Title: Automated tracking of avian parental care behavior

Authors:

Smith-Vidaurre, Grace\textsuperscript{1-4}, Molina, Tania\textsuperscript{5}, Jarvis, Erich D.\textsuperscript{1,6,7}, and Hobson, Elizabeth A.\textsuperscript{2}

Authors' affiliations:

1 Rockefeller University Field Research Center, Millbrook, NY, U.S.A.
2 Department of Biological Sciences, University of Cincinnati, Cincinnati, OH, U.S.A.
3 Department of Integrative Biology, Michigan State University, Lansing, MI, U.S.A.
4 Department of Computational Mathematics, Science, and Engineering, Michigan State University, Lansing, MI, U.S.A.
5 Independent researcher, Germantown, NY, U.S.A.
6 Laboratory of Neurogenetics of Language, Rockefeller University, New York, NY, U.S.A.
7 Howard Hughes Medical Institute, Chevy Chase, MD, U.S.A.

Corresponding author:

Grace Smith-Vidaurre
Field Research Center
Rockefeller University
495 Tyrrel Rd.
Millbrook, NY 12545
gsvidaurre@gmail.com
575-646-486
ORCID: 0000-0002-0155-8159
Abstract:

1. Parental care may be an important source of phenotypic variation for ecological and evolutionary processes. However, it can be difficult to collect and interpret data on parental care behaviors. To address these challenges, we developed a new hardware and software platform for automated behavioral tracking called ABISSMAL (Automated Behavioral Tracking by Integrating Sensors that Survey Movements Around a target Location).

2. ABISSMAL automatically collects data across low-cost sensors with built-in system monitoring and error logging. ABISSMAL also generates behavioral inferences with internal validation by integrating data across multiple movement sensors.

3. We successfully used ABISSMAL to track nest attendance activities performed by captive zebra finches (*Taeniopygia guttata*) that raised chicks through fledging. We highlight the behavioral inferences that ABISSMAL can derive from integrated datasets that represent discrete movement events, including types of behaviors, and the direction and magnitude of movements.

4. ABISSMAL streamlines the process of automated data collection, curation, and interpretation for researchers studying parental care across many experimental replicates and over long developmental timescales. ABISSMAL is a modular system that can be deployed with different combinations of sensors to suit different research questions and experimental setups. We made ABISSMAL open-access on GitHub with detailed documentation to facilitate widespread use and modification.
Data/Code for peer review statement: Software and documentation for ABISSMAL are publicly available on GitHub: [https://github.com/lastralab/ABISSMAL](https://github.com/lastralab/ABISSMAL). We uploaded the code that we used to pre-process data, integrate data, and make figures to GitHub. We published data associated with this manuscript on [figshare](https).

Keywords: Automated behavioral tracking, Avian parental care behavior, Infrared beam breakers, Integration across sensors, Motion-detection video recording, Movement detection, Radio-frequency identification technology, Raspberry Pi
Parental care can change offsprings' adult phenotypes. For instance, the diets that adult bees feed larvae can influence caste determination (Kamakura, 2011). In crocodilians, sex determination is impacted by incubation temperature (Lang & Andrews, 1994). Adults can also transmit behaviors to offspring through social learning that are important to survive, including foraging preferences (Slagsvold & Wiebe, 2011), and sequences of fine-scale motor movements to access specific foods (Zohar & Terkel, 1991). Parental care behaviors may therefore cause phenotypic variation in offspring that is critical for ecological and evolutionary processes (Klug & Bonsall, 2014; Laland et al., 2015; Uller, 2012).

However, in order to link variation in parental care behavior to ecologically and evolutionarily relevant variation in offspring phenotypes, we need to be able to quantify fine-grained variation in parental care behaviors throughout the course of offspring development. Collecting data on these behaviors is difficult because parental care can be infrequent, cryptic, and performed by one or up to several individuals. The types of behaviors that adults exhibit, and how often adults perform these behaviors, can also change as offspring develop. To accurately capture variation in parental care behavior, we require non-invasive continuous monitoring tools that can capture rare behavioral events (Iserbyt et al., 2018; Kalafut & Kinley, 2020).

