

## APPLICATION

# respR—An R package for the manipulation and analysis of respirometry data

Januar Harianto<sup>1</sup>  | Nicholas Carey<sup>2,\*</sup>  | Maria Byrne<sup>1,3</sup>

<sup>1</sup>Discipline of Anatomy and Histology, School of Medical Sciences, The University of Sydney, Sydney, NSW, Australia

<sup>2</sup>Scottish Association for Marine Science, Oban, Argyll, UK

<sup>3</sup>School of Life and Environmental Sciences, The University of Sydney, Sydney, NSW, Australia

## Correspondence

Januar Harianto

Email: januar.harianto@sydney.edu.au

## Funding information

NSW Environmental Trust Grant; Endeavour Postdoctoral Fellowship; Professor NGW & Mrs Ann Macintosh Memorial Scholarship from the University of Sydney

Handling Editor: Samantha Price

## Abstract

1. Respirometry is a ubiquitous practice in experimental biology, but there is a lack of standard practices when analysing the resulting data, limiting transparency and reproducibility. As respirometry datasets become increasingly large and analytical approaches more complex, manipulating the data remains a challenge and often intractable with existing tools.
2. Here we describe the *respR* R package, a collection of functions that implement a workflow-based approach to automate the analysis and visualisation of respirometry data. The package can be used for closed, intermittent flow, flow-through and open-tank respirometry and uses well-defined sets of rules to reliably and rapidly generate reproducible results.
3. We demonstrate how *respR* uses novel computing methods such as rolling regressions and kernel density estimates to reliably detect maximum, minimum and most linear sections of the data, and critical oxygen tension,  $P_{crit}$ .
4. Although designed specifically with aquatic respirometry in mind, the object-oriented approach of the package and the unit-less nature of its analytical functions mean that parts of the package can easily be used to estimate linear relationships from a range of applications in many research disciplines.

## KEYWORDS

aquatic respirometry, bioinformatics, experimental biology, linear models, metabolic rate, respirometry, software, statistics

## 1 | INTRODUCTION

Metabolic rate is a fundamental trait associated with virtually all biological functions and is key in predicting patterns in ecology and conservation biology, from populations (Barneche et al., 2014; Seibel & Drazen, 2007) to ecosystems (Brown, Gillooly, Allen, Savage, & West, 2004). It has been widely investigated in studies of the effects of external stressors on organisms and is increasingly used to study resilience in climate change-related studies where warming is expected to drive increased metabolism in ectotherms (e.g. Carey, Harianto, & Byrne, 2016; Delorme & Sewell, 2016; Pörtner, 2002). It is also by

far the most common metric used to assess physiological performance in whole organisms, tissues and cells (White & Kearney, 2013). While there are other methods such as monitoring heart or ventilatory rates, metabolic rate is typically quantified using respirometry, which measures the rate of oxygen uptake over time (Lighton, 2008).

Depending on the experiment, different metabolic rate parameters may be of interest to researchers. These include maximum metabolic rate (MMR, or  $\dot{M}O_{2,max}$ ), rates under high activity or exhaustive exercise and minimal metabolic rate ( $\dot{M}O_{2,min}$ ), which may be termed standard (SMR), basal (BMR) or resting metabolic rate, and typically represents the minimum metabolic cost of maintaining biological functioning (Chabot, Steffensen, & Farrell, 2016; White & Kearney, 2013). Routine metabolic rate (RMR) is similar

\*Nicholas Carey is a joint corresponding author as well as joint first author.

to  $\dot{M}O_{2,min}$ , but takes into account that in some organisms energy is expended on small, spontaneous movements to maintain posture or position (Rogers, Urbina, Reardon, McKenzie, & Wilson, 2016). Another metric of interest is the critical oxygen tension, which represents the lowest level of oxygen at which aerobic metabolism is independent of the ambient partial pressure of oxygen ( $P_{crit}$ ; Hochachka & Somero, 2002; Yeager & Ultsch, 1989).

Respirometry studies are increasingly outputting large, high-resolution data conducted over long periods (e.g. 20 hr; Norin & Malte, 2012). In many cases, processing the data involves an ad hoc selection of data points with poorly reported criteria, and subsequent manual processing of the data subset(s) using a spreadsheet program (e.g. Microsoft Excel) or an integrated development environment (IDE, e.g. R or Matlab). These approaches can be tedious and time-consuming especially when spreadsheet programs struggle with the vast datasets that are generated, while IDEs require a degree of expertise to use and have substantial learning curves. Dedicated software are also available to perform metabolic rate analyses, but many have costly licensing requirements (e.g. AutoResp by Loligo Systems), complicating or preventing their use on multiple machines, and are proprietary and closed-source, hindering scientific reproducibility and transparency.

A number of open-source R software packages have recently become available which are designed for, or are suitable to analyse data from respirometry. The `respirometry` package (<https://CRAN.R-project.org/package=respirometry>) contains a comprehensive collection of tools to explore and evaluate experimental parameters in aquatic respirometry and is useful for the design and diagnosis of experimental setups. The `rMR` package (<https://CRAN.R-project.org/package=rMR>) performs interval-based metabolic rate calculations and has a method to analyse  $P_{crit}$  using the “broken-stick” regression method (Yeager & Ultsch, 1989). `FishResp` (Morozov, McCairns, & Merilä, 2019) is focused on the analysis of intermittent respirometry and performs background respiration corrections. The package `LoLinR` (Olito, White, Marshall, & Barneche, 2017), while not specifically coded for respirometry, provides a statistically robust method of detecting a “best-fit” regression, and performs well at identifying truly linear subsets of a data series.

Here, we describe `respR`, an open-source R package designed to provide an efficient and reproducible workflow for the analysis of respirometry data (Table 1). The package contains utilities to: (a) analyse closed, intermittent, flow-through and open-tank respirometry data (see Lighton, 2008; Svendsen, Bushnell, & Steffensen, 2016), (b) determine volume and mass specific oxygen uptake rates, (c) automatically detect maximal and minimal rates,  $P_{crit}$ , and (d) detect the most linear sections of data using novel rolling regression and kernel density estimation techniques. In addition, the package contains ‘housekeeping’ functions for data import and export as supplementary material at all stages of respirometry analysis, including saving raw data, subsetting criteria, background corrections, units of measure and resulting metabolic rates. This streamlines the reporting of analytical methods and allows reviewers or other

investigators to fully reproduce or scrutinise the results. Additional functions useful in processing respirometry data are available, and are described in more detail in our online documentation (<https://januarhianto.github.io/respR/>). A comprehensive series of vignettes, which explain all package functions, discuss best practices and alternative methods are also available (Table 2).

