EasieRR: An open-source software for non-invasive heart rate variability assessment

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Abstract

1. The assessment of heart rate (HR) and heart rate variability (HRV) based on electrocardiograms (ECG) is considered a good proxy for stress in a wide range of animal species. However, problems can occur e.g., when measuring ECG in ambulatory settings such as recording of unrestrained animals using non-invasive devices. Artefacts caused by technical (i.e. bad electrode contact) or physiological (i.e. ectopic beats, non-cardiac muscle potentials) sources are common and can disturb the ECG signal. As HRV analysis is highly sensitive to artefacts in the interbeat interval (RR-interval) time series the process of visual inspection of the raw signal to detect and correct these is essential. Most of the commercially available software requires intensive training and extensive manual work to accomplish this task and/or is often not available to access for free.

2. EasieRR is an open-source, stand-alone software optimized for analysing ECG in non-restrained animals. The program allows a species-specific analysis and calculation of recommended standard HRV parameters in both, the time- and the nonlinear domain (RMSSD, SDNN, SD1 and SD2).

3. Visualization of data using Poincaré plots and tachograms of RR-intervals eases the validation of correct heart cycle interval detection and minimizes manual work for the user. Automatically detected peaks can be manually corrected via deletion, correction of spurious detections or marking of undetected peaks.

4. The HRV analysis can be exported using common formats (TXT, MAT). Figures can be plotted and exported in various formats (PDF, SVG, PNG, JPG, TIFF, EMF and EPS).

5. Included in EasieRR is the possibility for synchronization of ECG data with video in order to link cardiac responses to specific behavioural responses.

Keywords
artefact correction, behavioural ecology, electrocardiograms, heart rate variability, peak detection, Poincaré plot, stress in animals, time domain
1 | INTRODUCTION

Measures of heart rate (HR) and heart rate variability (HRV) have a long history as indicators of cardiac health, and stress in humans (Carney, Freedland, & Veith, 2005; Delaney & Brodie, 2000; Fleisher, 1996; Hall et al., 2004), and have gained considerable interest in animal behavioural studies during the last 30 years (Hopster, Werf, & Blokhuis, 1998; Kovács et al., 2014; Mohr, Langbein, & Nürnberg, 2002; von Borell, 2000). HRV is based on the analysis of normal fluctuations in the time intervals of consecutive heartbeats = interbeat intervals (IBIs) or RR-intervals. It reflects the interplay between sympathetic (SNS) and parasympathetic nervous system (PNS) and thus also provides information about cardiac vagal tone (Porges, 1995; von Borell et al., 2007). Analysing HRV is regarded as a suitable approach to determine the activity of the autonomic nervous system (ANS) in the context of stress, affect and emotion (Appelhans & Leucken, 2006; Boissy et al., 2007; Thayer, Åhs, Fredrikson, Sollers, & Wager, 2012), and is progressively emerging as a suitable indicator of welfare states in farm animal research.

Furthermore, animals of the same species cope differently with environmental challenges. As the ANS has a major impact on the regulation of fundamental physiological functions related to coping and stress resilience, many of these phenotypic differences in stress response are mediated by different activation of the ANS. ANS activity in different autonomic phenotypes of free-living streaked shearwater Calonectris leucomelas was linked to stress level (Muller, Vyssotski, Yamamoto, & Yoda, 2018), consistent across time and context. Evans et al. (2016) used HR and HRV to investigate the impact of ecological mechanisms on the hibernation process in free-ranging brown bears. These studies have speculated ANS may be a key mechanism driving phenotypic variation in animal populations, and is therefore a potentially important mediator in the evolution of life history (Evans et al., 2016; Muller et al., 2018).

The process of artefact correction of the electrocardiograms (ECG) signal has been shown to be essential for the appropriate analysis of HRV data (Berntson & Stowell, 1998; Shaffer & Combatalade, 2013). This makes HRV analysis especially challenging for data derived from unrestrained animals where, due to technical or physiological interference, a high occurrence of artefacts is likely. However, even when recording ECG during resting states, visual inspection is important to detect artefacts like atrioventricular blocks (AV blocks type 1-3), due to disturbances in impulse conduction at the heart. These type of artefacts can be relatively commonly found in horses, but also in other animals and if not excluded, can result in very high HRV. In veterinary and behavioural animal research, human HR monitors, such as POLAR® devices (POLAR Electro Oy) has been widely used in unrestrained animals. The major disadvantage of many of these devices has been that only RR-intervals were extracted but

