

Standard Operating Procedure #8:

Processing Acoustic Data

Version 1.0

In Bat monitoring protocol for the Great Lakes Inventory and Monitoring Network: Version 1.0

Katy Goodwin¹ and Karen Gilland²

¹ National Park Service
Great Lakes Inventory and Monitoring Network
2800 Lakeshore Drive East
Ashland, Wisconsin 54806

² Normandeau Associates, Inc.
4581 NW 6th Street, Suite A
Gainesville, Florida 32609

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Revision History Log

The following table lists all edits and amendments to this document since the original publication date. Information entered in the log must be complete and concise. Users of this standard operating procedure will promptly notify the GLKN protocol lead and/or project coordinator about recommended and required changes. The protocol lead and/or project coordinator must review and incorporate all changes, complete the revision history log, and change the date and version number on the title page and in the header of the document file.

Previous Version #	Revision Date	Author (with title and affiliation)	Location in Document and Concise Description of Revision	Reason for Change	New Version #
Add rows as needed for each change or set of changes tied to an updated version number					

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1.0 Introduction

The Great Lakes Inventory and Monitoring Network (GLKN) is implementing a monitoring program to document changes in bat populations in national park units in the upper Midwest. We are using acoustic monitoring as an index for assessing long-term bat population trends in response to ecosystem changes including climate, habitat, and disease such as white-nose syndrome (WNS). The goals of this monitoring program are to assess probable presence and absence of bat species, and to determine relative activity levels of those species for each national park unit over time. This Standard Operating Procedure (SOP) describes how acoustic files collected in the field are organized, processed, and run through automated species classification software, as well as manual vetting procedures. This document is largely based on the “Data Management Plan” provided to GLKN by Normandeau Associates (Normandeau), an environmental consulting firm contracted to analyze our data starting in 2016.

1.1 Preparing for Data Processing

The individual processing acoustic data will be provided with both the raw audio files (in WAV format) and the associated Microsoft Access NPS Bats database. Audio files must be organized in folders by park and site, using standard folder naming conventions to ensure they match the values in the database’s lookup tables. All cell, site, and deployment information should already be entered in the database. In addition, QAQC checks and any final corrections should already be completed. Refer to SOP #7 for data entry and quality assurance procedures.

1.2 Importing Audio File Information into the Database

Normandeau uses automated routines to read each audio file and update the database with information pertaining to that file. This information is stored specifically in the database table called *tblDeploymentDetection1* and includes the following:

- a record of each audio file linking it to its deployment (and thus its site and park)
- relevant information about the audio files (e.g., duration)
- an automated ID of the audio file (using Kaleidoscope Pro software)
- a manual ID for 1% of the audio files

Normandeau has created additional database tables that aid in the data processing procedures. These additional tables do not affect the functionality of the original database created by the NPS. The GLKN copy of the bat database does not include Normandeau's added tables. Therefore, if in future years GLKN staff are processing the data internally, rather than contracting with Normandeau, it will be necessary to obtain these tables as a final deliverables from Normandeau, or create equivalents.

One table created by Normandeau is called *tblAudioFile* and contains an inventory of all audio files supplied for processing. In this table each audio file is given a status code to determine if it can be used for analysis, based on whether the deployment meets the protocol specifications:

- Deployments are excluded from analysis if they do not have at least four valid nights of recording. A valid night is defined as having a minimum of six hours of recording. The duration of recording is determined by examining the summary file automatically generated by the Song Meter, which indicates the times at which the device turned on at night and off in the morning. If the summary file is not available, duration is determined from the date/time of the first and last audio files.
- Deployment length is cut off at a maximum of sixteen nights, with any audio files recorded after the sixteenth night excluded from analysis.
- Deployments are also excluded from analysis if fewer than four nights fell within the protocol sampling period (1 June–15 August). If at least four nights fell within these dates, and additional nights extended beyond, all nights in that deployment will be included.

To import information for each audio file into the table, run Normandeau's Microsoft Access module (automated routine) called *modAudioFileImport*. This module includes the use of the SoX program (see Appendix A). The module will perform the following steps:

1. Finds all WAV files for a given park or given park/site combination.
2. If needed, creates a recording night directory in the form yyyy-mm-dd and moves all WAV files to the directory by night of recording.

3. Runs SoX program to get WAV file recording details (i.e., bit rate, channels of audio, duration of audio).
4. Using SoX, splits any file with two channels of audio into two separate WAV files.
5. Inserts record into database table *tblAudioFile* with each file's details.
6. As part of this, records a Status Code indicating if the file is to be rejected from analysis and why (e.g., short deployment).