There are few tools available for scientists interested in quantifying variation in parental care behaviors. The most commonly used tools often require costly resource investment and are largely limited to data collection, with little support for cleaning, checking, and interpreting data. Continuous video recordings are used to capture parental care behaviors (Bendesky et al., 2017; Gilby et al., 2011; Iserbyt et al., 2018; Ogino et al., 2021; Smiley & Adkins-Regan, 2016), but manually scoring videos over developmental trajectories and across many
experimental replicates can become prohibitively time-consuming. Deep learning tools for automated video scoring also require building large manually scored training datasets (Ferreira et al., 2020; Mathis & Mathis, 2020).

Movement sensors are promising solutions for quantifying infrequent behaviors (Kalafut & Kinley, 2020; Smith & Pinter-Wollman, 2021), including parental care behavior. These sensors provide continuous monitoring that is important to collect data on rare events and can be programmed for automated data collection, which reduces the need for time-intensive manual scoring. Movement sensors are also financially accessible and can be deployed in a high-throughput manner across many experimental replicates. While movement sensors hold great potential, it can be difficult for biologists to apply these sensors to collect empirical data from live animals. First, these sensors do not often come with “out-of-the-box” software that can be easily deployed or modified to suit different practical applications. Second, using movement data collected by any one sensor to make inferences about behavioral variation poses great challenges.

For example, radio frequency identification (RFID) systems are increasingly used to streamline data collection of animals’ movements associated with parental care behaviors (Iserbyt et al., 2018; Maldonado-Chaparro et al., 2021; Prinz et al., 2016; Santema & Kempenaers, 2023). However, RFID systems alone do not contain built-in validation and can fail to detect passive integrated transponder (PIT) tags (Hughes et al., 2021; Iserbyt et al., 2018). RFID systems are also limited to collecting the timestamps when PIT tags were detected by an RFID antenna, and it can be very challenging to interpret patterns of behavioral variation from the timing of location-specific movement events.

In order to address these challenges associated with collecting and interpreting data on parental care behavior, we developed ABISSMAL, a unified hardware and software
platform for automated behavioral tracking. We named our tool “abysmal” as in “endless” to highlight the many possibilities made available by this modular, open-access tracking system. ABISSMAL automates data collection across multiple types of sensors with internal system monitoring and error logging, and also derives behavioral inferences with built-in validation by integrating data across sensors.

Below we describe the three main components of ABISSMAL: 1) a suite of sensors mounted around a nest container to track avian parental care behaviors, 2) software for automated data collection, system monitoring, and error logging, and 3) a set of computational analyses to derive behavioral inferences by integrating data across multiple movement sensors. We tested ABISSMAL with captive zebra finches to highlight how this tracking system helped us streamline the process of automated behavioral data collection, curation, and interpretation of movements associated with parental care behaviors.

ABISSMAL is an accessible tool with estimated hardware costs around several hundred dollars (USD, Supplementary Table 1), and this tracking system is open-access through a public GitHub repository (https://github.com/lastralab/ABISSMAL).

Materials and Methods: The core components of ABISSMAL

Ethics statement: Data collection and animal care for captive zebra finches was conducted under an IACUC protocol approved by Rockefeller University (protocol no. 21063-H).

1. Custom hardware to track avian parental care

ABISSMAL can collect data across four different types of sensors: 1) infrared beam breakers, 2) a radio frequency identification (RFID) system, 3) an infrared camera triggered by motion detection, and 4) a temperature probe. These sensors are mounted on a custom-built PVC
nest container in order to track activities associated with avian parental care behavior (Figure 1). The first three sensor types track movement around the entrance and inside of the nest container (Figure 1A-B). ABISSMAL later integrates data across these movement sensors in order to provide behavioral inferences. The infrared beam breakers sit on 3D-printed mounts to capture activity in front of the RFID antenna (the “outer” pair, which detects movement outside of the container) and behind the RFID antenna (the “inner” pair, which detects movement inside the container). The RFID antenna sits inside of the circular entrance of the nest container (Figure 1A). An infrared camera and LEDs are mounted on a 3D-printed cap (Figure 1B). The camera captures activities that occur inside of the nest container. The fourth sensor, the temperature probe, can be mounted inside of the nest container to record ambient temperature, which can be a critical feature of the physical environment during development (Figure 1C).

