

Original Software Publication

DigiRhythm: An R package for evaluating circadian rhythmicity in animals using the degree of functional coupling

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ABSTRACT

Studying animals' rhythmicity provides insights into their physiological and psychological states. The degree of functional coupling (DFC) is one of the algorithms available to assess rhythmicity in activity-related time series data, such as accelerometer or GPS data. However, DFC computation is complex, as it includes frequency spectrum analysis and statistical significance testing. This paper introduces digiRhythm, an R package that makes the DFC-based rhythmicity analysis easily accessible. Beyond the DFC, the package includes an additional set of tools, which are crucial for rhythmicity investigations, such as actogram generation, daily activity visualization, and diurnality index computation.

Metadata

While several tools allowing to analyze such rhythms exist, such as Mosaic [6] for proteome data and RhythmicAlly for ethological data [1],

Nr	Code metadata description	Metadata
C1	Current code version	2.4
C2	Permanent link to code/repository used for this code version	https://github.com/nasserdr/digiRhythm
C3	Permanent link to reproducible capsule	https://github.com/nasserdr/digiRhythm/blob/main/examples/code_snippets_manuscript.R
C4	Legal code license	General Public License (GPL)
C5	Code versioning system used	git
C6	Software code languages, tools and services used	R
C7	Compilation requirements, operating environments and dependencies	R ≥ 4.0.0
C8	If available, link to developer documentation/manual	
C9	Support email for questions	hassan-roland.nasser@agroscope.admin.ch

1. Motivation and significance

Biological rhythms reflect adaptive behavioral patterns influenced by internal and external stimuli [4]. The advances in sensor technology have enabled the collection of large-scale behavioral data, thus facilitating the study of these rhythms [5,14,18].

to date, none incorporate the Degree of Functional Coupling (DFC) method (for a comprehensive comparison, refer to the comparative table in supplementary materials). The DFC, originally introduced by Sinz and Scheibe [17], quantifies the synchronization between an organism's activity and the 24-hour environmental cycle. It is particularly useful for detecting stress or disease-induced disruptions in rhythmicity [4].

Despite its potential, the DFC method has remained underutilized because no open-source implementation has been previously available.

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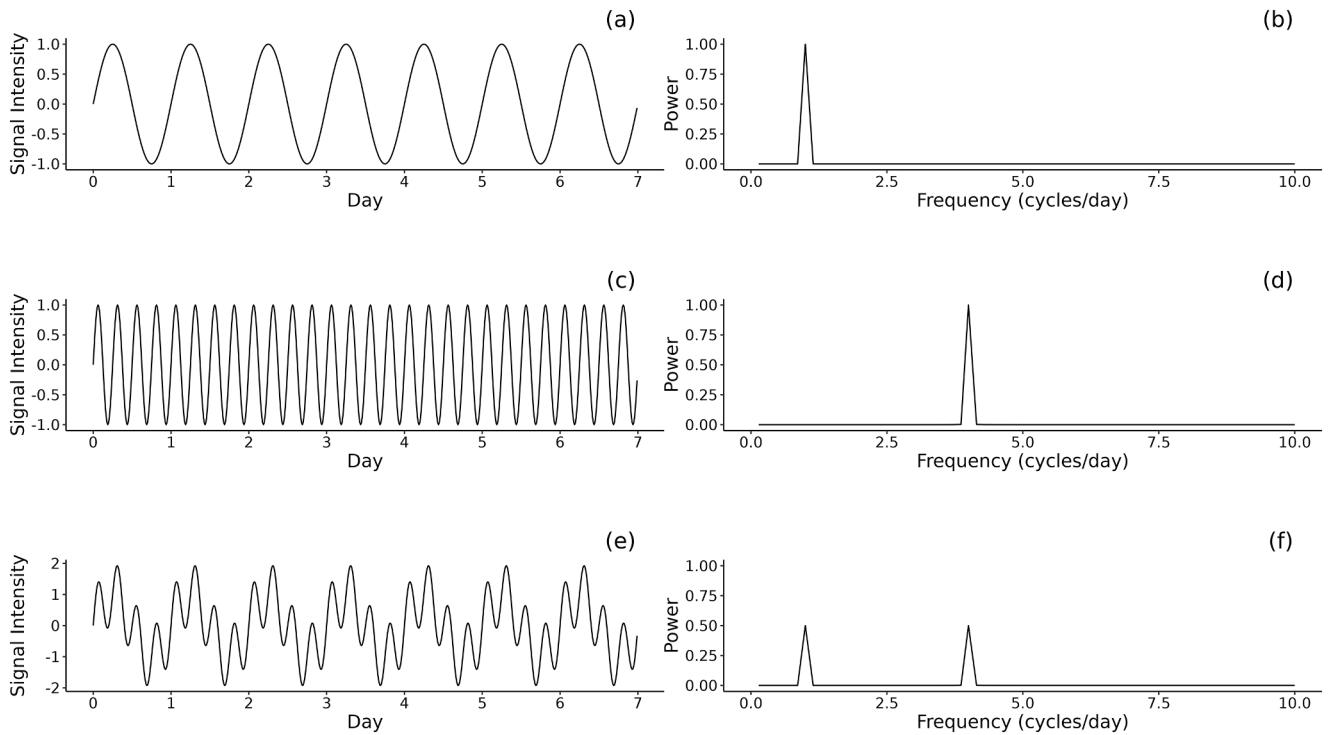