1.3 Species Classification and Noise Filtering Using Kaleidoscope Pro

Once audio file information has been added to the database, the next step is running the audio files through a specialized software program that filters out noise and performs automated species classifications. GLKN uses the program Kaleidoscope Pro (Wildlife Acoustics, Inc.), but other similar software programs are available. For analysis of 2015–2019 data, Kaleidoscope Pro Version 4.0.0 and the Bats of North America Classifier 3.1.0 were used. Although updated versions of the Kaleidoscope Pro program and classifier have been released, we have continued using the same older version to ensure consistency and comparability among years. Tests by Goodwin (2019) comparing three different Kaleidoscope Pro classifiers indicated that two newer versions (4.3.0 and 5.1.0) are more conservative, identifying a greater proportion of files as unknown bats rather than specific species. In contrast, the older version we have been using for this project (3.1.0) assigns more species-level identifications. Additionally, percent agreement of classifications on a file-by-file level was highest between the two newer versions. Thus, future data processing may be done with an updated version of Kaleidoscope Pro, so all previous years of data should also be re-analyzed using the updated version.

Kaleidoscope Pro is used for two main functions: 1) to filter out audio files containing noise from those containing bat calls, and 2) to obtain automated species-level classifications for files containing bat calls. Kaleidoscope Pro also offers the option to attribute audio files with additional user-defined metadata if desired (e.g., a description of the sample site). Because audio file names already include the Site IDs, GLKN protocol does not currently call for attributing files with additional metadata beyond what is automatically stored by the detector.

Unlike the SoX program, Kaleidoscope Pro cannot be run automatically by Microsoft Access scripts. Instead, Kaleidoscope Pro must be run manually outside of the database using the desktop application. Kaleidoscope Pro will read the audio files directly and produce output text files detailing the classification results (id.csv, idsummary.csv). These output files must then be imported into the database. A Microsoft Access script can be used for this step to import the results.

Kaleidoscope Pro Settings

Our data processing procedure generally follows the U.S. Fish and Wildlife Service (USFWS) guidelines (USFWS 2019) for Indiana Myotis (*Myotis sodalis*) and Northern Myotis (*Myotis septentrionalis*). These include converting full spectrum audio files to zero-crossing format; selecting the species to include in the classification filter using only species that occur in the study area; and selecting certain settings to use in the classification software.

Kaleidoscope Pro allows the user to customize a list of possible bat species that will be considered during the classification process. This permits exclusion of any species that are not found in the specific project area, to reduce the chance of obtaining false positives in the results. For the GLKN project, bat species with known ranges within each park's boundaries were identified based on published range maps (Harvey et al. 2011, Rodhouse et al. 2016, Kurta 2017). These species were

considered the “baseline” species list for each park. Additionally, we created an “expanded” species list for each park which added any species that have been documented relatively close to the park and that may possibly be present. To accommodate both versions of the species list, all audio files will be run through Kaleidoscope Pro two times. The first time we will use the baseline list as the list of possible species the program can consider, and the second time we will use the expanded list. The baseline and expanded species lists for each park are provided in Appendix B.

Kaleidoscope Pro also allows customization of other settings before processing audio files. Specific settings used for the GLKN program are provided in the next section and in Appendix C.

Kaleidoscope Pro Processing

Detailed, step-by-step instructions for processing audio files through Kaleidoscope Pro are provided in Appendix C. A general description of the procedure and steps involved is provided here:

1. Verify that your computer is set up for this task.
 - a. Make sure Kaleidoscope Pro is installed and Bat Analysis Mode is enabled.
 - b. Make sure you can access the dataset of WAV files from the computer.
 - c. Make sure the WAV files are organized into the correct folder structure (Park/Site/WAV/date).
2. Run audio files (WAV format) through Kaleidoscope Pro to convert from WAV format to zero-cross format. This is the first of three times you will run files through Kaleidoscope Pro. This step is simply converting files, not identifying them.
 - a. Select settings as specified in Appendix C for the Batch and Signal Parameters tabs.
 - b. Select “Disabled” for the Auto ID for Bats, Cluster Analysis, and Noise Analysis tabs.
 - c. Results:
 - i. New folder directories will be created, containing the converted zero-cross files.
 - ii. A few output text files will also be generated. The *log.txt* file lists all of the audio files that were processed and indicates whether the file conversion was successful. The *meta.csv* file gives details about each file. The *settings.ini* file documents what settings were used for processing.
3. Run the converted audio files (zero-cross format) through Kaleidoscope Pro two times to obtain species-level classifications: first using the “baseline” species list and second using the “expanded” species list. This step also includes filtering to remove noise files.

- a. Select settings as specified in Appendix C for the Batch and Signal Parameters tabs. On the Auto ID for Bats tab, select Bats of North American Classifier 3.1.0 and the “-1 More Sensitive (Liberal)” option from the dropdown menus. Then select the bat species to use as possible candidates, using the “baseline” or “expanded” list specific to each park (see Appendix B).
- b. Select “Disabled” for the Cluster Analysis and Noise Analysis tabs.
- c. Results:
 - i. No new files or new folder directories will be created.
 - ii. Files identified as noise will be moved to a separate new NOISE folder.
 - iii. Four output files will be generated. The *log.txt* file lists each file that was processed, indicates if there were any errors in processing it, and indicates if it was classified as noise. The *settings.ini* file documents what settings were used for processing. The final two files are *id.csv* and *idsummary.csv*, which both provide the species identification results. The *id.csv* file provides results on a file-by-file basis and includes details such as measurements of echolocation call characteristics. The *idsummary.csv* file provides a simple summary of the results and shows the number of detections per species per site.

