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Bioacoustics

The International Journal of Animal Sound and its Recording

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/tbio20

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To cite this article: Jessie C. Tanner , Joshua Justison & Mark A. Bee (2020) SynSing: open-source MATLAB code for generating synthetic signals in studies of animal acoustic communication, Bioacoustics, 29:6, 731-752, DOI: 10.1080/09524622.2019.1674694

To link to this article: https://doi.org/10.1080/09524622.2019.1674694

Published online: 10 Oct 2019.

BIOACOUSTICS
2020, VOL. 29, NO. 6, 731–752
https://doi.org/10.1080/09524622.2019.1674694

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SynSing: open-source MATLAB code for generating synthetic signals in studies of animal acoustic communication

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ABSTRACT
Synthetic, computer-generated signals are widely used in playback studies of animal acoustic communication. Depending on the goals of the experimenter, they can offer several significant advantages over playbacks of recordings of edited or unedited natural signals. However, there are few 'off the shelf' software options for the bioacoustician interested in synthesizing signals that combine ease of use with versatility. Here, we introduce SynSing, a free, open-source graphical user interface (GUI) for generating synthetic acoustic and seismic signals in MATLAB for use in playback studies of animal acoustic communication. Following a brief overview of the GUI, we describe how users can specify a variety of spectral properties (e.g., fundamental frequency, relative amplitudes and starting phases of harmonic and inharmonic components, frequency modulation) and temporal properties (e.g., pulse, note, or call duration and rate, onset and offset characteristics of amplitude envelopes) to generate individual signals or long sequences of repeated signals. We demonstrate SynSing's versatility by reconstructing synthetic signals from published studies of several frogs, a field cricket, a katydid, a grasshopper, and a spider. We also provide worked examples of simple birdsong, as well as pure tones, linear frequency modulated sweeps, and noise.

ARTICLE HISTORY
Received 20 June 2019
Accepted 24 September 2019

KEYWORDS
Synthetic stimuli; sound generation; software; anuran; orthopteran; acoustic signal

Introduction

The study of acoustic communication in animals relies upon playback of relevant, well-controlled signals (McGregor et al. 1992; Hopp and Morton 1998). Recording individual signals or small repertoires for use as playback stimuli can introduce problematic issues of pseudoreplication and limited external validity (Catchpole 1989; Kroodsma 1989; Searcy 1989; Kroodsma et al. 2001). To manipulate particular properties of animal signals and mitigate the problem of pseudoreplication, researchers sometimes excise parts of acoustic recordings, such as individual sound pulses or song phrases, and concatenate them together with silent intervals to make song models (Wagner 1996; Simmons et al. 2001; Rose et al. 2004; Linhart et al. 2012). The major drawbacks to this

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approach are that models created in this way are not completely manipulable and may contain acoustic artefacts introduced during editing. The use of completely synthetic signals generated *de novo* by the experimenter can avoid many of these pitfalls (Gerhardt 1992; McGregor et al. 1992).

Synthetic signals are particularly useful for testing focused hypotheses about the specific properties used in signal assessment by receivers because generating signals *de novo* allows for complete control of signal parameters (Gerhardt and Huber 2002). Additionally, synthetic signals are free of background noise that may mar recordings of natural signals and introduce ambiguity about what aspect of a recording elicits a particular receiver response. Because of these advantages, synthetic signals are now used in experiments with a diversity of taxa to test hypotheses, for example, about female mate choice (Baugh and Ryan 2010; Fowler-Finn and Rodríguez 2011; Reichert and Ronacher 2015), male-male aggression (Burmeister et al. 1999; Reichert 2014; Tumulty 2018), signal function (Grafe and Wanger 2007; Rebar et al. 2009), social communication (Norcross et al. 1994; King et al. 2014; Aubin et al. 2015), and receiver perception and recognition systems (Schüch and Barth 1990; Pires and Hoy 1992; Simmons and Bean 2000; Schul and Bush 2002).

Synthesizing acoustic signals *de novo* can be time-consuming, particularly for researchers that lack coding experience. The bioacoustician's toolbox currently includes a number of free applications for sound synthesis (e.g., CSound, and R packages seewave and soundgen) with varying degrees of functionality for creating synthetic stimuli for use in playback experiments with different taxa (Sueur et al. 2008; Lazzarini et al. 2016; Sueur 2018; Anikin 2019). Here, we introduce SynSing (pronounced like 'sensing'), a graphical user interface (GUI) in MATLAB™ for the production of synthetic acoustic and seismic signals, such as those produced by insects and anurans, in playback experiments. While SynSing is explicitly designed to simplify synthesis of animal signals that comprise repeated elements, such as the pulsed signals of many anurans and insects, it is also useful for generation of pure tones, FM sweeps, and masking noises that are frequently used as stimuli in bioacoustics experiments.

Software implementation

Users may download the current version of SynSing (v1.0), with accompanying User Guide and example stimuli, from GitHub: https://github.com/jessiectanner/SynSing/releases. While SynSing is free and open-source, MATLAB is subject to licencing requirements and users are referred to the Mathworks website for information. We note for those concerned about licencing costs that many research settings, such as universities, make MATLAB available to students and employees via site licences. SynSing v1.0 was designed using MATLAB's Graphical User Interface Development Environment ('GUIDE') in version 2018b (The Mathworks, Natick, MA, USA). We have confirmed that SynSing operates as expected in MATLAB versions 2017a, 2018a, and 2019a; its performance in versions of MATLAB earlier than 2017a or later than 2019a has not been evaluated. While SynSing works with any operating system on which MATLAB is available, small differences are to be expected in the appearance of the graphical user interface between platforms. Future versions of MATLAB may also incorporate changes to the underlying functions upon which SynSing v1.0 relies.

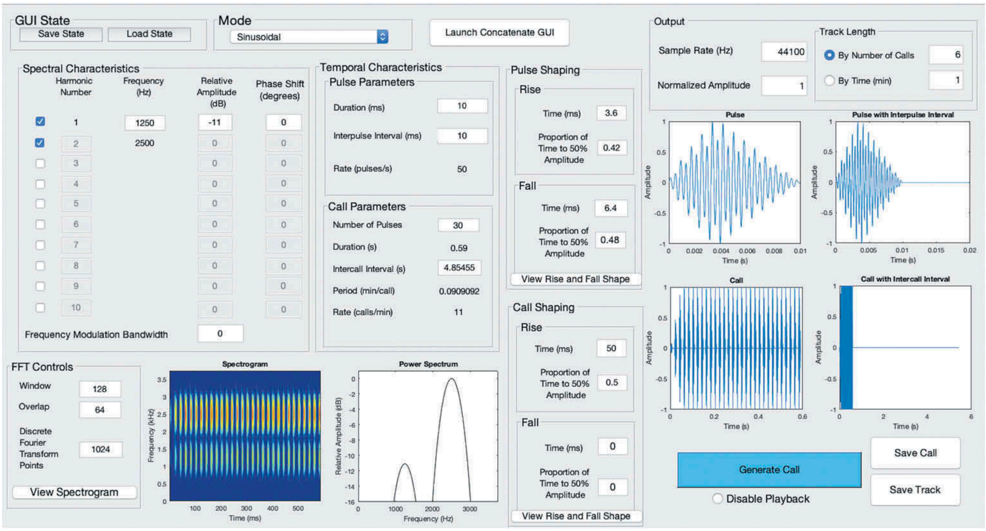

Figure 1. SynSing's appearance in sinusoidal mode with default settings.