Fig. 1. Illustration of the Lomb-Scargle periodogram (LSP). (a) and (c) show time series with frequencies of 24 h and 6 h, respectively. (b) and (d) present the corresponding LSP of (a) and (c), highlighting the frequency peak positions. (e) represents the sum of the two time series from (a) and (c). (f) displays the LSP of (e), revealing two distinct peaks, as (e) is composed of two underlying time series.

Researchers interested in applying DFC had to invest significant effort in fundamentally programming their own implementation. By developing digiRhythm as an open-source package, we aim to make this valuable analytical method accessible to the broader research community, providing an off-the-shelf solution that eliminates the technical barriers to its adoption.

1.1. Theory

The first part of DFC calculation entails the computation of the Lomb-Scargle Periodogram (LSP) and the significance of each frequency in this periodogram. The LSP [12] transforms time-series data into frequencies, revealing the power of oscillations, which will be used to estimate the rhythmicity (Fig. 1).

The LSP is computed using Eq. (1):

$$P_{LS}(f) = \frac{1}{2} \left\{ \frac{(\sum_n g_n \cos(2\pi f[t_n - \tau]))^2}{\sum_n \cos^2(2\pi f[t_n - \tau])} + \frac{(\sum_n g_n \sin(2\pi f[t_n - \tau]))^2}{\sum_n \sin^2(2\pi f[t_n - \tau])} \right\} \quad (1)$$

Where g_n is the activity sampled at time t_n , f is the frequency of oscillation (in hertz) and τ which depends on f and ensures time-shift invariance (Eq. (2)):

$$\tau = \frac{1}{4\pi f} \tan^{-1} \frac{\sum_n \sin(4\pi f t_n)}{\sum_n \cos(4\pi f t_n)} \quad (2)$$

The definition of the frequency grid is crucial in computing the LSP from Eq. (1). This grid ranges from 0 to f_{\max} , the maximum frequency power computable from the activity g_n and determined using the Nyquist-Shannon theorem [16]. The digiRhythm package implements the LSP (function: `lomb_scargle_periodogram`) and a user-friendly graphic for periodograms and identifying significant frequency peaks (Fig. 2).

Each frequency can emerge either from a noise component or from a signal component. The significance of the frequency peaks is computed using the false alarm probability (FAP) calculation method described by

Baluev [2]. The method calculates an upper limit to the false alarm probability based on the expected number of up-crossings of a given threshold level by the periodogram within the frequency range being examined. It accounts for both the periodogram value and the effective frequency bandwidth, making it particularly useful for distinguishing genuine periodic signals from random noise fluctuations. In the context of circadian rhythm analysis and the DFC method specifically, harmonic frequencies are those related to the 24-hour cycle (e.g. 1/24 h, 2/24 h, 3/24 h), representing oscillations that align with daily environmental patterns.

As described by Scheibe et al. [13], the DFC can be calculated as the ratio of the sum of significant harmonic frequency powers (*ssh*) to the sum of all significant frequency powers (*sumsig*) below the harmonic cutoff (Section 2.3) as shown in Eq. (3):

$$DFC = \frac{ssh}{sumsig} \quad (3)$$

The DFC reflects the extent to which an organism's activity aligns with harmonic frequencies. A DFC of 1 indicates strong synchronization, whereas lower values suggest deviations, potentially due to stress or disease.

A related metric, harmonic power (HP), is computed similarly but includes all frequencies regardless of if they are harmonic or not (Eq. (4)). Both DFC and HP are valuable for studying rhythmic behaviors, with DFC reflecting synchronization to the 24-hour cycle and HP indicating overall rhythmicity.

$$HP = \frac{ssh}{sumall} \quad (4)$$

Where *sumall* is the sum of all frequency powers (regardless of whether they are significant or not, harmonic or not) below the harmonic cutoff.

