microphones are produced by Sennheiser and Røde; Audio Technica offers some good quality and affordable microphones, which were used for this workshop. Some accessories are necessary for a successful recording, such as a windscreen to reduce environmental noise, a shockmount and handle to reduce vibrations transmitted to the microphone and a cable to connect recorder and microphone.

Notes for the Instructor

Download Raven Lite software at http://www.birds.cornell.edu/brp/raven/RavenOverview.html. You will have to register and use the free license code e-mailed to you to install the software.

Raven Pro with expanded functionality is available for purchase. Raven Pro allows categorizing sounds, i.e. if you record a songbird that makes several vocalizations, the software will group them in a table by similarity. It then makes a cluster diagram that shows the distance relationship of these sounds.

If you do not have a zoo or aquarium with vocal animals nearby, you can use recordings archived in a sound library (Table 2).

Table 1. Sound recording equipment used for exercise. All values in US dollars.

Type of Equipment	Make	Specifics	Approx. Price
Sound Recorder	Marantz	PMD660 (carry case)	$599 ($70)
		PMD670	$200-300
Microphone	Audio-Technica	AT8035 - Line/Gradient Shotgun Condenser microphone	$270
Grip	Auray	Universal Microphone Pistol Grip	$30
Mount	Auray DUSM-1	Universal Shock Mount	$40
XLR cable	Kopul Studio Elite 4000 Series	XLR M to XLR F Microphone Cable - 3' (0.91 m), Black	$20
Windscreen	Auray	WSS-2024 Professional Windshield for Shotgun Microphones	$90
Headphones	Sennheiser	PX 100-II On-Ear Stereo Headphones	$40
Memory Card	--	--	$10
Batteries	--	9 AA	$5
TOTAL	--	starting at	$700

Table 2. Some natural sound libraries accessible online.

Name	Web Address	Hosted By
Borror Laboratory of Bioacoustics	https://blb.osu.edu/	The Ohio State University
Bird Sounds Database	https://www.floridamuseum.ufl.edu/bird-sounds/	Florida Museum of Natural History
Macaulay Library	https://www.macaulaylibrary.org/	Cornell University
xeno-canto	https://www.xeno-canto.org/	Xeno-canto Foundation and Naturalis Biodiversity Center

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Appendix A

Manuals

Raven Lite: http://www.birds.cornell.edu/brp/RavenLite/RavenLite10UsersGuide.pdf

Recorder Marantz PMD670: https://www.avisoft.com/PMD670.pdf

Recorder Marantz PMD660: https://www.avisoft.com/PMD660.pdf

Recording Apps (for IOS Devices)

Awesome Voice Recorder: https://itunes.apple.com/us/app/awesome-voice-recorder/id892208399?mt=8. High quality audio recording in WAV format with Wave display for recording status, various audio quality options (11,025KHz, 22,050KHz, 44,100KHz,48,000KHz) and Mono/stereo recording option.

Voice Recorder Lite: https://itunes.apple.com/us/app/voice-recorder-lite-hd-audio-recording-playback/id955000203?mt=8. Audio recorder with three quality options, Low: 8KHz; Medium: 22.05KHz; High: 44.1KHz; WAV format; and external input device support. Allows playback, editing and sharing of recordings (e.g. Email recordings, Dropbox sharing and Upload to iCloud Drive).

RØDE Rec: https://itunes.apple.com/us/app/r%C3%B8de-rec/id528642521?mt=8. High resolution 24-bit, 48kHz stereo/mono Ørecording with live input monitoring and complete control of the iXY microphone, including high pass filter and LED behavior. Includes editing tools.

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