Importing Kaleidoscope Pro Results into Database

After audio files have been processed for noise filtering and species classification, results must be imported into the Microsoft Access database. Normandeau used another automated routine (module) in Access to accomplish this.

Run the Normandeau module called *modReadKProID*. This will update the database table called *tblDeploymentDetection1* to include all of the automated species classifications generated by Kaleidoscope Pro, drawing the data from the *id.csv* file. In addition, this module will update the database table called *tblAudioFile* with the automated species classifications. This is redundant (auto IDs are stored in two places) and will be used for quality control but will not be used for summary reports.

1.4 Manual Vetting of Kaleidoscope Pro Results

Kaleidoscope Pro and other similar bat acoustic analysis programs are not 100% accurate in assigning species classifications. The classification results are expected to include some errors due to factors such as similarity of echolocation calls among different species, variation of calls within the same species, poor quality or truncated calls, clutter effects, or multiple bats recorded together (Britzke et al. 2013, Reichert et al. 2018). Therefore, manual review (manual vetting) of call files is an important secondary step to verify the results produced by the software.

Due to the large scope of the GLKN bat project and the enormous quantity of data collected, it will not be possible to manually review all of the audio files containing bat calls. Instead, we will perform manual vetting on a small subset of files, 1% at minimum. From each year's dataset, we will randomly select 1% of the files that Kaleidoscope Pro identified to the species level (i.e., excluding Noise and No ID files). To ensure all parks and all species are proportionally represented in the manual vetting, files are not randomly selected at large. Instead, we will randomly select 1% of files from each park/species combination, with a minimum of 10 files selected for each park/species combination. This procedure will apply to the baseline analysis; for the expanded analysis additional files will be selected only for the park/species combinations not already represented by the baseline analysis.

Manual vetting must be done by an experienced bat biologist who has had specific training on this process. Ideally, the same individual will conduct all the manual vetting, to ensure consistency and comparability among parks and among years of data. This will help avoid bias associated with different reviewers (Fritsch and Bruckner 2014). GLKN reports must include documentation of who completed the manual reviews and specify if multiple individuals were involved.

The process of selecting the files to be reviewed should be performed by a different individual, not the person who will be doing the manual vetting. All files selected for manual vetting will have identifying information removed from the file name (e.g., park location, date). The manual reviewer will be provided with a list of possible species for each file, which matches the list of possible species that Kaleidoscope Pro was allowed to consider (see Section 1.3 and Appendix B). However, the manual reviewer will not know how the file was classified by Kaleidoscope Pro. These procedures will help ensure that the manual vetting is an independent and “blind” test.

The manual reviewer is provided with the original full spectrum audio file and can examine the file by looking at a full spectrum spectrogram as well as a zero-cross spectrogram if needed. The manual reviewer will assign an identification to each audio file, assuming that it contains bat calls of sufficient quality. The manual reviewer is permitted to identify a call to one particular species, to a pair of similar species (e.g., two species in the genus *Myotis*), or to a broader group of species (e.g., unknown high frequency or unknown low frequency). The reviewer may also determine that the file does not contain bat echolocation calls, in which case it will be identified as non-bat or noise. Identifications should be based on call characteristics such as shape, frequency, and duration, using published reference materials and call libraries.

The specific steps to prepare for and complete manual vetting are given below.

1. Create a list of the files to be manually vetted. This will be done in the database, by running the Microsoft Access module created by Normandeau called *modSelectForManualID*. The module does the following:
 - a. Randomly selects 1% of audio files or 10 files, whichever is greater, for a given park and species combination.
 - b. Makes a copy of selected audio files, renaming each file to a random 6-digit number. Random file names now contain no information about the location or date of the original file. Example: 102650.wav, 115419.wav.
 - c. Creates a record in the database table called *tblManualQC* that relates the new random file name to the audio file's record in *tblDeploymentDetection1*. This allows us to correctly import the manual identification data later.
 - d. Places selected files into new folders. Files are grouped into folders according to the list of possible species given to Kaleidoscope Pro for that file. For example, all selected files from the parks MISS and SACN would be placed into the same folder, because these two parks have the same list of possible species.
2. Trained bat biologist performs manual vetting.
 - a. Biologist is provided with the selected files, named with random 6-digit numbers and placed in folders according to possible species.
 - b. Biologist opens each file and examines the spectrogram. This can be done using Kaleidoscope Viewer, SonoBat, or another similar call viewing software program.
 - c. Biologist makes a determination of whether the file contains bat echolocation calls or not. If it does, biologist assigns an identification.
 - d. Identifications can be species-specific, a pair/group of species, non-bat, or unknown bat.
3. Import manual vetting results into database. This will be done by running Normandeau's Microsoft Access module called *modImportManualID*. This module automatically updates the database table called *tblManualQC*, adding the manual reviewer's species identifications and any additional notes for each file.