Overview

SynSing (Figure 1) has two modes of sound generation that can be used to produce songs or calls with repeated acoustic elements, such as notes or pulses (hereafter referred to as 'pulses'). In Sinusoidal Mode, SynSing constructs sound pulses using sine waves, which are added together to create up to 10 customisable harmonics. The amplitude of each harmonic may be independently specified relative to the amplitude of the dominant frequency (i.e., the harmonic of greatest relative amplitude). The starting phase of each harmonic, relative to the start of the sound, also may be specified independently. Additionally, SynSing supports frequency modulation of sound pulses, which enables the creation of sounds that sweep linearly either up or down in frequency. In Broadband Noise Mode, SynSing creates bursts of white noise, then applies a user-controlled bandpass filter. In both modes, sounds are constructed with custom pulse durations, interpulse silent intervals, and onset and offset shape characteristics. Pulses and interpulse intervals can then be concatenated to construct 'calls' that contain a user-specified number of pulses. Like pulses, calls can be given custom onset and offset characteristics. Finally, calls are concatenated together at user-specified rates to form audio 'tracks' of a minimum specified duration, measured either in a number of repeated calls or in minutes. The spectral and temporal characteristics of calls and their constituent pulses may be viewed using built-in plots that display spectrograms and power spectra (in Sinusoidal Mode) or filter frequency responses (in Broadband Noise Mode). An FFT Controls module gives the user control over the window size, window overlap, and number of Fast Fourier Transform (FFT) points used to generate spectrograms and power spectra. Additionally, SynSing plots waveforms of generated pulses and calls and the amplitude envelope shaping functions used to generate them. By default, SynSing plays back the synthetic stimulus during sound generation; this feature may be disabled using the Disable Playback radio button. Audio files can be saved as uncompressed .WAV files.

Additionally, we have built SynSing to alert the user by displaying warning messages when the current settings might cause MATLAB to generate error messages or produce unexpected output. Next, we provide a brief description of the program's general setup, and then we discuss the capabilities and limitations of SynSing in Sinusoidal and Broadband Noise Modes of sound generation. We conclude with some worked examples showing how SynSing is able to recreate synthetic animal signals and other acoustic stimuli used in previously published bioacoustic research.

General setup

Using a dropdown menu, users must specify whether sounds should be generated in Sinusoidal Mode, by modifying sine waves (pure tones), or else in Broadband Noise Mode, by modifying white noise. Additionally, users define the sample rate in Hz (samples per second). Choosing an appropriate sample rate is a crucial aspect of synthesizing digital (as opposed to analogue, continuous) signals because insufficient sample rates result in a loss of information. In general, the sample rate should exceed the Nyquist Frequency, defined as twice the highest frequency in the signal, in order to be reproduced digitally (Clements 1998); a signal whose maximum frequency was 3.9 kHz could thus be faithfully reproduced at a sample rate of 7.8 kHz or higher. SynSing's default sample rate in Sinusoidal Mode is 44.1 kHz, chosen because it is the sample rate at which compact discs (CDs) and mp3s are produced. Digital audio signals designed for human consumption are reproduced at this sample rate because it is the lowest power of 2 that exceeds two times the maximum frequency humans can hear (~20 kHz). SynSing's default sample rate in Broadband Noise Mode is 88.2 kHz, chosen to exceed the Nyquist frequency for the bow-winged grasshopper (*Chorthippus biguttulus*) signal that is produced using the default settings when SynSing is in Broadband Noise Mode. Note that increasing the sample rate necessarily increases the file size that SynSing outputs. Users control the Normalised Amplitude of the output signal using the text box in the Output module.

Spectral properties

The primary difference between Sinusoidal Mode and Broadband Noise Mode is in the specification of spectral characteristics. Below we discuss user inputs to the Spectral Characteristics module for each mode separately before discussing how SynSing incorporates temporal characteristics and shapes signal envelopes in both modes.

Sinusoidal mode

In Sinusoidal Mode, signals may be designed to have up to 10 custom harmonics, or multiples of the fundamental (lowest) frequency (Figure 2(a)). SynSing must be given the fundamental frequency in Hz. Additional harmonics may be specified using check boxes. Their frequencies are automatically calculated by SynSing using their harmonic number and the fundamental frequency. Harmonics may be added or removed by checking and unchecking the boxes next to the harmonic number of each. SynSing allows for the creation of a 'missing' fundamental frequency, such as occurs in the American bullfrog, *Rana catesbeiana* (Schwartz and Simmons 1990; Bee and Gerhardt 2001b). Though the

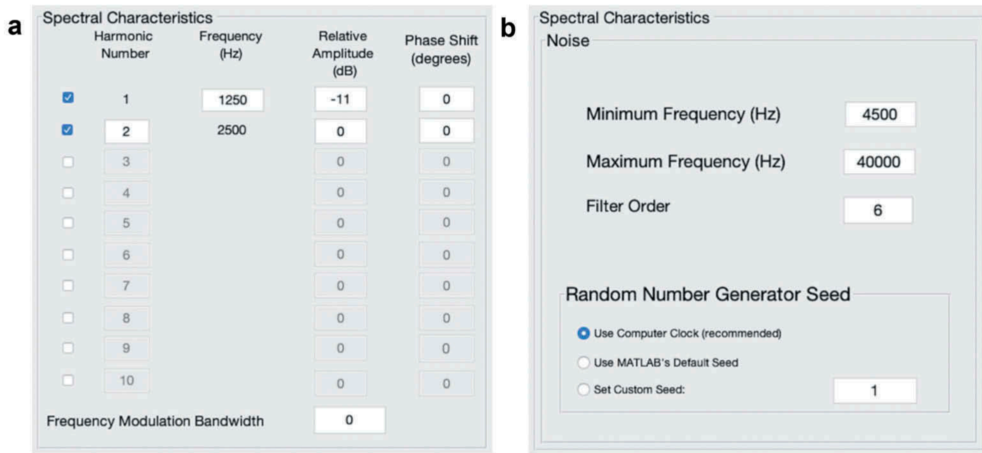

Figure 2. The spectral characteristics module for (a) Sinusoidal Mode and (b) Broadband Noise Mode.