## 2 | PACKAGE OVERVIEW

The `respR` package streamlines the analysis of respirometry data by partitioning the data processing workflow into several independent actions: (a) initially, data are imported, checked for common errors, plotted for visual inspection and exported as an object; (b) the object is then analysed to estimate the desired rate parameter; (c) diagnostic plots are automatically generated and the data can be further explored using the generic `S3 print()`, `plot()` and `summary()` commands to verify the results; (d) adjustments to the data from background respiration are applied; and (e) volume- or mass-specific rate conversions, specific to the respiratory chamber and specimen(s), are calculated where necessary (Figure 1). By design, each step of the workflow requires minimal manual manipulation of the data, and the user can pass the output of each function to the next. This object-oriented approach makes `respR` easy to use, even for novice users of R, and allows R programmers to extend the functionality of the package easily. Note, we use  $\dot{O}_2$  in the text to represent the unit-less rate of  $O_2$  use with regard to the time unit, and to distinguish it from other commonly reported metrics (e.g.  $\dot{M}O_2$ ,  $\dot{V}O_2$ ). Installation of `respR` can be performed directly via the R console using `devtools`:

```
# install.packages("devtools") # uncomment to run
devtools::install_github("januarhianto/respR") # install
library(respR) # load
```

To illustrate the main features of the package, five example datasets (`sardine.rd`, `urchin.rd`, `intermittent.rd`, `squid.rd` and `zeb_intermittent.rd`) are distributed with `respR`.

## 3 | DATA IMPORT AND EXPLORATION

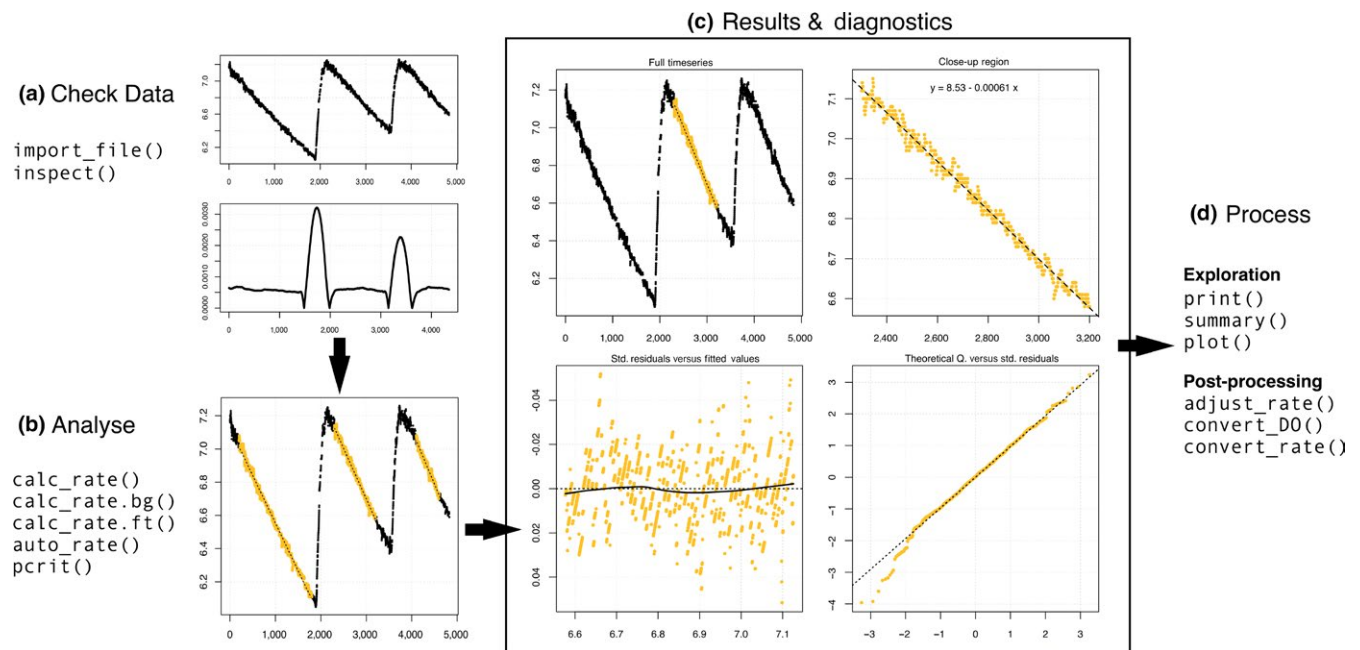
Data should be formatted correctly before use in `respR`. The function `inspect()` extracts a two-column data frame from a multicolumn dataset and performs error checks while plotting the data for quick data visualisation. Time data, being a continuous variable, should be sequential, without duplicates. Ideally, sampling frequency will be monotonic, but in many instances this may not be the case, such as experiments with irregular sampling intervals, or because machine drift and/or data dropouts frequently occur during regular sampling. The information obtained from the checks allow us to make informed decisions on how to best analyse the data, and fix any cryptic errors (e.g. non-numeric data) easily. In particular, knowing whether a timeseries is irregular, or not, will allow us to decide whether to use time-based data extraction methods to obtain data segments of predetermined time intervals.