Additional call files beyond the minimum 1% may be reviewed for specific purposes such as endangered species compliance, assessing suspicious records outside of a species' documented range, or developing occupancy models. In these cases, instead of a random sample, it may be more appropriate to specifically focus on the call files that offer the greatest chance of verification. This would include files that contain search-phase calls (as opposed to feeding buzzes or social calls), files

that have longer call sequences (containing a greater number of individual call pulses), and files assigned a higher confidence score by the software. For Kaleidoscope Pro, the confidence score or classification margin is found in the column titled “Margin” of the id.csv output file. If analyses will include occupancy modeling, this will require a significant investment of time and effort in manual vetting. According to NABat guidance, the reviewer should continue vetting files until achieving a confident identification of at least one call file per species per deployment night at each sample site (Reichert et al. 2018). Alternative modeling methods such as those presented by Banner et al. (2018) should be considered to potentially reduce the manual vetting effort.

Literature Cited

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Appendix A. Using the SoX Program

Sound eXchange (SoX; Version #14.4.2; [SourceForge.net](https://sourceforge.net)) is a public domain (free) software program. This software has no GUI interface but it is easy to create Microsoft Access routines that will run the program and retrieve the outputs to import into the bat project database.

For the GLKN project, SoX is used to obtain information about each audio file such as bit and sample rate, format, number of channels of audio, and duration. The command to generate this audio file information is as follows:

```
sox -i <filename>
```

Some data collected on the GLKN project was recorded with two microphones in use simultaneously, which created WAV files with two channels of audio combined in one file. Kaleidoscope Pro would be capable of separating the two channels during the species classification process. However for this project the SoX software was used to separate the two channels of audio prior to species classification in Kaleidoscope Pro. The command to split channels is as follows:

```
sox <inputfile>.wav <outputfile>.wav remix <channel#>
```

The output filename will be formatted as:

```
<input filename>c<channel#>.wav
```


Appendix B. Baseline and Expanded Species Lists

The following tables list the bat species included as possible candidates during Kaleidoscope Pro species classification for each park. The first table shows the “baseline” species group and the second table shows the “expanded” species group (additions highlighted). Nomenclature follows Simmons and Cirranello (2020).

Table B1. Bat species included in baseline analysis.

Species Name	Park								
	APIS	GRPO	INDU	ISRO	MISS	PIRO	SACN	SLBE	VOYA
Big Brown Bat <i>Eptesicus fuscus</i>	X	X	X	X	X	X	X	X	X
Eastern Red Bat <i>Lasiurus borealis</i>	X	X	X	X	X	X	X	X	X
Hoary Bat <i>Lasiurus cinereus</i>	X	X	X	X	X	X	X	X	X
Silver-haired Bat <i>Lasionycteris noctivagans</i>	X	X	X	X	X	X	X	X	X
Little Brown Myotis <i>Myotis lucifugus</i>	X	X	X	X	X	X	X	X	X
Northern Myotis <i>Myotis septentrionalis</i>	X	X	X	X	X	X	X	X	X
Indiana Myotis <i>Myotis sodalis</i>			X						
Evening Bat <i>Nycticeius humeralis</i>			X						
Tricolored Bat <i>Perimyotis subflavus</i>			X		X		X		

Table B2. Bat species included in expanded analysis. Asterisk (*) indicates species added to a park’s species list.

Species Name	Park Location								
	APIS	GRPO	INDU	ISRO	MISS	PIRO	SACN	SLBE	VOYA
Big Brown Bat <i>Eptesicus fuscus</i>	X	X	X	X	X	X	X	X	X
Eastern Red Bat <i>Lasiurus borealis</i>	X	X	X	X	X	X	X	X	X
Hoary Bat <i>Lasiurus cinereus</i>	X	X	X	X	X	X	X	X	X
Silver-haired Bat <i>Lasionycteris noctivagans</i>	X	X	X	X	X	X	X	X	X