An additional metric to characterize the circadian rhythm is the diurnality index (DI). It measures the ratio of diurnal and nocturnal activity. The calculation of DI is given in Eq. (5) as proposed by

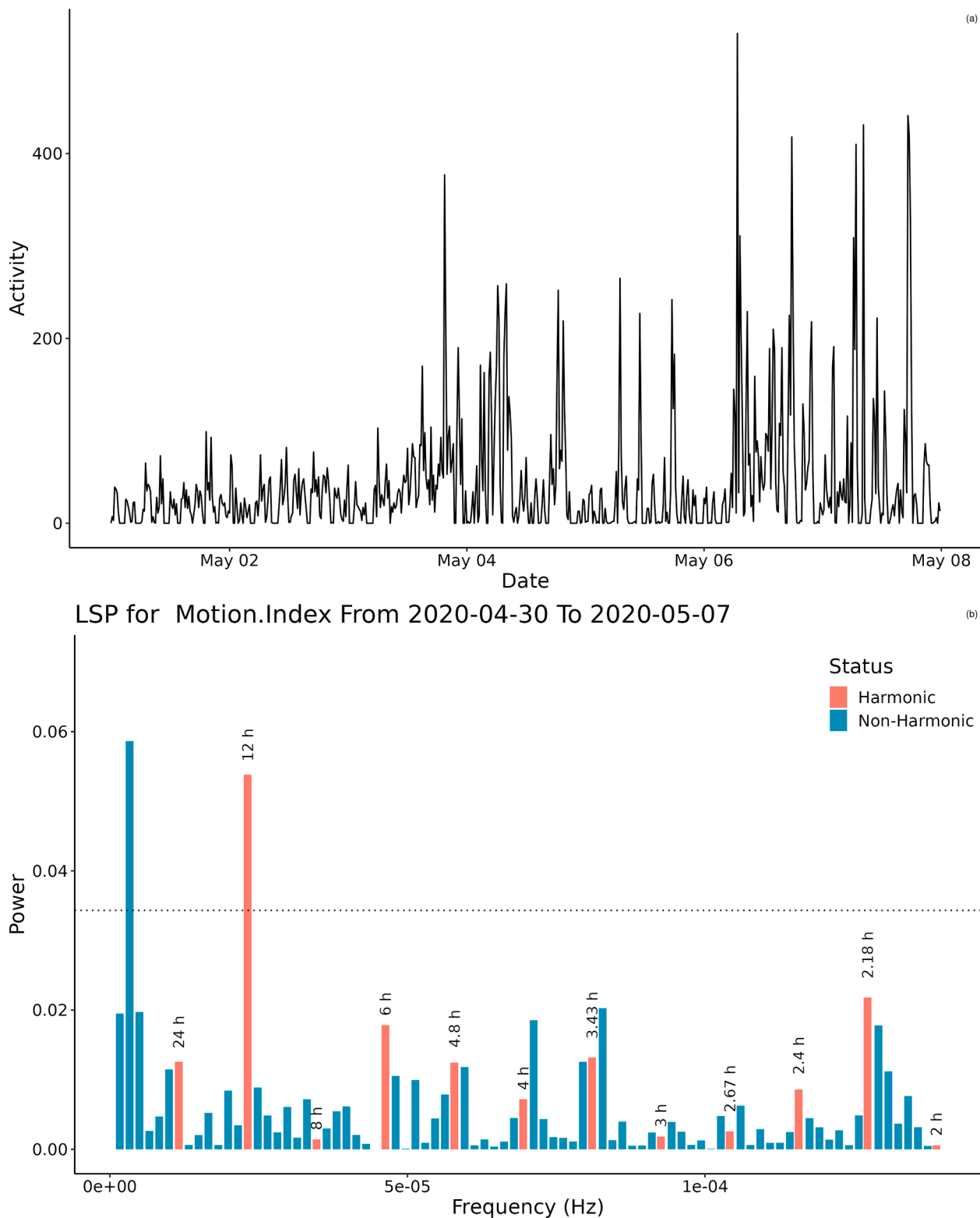


Fig. 2. Lomb-Scargle periodogram (LSP): (a) Sample time series data from an accelerometer installed on a cow. (b) Corresponding LSP. In (b), frequency bars are color-coded: green for non-harmonic frequencies and red for harmonic frequencies. The dashed bar indicates the threshold above which a frequency is considered significant. The `lomb_scargle_periodogram` function provide the possibility to produce the graph in (b) making it easier to investigate individual harmonics.

```

> library(digiRhythm)
> data("df516b_2", package = 'digiRhythm')
> df <- df516b_2
> head(df)
      datetime Motion.Index Steps
1 2020-05-01 00:00:00         0    0
2 2020-05-01 00:15:00         7    0
3 2020-05-01 00:30:00         3    0
4 2020-05-01 00:45:00        39   13
5 2020-05-01 01:00:00        37   16
6 2020-05-01 01:15:00        33   14

> is_dgm_friendly(df, verbose = TRUE)
v Correct time format: First column has a POSIXct Format
v Number of days good for DFC: 46 days >= 2 days
v Correct numeric format - Column 2 ==> Motion.Index
v Correct numeric format - Column 3 ==> Steps
The data is digiRhythm friendly
[1] TRUE

```

Fig. 3. (a) Code snippet for loading a sample dataset from the digiRhythm library. The output demonstrates the dataset format, where the first column represents a datetime (POSIX), and the subsequent columns are numeric values: the motion index and the number of steps. (b) The `is_dgm_friendly` function is used to verify whether a given dataset is compatible with all functions in the digiRhythm library.

