

1 **Supplementary files (R scripts)**

2

3 **File S1.** R-script for running the stickleback example. Comments and information about the
4 scripts (not run) are preceded by a hash symbol (#) and shaded in grey. Our thanks to an
5 anonymous reviewer of a previous draft who provided insightful and helpful commentary of the
6 code which we have also incorporated here.

7

8 #####

9 # Species: Gasterosteus aculeatus (three-spined stickleback) #

10 # Chamber info: AutoResp, n=4, V=250ml #

11 # Trait.1: Standard metabolic rate (SMR) #

12 # Trait.2: Active metabolic rate (AMR) #

13 # Background respiration: pre and post #

14 #####

15

16 # Loading the package 'FishResp' into R environment

17 `library(FishResp)`

18

19 # Specifying paths to the files containing raw respirometry data for Case Study 1

20 `pre.path = system.file("extdata/stickleback/pre_raw.txt.xz", package = "FishResp")`

21 `post.path = system.file("extdata/stickleback/post_raw.txt.xz", package = "FishResp")`

22 `SMR.path = system.file("extdata/stickleback/SMR_raw.txt.xz", package = "FishResp")`

23 `AMR.path = system.file("extdata/stickleback/AMR_raw.txt.xz", package = "FishResp")`

24

25 # Filling the information about animals, respirometry chambers and DO units

26 `info <- input.info(ID = c("Stickleback_1", "Stickleback_2",`
27 `"Stickleback_3", "Stickleback_4"),`

28 `Mass = c(1.86, 1.92, 2.23, 1.80),`

29 `Volume = c(250, 250, 250, 250),`

30 `DO.unit = "mg/L")`

31

32 # Note, only three DO units are available in FishResp: "mg/L", "mmol/L", or "ml/L".

33 # Other DO units should be converted to FishResp compatible ones using the functions

34 # 'convert.respirometry' or 'convert.rMR' (see File S3 and the R package documentation)

35

36 # Importing background respiration tests (pre and post)

37 `pre <- import.test(pre.path,`

38 `info.data = info,`

39 `logger = "AutoResp",`

40 `n.chamber = 4,`

41 `plot.temperature = TRUE,`

42 `plot.oxygen = TRUE)`

43

44 `post <- import.test(post.path,`

45 `info.data = info,`

46 `logger = "AutoResp",`

```

47         n.chamber = 4,
48         plot.temperature = TRUE,
49         plot.oxygen = TRUE)
50
51 # Importing raw data for SMR and AMR measurements.
52 SMR.raw <- import.meas(SMR.path,
53                       info.data = info,
54                       logger = "AutoResp",
55                       n.chamber = 4,
56                       date.format = "DMY",
57                       start.measure = "22:00:00",
58                       stop.measure = "06:00:00",
59                       plot.temperature = TRUE,
60                       plot.oxygen = TRUE)
61
62 AMR.raw <- import.meas(AMR.path,
63                       info.data = info,
64                       logger = "AutoResp",
65                       n.chamber = 4,
66                       date.format = "DMY",
67                       plot.temperature = TRUE,
68                       plot.oxygen = TRUE)
69
70 # Correcting raw data for background respiration
71 SMR.clean <- correct.meas(info.data = info,
72                          pre.data = pre,
73                          meas.data = SMR.raw,
74                          method = "pre.test")
75
76 AMR.clean <- correct.meas(info.data = info,
77                          post.data = post,
78                          meas.data = AMR.raw,
79                          method = "post.test")
80
81 # QC graphical tests of raw data before and after correction
82 # Here, we recommend maximizing a plot window in R
83 QC.meas(SMR.clean, "Temperature")
84 QC.meas(SMR.clean, "Total.O2.phases")
85 QC.meas(SMR.clean, "Corrected.O2.phases")
86 QC.meas(SMR.clean, "Total.O2.chambers")
87 QC.meas(SMR.clean, "Corrected.O2.chambers")
88
89 QC.meas(AMR.clean, "Temperature")
90 QC.meas(AMR.clean, "Total.O2.phases")
91 QC.meas(AMR.clean, "Corrected.O2.phases")
92 QC.meas(AMR.clean, "Total.O2.chambers")