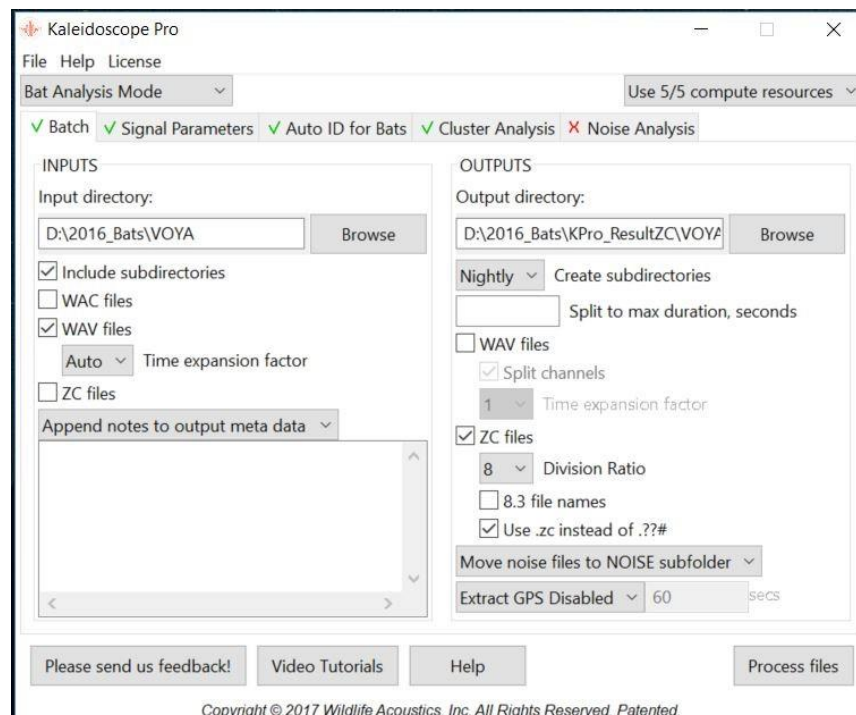
Table B2 (continued). Bat species included in expanded analysis. Asterisk (*) indicates species added to a park's species list.

Species Name	Park Location								
	APIS	GRPO	INDU	ISRO	MISS	PIRO	SACN	SLBE	VOYA
Little Brown Myotis <i>Myotis lucifugus</i>	X	X	X	X	X	X	X	X	X
Northern Myotis <i>Myotis septentrionalis</i>	X	X	X	X	X	X	X	X	X
Indiana Myotis <i>Myotis sodalis</i>			X					X*	
Evening Bat <i>Nycticeius humeralis</i>			X		X*		X*		
Tricolored Bat <i>Perimyotis subflavus</i>	X*	X*	X	X*	X	X*	X	X*	X*

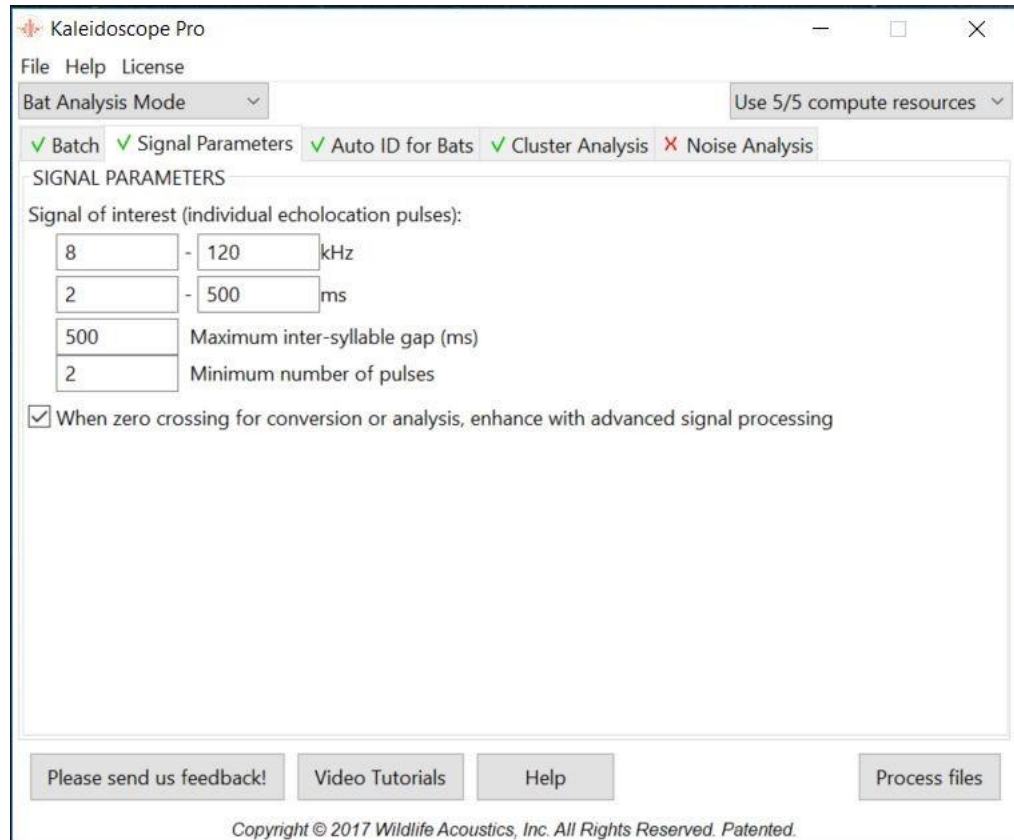
Appendix C. Step-by-Step Instructions for Kaleidoscope Pro Processing