Hoogenboom et al. [10]:

$$DI = \frac{\frac{C_d}{T_d} + \frac{C_n}{T_n}}{\frac{C_d}{T_d} + \frac{C_n}{T_n}} \quad (5)$$

C_d and C_n represent the sum of activity during the daytime and nighttime, respectively. T_d and T_n are the number of activity samples recorded during the daytime and nighttime.

2. Software description

The digiRhythm package provides a set of functionalities bridging the implementation gap for rhythmicity analysis, extending beyond the calculation of the DFC and HP. Developed in collaboration with animal behavior scientists, the package includes features adjusted to the needs of livestock researchers interested in rhythmicity assessment. A key design consideration was flexibility in data visualization and ability to access the output of each function: Thus, all functions output graphs are in **ggplot** format. This allows researchers to customize the aesthetics of the plots and access the underlying data within the **ggplot** objects, ensuring researchers can access the data for further scientific analysis or modify the plots directly using **ggplot** aesthetics.

2.1. Input data formatting

The digiRhythm package necessitates that input data frames adhere to a specific structure, whereby the first column should be a consecutive datetime in POSIX (Portable Operating System Interface) format (regardless of whether the sampling interval is even or uneven) and the subsequent columns are numeric activity data (e.g. Fig. 3a). We made a deliberate design choice to standardize the required format rather than implementing numerous preprocessing functions. This decision allows us to maintain focus on robust analytical capabilities while ensuring consistent performance across datasets. The standardized format (datetime in POSIX format followed by numeric activity columns) can be achieved through common data manipulation techniques in R, and we provide the '`is_dgm_friendly`' function to help users verify their data compatibility.

Activity data can be any quantitative measurement representing animal movement or behaviour (e.g., motion index from

accelerometers, step counts, activity counts from pedometers or specialized collars) organized in numeric columns within the data frame. Users must ensure their raw sensor data is appropriately transformed into activity metrics before using the package.

Fig. 3a presents sample activity data frame and is bundled with the digiRhythm package. The columns represent datetime, the motion index, and the number of steps measured during each 15-minute time interval. This particular dataset (in addition of few other examples) is available (<https://zenodo.org/doi/10.5281/zenodo.11083256>; name: "516b_2.csv"). The details of the underlying experiment and data source are beyond the scope of this paper; however, interested readers can find further information in Heirbaut et al. [9] and Heirbaut et al. [8]. To confirm that the data structure follows the required format, we have included a utility function capable of verifying its compatibility with digiRhythm. This check can be performed using the `is_dgm_friendly` function (Fig. 3b):

This function takes a data frame as an input argument and verifies its digiRhythm compatibility, returning a Boolean value (True or False). The data is considered digiRhythm-friendly if it meets all the following conditions:

1. The data frame is not a NULL object.
2. The data frame contains at least two columns.
3. The first column is formatted as POSIX standard and the subsequent columns are numeric.

The verbose argument serves as a tool to inform the user about the reasons for which the library considers the data as compatible or not. When set to `verbose = TRUE`, the function offers detailed feedback on every condition it assesses thus providing specific guidance to make the dataset compatible with the library's functionalities.

A warning message will be generated if the data encompasses <2 days, as the DFC and HP functionalities require at least two days of data to detect information about the 24 h harmonic. However, other functionalities may still be used.

2.2. Software functionalities

At present, the digiRhythm library includes the following features:

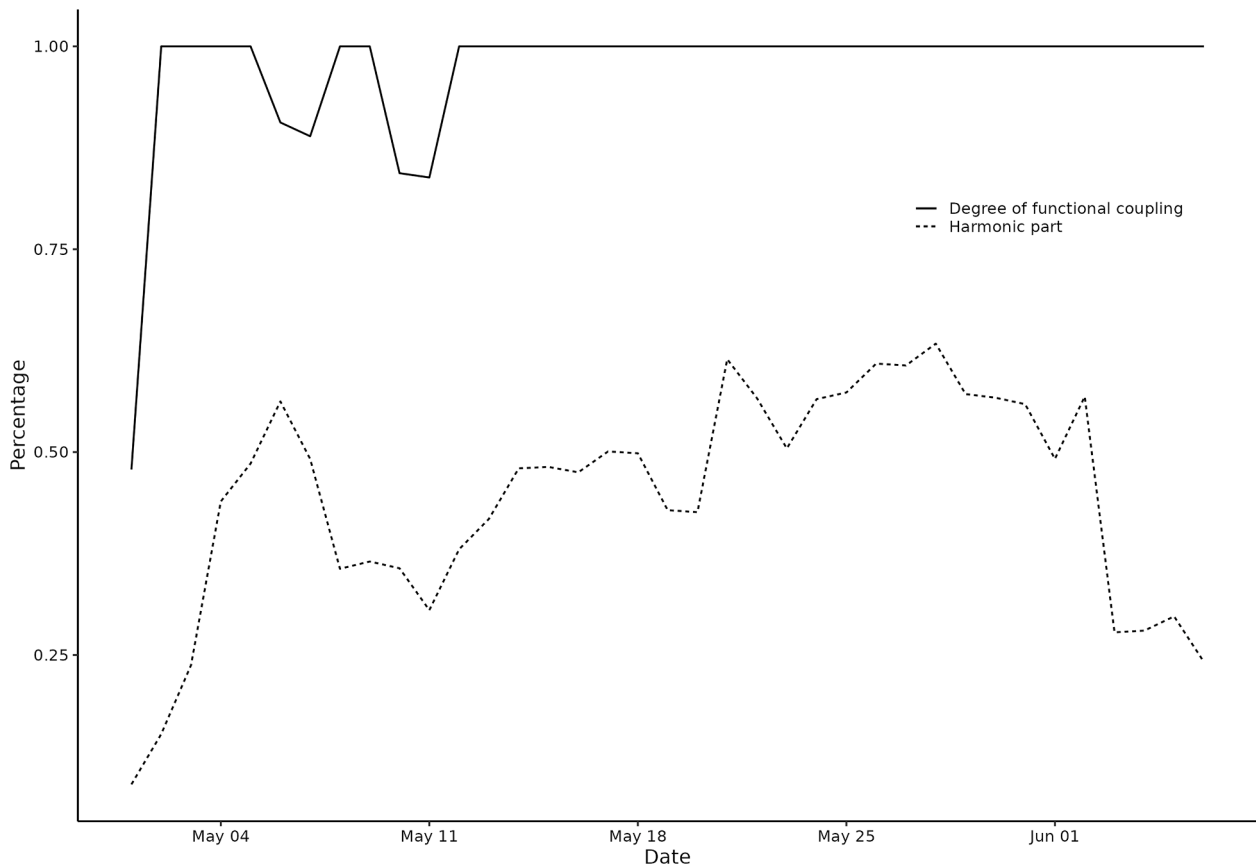


Fig. 4. A sample output of the `dfc` function using the `digiRhythm` library. The degree of functional coupling and the harmonic part of the dataset `df516b_2`.

1. **dfc**: This function allows for both computation and graphical representation of the DFC and the HP.
2. **lomb_scargle_periodogram**: This function not only computes the Lomb-Scargle periodogram (LSP) but also provides a visualization, containing graphical elements to identify significant/non-significant as well as harmonic and non-harmonic peaks.
3. **actogram**: This tool is designed for actogram visualization. Activities are visualized as tiles in a heat-map with the time of day on the x-axis and the date on the y-axis.
4. **daily_average_activity**: This feature facilitates the calculation and visualization of the daily average activity.
5. **diurnality**: With this function, users can compute and graphically present the diurnality index. Here, the day and nighttime are fixed for the whole dataset.
6. **diurnality_customTimes**: an alternative of the diurnality index where the user can specify variable start and end of day and nighttime.

2.3. The DFC and the `lomb_scargle_periodogram` functions

The DFC and HP are both implemented in the `dfc` function. The algorithm iterates over a sliding window of 2 days minimum (the user can determine the length of the sliding window in days), computes the LSP and the false alarm probabilities, then classify frequencies into harmonic/non-harmonic and significant/non-significant (Fig. 3) to compute `sumall`, `sumsig` and `ssh`. DFC and HP are computed respectively using Eq. (1) and Eq. (2). This function processes a `digiRhythm`-compatible data frame by calculating the DFC for the chosen variable targeted for analysis. Initially, it evaluates the start and end dates to ensure enough days are available for the computation. Indeed, the number of days in the dataframe should be bigger or equal to the sliding window. If the user selected a sliding window of 7 days, the function

applies an LSP on that sliding 7-days window. For instance, the DFC and HP are computed for the first 7 days, then for days 2 to 8, and so on. A typical combined DFC and HP output (with a sliding 7-day window) is illustrated in Fig. 6.