These four types of sensors provide different types of information. The three types of movement sensors provide continuous monitoring of movements. Each sensor independently records the timing and location of movement. The outer and inner pairs of infrared beam breakers with 3mm LEDs and ranges of 25 cm (Adafruit Industries LLC, New York, NY, USA) detect the time at which their respective beams of infrared light are broken. The RFID antenna is connected to a CognIoT 125 kHz RFID reader (Bostin Technology Services Ltd, Lichfield, Staffordshire, UK) that detects when unique passive integrated transponder (PIT) tags attached to each individual are in close proximity (a few millimeters). The Waveshare H wide-angle infrared camera (Waveshare, Futian District, Shenzhen, China) is programmed to record short videos by motion detection in all three color channels, and we use the onset of video recording events as the timing of movement. Recording videos by motion detection also reduces the number of videos that need to be stored and scored to obtain finer-scale
behavioral information (Prinz et al., 2016). The waterproof temperature probe (Low Voltage Labs LLC, Vancouver, WA, USA) can be programmed to return temperature readings with coarse or fine-grained temporal resolution. All sensors, including the temperature probe, are connected to a Raspberry Pi computer (Raspberry Pi Ltd, Milton, Cambridge, UK) (Supplementary Figure 1). Raspberry Pi computers are increasingly used for behavioral tracking (Alarcón-Nieto et al., 2018; Jolles, 2021; Maldonado-Chaparro et al., 2021; Prinz et al., 2016; Youngblood, 2020) and facilitate long-term data collection as well as directly comparing timestamps across multiple sensors. Supplementary sections 1-3 provide more information about ABISSMAL hardware. Supplementary sections 4-5 contain more information on parameters used for data collection and data formats.
Figure 1: The first component of ABISSMAL is a suite of four sensor types mounted on a custom-built nest container. Panels A and B show three types of sensors that capture movement at the entrance and inside of the nest container. Panel C shows a temperature probe mounted inside of the container.
2. Software for automated data collection, system monitoring, and error logging

The second component of ABISSMAL is software that provides automated data collection, system monitoring, and error logging through Python version 3 (Van Rossum & Drake, 2009) and the bash shell (GNU, 2007) (Figure 2). ABISSMAL's software facilitates data collection across the four different sensor types, while the automated monitoring and logging can help streamline long-term data collection and troubleshooting across parallel experimental replicates. Movement events and temperature data recorded across the different sensors are saved inside spreadsheets each day, and are also stored in log files to provide back-up data and troubleshoot errors. Our system monitoring module automates the daily transfer of spreadsheets (.csv format), videos (.mp4 format), and log files from the Raspberry Pi to an external hard drive using cron (a utility for task scheduling). The data collection and system monitoring modules are set up to automatically run in the background on different screens once the tracking system initiates. We also include optional software for sending automated text message alerts through Twilio when errors are encountered (users will need their own Twilio account). Our software can be automatically set up using a script that installs software dependencies and configures the Raspberry Pi for compatibility with ABISSMAL.
Figure 2: Here we highlight ABISSMAL’s software for automated data collection across different sensors (an RFID system, infrared beam breakers, a camera, and a temperature probe), as well as automated system monitoring and error logging. The types of errors logged by the system are described in documentation on GitHub.
3. Computational analyses to integrate data and make behavioral inferences

The use of multiple movement sensors in ABISSMAL increases the likelihood of detecting movements associated with parental care behaviors and also provides redundant datasets when a sensor fails (Figure 3). However, it can be challenging to link movements to behavioral activities when using data from a single sensor alone. The third component of ABISSMAL is a set of computational analyses to detect discrete movements and to make behavioral inferences about different movement events. We detect movement events and link these events with behavioral activities by using custom functions that pre-process and integrate data collected across movement sensors (Figure 4). This integration across multiple sensors provides higher confidence when linking movement events to behaviors, as well as information about the direction of movement and the type of behavior that occurred (Figure 4).