default settings allow for harmonics 1 through 10 to be specified, users may specify other harmonics using the text boxes to input harmonic numbers. Users should note that it is possible to add acoustic energy 'between' harmonics (e.g., subharmonics) by specifying harmonic numbers with decimal points (e.g., a signal with a fundamental frequency of 1 kHz can be made with energy at 4.5 kHz by adding a harmonic with number 4.5). This flexibility may be useful for users interested in various bioacoustic phenomena, such as biphonation (e.g., the two-voice system of birds), which can result in animal signals that contain harmonically unrelated sounds (Aubin et al. 2000), vocalisations characterised by non-linear phenomena (Wilden et al. 1998), or the perception of mistuned harmonics (Simmons and Bean 2000).

Across species and across individuals within a species, animal signals commonly vary in how acoustic energy is distributed across the frequency spectrum. In Sinusoidal Mode, users can shape the spectrum of synthesised sounds by adjusting the relative amplitude of each harmonic. The relative amplitude in decibels (dB) may be specified independently for each harmonic using the adjacent text boxes. We believe that best practice is to set the relative amplitude for the dominant frequency (i.e., the harmonic with the most acoustic energy) to 0. Other relative amplitudes may then be defined in terms of dB attenuation relative to the dominant frequency (i.e., by inputting negative numbers). SynSing's default settings upon opening in Sinusoidal Mode produce a synthetic call of Cope's gray treefrog (*Hyla chrysoscelis*), which has a dominant frequency of 2.5 kHz and a fundamental frequency of 1.25 kHz. Because the relative amplitude of the fundamental frequency is, on average, 11 dB lower than that of the dominant frequency, we have specified the relative amplitude of the second harmonic as 0 dB and the relative amplitude of the fundamental frequency as −11 dB.

Users may wish to produce signals whose harmonics are phase shifted relative to sound onset or relative to one another. Such manipulations are potentially useful in studies of sound pattern recognition and source localisation (Masterton et al. 1975; Simmons et al. 1993; Michelsen et al. 1994; Bodnar 1996; von Helversen and von Helversen 1998). By default, all sounds start in sine phase at an amplitude of 0 at time 0. Phase shifts of each harmonic may be specified, in degrees, using the text boxes on the right-hand side of the

Spectral Characteristics module. Phase shifts are specified relative to the onset of the sound. When the phase shifts of each specified harmonic are equal (e.g., when they are all set to the default 0 degrees) the harmonics will be phase-locked.

Finally, SynSing supports linear frequency modulation of spectral components, such as characterises the signals of the Pacific field cricket (*Teleogryllus oceanicus*) and the coquí frog (*Eleutherodactylus coqui*) (Walker and Cade 2003; Benevides and Mautz 2014). To add a linear FM sweep to sound pulses, users can specify the Frequency Modulation Bandwidth in Hz in the text box at the bottom of the Spectral Characteristics module. SynSing uses this bandwidth, with centre frequency at the user-specified fundamental frequency, to determine how the signal should be frequency modulated. That is, the signal's frequency sweeps from $f_1 - 0.5 \times$ bandwidth to $f_1 + 0.5 \times$ bandwidth across the duration of the pulse, such that at the midpoint of the pulse, the frequency is equal to the specified fundamental frequency. To create a signal that sweeps downward in frequency, the bandwidth may be specified as a negative value. All frequency components remain harmonically related over the duration of a frequency-modulated sound.

Broadband noise mode

Many animal sounds, such as the elements comprising the songs of some katydids (e.g., *Neoconocephalus ensiger*, Faure and Hoy 2000c) and grasshoppers (e.g., *Chorthippus biguttulus*, von Helversen and von Helversen 1997) are noisy and broadband. Signals such as these can be synthesised using SynSing's Broadband Noise Mode (Figure 2(b)). In Broadband Noise Mode, SynSing generates white noise, which is by definition a random sample of values from the same probability distribution with a flat spectral density. Users should note that MATLAB always reverts to the same random number generator seed at startup by default. Therefore, to avoid issues of pseudoreplication associated with the use of pseudorandom number generators, SynSing by default chooses a new seed based on the computer's clock in Broadband Noise Mode and draws a new sample of random numbers independently each time that a noise burst is generated. Alternatively, users may choose to use MATLAB's default seed or set a custom seed; this feature may be used to create reproducible, identical realisations of noise.

Following the generation of bursts of white noise, SynSing shapes the spectrum of the noise using the user-specified minimum and maximum frequencies of noise desired by applying a bandpass Butterworth filter, which attenuates the noise outside of these bounds (the passband). We chose the Butterworth filter for its flat frequency response within the passband. Users may specify the filter order, which controls how rapidly the frequency response attenuates at the limits of the passband. A filter order of 1 creates a filter that attenuates 6 dB per octave. We set the default filter order to 6, which creates relatively steep attenuation (36 dB per octave) of the frequency response at the limits of the passband.

Temporal properties

Biological information of relevance to many animals is often encoded in the temporal properties of acoustic signals (Gerhardt and Huber 2002; Bradbury and Vehrencamp 2011). SynSing gives the user the ability to independently specify a variety of temporal characteristics of pulses and calls of known biological relevance in many species (Figure 3).

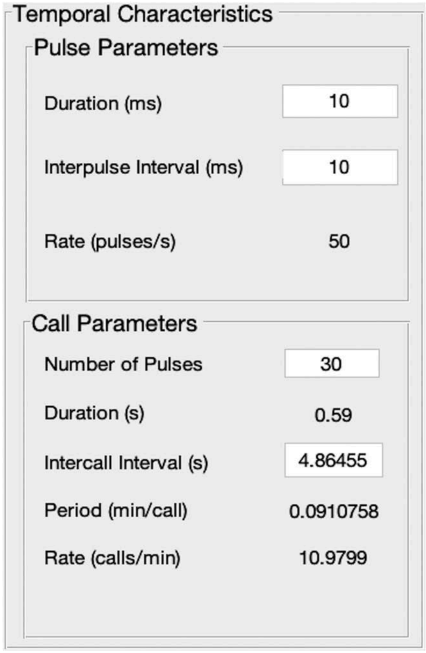

Figure 3. The Temporal Characteristics module includes the temporal parameters for both pulses and calls.