### TABLE 1 Common software available for ECG processing and HRV analysis compared to EasieRR

| Software name          | Supported domains of HRV analysis | Supported data formats | Peak detection algorithm | Artefact detection and processing | Analysis reports and export of results | Availability      |
|------------------------|----------------------------------|------------------------|--------------------------|-----------------------------------|---------------------------------------|------------------|
| Kubios HRV (Premium)   | Time, frequency, nonlinear domain| Inter-beat-interval data from HR monitors or text file, ECG data* | QRS detection based on Pan-Tompkins | Automatic (threshold-based) artefact detection algorithm, cubic spline interpolation, manual correction | Text file (import into MS Excel or SPSS), MAT-file, PDF report, SPSS-friendly batch process | Standard version: open access/ premium version: purchase via Kubios website |
| Acq-Knowledge          | Time, frequency, nonlinear domain| NA (software only reads its own data) | Modified Pan-Tompkins QRS detector; ECG QRS Peak event | Cubic spline interpolation, manual correction using waveform transformations | HRV statistics as MS Excel file, Poincaré plot as Acq file | Purchase via BIOPAC website |
| ARTiiFact              | Time, frequency domain           | Inter-beat-interval data, ECG data | Global or local threshold detection | Distribution based artefact detection algorithm, manual correction: deletion, linear interpolation, cubic spline interpolation; batch processing, | MS Excel file, text file, PDF report, batch processing possible | Open-access, download via ARTiiFact website |
| EasieRR                | Time, nonlinear domain           | ECG data               | Peak prominence           | Manual correction: deletion, insertion or movement of peaks | Text file and MAT-file, Figures in all commonly used formats | Open-source, download via figshare or Github |

Abbreviations: ECG, electrocardiograms; HRV, heart rate variability.

*Only for Kubios HRV Premium version.
For HRV analysis (see Table 1). However, these programs require experience in the field and can be time-consuming to learn to use correctly. Recently, advanced technologies used in medical science capable of assessing the ECG from electrical differences on the body surface (e.g. with a sensor attached via a belt) have found application in animal science (e.g. BioHarness®; Zephyr Technology Corporation). This progression in technology has not only made raw ECG data accessible, but also confronts researchers working on physiology and behaviour in unrestrained animals with the challenge of correct pre-processing of data before HRV analysis.

Commercial HRV analysis programs such as AcqKnowledge 4.4 (BIOPAC System Inc.), Nevrokard® (Nevrokard) or Kubios HRV Premium (University of Eastern Finland; Niskanen, Tarvainen, Ranta-aho, & Karjalainen, 2004), but also Open Source software such as Artiifact (Kaufmann, Sütterlin, Schulz, & Vögele, 2011) are available for HRV analysis (see Table 1). However, these programs require experience in the field and can be time-consuming to learn to use correctly. In this article, we describe an open-source software with an intuitive graphical user interface (GUI) that imports ECG from any csv file or txt files exported from AcqKnowledge software, detects the prominent peaks, makes correction of spurious detections easy and transparent, and calculates a variety of HRV parameters in the time- and nonlinear domain.

2. EasieRR: COMPUTATIONAL BACKGROUND AND THEORY

EasieRR is an open-source software developed to assist researchers in the use of HR parameters and their processing and analysis. Special emphasis was put on an intuitive GUI to ease detection and manual correction of artefacts.

EasieRR has been programmed using MATLAB2018b (Mathworks) and compiled as a stand-alone application with the MATLAB compiler 7.0 for Microsoft Windows operating systems (Version 7 and upwards). EasieRR is hosted at https://figshare.com/projects/EasieRR/68831 and distributed under the terms of the GNU General Public License.