The data pre-processing and integration functions are written using R and the tidyverse (R Core Team, 2023; Wickham et al., 2019). Each function is unit-tested through a battery of automated tests with simulated data and the package testthat to ensure that the functions produce their expected outcomes (Wickham, 2011). Supplementary section 6 contains more information about each function.
Figure 3: This plot shows raw data collected over 4 days (Days 4 through 7 out of 50 total) across three types of movement sensors for one pair of captive zebra finches (see Results). Nocturnal periods are shaded in grey.
Figure 4: We provide a description of four ABISSMAL functions for data processing and integration across movement sensors, as well as a graphical representation of the input and output data per function. An additional function for combining raw data across dates per sensor type (including the temperature probe) is not shown.
Results: Testing ABISSMAL with captive zebra finches

1. Setting up the tracking system for data collection

We used ABISSMAL to collect data from captive zebra finch pairs at the Rockefeller University Field Research Center. Zebra finches are small Australian songbirds that readily breed inside artificial containers in captivity. When bred in opposite-sex pairs, both parents will contribute to parental care activities (Smiley & Adkins-Regan, 2016). In naturalistic aviary settings, adults will allofeed unrelated fledglings (Ogino et al., 2021). We chose opposite-sex pairs that had already raised chicks together, and fitted each adult with an EM4102 passive integrated transponder (PIT) tag leg band (2.3mm inner diameter, Eccel Technology, Groby, Leicester, UK) to facilitate tracking individual identity through the RFID system. We placed each pair of birds in cages that were fitted with a custom-built nest container and placed inside of sound attenuation chambers (Figure 1; supplementary section 2). We used ABISSMAL to monitor the birds’ movements around each nest container, as well as ambient temperature inside of the containers. All birds were kept on a 12:12 hour light:dark cycle with ad libitum access to food and water in temperature-controlled rooms. We collected data from 5 different pairs over 7 rounds of breeding in all. Some of these rounds of data collection were shorter (e.g. captured egg-laying only) and represented testing rounds with earlier versions of our hardware and software. Two pairs that were each bred twice raised 1 – 4 chicks through fledging in each breeding round, which allowed us to ensure that our custom hardware in this version of ABISSMAL did not compromise chick survival. Throughout our figures, we use data from one pair that laid 5 eggs and raised 4 chicks in their second round of breeding. ABISSMAL captured movements associated with the nest container throughout the diurnal and nocturnal periods over 50 days of data collection (Figures 3, 5, 6). These birds laid 5 eggs over days 7 – 11 (Figures 5 and 6). Four of these eggs hatched over days 22 – 25, and
all four chicks fledged from days 40 – 41 (Figures 5 and 6). The adults started laying another clutch of eggs shortly after their chicks fledged.
2. Deriving behavioral inferences