Pulse duration, interpulse interval, pulse rate, and pulse number

Pulse parameters must include at minimum a duration and an interpulse interval, both measured in milliseconds. SynSing uses the duration and interpulse interval with which pulses are produced to calculate the pulse rate and displays the result in pulses per second. Call parameters must include a whole number of pulses to be concatenated together into a call and the intercall silent interval (seconds). SynSing then calculates the duration of the call (pulse period × the number of pulses – one interpulse interval), the call period (call duration + intercall interval), and the call rate (1/call period).

Note that while SynSing is designed to produce calls that comprise multiple sound pulses (e.g., *Hyla chrysoscelis*; Ward et al. 2013) or consist of individual notes repeated a characteristic number of times to form a call (e.g., *Rana catesbeiana*; Bee and Gerhardt 2001) some animal signals comprise a single pulse. In this case, a sound pulse, a note, and a call may all be synonymous, and successfully using SynSing will depend on the user's ability to translate between pulses, notes, and calls to achieve a desired outcome. To construct such a call using SynSing, we recommend specifying temporal pulse parameters and, in the Call Parameters module, specifying the Number of Pulses as 1. Additionally, some signals such as those of the Pacific field cricket (Walker and Cade 2003) and Puerto Rican coquí frog (Benevides and Mautz 2014) may consist of more than one type of pulse having different spectral and temporal characteristics. To produce such calls, we have used SynSing to create signals with two parts in separate steps and concatenated the two files together using a separate GUI, called Concatenate, which we have included as part of SynSing's download package. The Concatenate GUI may be accessed by clicking the Launch Concatenate GUI button at the top of the SynSing window. Users could alternatively conduct similar concatenation operations using the copy/paste functions of their favourite audio editing software (e.g., Adobe Audition or Audacity).

Amplitude envelopes of pulses and calls

In some communication systems, receivers attend to the shapes of amplitude envelopes of signals (e.g., Gerhardt and Schul 1999). SynSing supports linear and exponential shaping of the rise (onset) and fall (offset) of both pulses and calls, which function similarly (Figure 4). To shape the onset of a sound pulse, users may use the text boxes in the Rise module to specify the rise time (milliseconds) and the proportion of that time at which the pulse should reach half its maximum amplitude (Figure 5). Linear rise and fall shapes may be specified by setting the Proportion of Time to 50% Amplitude to 0.5; exponential rise and fall shapes result from any other value. For example, SynSing's default settings to create a *Hyla chrysoscelis* pulse give a rise time of 3.6 ms and a Proportion of Time to 50% Amplitude as 0.42. SynSing then calculates an exponential function that reaches its maximum value of y = 1 at 3.6 ms, and reaches y = 0.5 at 1.512 s (= 0.42 × 3.6 ms). That function is then used to shape the onset of the pulse. Similarly, the fall (offset) of the call may be specified by giving the duration (ms) from the end of the pulse over which to shape the envelope. SynSing calculates the exponential function in the same way it handles rise shape specifications, then inverts the function. Thus, the time entered in the Fall module should be specified as the time elapsed from the sound onset when the amplitude should begin to decline from its maximum, while the Proportion of Time to 50% Amplitude should be specified relative to the beginning of the fall. Users may click on View Rise and Fall Shape to display the exponential functions to be applied in a new window. Shaping of

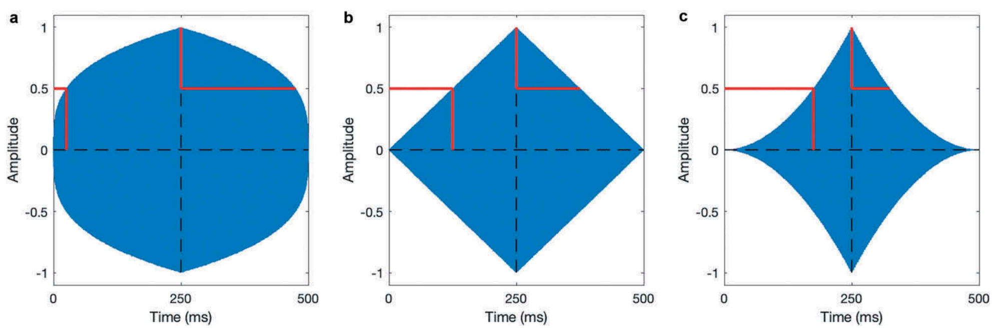

Figure 4. Waveforms illustrating three pulses of equal duration (500 ms) with differently shaped amplitude envelopes. All three pulses have rise times of 250 ms and fall times of 250 ms. Black dashed lines mark amplitude = 0 and time = 0.5 (250 ms). The red guide lines are provided to show where the pulse envelope intersects with the amplitude = 0.5 line, relative to the duration of the rise and fall. (a) A relatively rapid pulse onset generated with a Proportion of Time to 50% Amplitude of 0.1; that is, the pulse reaches 50% of its maximum amplitude at 25 ms (= 0.1 × 250 ms) after the onset of the sound. For the pulse fall, we set the Proportion of Time to 50% Amplitude to 0.9 (the complement of the Proportion of Time to 50% Amplitude for the pulse rise) to generate a symmetrically shaped pulse whose amplitude declines to 50% amplitude 225 ms (= 0.9 × 250 ms) after the beginning of the fall. (b) A linearly shaped pulse, generated by setting the Proportion of Time to 50% Amplitude to 0.5 for both the rise and fall. (c) A relatively slow pulse onset generated with a Proportion of Time to 50% Amplitude of 0.7; for the pulse fall, we set the Proportion of Time to 50% Amplitude to 0.3.

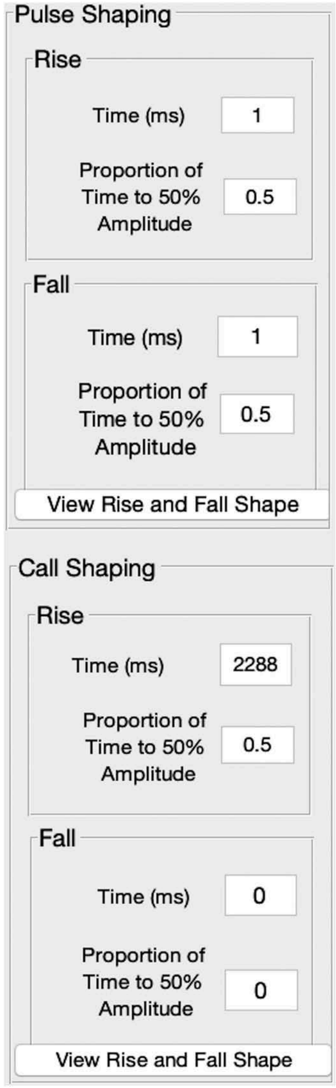

Figure 5. Pulse Shaping and Call Shaping modules allow the user to control the characteristics of the amplitude envelopes.

call envelopes functions analogously, but this shaping is applied to the call as a whole; this feature may be useful to create animal signals that increase in amplitude over part of the call, as in *H. chrysoscelis* (Figure 7(a)), or over the entire duration of the call, as in *C. biguttulus* (Figure 7(f)). Note that specifying very rapid onsets or offsets of either pulses or calls may produce undesirable acoustic artefacts (e.g., spectral splatter) during playback.