A typical workflow for computing the DFC and HP is outlined herein. Once the data is loaded and its compatibility with the library is verified, the data—originally sampled at 1-min intervals—is resampled to 15-min intervals using a utility function called `resample_dgm`. In this context, ‘resampling’ refers to a simple summation process. For this example, we used the default parameters mainly used in the literature [4,7,13,15] as follows:

1. The data are sampled to min15 minutes.
2. The sliding window is set to 7 days.
3. The highest harmonic (**harm_cutoff**) is the 12th harmonic (the one that corresponds to 24 hours / 12 = 2 hours).

It’s important to note that, in accordance with the Nyquist-Shannon sampling theorem [16], the highest resolvable harmonic frequency must be less than half the sampling frequency. Consequently, the minimum resolvable harmonic period should be at least twice the sampling interval. For instance, with a 15-min sampling period, the smallest discernible harmonic is 30 min, corresponding to the 48th harmonic ($24 \text{ h} / 48 = 30 \text{ min}$).

The returned data frame, `my_dfc`, contains the DFC and HP measured within a sliding window of seven days. One can inspect these data for further investigation (such as exploratory data analysis or statistical modelling) as follows:

In this code, the variable `my_dfc` is a `ggplot2` object [19], which contain both the visualization (accessed directly as `my_dfc`) and the calculated DFC and HP values (accessed as `my_dfc$data`). Because the returned object is a `ggplot2`, the plot can directly be modified, by adding

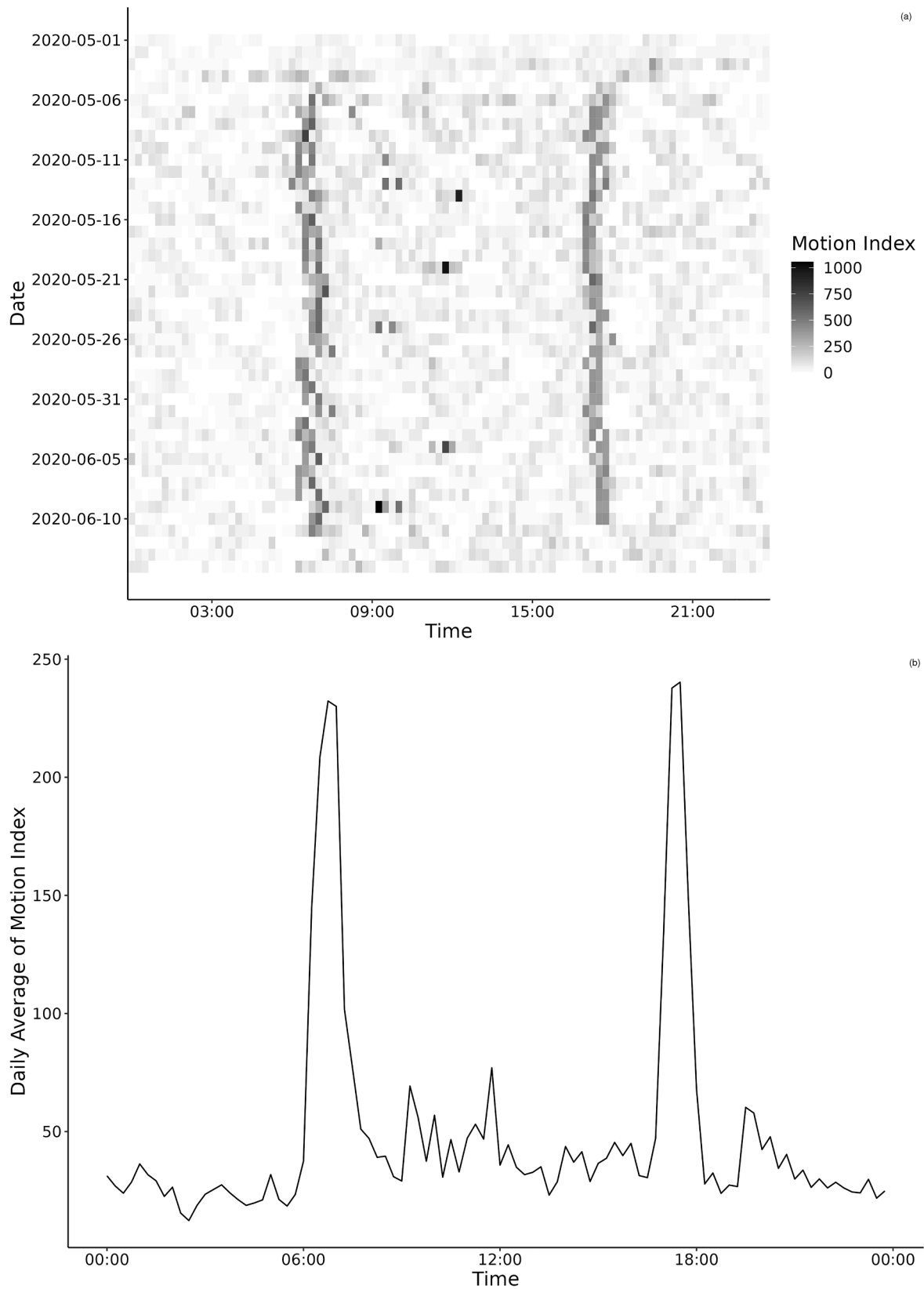


Fig. 5. (a) Actogram displaying raw activity data. (b) Daily average activity computed from the raw data and displaying the average activity at a specific time point from all input days. In both (a) and (b), the df516b_2 dataset is presented.