We used ABISSMAL’s computational analyses to detect discrete movement events from the raw data collected across sensors, and to generate behavioral inferences by integrating data across sensors. We detected perching events in the raw data and pre-processed the raw data collected by each movement sensor (Figure 4, Figure 5A). We integrated the pre-processed datasets across sensors by finding clusters of detections that occurred close together in time (Figure 4). This integration was performed for 4 different combinations of sensors, in order to highlight the built-in redundancy provided by using multiple sensors to track movements. The general pattern of how the number of daily activities changed over time was consistent across sensor combinations, with the exception of the RFID and beam breaker dataset that did not have video data (Figure 5B). We then focused on detection clusters from the integrated dataset across all sensors to make behavioral inferences. For each of these clusters we used the order in which sensors triggered to score the direction of movements that occurred at the container entrance (entrances and exits, Figure 4, Figure 5C). We integrated perching events detected from the raw RFID data (another type of behavior at the container entrance), and scored movements that were captured by video recordings only as movements that occurred inside of the container (Figure 5C). We used information about perching events and movement inside of the container to determine when these behaviors occurred together (Supplementary Figure 2). For detected clusters with video data, we also used the number of pixels that changed across color channels to calculate the magnitude of movement. We assessed how movements of different sizes changed over time (Supplementary Figure 3), and how these movement categories mapped back onto behavioral inferences (Supplementary Figure 4). Supplementary section 7 contains more information about behavioral inferences.
Figure 5: We show data collected by ABISSMAL for 1 pair of birds over different stages of data processing. Panel A contains the pre-processed data from movement sensors prior to data integration. Panel B shows 4 datasets of inferred activities that were obtained by integrating pre-processed data across different sensor combinations. In panel C we show the fully integrated dataset (across all sensors) split by four different behavioral inferences. Early-life events are shaded in grey across panels.
3. Assigning movements to individuals

We used the fully integrated dataset to assess how ABISSMAL captured movements performed by each individual. When RFID data was present (e.g. movements that occurred at the container entrance), we used the PIT tag(s) detected to assign the movement event to one or both individuals (Figure 6), including which individual initiated or ended the movement event. We found that more movement events through the nest container entrance were assigned to the male than the female for this pair of birds, and this pattern was consistent over time (Figure 6). This difference in the number of inferred activities assigned to each adult does not mean that the female was performing fewer parental care activities, but rather that this individual moved less often through the nest container entrance. Using multiple sensor types through ABISSMAL also allowed us to capture movement events in the fully integrated dataset that were not assigned to either adult (Figure 6). The greatest number of unassigned movements occurred for inferred movements inside of the container that were captured by video recording events, which cannot resolve individual identity in the current version of ABISSMAL.
**Figure 6:** Here we show how two types of inferred movements from the fully integrated dataset were assigned back to individuals (the same dataset as Figure 5C). We show early-life events in grey shading.
Discussion

How parents care for their offspring may be critically important for ecological and evolutionary processes (Klug & Bonsall, 2014; Laland et al., 2015; Uller, 2012), but parental care behaviors can be difficult to capture. We developed a new platform of unified hardware and software called ABISSMAL, which provides automated behavioral tracking with built-in system monitoring and error logging. ABISSMAL also provides the capacity to make behavioral inferences in order to streamline data collection, curation, and interpretation for researchers studying parental care. We successfully used ABISSMAL to highlight the process of data collection, integration, and making behavioral inferences for one pair of captive zebra finches that laid eggs and raised chicks over 50 days.

ABISSMAL provides a comprehensive overview of movements associated with parental care behavior by collecting and integrating data across multiple sensors. Collecting data across multiple sensors provides redundancy when any one sensor fails, and therefore higher confidence that the majority of movements around the entrance and inside of the nest container are recorded. Integrating data across multiple sensors also provides internal validation while drawing behavioral inferences from series of detections across sensors that represent movement events. The datasets of inferred behavioral activities returned by ABISSMAL can be used to assess general patterns of activities by adult birds around a nest container before and throughout offspring development.