Step 1: Convert audio files from WAV format to zero-cross (ZC) format

1. This step is simply converting file format, not identifying to species.
2. All audio files should be organized and placed in the correct folder structure: Park folder/Site folder/WAV folder/Date folder/.wav files.
 - a. Example: 2016_Bats/VOYA/VOYA032A/WAV/2016-08-06/ *.wav
 - b. The date folder is optional and will be created if not present.
3. Create a new empty folder to receive the output data, with subfolders for each park. Ensure these folders all have write permission.
 - a. Example: 2016_Bats/KPro_ResultZC/VOYA
4. Convert files to zero-cross format, running a single park at a time.
 - a. Open Kaleidoscope Pro and make sure Bat Analysis Mode is selected in top left.
 - b. Use these settings for the Batch tab:



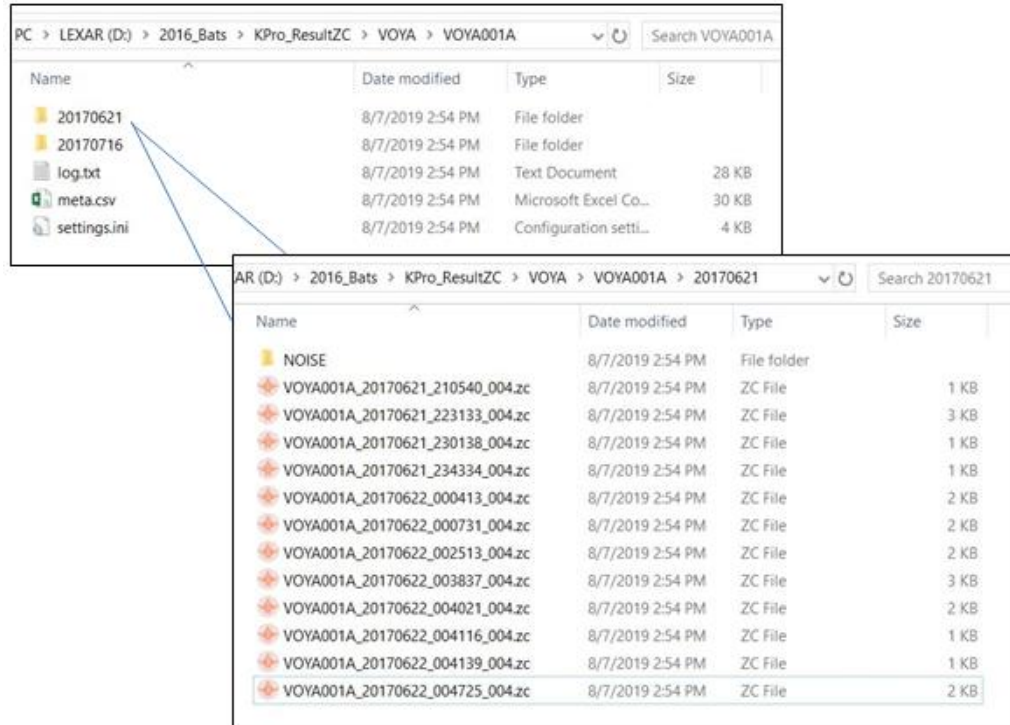
- c. Use these settings for the Signal Parameters tab:



- d. Select “Disabled” for the Auto ID for Bats, Cluster Analysis, and Noise Analysis tabs.
- e. Click the “Process Files” button in the lower right corner. Processing may take a long time (4–6 hours) for the typical number of sites and dates within a single park. You should see a progress window as shown below. The count of ZC files in the outputs section should be constantly changing. If the count has stopped changing, check for an error window. DO NOT allow this processing to run overnight; run it during the day when you can monitor it and make sure it is running properly. The program sometimes generates errors/exceptions that pause the program and that require a human to manually click a button to resume the program.



- f. When processing concludes, you will see a new set of ZC files within the nightly subfolders, which are within the ZC results folder you created in Step 2 (illustrated below). If any files had long durations, they will be split into multiple files with a maximum duration of 15 seconds. If any files were filtered out as noise, they will be placed in a new automatically generated NOISE folder. There will also be three output files called log.txt (lists the result of each file conversion), meta.csv (gives details about each file), and settings.ini (records what settings were used).