**TABLE 1** Main functions available in *respR*

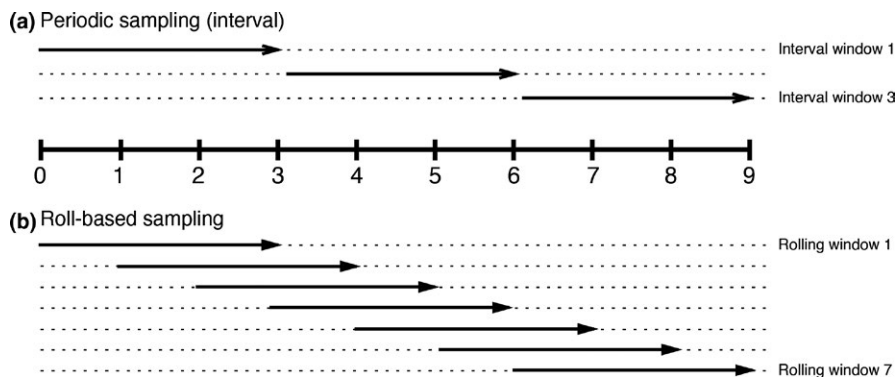
Treatment A	Treatment B
<code>import_data()</code>	Automatically import and format raw data files from commonly used respirometry devices
<code>inspect()</code>	Checks data for errors, extracts a two-column dataframe for analysis, and plots the data for quick visualisation
<code>calc_rate.bg()</code>	Calculates background oxygen uptake rate
<code>calc_rate()</code>	Calculates oxygen uptake rate from precise segment(s) of the data
<code>calc_rate.ft()</code>	Calculates oxygen uptake rate in flowthrough experiments
<code>auto_rate()</code>	Calculates 'max', 'min', 'interval' or 'best-fit' rates
<code>adjust_rate()</code>	Corrects data for background changes in oxygen concentration
<code>convert_DO()</code>	Converts between units of oxygen concentration
<code>convert_rate()</code>	Converts experimental rates to specific units
<code>pcrit()</code>	Calculates critical oxygen tension, $P_{crit}$
<code>subsample()</code>	Reduces the size of a large data frame, by extracting every $n$ -th row
<code>subset_data()</code>	Extract a subset of a data frame
<code>intermittent.rd.rda</code>	Respirometry data for a single sea urchin specimen ( <i>Heliocidaris erythrogramma</i> ) collected using intermittent respirometry. Data were collected over 80 min
<code>sardine.rd.rda</code>	Respirometry data for a single sardine specimen, <i>Sardinops sagax</i> . Data were collected over 2 hr
<code>squid.rd.rda</code>	Respirometry data for a single squid specimen, <i>Doryteuthis opalescens</i> , to determine $P_{crit}$ . Data were collected over a 9-hr period
<code>urchins.rd.rda</code>	Multi-column dataset containing respirometry and background respirometry data for 16 sea urchin specimens ( <i>Heliocidaris erythrogramma</i> ). Data for each urchin were collected over 45 min
<code>zeb_intermittent.rd.rda</code>	Multiple measurements (106 replicates, plus initial and end background measurements) of oxygen consumption in a zebrafish, <i>Danio rerio</i> , obtained using intermittent flow respirometry

**TABLE 2** R Vignettes for the *respR* package, with worked examples, discussions and comparisons to other R package functions. Available online: <https://januarhianto.github.io/respR/articles/respR.html>

Treatment A	Treatment B
Getting Started	Introduction to respirometry and <i>respR</i>
Importing your data	How to import and format data using <i>respR</i>
Closed-chamber respirometry	A typical workflow example that introduces the main functions of <i>respR</i>
<code>auto_rate()</code> : Automatic detection of metabolic rates	Introduction to the <code>auto_rate()</code> function
Performance of <code>auto_rate()</code> in detecting linear regions	Assessing the accuracy of <code>auto_rate()</code> using custom performance tests
Comparative performance of <code>auto_rate()</code> and <i>LoLinR</i>	A comparison of <code>auto_rate()</code> with a similar function in <i>LoLinR</i> (Olito et al., 2017)
Intermittent-flow respirometry: Simple example	How to analyse simple intermittent-flow data
Intermittent-flow respirometry: Complex example	A more complex example on the analysis of intermittent-flow data from a larger experiment
Flowthrough respirometry	How to analyse flowthrough respirometry data
$P_{crit}$ analysis	How to estimate $P_{crit}$
Reproducibility	Using <i>respR</i> to promote open, reproducible respirometry analyses
<i>respR</i> and the tidyverse	A quick guide on how to integrate <i>respR</i> functions with the popular <i>dplyr</i> package, and more
A comparison of <i>respR</i> with other R packages	Comparing outputs of <i>respR</i> to other R package functions, and a discussion of the advantages of using <i>respR</i>
When to use <i>respR</i>	A discussion of what <i>respR</i> offers and when users should consider using the package for respirometry analyses



**FIGURE 1** Diagram showing steps in a typical workflow in the analysis of respirometry data using `respR`. (a) Data are first checked for errors before (b) main functions are used to extract and analyse segments of the data. (c) Summarised results and diagnostic plots provide immediate visual feedback on the outcome of the analyses. (d) Once the rate estimates are obtained, they can be converted into volume and/or mass-specific rates



**FIGURE 2** Illustration showing the sampling method used by `auto_rate()` during (a) interval regression analysis, where sample windows do not overlap and (b) rolling regression analysis, where sample windows overlap and move forward by one sample unit at a time

Using `inspect()` is optional—the main functions in our package will readily work with data frames that were not processed by the function. Running `inspect()` is an exploratory step that flags potential issues before analysis. Error check results are summarised in the R console and all tests are saved in the output object with the locations (i.e. row numbers), if any, of the errors:

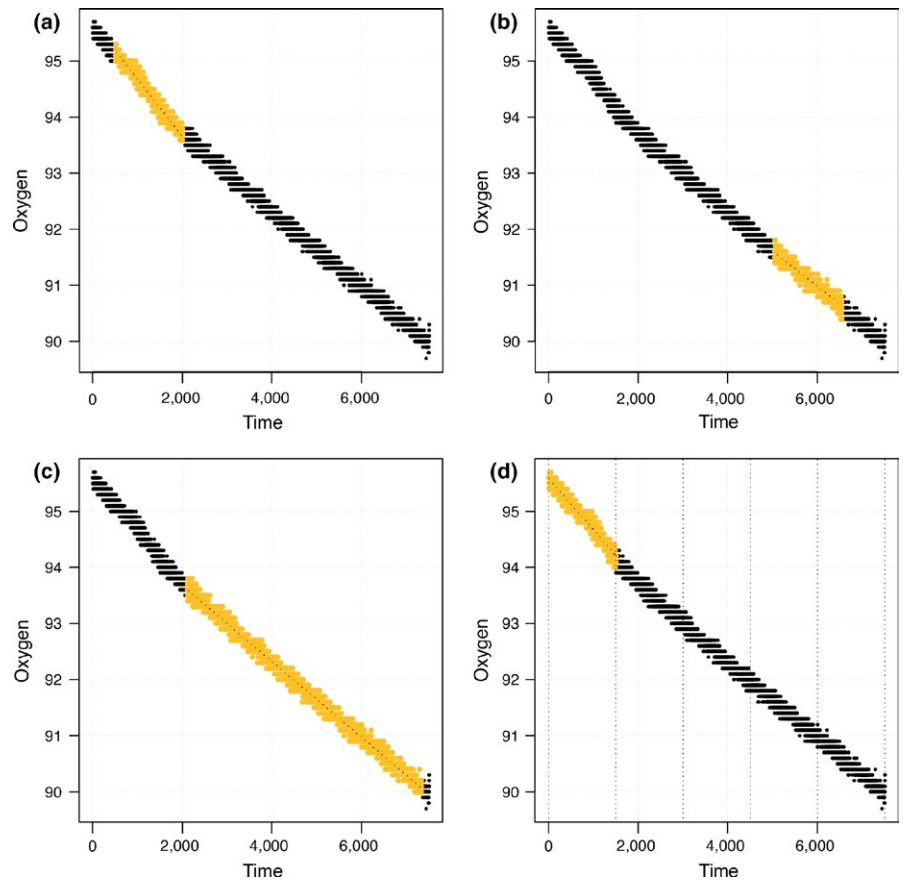
```
inspect(squid.rd, plot = F)
```

```
#> No issues detected while inspecting data frame.
#>
#> # inspect # -----
#>
#>      Time  o2
#> NA/NAN  pass pass
#> sequential  pass  -
#> duplicated  pass  -
#> evenly-spaced pass  -
```

## 4 | DATA ANALYSIS

### 4.1 | Calculating background rate

Background oxygen consumption rate is often measured to account for the contribution of microbial respiration, and applied as a correction to experimental rates (Rogers et al., 2016). Background rates typically account for a small percentage of experimental rates, and multiple 'blank' experiments are usually conducted and the rates averaged to obtain a more accurate estimate of the correction (e.g. Carey & Sigwart, 2014; Daoud, Chabot, Audet, & Lambert, 2007). The function `calc_rate.bg()` uses simple linear regression to process multiple background rate measurements simultaneously. Data segments can be truncated before analysis by time period or row numbers. The results are stored as an object for later use.



**FIGURE 3** Depending on the method input, the function, `auto_rate()` is able to show: (a) the maximum rate detected; (b) the minimum rate detected; (c) the most linear region as determined by kernel density estimates; and (d) calculation of rate by fixed-width intervals. Data obtained from analysis of the `sardine.rd` dataset

## 4.2 | Processing respirometry data

Typically, the analysis of respirometry data requires the selection one or more linear sections of the data for more accurate calculations of  $\dot{O}_2$  (e.g. Carey et al., 2016; Chu & Gale, 2017; Gordon, Chin, & Vojkovich, 1989). The function `calc_rate()` can manually extract and process data subset from respirometry data. Data subsets can be selected by (a) time period, (b) row numbers, (c)  $O_2$  decrease or (d) proportion, which should accommodate most, if not all, manual data selection requirements and allow for consistent reporting of methods and results. Rates are determined using linear regression.

For more complex respirometry parameters, the function `auto_rate()` uses a novel method of combining rolling regression and kernel density estimate algorithms to detect patterns in respirometry data. First, `auto_rate()` always performs a rolling linear regression on the data before additional methods are applied. The rolling regression runs all possible ordinary least-squares linear regressions ( $y = \beta_0 + \beta_1 X + \epsilon$ ) of a fixed sample width across the entire data series, and is expressed as:

$$y_t(n) = X_t(n)\beta(n) + \epsilon_t(n), t = n, \dots, T$$

where  $n$  is the window of width  $n < T$ ,  $T$  is the total length of the dataset,  $y_t(n)$  is the vector of observations (e.g. oxygen concentration),

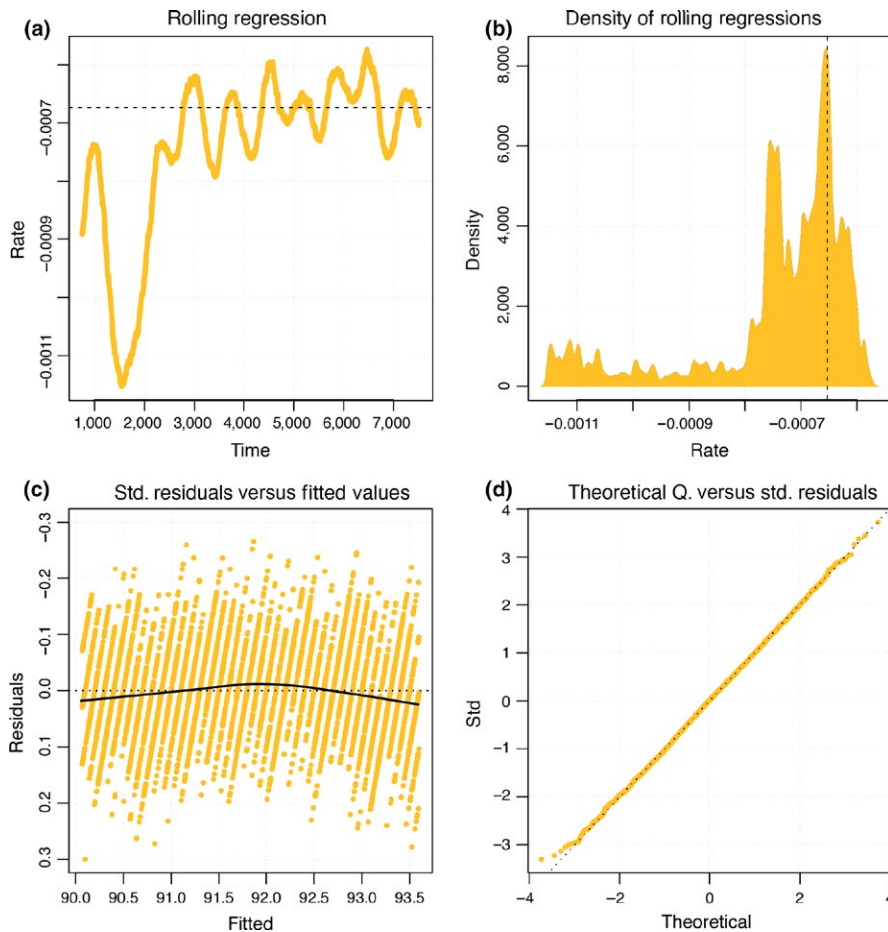
$X_t(n)$  is the matrix of explanatory variables,  $\beta(n)$  is a vector of regression parameters and  $\epsilon_t(n)$  is a vector of error terms. Thus, a total of  $(T - n) + 1$  number of overlapping regressions are fitted. Here, we can obtain maximum and minimum rate values of width  $n$  by ranking the regressions, and if a periodic, interval-based regression is required, the function automatically selects regression metrics for non-overlapping sections of the data (Figure 2).