Saving a call, audio track, or GUI state

Users may export a single call using the Save Call button in the lower right corner (Figure 6). To create a single call without an intercall interval, users may enter 0 in the intercall interval

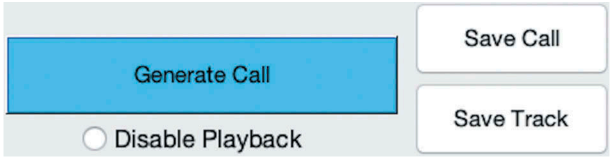

Figure 6. The Generate Call, Save Call, and Save Track buttons control the generation and export of stimuli from SynSing. The Disable Playback radio button allows the user to turn off SynSing's audio playback of the sound being generated.

text box. If the specified intercall interval is not equal to 0, the saved call includes the following intercall interval and thus would be suitable for use with the 'loop' feature of some digital playback systems.

SynSing also supports the creation of an Audio Track of a given length, specified in minutes, which may be useful for conducting playback trials of a predetermined duration. Clicking the Save Track button in the lower right allows the user to export a sequence of calls with a user-specified Track Length, set in the Output module, measured as either a number of repeated calls or as a minimum duration. If the user selects the radio button labelled By Number of Calls, SynSing adds together the specified number of call periods. If the user selects the radio button labelled By Time, SynSing concatenates call periods together until the duration of the signal meets or exceeds the minimum Track Length specified in minutes; for example, to generate a stimulus for a playback experiment with a predetermined maximum trial length of five minutes, enter 5 in the Track Length text box. Note that the output audio will be at *minimum* the user-specified duration; the output will be exactly the user-specified length only if the specified track length is an integer multiple of the call period.

We expect that users are likely to routinely generate synthetic signals for particular target species and therefore anticipate that saving user inputs to SynSing for future reference or to regenerate standard or commonly used signals may be useful for experimenters. SynSing supports saving a copy of the GUI state, which generates a MAT-file (.mat) that stores current inputs to the GUI. Clicking on the Save State button in the upper left corner allows the user to choose a location and a filename to save the GUI state. Loading a previously generated .mat file allows for the expedited generation of stimuli identical or similar to those created using SynSing in the past. Clicking the Load State button in the upper left corner allows the user to choose a previously saved .mat file from the file directory.

Examples

In this section, we detail some example stimuli generated using SynSing. The stimuli described here were chosen to demonstrate the features and versatility of the GUI. Audio files, as well as associated MAT-files containing the GUI inputs used to construct these example stimuli, are provided as part of the SynSing download package.

Gray treefrogs

Among the best studied frogs in terms of sound pattern recognition, source localisation, species recognition and sexual selection are the North American treefrogs

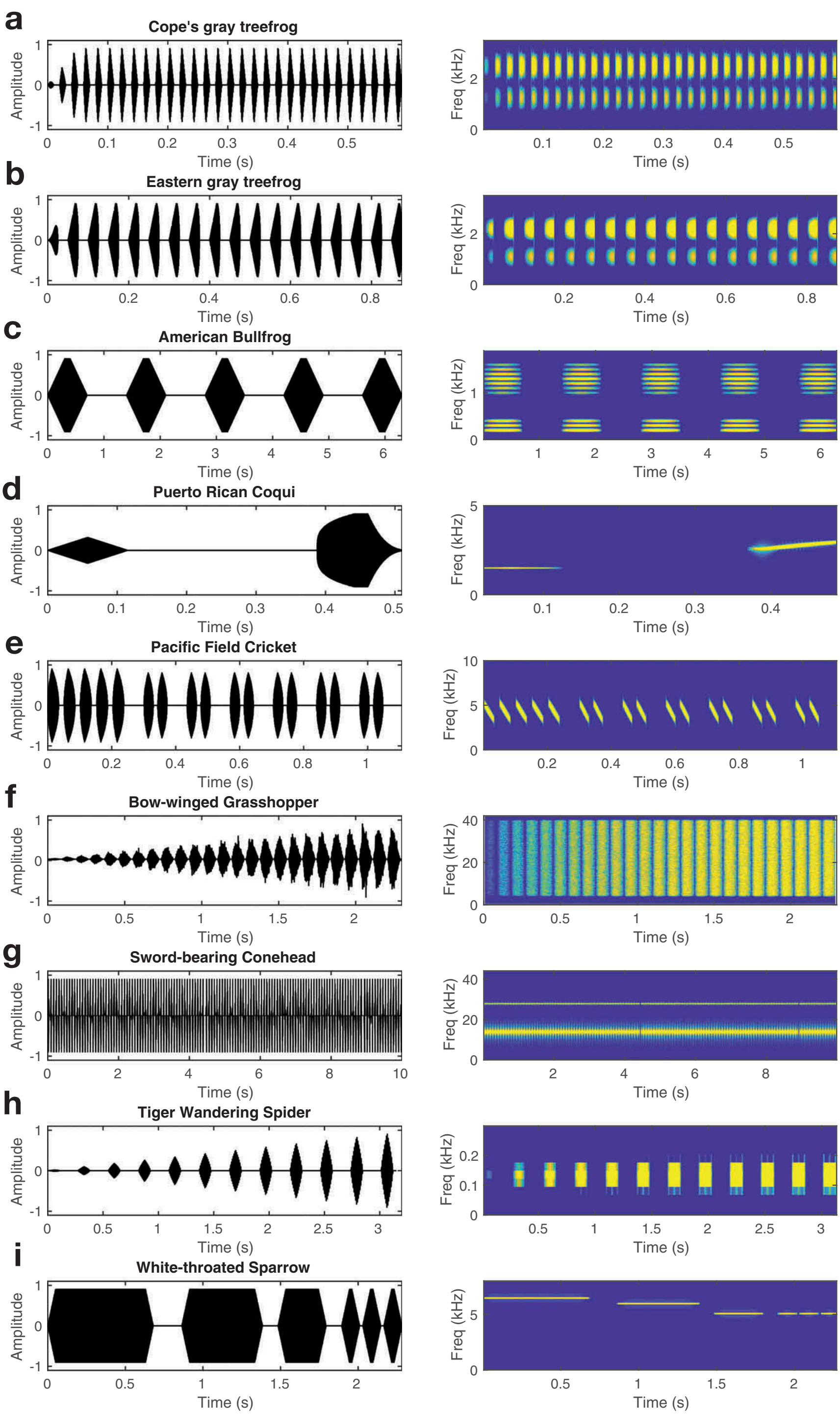

Figure 7. Waveforms (left column) and spectrograms (right column) of synthetic animal signals generated with SynSing. All visual depictions show a single complete 'call' with the exception of (g), which depicts a 10 second sample of the signal of the sword-bearing conehead katydid. Note that the signals of the Puerto Rican coqu (d), the Pacific field cricket (e), and the white-throated sparrow (i) were generated in more than one step using SynSing and then concatenated together using the Concatenate GUI.