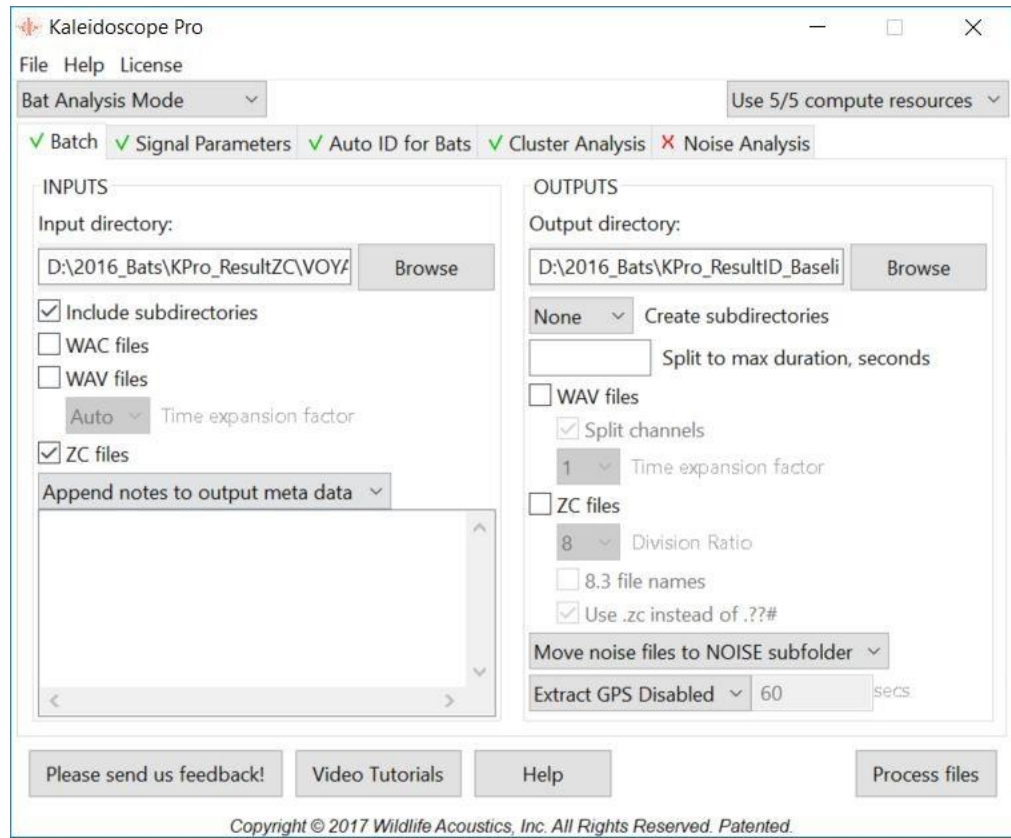


5. Repeat these steps for each park.

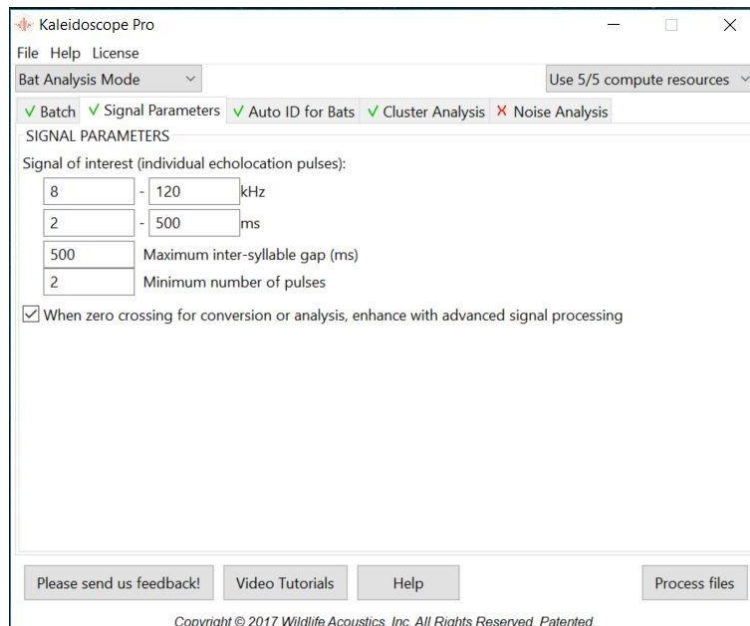
Step 2: Obtain species identifications on zero-cross (ZC) files

1. All audio files should be organized and placed in the correct folder structure. Unlike above for the file conversion process, now the input files will be the zero-cross formatted files. The folder structure should be: ZC Results folder/Park folder/Site folder/Date folder/.zc files.
 - a. Example: 2016_Bats/VOYA/VOYA032A/2016-08-06/*.zc
2. Create new empty folders to receive the output data, one each for baseline and expanded analyses, with subfolders for each park. Ensure these folders all have write permission.
 - a. Example: 2016_Bats/KPro_ResultID_Baseline/VOYA and 2016_Bats/KPro_ResultID_Expanded/VOYA
3. Run automated species classification, running a single park at a time. Run baseline analysis separately from expanded analysis.