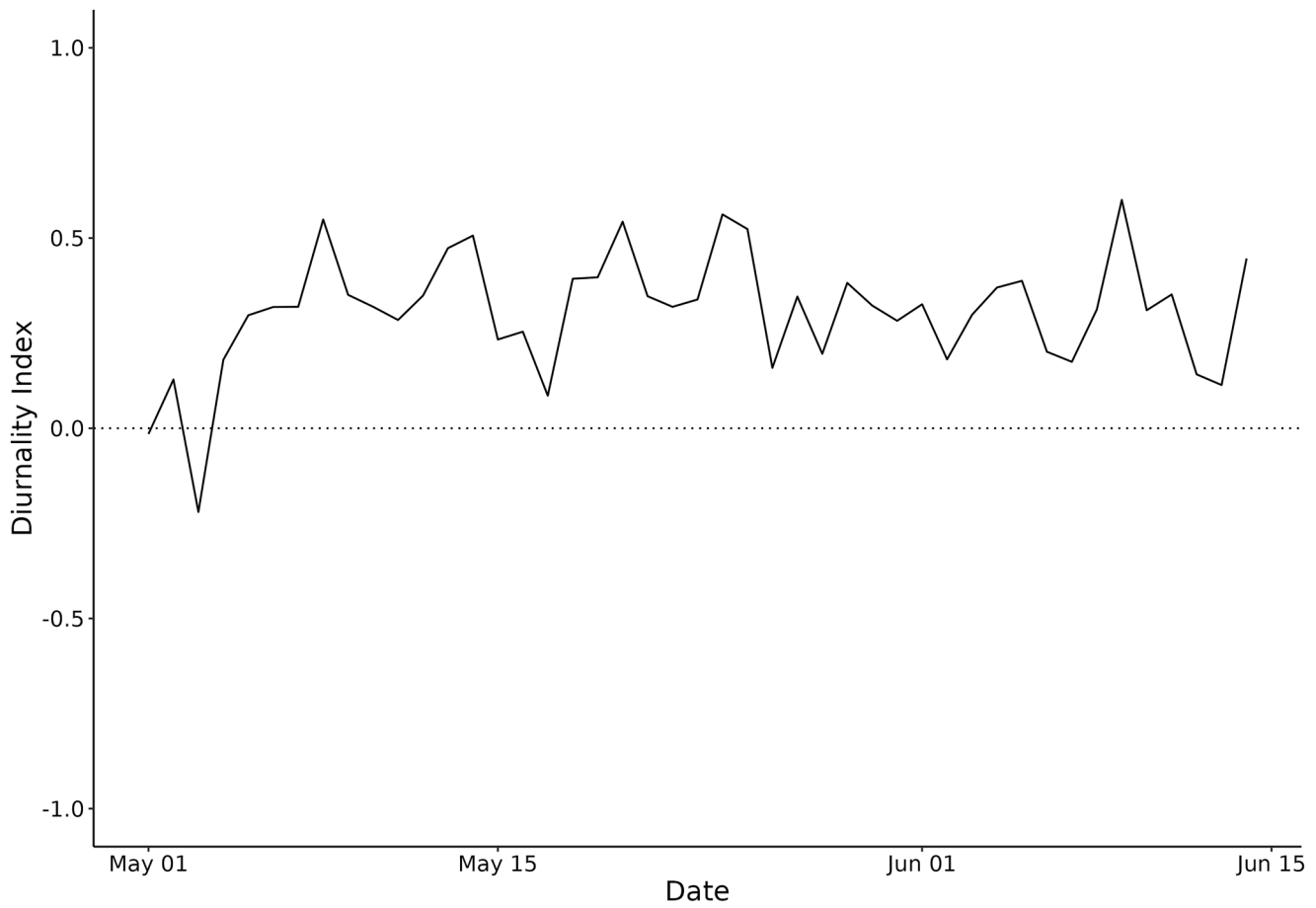


Fig. 6. Diurnality index. A sample output of the diurnality index function using the digiRhythm library, calculated using the dataset df516b_2.

a layer to the aesthetics of the `my_dfc` object and plotting it again.

Although the `dfc` function can analyze data with any sampling frequency, and a variable sliding window of 2 days minimum, several authors [7,11,17] have suggested that optimal insights can be achieved using a 7-days sliding window with a 15-min sampling period.