(*Hyla*, Hylidae), which have been extensively investigated over the past 50 years using synthetic acoustic signals (Gerhardt 2001; Gerhardt and Huber 2002; Bee 2015; Bee and Christensen-Dalsgaard 2016). Within this group, the two members of the cryptic gray treefrog species complex, Cope's gray treefrog (*Hyla chrysoscelis*) and its tetraploid sister species, the eastern gray treefrog (*Hyla versicolor*), have been particularly well studied.

Both gray treefrog species produce pulsatile advertisement calls with similar spectral profiles consisting of a fundamental frequency (f_1) and a dominant second harmonic (f_2). SynSing is able to reproduce the synthetic calls used in previous studies of these two species. The default settings in Sinusoidal Mode produce the synthetic advertisement call of Cope's gray treefrog used as the 'standard' signal in Tanner et al. (2017; Figure 7(a)), which had a fundamental frequency of 1250 Hz and a dominant frequency of 2500 Hz with an amplitude 11 dB higher than that of the fundamental frequency. The call comprises 30 pulses, with pulse durations and interpulse intervals of 10 ms and a resulting pulse rate of 50 pulses/s. The rise and fall shapes of each individual pulse are nearly, but not quite, linear. The first 50 ms of the entire call was shaped with a linear rise. The advertisement call of the eastern gray treefrog is slightly lower in frequency (f_1 = 1100 Hz, f_2 = 2200 Hz), and consists of fewer pulses (about 18 pulses/call) that are longer in duration (25 ms) and separated by longer interpulse intervals (25 ms), resulting in a slower pulse rate (20 pulses/s) at 20°C (Gerhardt and Schul 1999). Females of the eastern gray treefrog are also selective for the species-specific pulse shape (Gerhardt and Schul 1999). In the *H. versicolor* example stimulus (Figure 7(b)), pulses have a linear rise time of 17.5 ms and an exponential fall-time of 5 ms, chosen to recreate the 'standard' pulse used by Gerhardt and Schul (1999) to approximate a natural pulse shape.

American bullfrog

The vocal behaviour of the American bullfrog, *Rana catesbeiana*, has been studied primarily in the context of neural processing and receiver mechanisms for call recognition (Megela Simmons 1984; Megela-Simmons et al. 1985; Freedman et al. 1988; Simmons 1988; Schwartz and Simmons 1990; Simmons et al. 2000), vocal interactions and chorusing behaviour (Boatright-Horowitz et al. 2000; Megela Simmons et al. 2008; Bates et al. 2010), territorial aggression (Wiewandt 1969; Bee 2001, 2002, 2003), and neighbour-stranger discrimination (Bee and Gerhardt 2001a, 2001b, 2001c; Bee 2002, 2004). Many of these studies have used synthetic calls. The call of the American bullfrog consists of a series of repeated notes with relatively complex spectral characteristics. It is characterised by its 'missing' fundamental frequency of approximately 100 Hz and multiple harmonics. In this example (Figure 7(c)), we have created a synthetic call used as the 'training stimulus' to investigate territorial aggression and neighbour-stranger discrimination in Bee and Gerhardt (2001a).

The synthetic bullfrog call consists of 10 harmonics ($f_{2\text{-}4}$ and $f_{10\text{-}16}$) with relative amplitudes between 5 and 20 dB below that of the dominant frequency (f_2). Note that in real signals, the first harmonic, f_1, is near 100 Hz and is missing. This could be specified by setting the value of harmonic #1 to 100 Hz and then unchecking the box to create the missing fundamental frequency. However, doing so would only allow a user to specify 9 additional harmonics. To generate this example stimulus, we employed a different approach that allows the user to synthesise a call with 10 harmonics. By default, SynSing numbers harmonics consecutively (1–10), but they may be customised by entering text into the 'Harmonic Number' boxes. We therefore specified a value for harmonic #1 of 200 Hz (which is really f_2), and then used appropriate multiples to specify the remaining 9 harmonic numbers (i.e., we specified harmonic numbers 1, 1.5, 2, 5, 5.5, 6, 6.5, 7, 7.5, and 8 to generate a call with energy at 200, 300, 400, 1000, 1100, 1200, 1300, 1400, 1500, and 1600 Hz). Finally, the amplitude envelopes of the notes within a call consist of prolonged, linear rise- and fall-times. In this example, a single note has a duration of 700 ms, linear rise and fall times of 300 ms, and an internote (i.e., interpulse) interval of 700 ms.

Puerto Rican coquí frog

The Puerto Rican coquí frog, *Eleutherodactylus coqui*, is named onomatopoetically for its two-note call. The first ('co') note is primarily an aggressive signal directed at male competitors, while the second ('qui') note functions as an advertisement signal directed at females (Narins and Capranica 1976). The acoustic communication of the coquí frog has been studied in the context of signal production, detection, and recognition mechanisms (Narins and Capranica 1976, 1980; Narins 1982, 1992; Lopez and Narins 1991; Meenderink et al. 2010; Narins and Meenderink 2014) and mate choice (Lopez and Narins 1991). Additionally, *E. coqui* has been introduced to Hawaii, where its vocal behaviour and associated impact on the communication systems of other species have also been studied (O'Neill and Beard 2011; Benevides and Mautz 2014; Zuk et al. 2017).