2.1 Peak detection

EasieRR applies by default a band pass filter (4. Order 0 degree Butterworth filter with low cut-off at 1 Hz and high cut-off at 20 Hz), hence removing high frequency peaks in the signal as well as removing any voltage offset. These two cut-off frequencies have been shown to be applicable for HRV analysis in goats, but can be modified by the user for optimal use on other species. The extraction of time intervals between heart beats is traditionally done by detecting R-peaks (RR-intervals), because they are usually the most distinct peaks (Lippmann, Stein, & Lerman, 1994). However, as long as variability within the HR cycles is calculated, other peaks and thus intervals i.e. S-S intervals can be used in case they are more prominent. It is essential that the type of peak used for detection is consistent within and between all individuals of an experiment (G.G. Berntson, pers. comm.). There are various algorithms for R-peak detection applied within different programs, i.e. global/local threshold detection (Berntson, Quigley, Jang, & Boysen, 1990), Pan-Tompkins QRS detector (Pan & Tompkins, 1985) or template matching (Friese et al., 1990). In EasieRR, the peak detection used for heart cycle interval determination is based on the peak prominence. This allows for robust R-peak detection when combined with the band pass filtering and a predetermined minimum timespan between RR-intervals (i.e. heart refractory time of the species used).

2.2 Artefact detection and processing

Currently available software uses different approaches to correct artefacts such as deletion of spurious RR-intervals, interpolation of missing or extra beats, i.e. cubic spline interpolation, linear interpolation, but also manual correction (see Table 1). To maximize transparency and reliability, EasieRR does not detect artefacts automatically. Instead, the GUI is specifically designed for easy visual examination and detection of artefacts. For this purpose, EasieRR is displaying the raw (and band pass filtered) ECG signal, a tachogram as well as a Poincaré plot simultaneously. Significant deviations between the lengths of successive interbeat intervals are often caused by spurious peak detection and artefacts. These divergent intervals are easily recognizable as distinct peaks in the tachogram and can also be seen as outliers in the typically elliptical shaped Poincaré plot. Figures 1–3 display in detail the procedure of artefact detection and correction using the three correction options: move mark, insert mark, mark outlier.

In contrast to most existing software which use interpolation algorithms for outlier correction (i.e. Kubios), EasieRR is based on a deletion algorithm. This method has been shown to be best suited for time-domain measures (Rincon Soler, Silva, Fazan, & Murta, 2017) and analysis of shorter sequences of ECG recordings (Lippman et al., 1994). The latter is often the case when data is obtained in ambulatory settings. Besides deleting outliers, EasieRR also allows selection of missed peaks and movement of misplaced marks.

2.3 Analysis in the time and nonlinear domain

Assessment of HRV has been commonly done using time-domain parameters which are based on the calculation of successive RR-intervals, also called normal-to-normal (NN) intervals. EasieRR calculates the time-domain measures SDNN (standard deviation of NN-intervals), RMSSD (root mean square of successive differences between normal heartbeats) and mean HR. While HR is a good indicator for overall arousal or activity, it does not allow to draw inferences on the activity of the two autonomic nervous branches, the PNS and the SNS. In contrast, RMSSD has been found
to reflect PNS-mediated HRV and can quantify the instantaneous beat-to-beat variance in HR. SDNN rather reflects the long-term variability of beat-to-beat intervals and is usually interpreted as an indicator of the sympatho-vagal balance (Task Force of the European Society of Cardiology & the North American Society of Pacing & Electrophysiology, 1996).
In recent years, the use of nonlinear methods has been gaining more interest. Nonlinear methods like the Poincaré plot make it possible to include non-stationary data in the analysis (Guzik et al., 2007).

A Poincaré plot is a scatter plot where each RR-interval is plotted as a function of the previous RR-interval. This typically results in an elliptical-shaped scatter plot tilted 45 degrees counter clockwise relative...
to the x-axis (see Figure 4). From these data points, it is possible to calculate the standard deviation in two dimensions: Along the minor axis of the ellipse (SD1) and the major axis (SD2). SD1 is describing the instantaneous variability of the RR-interval time series (see RMSSD) and reflecting parasympathetic efferent activity at the sinus node. SD2 is describing the long-term variability of the RR-interval time series.
Commonly these two standard deviations are visualized within the Poincaré plot via an ellipse where the minor axis illustrates the SD1 and the major axis illustrates SD2. Thus the major advantages of using Poincaré plots is their suitability as a quantitative visual tool allowing immediate recognition of artefacts (Myers, Workman, Birkett, Ferguson, & Kienzle, 1992) as well as estimation of the activity of the PNS (Kamen, Krum, & Tonkin, 1996; Woo, Stevenson, Moser, & Middlekauff, 1994).

To validate EasieRR, we analysed different HRV parameters (Table 2) using identical ECG data lasting 20 s for the programs described in Table 1. While all programs calculated almost identical values for HR and mean-RR, there are small deviations in the time-domain and nonlinear parameters. This is probably due to the different underlying algorithms for artefact correction used in the different programs.