The `auto_rate()` function can also automatically select the most stable (i.e. most linear) sections of noisy data by applying additional analytical techniques. First, we take advantage of the key assumption that linear sections of a data series are reflected by stable parameters across the rolling estimates, a property that is often applied in financial statistics to evaluate model stability and make forward predictions on time-series data (Zivot & Wang, 2006). We use kernel density estimation (KDE) techniques, often applied in various inference procedures such as machine learning, pattern recognition and computer vision, to automatically aggregate stable (i.e. linear) segments as they naturally form one or more local maximums ('modes') in the probability density estimate.

Kernel density estimation requires no assumption that the data is from a parametric family, and learns the shape of the density automatically without supervision. KDE can be expressed as:

$$\hat{f}(x) = \frac{1}{nh^d} \sum_{i=1}^n K\left(\frac{x - X_i}{h}\right)$$





**FIGURE 4** Diagnostic plots produced by the function, `auto_rate()`: (a) the rolling regression of rate as a function of time, with dotted lines indicating the region used to estimate the parameter; (b) a kernel density plot that shows only when the `linear` method is called; (c) a residual plot of the regression; and (d) a normal Q-Q plot of the residuals of the regression. Data obtained from analysis of the `sardine.rd` dataset

where  $f$  is the density function from an unknown distribution  $P$  for  $X_1, \dots, X_n$ ,  $K$  is the kernel function and  $h$  is the optimal smoothing bandwidth. The smoothing bandwidth is computed using the solve-the-equation plug-in method (Jones, Marron, & Sheather, 1996; Sheather & Jones, 1991) which works well with multimodal or non-normal densities (Raykar & Duraiswami, 2006).

We then use  $h$  to select all values in the rolling regression output that match the range of values around each mode ( $\theta_n$ ) of the KDE (i.e.  $\theta_n \pm h$ ). These rolling estimates are grouped and ranked by size, and the upper and lower bounds of the data windows they represent are used to reselect linear segments from the original data series. The rolling estimates are then discarded while the ranked data segments are analysed using linear regression. Summary diagnostics and visual plots provide supporting information on the technique and the validity of the results (Figures 3 and 4). The performance of this particular method is further discussed in Appendix S1.

### 4.3 | Estimating rate from flow-through respirometry

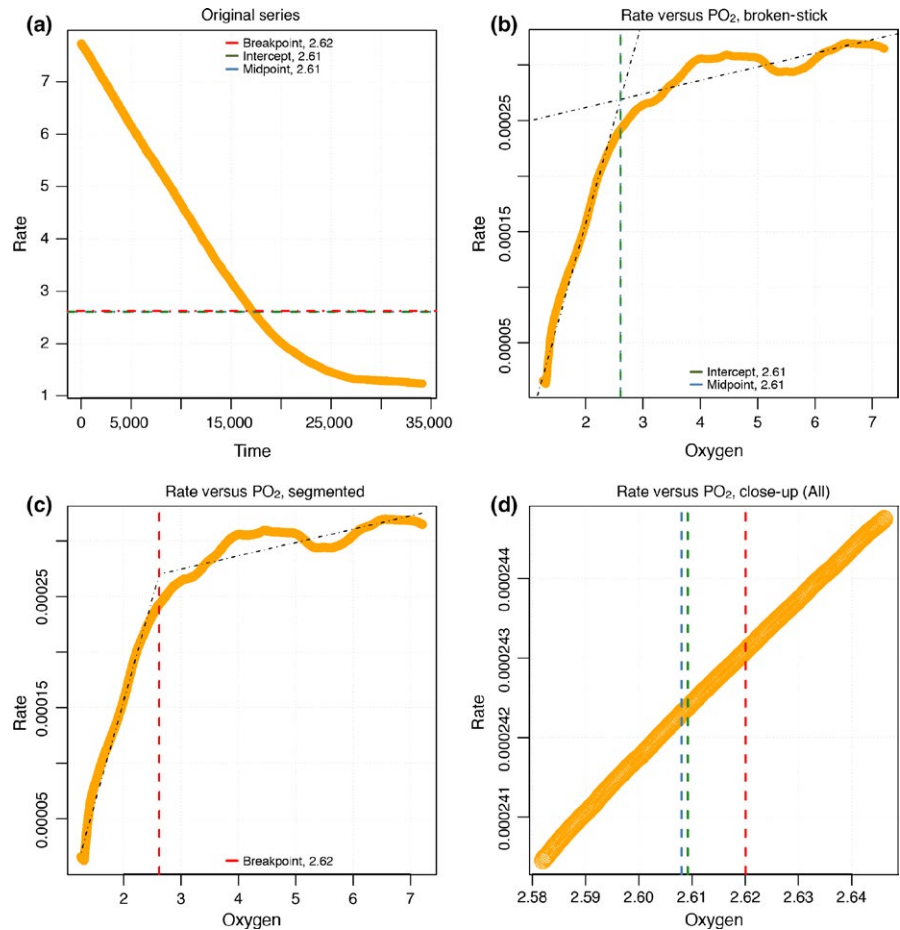
The function `calc_rate.ft()` is used to specifically analyse flow-through respirometry data, and is similar to `calc_rate()` in functionality. However, it accepts additional inputs of incurrent and excurrent oxygen concentration, and flow rate, based on the equation:

$$\dot{O}_2 = (C_i O_2 - C_e O_2) FR$$

where  $\dot{O}_2$  is the rate of  $O_2$  uptake over time,  $C_i O_2$  and  $C_e O_2$  are the incurrent and excurrent  $O_2$  concentrations and  $FR$  is the flow rate of water through the system (Lighton, 2008).