Further, the ultimate rhythmicity analysis will be based on the `dfc` function (which acts as a wrapper for the `lomb_scargle_periodogram`), we also provided an interface to the `lomb_scargle_periodogram` function so that users can delve further into the LSP, should they wish to explore it in greater detail. An example of such case-study is shown in Fig. 4 [7], where the authors investigated the number of significant and non-significant frequencies per farm.

2.4. Actogram and Average activity

The `actogram` function is used to produce a 2D heatmap (Fig. 5a) of activity data, with time of day on the x-axis and dates on the y-axis. Tile colours represent activity intensity.

The `daily_average_activity` function calculates average activity (Fig. 5b) using Eq. (6):

$$\bar{A} = \frac{1}{d} \sum_d A(T_{id}) \quad (6)$$

Where, T_i represents a specific time of day, d stands for the total number of days, and $A(T_{id})$ represents the activity level at time T_i on day d .

2.5. Diurnality index (DI)

The `diurnality` function of the digiRhythm package can be used to

calculate the ratio of diurnal and nocturnal activity with the DI, as described in Section 1. By default, daytime is defined as 06:30AM–04:30PM, and nighttime as 06:00PM–05:00AM. Users can adjust these periods and exclude specific times (e.g., milking periods) to account for external influences on activity. The function outputs a plot (Fig. 6) showing the DI and activity distribution, providing insights into temporal behavioral patterns.

The `diurnality_CustomTimes` function extends the `diurnality` function by allowing dynamic definitions of day and night periods for each day. This is particularly useful for datasets spanning across multiple seasons, daylight-saving time, or geographical locations with varying daylight durations. Users provide a data frame with date and timestamp columns defining daily start/end times for day and night. If no exclusions are needed, timestamps can align with sunrise and sunset.

The `diurnality_CustomTimes` function computes DI for each day based on its specific time periods, offering a more accurate reflection of behavior by accounting for changes in daily routines or daylight hours.

3. Illustrative examples

A detailed example can be found on: https://github.com/nasserrdr/digiRhythm/blob/6791a98e31229475f33efe012596165a8aa12f08/examples/code_snippets_manuscript_softwareX.R

This code can be run from any computer having an Rstudio software. We included comments helping the reader reproduce each image of this manuscript along with a short explanation.

4. Impact

This software addresses a critical need in animal activity research by

enhancing the accessibility of the DFC method. By providing an out-of-the-box implementation, it reduces the complexity of spectral analysis, allowing researchers to focus on biological interpretation. The improved accessibility, is expected to drive broader adoption of the DFC method within the scientific community, accelerating progress in animal behavior research. Furthermore, the library enables direct comparisons between DFC and existing methodologies, empowering researchers to rigorously assess each approach's strengths and limitations, leading to more robust and reliable analyses. Preliminary evidence of DFC adoption [3,4,7,11,14,15], including its integration into multiple ongoing research projects, highlights the library's potential to advance animal activity research.

5. Conclusions

The digiRhythm package represents a significant advancement in rhythmicity analysis tools, providing researchers with user-friendly methods for evaluating behavioral patterns in animals. While initially focused on farm animals, its analytical approach holds potential for broader application across organisms, offering visualization features such as actograms and daily average activity plots that enhance interpretation of behavioral data.

Unlike other packages, digiRhythm's most distinctive contribution is the implementation of the Degree of Functional Coupling (DFC) method, a capability previously unavailable in open-source software that quantifies synchronization between an organism's activity and environmental cycles. The package also offers specialized features including harmonic power (HP) calculation and customizable diurnality indices that accommodate geographical and seasonal variations, with functions allowing dynamic definition of day and nighttime periods. digiRhythm has notable limitations compared to more comprehensive packages: it currently lacks metrics such as cosinor analysis, offers a narrower range of statistical algorithms than alternatives like Nitecap or CATkit. Researchers working with omics data or requiring more diverse statistical approaches may need to supplement digiRhythm with additional analytical tools.

The package's open-source nature encourages future development, with promising research directions including exploration of different data sources (GPS, feeding station access), investigation of the influence of DFC parameters (sliding window, sampling period, harmonic cutoff), alternative FAP calculation methods, accommodating ultradian and infradian, and frequency-dependent significance levels. These enhancements will further strengthen digiRhythm's contribution to chronobiological research and invite community contributions for continuous improvement.

CRedit authorship contribution statement

Hassan-Roland Nasser: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Marianne Cockburn:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation. **Marie Schneider:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Marie Schneider reports financial support was provided by Johann

Heinrich von Thünen Institute Federal Research Institute for Rural Areas Forestry and Fisheries. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.softx.2025.102184](https://doi.org/10.1016/j.softx.2025.102184).

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