Each note of the coquí's call is characterised by a single harmonic with frequency modulation. Here we used SynSing to reproduce an experimental stimulus used to construct an artificial chorus of *E. coqui* by Zuk et al. (2017; Figure 7(d)). We constructed each note separately using SynSing's Intercall Interval text box to add the internote interval (i.e., interpulse interval) of 273 ms to the 'co' note and the 2.6 s intercall interval to the 'qui' note. The 'co' note has a centre frequency of 1497 Hz and sweeps downward over a bandwidth of 55 Hz, while the 'qui' note has a centre frequency of 2702 Hz and sweeps upward over a bandwidth of 282 Hz. We implemented the FM sweeps by specifying −55 Hz in the Frequency Modulation Bandwidth text box while generating the 'co' note, and 282 Hz while generating the 'qui' note. The amplitude envelope of the 115 ms-long 'co' note was symmetrical and linear with rise and fall times of 57 ms. The amplitude envelope of the 133 ms-long 'qui' note was asymmetrical and exponential, with a rise time of 53 ms that reached 50% of its maximum amplitude at 4 ms from the onset of the note (Proportion of Time to 50% Amplitude = 0.075) and a fall time of 60 ms that declined to 50% of the maximum amplitude within 14 ms of the beginning of the fall (Proportion of Time to 50% Amplitude = 0.233). We attenuated the 'co' note by 10 dB relative to the 'qui' note using the Normalised Amplitude feature, by converting −10 dB to a proportion ($10^{-10/20} = 0.3162$) and inputting that value into the text box. Finally, we concatenated the two notes together, using the Concatenate GUI. This attenuation and concatenation could alternatively have been done using acoustic editing software such as Adobe Audition or Audacity.

Pacific field cricket

The Pacific or Australian field cricket, *Teleogryllus oceanicus*, produces both long-range calling song that functions as an advertisement signal and a short-range courtship song. Research on the acoustic communication system of *T. oceanicus* has addressed mechanisms of song production and signal detection, recognition, and localisation (Pollack et al. 1984; Doolan and Pollack 1985; Pollack 1986; Balakrishnan and Pollack 1996); signal function and female mating preferences (Simmons et al. 2001; Simmons 2004; Rebar et al. 2009); acoustically-mediated phenotypic plasticity (Bailey and Zuk 2008; Bailey 2011; Balenger and Zuk 2015; Lierheimer and Tinghitella 2017; Gurule-Small and Tinghitella 2018); and the evolutionary gain and loss of sexual signals (Zuk et al. 2006; Tinghitella and Zuk 2009; Tinghitella et al. 2018; Tanner et al. 2019b).

We constructed synthetic calling song using the signal characteristics published in Tanner et al. (2019a; Figure 7(e)). The long-distance calling song comprises two parts. The first part is a trill-like 'long chirp' segment that consists of five pulses. The second part is a series of 'short chirp' couplets produced at a lower amplitude than the pulses of the long chirp. We constructed this synthetic signal by generating the long chirp and short chirp segments as two different 'calls', each of which had a pulsed structure. Note that the short chirps segment of the call was produced by defining a single short chirp 'couplet' as a call. We specified the interpulse interval as the silent interval between pulses of the couplet, and the intercall interval as the silent interval between short chirps. The couplet was then repeated 6 times by generating an audio track of 6 calls using the By Number of Calls radio button and associated text box in the Track Length module.

In both call segments, pulses were given a downsweep of bandwidth 1000 Hz around a centre frequency of 4810 Hz. Because the short chirps are lower amplitude than the long chirp in *T. oceanicus* signals, we used the Normalised Amplitude feature to set the maximum amplitude of the short chirps to 0.8. As a result, the amplitude of the short chirps was 80% that of the long chirp, whose normalised amplitude we left at the default value, 1. After generating these two call segments, we concatenated the two audio files together using our Concatenate GUI.

Bow-winged grasshopper

The acoustic communication system of the bow-winged grasshopper, *C. biguttulus*, has been particularly well studied in the context of signal detection and recognition mechanisms (von Helversen and von Helversen 1997; Ronacher et al. 2000; Machens et al. 2003). Females of *C. biguttulus* produce acoustic responses to male songs that can be used to assay female preferences (Klappert and Reinhold 2003; Wirmer et al. 2010; Reichert and Ronacher 2015).

Male bow-winged grasshoppers produce a pulsed signal whose spectral properties are approximated using SynSing in Broadband Noise Mode with energy between 4.5 and 40 kHz. SynSing's default settings in Broadband Noise Mode generate the *C. biguttulus* example based on experimental stimuli used by Reichert and Ronacher (2015; Figure 7(f)). The maximum frequency at which the *C. biguttulus* signal contains acoustic energy is 40 kHz. As such, we have set the default sample rate in Broadband Noise Mode at 88.2 kHz, or twice that of the Sinusoidal Mode. Specialised playback equipment may be required to faithfully reproduce this and other such signals with energy at ultrasonic frequencies. The *C. biguttulus* example stimulus comprises 25 pulses, each 80 ms in duration, with interpulse intervals of 12 ms. Each pulse was shaped with a linear onset and offset of 1 ms. The call was given a linear rise across its entire duration.

Sword-bearing conehead katydid

The acoustic communication system of the sword-bearing conehead katydid, *Neoconocephalus ensiger*, has been investigated in the context of receiver preferences and signal evolution (Schul and Patterson 2003; Frederick and Schul 2016; Murphy and Schul 2016), signal detection and recognition mechanisms (Faure and Hoy 2000a,2000b), and acoustically mediated predator avoidance behaviours (Faure and Hoy 2000b,2000c; Ter Hofstede and Fullard 2008; Ter Hofstede et al. 2008).

Neoconocephalus ensiger signals are produced at a pulse rate of approximately 15 sound pulses per second, which are separated by interpulse intervals of approximately 40 ms. In Sinusoidal Mode, we constructed stimuli after the details provided in Kong et al. (2015; Figure 7(g)), with a dominant frequency of 14 kHz and a second harmonic, at 28 kHz, at an amplitude of −18 dB relative to the 14 kHz component. Pulses were given a linear rise over the first 15 ms and a linear fall over the last 5 ms.

Tiger wandering spider

In addition to acoustic signals, SynSing may be used to generate vibrational signals. Male tiger wandering spiders, *Cupiennius salei*, produce vibratory courtship displays using rhythmic movements of both the pedipalps and the abdomen, which propagate through plants on which the spiders live (Barth 1985; Barth et al. 1988). Abdominal vibrations presented alone are sufficient to elicit responses from females, which readily respond to playback of synthetic signals (Schüch and Barth 1990). The vibrational communication behaviours of *C. salei* have been investigated with regard to female preferences, vibration reception mechanisms, and properties of signal propagation (Rovner and Barth 1981; Barth 1985, 1998; Speck-Hergenröder and Barth 1987; Barth et al. 1988; Anton and Barth 1993; Shimizu and Barth 1996; McConney et al. 2007).