### 4.4 | Estimating critical oxygen tension, $P_{crit}$

We provide two methods of estimating  $P_{crit}$ . The first is a “broken-stick” regression (BSR) approach, adopted from Yeager and Ultsch (1989) in which two segments of the data are iteratively fitted and the intersection with the smallest sum of the residual sum of squares between the two linear models is the estimated critical point. The second method is a wrapper for the segmented, or nonlinear ‘broken-line’ regression approach, presented by Muggeo (2003) and available as part of the `segmented` R package (Muggeo, 2008), which estimates the critical point by iteratively fitting two intersecting models on the data and selecting the point that minimises the ‘gap’ between the two fitted lines. Both methods are well-used in the literature (Chu & Gale, 2017; Hansen, Byriel, Jensen, Steffensen, & Svendsen, 2017; Labra, Bogdanovich, & Bozinovic, 2016; Regan & Richards, 2017; Svendsen et al., 2016). Note that the accuracy of the BSR method to describe non-linear traits is increasingly being debated (Marshall, Bode, & White, 2013) and is discussed in greater detail in our vignettes (see Appendix S1).



**FIGURE 5** Output plots of the function `p_crit()`, used in the calculation of critical oxygen tension,  $P_{crit}$ . (a) To determine  $P_{crit}$  in a regular  $O_2$  time series, the function performs a rolling regression against the rolling mean of the data. This step is skipped if similar data is already provided, and the argument `has.rate = TRUE` is used in the function. This produces (b, c) data that represents the rate of  $O_2$  consumption as a function of  $O_2$  concentration. Regression lines are then fitted iteratively, based on the broken-stick methods of Yeager and Ultsch (1989) and the segmented approach of Muggeo (2003), until convergence is achieved. The output then highlights the critical points as indicated by the coloured lines. (d) A close-up plot of the results in (b) and (c) is provided for the user to visualise the positions of the dotted lines relative to the x-axis

As  $P_{crit}$  is the break point in the relationship between ambient dissolved oxygen ( $O_2$ ) and rate of oxygen uptake ( $\dot{O}_2$ ), the user has two options to input data. With an  $O_2 \sim$  timeseries dataset, the function will automatically perform both a rolling mean and a rolling regression of  $O_2$  data over time to produce the required  $\bar{O}_2 \sim \dot{O}_2$  dataset (Figure 5). This computationally intensive, but fast, method is unique to `respR` as most other methods use non-overlapping intervals to characterise their data, but rolling metrics are more sensitive to  $P_{crit}$  analysis due to their high resolution. Alternatively, the user can input previously-determined rate  $\sim \dot{O}_2$  data and the function will determine  $P_{crit}$  using the input data directly.

## 5 | CORRECTING, CONVERTING AND SCALING DATA

### 5.1 | Corrections for background respiration

The function `adjust.rate()` can be used to correct  $\dot{O}_2$  estimates for background respiration. As the oxygen consumption units are not mass specific at the point of correction, the function adjusts  $\dot{O}_2$  directly without bias using the equation:

$$\dot{O}_{2(\text{correction})} = \dot{O}_2 - (\phi_d + \dot{O}_{2(bg)})$$

where  $\phi_d$  is the air–water oxygen flux as determined by Fick's Law (Leclercq, Gattuso, & Jaubert, 1999) and  $\dot{O}_{2(bg)}$  is background respiration.

**TABLE 3** Units available for conversions in `convert_DO()` and `convert_rate()` functions in `respR`

Parameter	Used in	Units
Oxygen; $O_2$	<code>convert_DO()</code> , <code>convert_rate()</code>	$\text{mg L}^{-1}$ , $\mu\text{g L}^{-1}$ , $\mu\text{mol L}^{-1}$ , $\text{mL L}^{-1}$ , $\mu\text{g kg}^{-1}$ , $\text{mg kg}^{-1}$ , $\mu\text{mol kg}^{-1}$ , $\text{mmol kg}^{-1}$ , $\text{mL kg}^{-1}$ , $\text{hPa}$ , $\text{kPa}$ , $\text{mmHg}$ , $\text{inHg}$
Mass	<code>convert_rate()</code>	$\mu\text{g}$ , $\text{mg}$ , $\text{g}$ , $\text{kg}$
Time	<code>convert_rate()</code>	second, minute, hour

### 5.2 | Rate conversions

Because `respR` does not make any underlying assumptions on the units used to calculate metabolic rate, we provide two functions, `convert_DO()` and `convert_rate()`, to transform  $\dot{O}_2$  into volume-adjusted or mass-specific units. These functions are comprehensive and can currently convert between 168 combinations of units and has support for changes in temperature, salinity and pressure (Table 3). This unique approach has the advantage of allowing rate calculations for data that may not be entirely supported by the `respR` workflow. For example, measurements of respiration can be scaled to surface area (e.g. diatoms & coral, Iversen & Ploug, 2013; Naumann, Orejas, & Ferrier-Pagès, 2014),

volume (e.g. copepod eggs Hammervold, Glud, Evjemo, Hagemann, & Hansen, 2015) or density (e.g. sea urchin larvae Stumpp, Wren, Melzner, Thorndyke, & Dupont, 2011), all of which are not yet supported in *respR*'s conversion functions. Thus, any respirometry data may still be analysed without any restriction until conversion is required, in which users at this point perform those conversions manually.

The function `convert_DO()` is used to convert units of oxygen concentration only (e.g. mg/L or %; Table 2). For ease of use, we implemented a simple fuzzy string-matching algorithm to match relevant units (e.g. "mg/L," "mg/l," "mg L<sup>-1</sup>" and "mg l<sup>-1</sup>" are identified as the same units). The function `convert_rate()` is conceptually similar to `convert_DO()`, but it focuses on volumetric and mass-specific conversions (e.g. mgh<sup>-1</sup> kg<sup>-1</sup>).