### TABLE 2 Comparison of basic HRV parameters calculated using EasieRR and other commonly used HRV software. (ECG data was the same for all software; X = not available in the software analysis)

| Software name          | Imported data format | bpm  | Mean RR (ms) | RMSSD | SD1   | SD2   | SDNN  |
|------------------------|----------------------|------|--------------|-------|-------|-------|-------|
| EasieRR                | ECG                  | 188.57| 318.18       | 7.57  | 5.37  | 23.60 | 17.13 |
| Kubios HRV Premium     | ECG                  | 188.25| 318.71       | 5.45  | 3.86  | 21.91 | 15.72 |
| AcqKnowledge ECG       | ECG                  | 188.02| 319.11       | 7.64  | X     | X     | 17.13 |
| ARTiiFact               | ECG                  | 188.63| 318.08       | 10.53 | X     | X     | 17.87 |
| Kubios                 | RR data series, generated with Kubios Premium | 188.77| 318.17       | 6.69  | X     | X     | 17.07 |

Abbreviations: bpm, beats per minute; ECG, electrocardiograms; HRV, heart rate variability; mean RR, mean interbeat interval; RMSSD, root mean square of successive differences between interbeat intervals; SD1, standard deviation along minor axis of the ellipse; SD2, standard deviation along major axis of the ellipse; SDNN, standard deviation of interbeat intervals.

### 3 PROGRAM DESCRIPTION

The GUI is split into three separate interactive windows (Figure 5): an upper window displaying the ECG signal (raw and filtered) which allows to visually inspect automatically detected QRS complexes and to correct artefacts. The lower left window shows the corresponding tachogram facilitating the efficient location of artefacts and a Poincare plot at the lower right.

The EasieRR GUI is operated via a range of buttons. The ‘Choose data range’ button allows the user to select a sequence to be analysed. Time-domain as well as nonlinear parameters are then automatically calculated and the corresponding Poincaré plot is generated in the lower right window. For artefact correction, the GUI offers three
buttons with the options of either manually mark (delete) artefacts, move marks of spuriously detected peaks or insert marks for missed peaks. Their corresponding 'Undo' buttons allow the user to reverse their last action. After each artefact correction, the 'Recalculate' button will create a new tachogram as well as Poincaré plot. After artefact correction is finished the data range can be saved using the 'save range' button and viewed with the 'Check data' button. When the analysis is finalized, pressing the 'save to file' button will save all analysed time ranges. A more detailed user manual can be found in the documentation hosted here: https://figshare.com/projects/EasieRR/68831.

3.1 | Synchronization with behaviour

Heart rate variability data can also be synchronized with observed behaviour via import it into common software for behavioural video analysis (e.g. The Observer®). More information is provided in the user manual available online.

4 | CONCLUSION AND FUTURE DIRECTIONS

EasieRR is a free software for evaluating ECG in non-restrained animals and allows for calculation of recommended standard HRV parameters in both, the time- and the nonlinear domains. The intuitive GUI facilitates the detection and correction of artefacts through the visualization of RR-intervals in tachogram and Poincaré plot.

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AUTHORS’ CONTRIBUTIONS
J.H.R. has written the EasieRR software code in Matlab, reviewed the literature, proposed an article outline, wrote the initial draft, and revised K.R. and J.L.’s later article drafts. K.R. wrote mainly the methods and GUI overview sections of the manuscript, made the tables and figures, revised drafts following feedback from J.H.R. and J.L. and alpha-tested the software for bugs. J.L. reviewed the literature, wrote the introduction and revised K.R. and J.R.’s drafts of the manuscript.

COMPETING INTERESTS
The authors have no competing interests.

DATA AVAILABILITY STATEMENT
The core software, a user manual, example data files, and a macro for easier import of HRV data into The Observer XT 13
(Noldus Information Technology) and Microsoft Excel 2010 are accessible here: https://github.com/KatrinaRosenberger/EasieRR and (https://doi.org/10.6084/m9.figshare.9804740 (Rasmussen, Rosenberger, & Langbein, 2019). The software source code has been archived and made accessible on Zenodo: https://doi.org/10.5281/zenodo.3725502 (Rasmussen, Rosenberger, & Langbein, 2020).

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