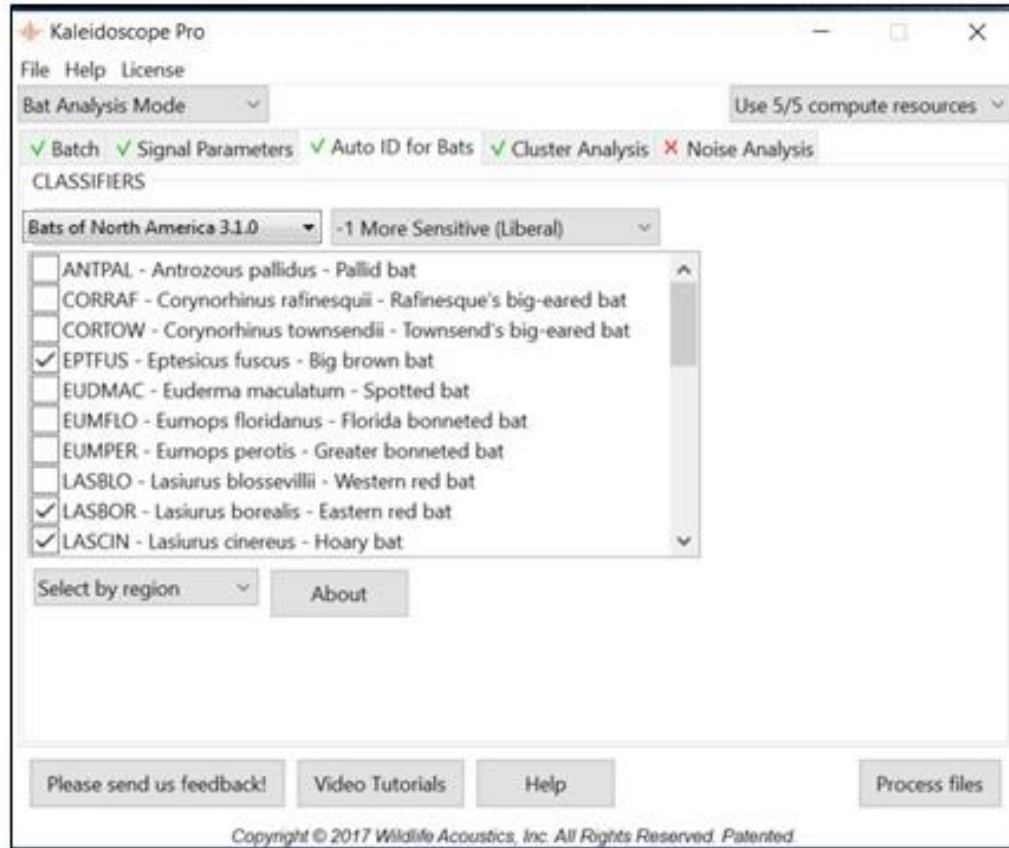
- a. Open Kaleidoscope Pro and make sure Bat Analysis Mode is selected in top left.
- b. Use these settings for the Batch Tab:



- c. Use these settings for the Signal Parameters tab:



- d. Use these settings for the Auto ID for Bats tab. Use the **Bats of North America 3.1.0 Classifier and the -1 More Sensitive (Liberal)** option. Select the check boxes for the bat species to use as possible candidates, using the “baseline” or “expanded” list specific to each park (see Appendix B).



- e. Select “Disabled” for the Cluster Analysis, and Noise Analysis tabs.
- f. Click the “Process Files” button. Processing may take up to an hour per park. Typically, this step is much faster than the conversion from WAV to ZC files, but it can still take a considerable amount of time if there are a lot of files. You will see a processing status window as shown below. The count of classified vs. unknown vs. noise files in the outputs section should be constantly changing. If the count has stopped changing, check for an error window. DO NOT allow this processing to run overnight; run it during the day when you can monitor it and make sure it is running properly. The program sometimes generates errors/exceptions that pause the program and that require a human to manually click a button to resume the program.
- g. When processing concludes, you will see a group of output files within the results folder you created in Step 2 (illustrated below). The *log.txt* file lists each file that was processed, indicates if there were any errors in processing it, and indicates if it was classified as noise. The *meta.csv* file gives details about each file. The *settings.ini* file

documents what settings were used for processing. The final two files are *id.csv* and *idsummary.csv*, which both provide the species identification results. The *id.csv* file provides results on a file-by-file basis and includes details such as measurements of echolocation call characteristics. The *idsummary.csv* file provides a simple summary of the results and shows the number of detections per species per site.

***NOTE: These output files are automatically assigned file names. These names are exactly the same every time; there is no prefix assigned. Make sure you are re-naming files or storing them in separate folders to differentiate between the results for each park and between the baseline vs. expanded analysis.

***NOTE: It appears that the *idsummary.csv* file is not always accurate so do not trust it to always be correct; double check your data!

AR (D:) > 2016_Bats > KPro_ResultID_Baseline > VOYA > VOYA001A			
Search VOYA001A			
Name	Date modified	Type	Size
 id.csv	8/7/2019 3:22 PM	Microsoft Excel Co...	39 KB
 idsummary.csv	8/7/2019 3:22 PM	Microsoft Excel Co...	1 KB
 log.txt	8/7/2019 3:22 PM	Text Document	13 KB
 meta.csv	8/7/2019 3:22 PM	Microsoft Excel Co...	23 KB
 settings.ini	8/7/2019 3:22 PM	Configuration setti...	4 KB

4. Repeat these steps for both baseline and expanded analysis and for each park.