## 6 | CONCLUDING REMARKS AND FUTURE IMPROVEMENTS

The package *respR* implements a number of methods not available in any other R package or software. These include the use of rolling regression techniques to estimate  $\dot{M}O_{2,max}$ ,  $\dot{M}O_{2,min}$  and  $P_{crit}$ , the use of kernel density estimation techniques to detect and rank linear segments of the data, support for unevenly spaced time data, and the ability to extract and analyse data precisely by time, row, or O<sub>2</sub> concentration. It is important to note, however, that while *respR* contains automated methods to assist in processing difficult data, no amount of data manipulation can fix poor data. Users should still ensure that their data has been collected using acceptable methods (e.g. closed respirometry setups should always be equipped with mixing devices, see Clark, Sandblom, & Jutfelt, 2013) before performing analyses of any kind. Nevertheless, *respR* adheres to well-defined rules to obtain rates of interest, is extensively documented online, and its methods and results are transparent and fully reproducible. The *respR* package allows for more effective and less time-consuming handling and processing of the broad datasets that are increasingly common in respirometry research.

## ACKNOWLEDGEMENTS

This work was supported by an NSW Environmental Trust grant (M.B.), and Endeavour Postdoctoral Fellowship (N.C.) and a Professor NGW & Mrs Ann Macintosh Memorial Scholarship from the University of Sydney (J.H.). Some experimental data were obtained from experiments performed at the Sydney Institute of Marine Science (SIMS). This manuscript is SIMS contribution number 240. We thank our reviewers, whose comments have greatly improved the manuscript.

## AUTHORS' CONTRIBUTIONS

J.H., N.C. and M.B. conceived the ideas and designed the methodology; J.H. wrote the code, N.C. edited code and analysed the example data; J.H., N.C. and M.B. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

## DATA ACCESSIBILITY

The *respR* R package is open-source and available on GitHub (<https://github.com/januarhariano/respR>). Package vignettes are also open-source, accessible at <https://januarhariano.github.io/respR/index.html>. Installing and loading *respR* will automatically load all example data used in this paper. Alternatively, the data may be downloaded manually from GitHub at <https://github.com/januarhariano/respR/tree/master/data>. Since the package is updated regularly, all code, data and documentation used to reproduce this manuscript have been archived at <https://doi.org/10.5281/zenodo.2548602> as *respR* v.1.5.1.

## ORCID

Januar Harianto  <https://orcid.org/0000-0002-4803-108X>

Nicholas Carey  <https://orcid.org/0000-0001-6715-8230>

## REFERENCES

- Barneche, D. R., Kulbicki, M., Floeter, S. R., Friedlander, A. M., Maina, J., & Allen, A. P. (2014). Scaling metabolism from individuals to reef-fish communities at broad spatial scales. *Ecology Letters*, 17, 1067–1076. <https://doi.org/10.1111/ele.12309>
- Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M., & West, G. B. (2004). Toward a metabolic theory of ecology. *Ecology*, 85, 1771–1789. <https://doi.org/10.1890/03-9000>
- Carey, N., & Sigwart, J. D. (2014). Size matters: Plasticity in metabolic scaling shows body-size may modulate responses to climate change. *Biology Letters*, 10, 20140408. <https://doi.org/10.1098/rsbl.2014.0408>
- Carey, N., Harianto, J., & Byrne, M. (2016). Sea urchins in a high CO<sub>2</sub> world: Partitioned effects of body size, ocean warming and acidification on metabolic rate. *Journal of Experimental Biology*, 219, 1178–1186. <https://doi.org/10.1242/jeb.136101>
- Chabot, D., Steffensen, J. F., & Farrell, A. P. (2016). The determination of standard metabolic rate in fishes. *Journal of Fish Biology*, 88, 81–121. <https://doi.org/10.1111/jfb.12845>
- Chu, J. W. F., & Gale, K. S. P. (2017). Ecophysiological limits to aerobic metabolism in hypoxia determine epibenthic distributions and energy sequestration in the northeast Pacific ocean. *Limnology and Oceanography*, 62, 59–74. <https://doi.org/10.1002/lno.10370>
- Clark, T. D., Sandblom, E., & Jutfelt, F. (2013). Aerobic scope measurements of fishes in an era of climate change: Respirometry, relevance and recommendations. *Journal of Experimental Biology*, 216, 2771–2782. <https://doi.org/10.1242/jeb.084251>
- Daoud, D., Chabot, D., Audet, C., & Lambert, Y. (2007). Temperature induced variation in oxygen consumption of juvenile and adult stages of the northern shrimp, *Pandalus borealis*. *Journal of Experimental Marine Biology and Ecology*, 347, 30–40. <https://doi.org/10.1016/j.jembe.2007.02.013>
- Delorme, N. J., & Sewell, M. A. (2016). Effects of warm acclimation on physiology and gonad development in the sea urchin *Evechinus chloroticus*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 198, 33–40. <https://doi.org/10.1016/j.cbpa.2016.03.020>
- Gordon, M. S., Chin, H. G., & Vojtkovich, M. (1989). Energetics of swimming in fishes using different methods of locomotion: I. Labriform swimmers. *Fish Physiology and Biochemistry*, 6, 341–352. <https://doi.org/10.1007/bf01875604>