ABISSMAL can be used to assign movement events to unique individuals in a breeding pair, which facilitates behavioral tracking in species that exhibit biparental care. However, questions about parental care behavior that rely on tracking individual identity and fine-scale behaviors with high confidence may require additional computational processing in later versions of ABISSMAL. The current version of ABISSMAL uses an RFID system to assign
activities to individuals, but this individual identity assignment is subject to the RFID antenna failing to detect PIT tags, and is also currently limited to movements that occurred at the container entrance. Since we tracked birds’ movements with multiple sensors, we were able to capture how often birds moved through the entrance of the nest container without triggering the RFID antenna (Figure 6), which could reflect the RFID antenna failing to detect PIT tags due to individual variation in movements (Hughes et al., 2021). ABISSMAL also captured movements that occurred inside of the nest container, which were captured by video recording events only and could not be assigned to individuals (Figure 6). These short videos recorded by ABISSMAL could be used in image processing pipelines to assign behaviors that occurred inside of the container back to individuals. In future work, validating datasets of inferred behavioral activities and individual identity assignments generated by ABISSMAL against behavioral datasets scored from videos by human observers will be important to account for biases that can arise from automated data collection and processing (Smith & Pinter-Wollman, 2021), as well as to assess our confidence while using ABISSMAL for finer-grained behavioral inferences, such as calculating the duration of nest visits or incubation events.

Quantifying variation in avian parental care behavior has traditionally relied on video scoring that can become prohibitively time-consuming when collecting data across many individuals and over long developmental timelines. ABISSMAL streamlines the process of automated data collection, curation, and interpretation for parental care behaviors. ABISSMAL makes it possible to deploy movement sensors for automated data collection in a high-throughput way, and also provides the capability to integrate movement data collected across these sensors in order to generate behavioral inferences. The built-in system monitoring and error logging, as well as the capacity for deriving behavioral inferences from large datasets,
are features of ABISSMAL that will be particularly useful for capturing parental care and other
social behaviors across many experimental replicates and over long developmental
timescales. ABISSMAL is a unified platform but is also modular, and can be used with any
combination of the two pairs of infrared beam breakers, RFID system, infrared camera, and
temperature probe sensors. Our software for automated data collection, system monitoring,
and error logging will require the least amount of modification for different questions, study
species, and research settings. All core components of ABISSMAL will require modification
when adapting the tracking system to use more sensors across any of the four types listed
above, or when adding a new type of sensor for data collection. ABISSMAL is an open-access
tool that we made freely available through the GitHub repository lastralab/Abissmal with
extensive documentation to support widespread use and modification
Acknowledgments and funding: We thank M. Youngblood, A. Maldonado Chaparro, G. Alarcon Nieto, and K. Kalafut for advice on using Raspberry Pi and RFID systems for behavioral tracking. We are grateful to M. Youngblood for lending us equipment for initial testing. We thank B. Maloney, S. Marcus, A. van der Marel, A. Keyte, and Hobson lab members for feedback that improved earlier versions of this manuscript. We thank C. Vargas for additional Raspberry Pi equipment, O. Tchernichovski for access to sound attenuation chambers, G. Permuy, L. Tchernichovski, and M. Maresca for their support with animal care at the field center, and J. Catalfamo and G. Holmes for their support with infrastructure at the field center. The project was supported by an NSF Postdoctoral Research Fellowship in Biology to G.S.V. (grant no. 2010982) and Howard Hughes Medical Institute funds to E.D.J.

Author contributions: Conceptualization, G.S.V., T.M., E.A.H., and E.D.J.; Methodology, G.S.V., T.M., and E.A.H.; Software, G.S.V. and T.M.; Validation, G.S.V. and T.M.; Investigation, G.S.V. and T.M.; Data Curation, G.S.V. and T.M.; Formal Analysis, G.S.V. and T.M.; Visualization, G.S.V. and T.M.; Project Administration, G.S.V. and T.M.; Writing – Original Draft, G.S.V.; Writing – Review & Editing, G.S.V., T.M., E.A.H., and E.D.J.; Funding Acquisition, G.S.V. and E.D.J.; Resources, E.D.J.; Supervision, E.A.H. and E.D.J. All authors contributed critically to the drafts and gave final approval for publication.

Data availability statement: We have made data publicly available on figshare (https://figshare.com/articles/dataset/Smith-Vidaurre_et_al_2023_ABISSMALMethodsPaper/24555883) to facilitate reproducing our results. We published the raw data used in this manuscript that was collected for 1 pair of captive zebra finches over 50 days of data collection. We also published the pre-processed and integrated versions of this data.
References