Schüch and Barth (1990) assayed female responses to synthetic abdominal vibrations that varied systematically in multiple signal traits and showed that females have closed preference functions for all temporal traits examined. We produced a synthetic *C. salei* signal with the temporal properties that elicited the reported maximum number of female responses (Figure 7(h)). The stimulus has a fundamental frequency of 133 Hz, a pulse duration of 105 ms with interpulse intervals of 169 ms, and consists of 12 pulses. We shaped the amplitude envelope of the signal with a linear rise over its entire duration to visually approximate the natural amplitude envelope.

White-throated sparrow

Our above examples detail how SynSing may be used to re-create synthetic signals in studies of arthropods and anurans, taxa in which synthetic signals are regularly used.

SynSing may also be useful for synthesizing simple vocalisations of birds or mammals, provided they do not contain non-linear frequency modulation, which is not supported by version 1.0. As an example, we generated the spectrally simple song of the white-throated sparrow, *Zonotrichia albicollis* following mean values presented by Borror and Gunn (1965; Figure 7(i)). The synthetic song consists of a series of three relatively long pure tone 'whistles' (680 ms at 6500 Hz, 520 ms at 5990 Hz, and 310 ms at 5100 Hz) followed by a triplet of shorter, 116 ms pulses with interpulse intervals of 20 ms. For this example, we shaped all pulses with a linear rise and fall times of 50 ms. We separated the notes by silent intervals of 184 ms, 106 ms, and 90 ms respectively.

Other uses

SynSing may also be used to generate stimuli not intended to mimic the signals of animals, but which are nevertheless experimentally useful in studies of bioacoustics or psychoacoustics in many taxa, including birds and mammals. For example, Kastelein et al. (2009) used pure tone stimuli, including one similar to the 900 ms, 250 Hz tone in Figure 8(a), to measure audiograms in harbour seals, *Phoca vitulina*. Wong et al. (2019) recorded distortion product otoacoustic emissions (DPOAEs) in budgerigars,

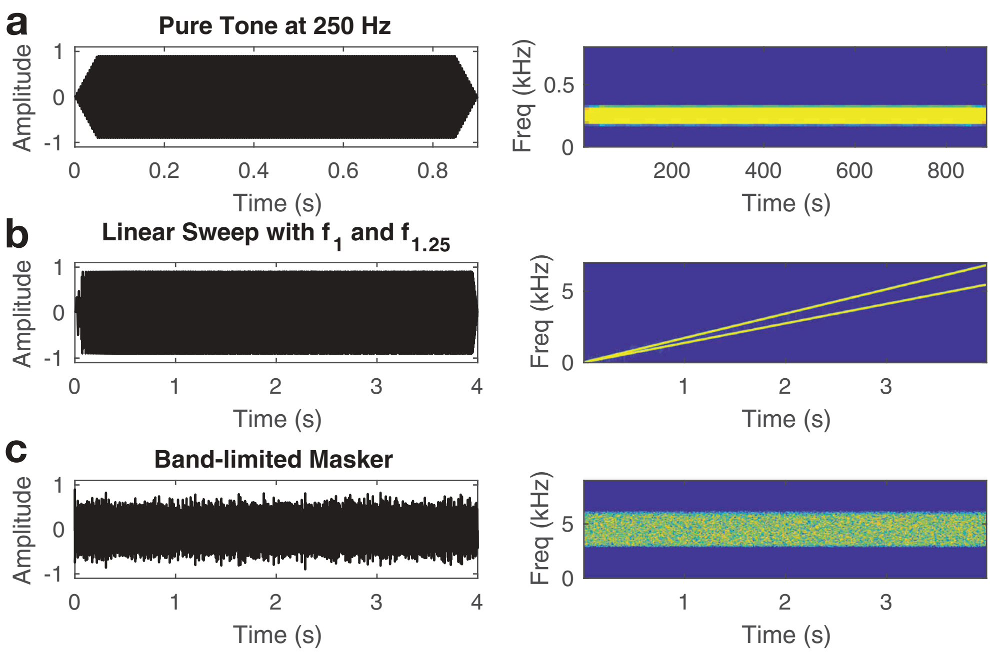

Figure 8. Waveforms (left) and spectrograms (right) of additional example stimuli. (a) A 250 Hz pure tone shaped with a 50 ms linear onset and offset, similar to one used to measure audiograms in harbour seals. (b) A linear sweep similar to one used to study hearing in budgerigars. The f_1 component sweeps between 500 Hz and 6 kHz over 4 s, and a second frequency component is equivalent to $f_{1.25}$. (c) Noise in a 3–6 kHz passband generated with a filter order of 10, similar to a stimulus used to mask cricket song in a study of *Ormia ochracea*.

Melopsittacus undulatus, using a stimulus consisting of two frequency components, f_1 and $f_{1.25}$, where f_1 swept from 0.5 kHz to 6 kHz across its 4 s duration (Figure 8(b)). Finally, in Broadband Noise Mode, SynSing may be used to generate masking noises, which are commonly used in behavioural assays (e.g., Narins 1982; Ronacher et al. 2000; Reichert and Ronacher 2015). For example, we have generated 4 s of band-limited noise (between 3 and 6 kHz) similar to that used by Lee and Mason (2017) to mask cricket song in a study of the acoustically-orienting parasitoid fly *Ormia ochracea* (Figure 8(c)).

Conclusion

The use of synthetic stimuli has a long history and a bright future in playback studies of animal communication. Our intention with SynSing is to introduce a new tool that makes the synthesis of animal sounds for use in playbacks more accessible to larger number of researchers. While the program is not without its little quirks and limitations, we believe that most users willing to roll up their sleeves and spend time exploring its features will find it reasonably versatile and easy to use. Note that no tool, including SynSing, should be regarded as an adequate substitute for the user's own good judgement and knowledge of the signals of interest, the intended receivers' sensory systems, and some basic digital signal processing. We end with a cautionary reminder that no matter how easy or versatile a program for signal synthesis might be, the only thing that ever matters in a playback test is what comes out of the speaker. Users of SynSing are encouraged to always make an analysis of broadcasts of SynSing's output through their playback system the ultimate arbiter of its functionality.

Acknowledgements

The authors thank Michael S. Reichert, Norman Lee, and Justa L. Heinen-Kay for beta-testing the GUI and Alejandro Vélez and Søren V. Jørgensen for contributing base code. J.C.T. was supported by a National Science Foundation Graduate Research Fellowship under Grant No. 00039202 and a Ford Foundation Predoctoral Fellowship. This material is based upon work supported by an NSF Postdoctoral Research Fellowship in Biology (1811930) to J.C.T. and by a National Science Foundation grant (IOS-1452831) to M.A.B.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Directorate for Biological Sciences [1811930]; Division of Graduate Education [00039202]; Division of Integrative Organismal Systems [1452831]; Ford Foundation [Predoctoral Fellowship].

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