- Hammervold, S. H., Glud, R. N., Evjemo, J. O., Hagemann, A., & Hansen, B. W. (2015). A new large egg type from the marine live feed calanoid copepod *Acartia tonsa* (Dana)—Perspectives for selective breeding of designer feed for hatcheries. *Aquaculture*, 436, 114–120. <https://doi.org/10.1016/j.aquaculture.2014.11.003>
- Hansen, A. K., Byriel, D. B., Jensen, M. R., Steffensen, J. F., & Svendsen, M. B. S. (2017). Optimum temperature of a northern population of Arctic charr (*Salvelinus alpinus*) using heart rate Arrhenius breakpoint analysis. *Polar Biology*, 40, 1063–1070. <https://doi.org/10.1007/s00300-016-2033-8>
- Hochachka, P. W., & Somero, G. N. (2002). *Biochemical adaptation: Mechanism and process in physiological evolution*. Cambridge, UK; New York: Oxford University Press.
- Iversen, M. H., & Ploug, H. (2013). Temperature effects on carbon-specific respiration rate and sinking velocity of diatom aggregates—Potential implications for deep ocean export processes. *Biogeosciences*, 10, 4073–4085. <https://doi.org/10.5194/bg-10-4073-2013>
- Jones, M. C., Marron, J. S., & Sheather, S. J. (1996). A brief survey of bandwidth selection for density estimation. *Journal of the American Statistical Association*, 91, 401–407. <https://doi.org/10.1080/01621459.1996.10476701>
- Labra, F. A., Bogdanovich, J. M., & Bozinovic, F. (2016). Nonlinear temperature effects on multifractal complexity of metabolic rate of mice. *PeerJ*, 4, e2607.
- Leclercq, N., Gattuso, J.-P., & Jaubert, J. (1999). Measurement of oxygen metabolism in open-top aquatic mesocosms: Application to a coral reef community. *Marine Ecology Progress Series*, 177, 299–304.
- Lighton, J. R. B. (2008). *Measuring metabolic rates: A manual for scientists*. Oxford, UK: Oxford University Press.
- Marshall, D. J., Bode, M., & White, C. R. (2013). Estimating physiological tolerances—A comparison of traditional approaches to nonlinear regression techniques. *Journal of Experimental Biology*, 216, 2176–2182. <https://doi.org/10.1242/jeb.085712>
- Morozov, S., McCairns, R. J. S., & Merilä, J. (2019). FishResp: R package and GUI application for analysis of aquatic respirometry data. *Conservation Physiology*, 7. <https://doi.org/10.1093/conphys/coz003>
- Muggeo, V. M. R. (2003). Estimating regression models with unknown break-points. *Statistics in Medicine*, 22, 3055–3071. <https://doi.org/10.1002/sim.1545>
- Muggeo, V. M. R. (2008). segmented: An R package to fit regression models with broken-line relationships. *R News*, 8, 20–25.
- Naumann, M. S., Orejas, C., & Ferrier-Pagès, C. (2014). Species-specific physiological response by the cold-water corals *Lophelia pertusa* and *Madrepora oculata* to variations within their natural temperature range. *Deep Sea Research Part II: Topical Studies in Oceanography*, 99, 36–41. <https://doi.org/10.1016/j.dsr2.2013.05.025>
- Norin, T., & Malte, H. (2012). Intraspecific variation in aerobic metabolic rate of fish: Relations with organ size and enzyme activity in brown trout. *Physiological and Biochemical Zoology*, 85, 645–656. <https://doi.org/10.1086/665982>
- Olito, C., White, C. R., Marshall, D. J., & Barneche, D. R. (2017). Estimating monotonic rates from biological data using local linear regression. *The Journal of Experimental Biology*, 220, 759–764. <https://doi.org/10.1242/jeb.148775>
- Pörtner, H. (2002). Climate variations and the physiological basis of temperature dependent biogeography: Systemic to molecular hierarchy of thermal tolerance in animals. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 132, 739–761. [https://doi.org/10.1016/s1095-6433\(02\)00045-4](https://doi.org/10.1016/s1095-6433(02)00045-4)
- Raykar, V. C., & Duraiswami, R. (2006). Fast optimal bandwidth selection for kernel density estimation. *Proceedings of the 2006 SIAM International Conference on Data Mining*, Bethesda, MD, USA, 524–528.
- Regan, M. D., & Richards, J. G. (2017). Rates of hypoxia induction alter mechanisms of O<sub>2</sub> uptake and the critical O<sub>2</sub> tension of goldfish. *Journal of Experimental Biology*, 220, 2536–2544. <https://doi.org/10.1242/jeb.154948>
- Rogers, N. J., Urbina, M. A., Reardon, E. E., McKenzie, D. J., & Wilson, R. W. (2016). A new analysis of hypoxia tolerance in fishes using a database of critical oxygen level (Pcrit). *Conservation Physiology*, 4, cow012. <https://doi.org/10.1093/conphys/cow012>
- Seibel, B. A., & Drazen, J. C. (2007). The rate of metabolism in marine animals: Environmental constraints, ecological demands and energetic opportunities. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 362, 2061–2078. <https://doi.org/10.1098/rstb.2007.2101>
- Sheather, S. J., & Jones, M. C. (1991). A reliable data-based bandwidth selection method for kernel density estimation. *Journal of the Royal Statistical Society Series B (Methodological)*, 53, 683–690. <https://doi.org/10.1111/j.2517-6161.1991.tb01857.x>
- Stumpp, M., Wren, J., Melzner, F., Thorndyke, M. C., & Dupont, S. T. (2011). CO<sub>2</sub> induced seawater acidification impacts sea urchin larval development I: Elevated metabolic rates decrease scope for growth and induce developmental delay. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology*, 160, 331–340. <https://doi.org/10.1016/j.cbpa.2011.06.022>
- Svendsen, M. B. S., Bushnell, P. G., & Steffensen, J. F. (2016). Design and setup of intermittent-flow respirometry system for aquatic organisms. *Journal of Fish Biology*, 88, 26–50. <https://doi.org/10.1111/jfb.12797>
- White, C. R., & Kearney, M. R. (2013). Determinants of inter-specific variation in basal metabolic rate. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, 183, 1–26. <https://doi.org/10.1007/s00360-012-0676-5>
- Yeager, D. P., & Ultsch, G. R. (1989). Physiological regulation and conformation: A BASIC program for the determination of critical points. *Physiological Zoology*, 62, 888–907. <https://doi.org/10.1086/physzool.62.4.30157935>
- Zivot, E., & Wang, J. (2006). *Modeling financial time series with S-PLUS®* (2nd ed.). New York: Springer-Verlag.

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**How to cite this article:** Harianto J, Carey N, Byrne M.

respR—An R package for the manipulation and analysis of respirometry data. *Methods Ecol Evol*. 2019;00:1–9.

<https://doi.org/10.1111/2041-210X.13162>