```

```

93 QC.meas(AMR.clean, "Corrected.O2.chambers")
94
95 # Activity of animals during SMR measurements
96 QC.activity(SMR.clean, compare = FALSE)
97
98 # Mass-specific metabolic rate before and after correction for background
99 # respiration (note, metabolic rate is calculated without any slope filtration)
100 QC.activity(SMR.clean, compare = TRUE)
101
102 # Extraction of target slopes for SMR and AMR
103 SMR.slope <- extract.slope(SMR.clean,
104                             method = "min",
105                             n.slope = 3,
106                             r2 = 0.95,
107                             length = 1200)
108
109 AMR.slope <- extract.slope(AMR.clean,
110                             method = "all",
111                             r2 = 0.95,
112                             length = 600)
113
114 # Detailed graphical description of extracted slopes for both SMR and AMR
115 QC.slope(SMR.slope, SMR.clean, chamber = "CH1", current = 1200, alter = 600)
116 QC.slope(SMR.slope, SMR.clean, chamber = "CH2", current = 1200, alter = 600)
117 QC.slope(SMR.slope, SMR.clean, chamber = "CH3", current = 1200, alter = 600)
118 QC.slope(SMR.slope, SMR.clean, chamber = "CH4", current = 1200, alter = 600)
119
120 QC.slope(AMR.slope, AMR.clean, chamber = "CH1", current = 600, alter = 300)
121 QC.slope(AMR.slope, AMR.clean, chamber = "CH2", current = 600, alter = 300)
122 QC.slope(AMR.slope, AMR.clean, chamber = "CH3", current = 600, alter = 300)
123 QC.slope(AMR.slope, AMR.clean, chamber = "CH4", current = 600, alter = 300)
124
125 QC.slope(AMR.slope, AMR.clean, chamber = "CH1",
126           current = 600, alter = 300, residuals = TRUE)
127 QC.slope(AMR.slope, AMR.clean, chamber = "CH2",
128           current = 600, alter = 300, residuals = TRUE)
129 QC.slope(AMR.slope, AMR.clean, chamber = "CH3",
130           current = 600, alter = 300, residuals = TRUE)
131 QC.slope(AMR.slope, AMR.clean, chamber = "CH4",
132           current = 600, alter = 300, residuals = TRUE)
133
134 # Reducing the length of measurements for each AMR slope
135 AMR.slope <- extract.slope(AMR.clean,
136                             method = "all",
137                             r2 = 0.95,
138                             length = 300)

```

```

139
140 # Calculation of background respiration rate, absolute and
141 # mass-specific metabolic rate for SMR and AMR
142 SMR <- calculate.MR(SMR.slope,
143                     density = 1000,
144                     plot.BR = TRUE,
145                     plot.MR.abs = TRUE,
146                     plot.MR.mass = TRUE)
147
148 AMR <- calculate.MR(AMR.slope,
149                     density = 1000,
150                     plot.BR = TRUE,
151                     plot.MR.abs = TRUE,
152                     plot.MR.mass = TRUE)
153
154 # Exporting the final dataset as a .txt file: SMR and AMR datasets
155 # are merged into one dataframe. In addition, absolute,
156 # mass-specific and factorial metabolic rate are calculated.
157 results <- export.MR(SMR, AMR,
158                     file = "results.txt",
159                     simplify = TRUE,
160                     MS = TRUE,
161                     plot.MS.abs = TRUE,
162                     plot.MS.mass = TRUE,
163                     plot.MS.fact = TRUE)
164
165 ##### The end #####

```

166 **File S2.** R-script for running the guppy example (Case Study 2)

```
167
168 #####
169 # Species: Poecilia reticulata (guppy) #
170 # Chamber info: AutoResp, n=8, V=21.8 ml #
171 # Trait: Standard metabolic rate (SMR) #
172 # Background respiration: pre and post #
173 #####
174
175 # Loading the package 'FishResp' into R environment
176 library(FishResp)
177
178 # Specifying paths to the files containing raw respirometry data for Case Study 2
179 pre.path = system.file("extdata/guppy/pre_raw.txt.xz", package = "FishResp")
180 post.path = system.file("extdata/guppy/post_raw.txt.xz", package = "FishResp")
181 SMR.path = system.file("extdata/guppy/SMR_raw.txt.xz", package = "FishResp")
182
183 # Filling the information about animals, respirometry chambers and DO units
184 info <- input.info(ID = c("Guppy_M1", "Guppy_M2", "Guppy_M3", "Guppy_M4",
185 "Guppy_F1", "Guppy_F2", "Guppy_F3", "Guppy_F4"),
186 Mass = c(0.097, 0.086, 0.065, 0.090, 0.253, 0.428, 0.181, 0.222),
187 Volume = c(21.8, 21.8, 21.8, 21.8, 21.8, 21.8, 21.8, 21.8),
188 DO.unit = "mg/L")
189
190 # Importing background respiration tests (pre and post)
191 pre <- import.test(pre.path,
192 info.data = info,
193 logger = "AutoResp",
194 n.chamber = 8,
195 plot.oxygen = TRUE,
196 plot.temperature = TRUE)
197
198 post <- import.test(post.path,
199 info.data = info,
200 logger = "AutoResp",
201 n.chamber = 8,
202 plot.oxygen = TRUE,
203 plot.temperature = TRUE)
204
205 # Importing raw data for SMR measurements.
206 SMR.raw <- import.meas(SMR.path,
207 info.data = info,
208 logger = "AutoResp",
209 n.chamber = 8,
210 start.measure = "22:00:00",
211 stop.measure = "06:00:00",
```

```

212         plot.oxygen = TRUE,
213         plot.temperature = TRUE)
214
215 # Correcting raw data for background respiration
216 SMR.clean <- correct.meas(info.data = info,
217                          pre.data = pre,
218                          post.data = post,
219                          meas.data = SMR.raw,
220                          method = "exponential")
221
222 # QC graphical tests of raw data before and after correction
223 # Here, we recommend maximizing a plot window in R
224 QC.meas(SMR.clean, "Temperature")
225 QC.meas(SMR.clean, "Total.O2.phases")
226 QC.meas(SMR.clean, "Corrected.O2.phases")
227 QC.meas(SMR.clean, "Total.O2.chambers")
228 QC.meas(SMR.clean, "Corrected.O2.chambers")
229
230 # Activity of animals during SMR measurements
231 QC.activity(SMR.clean, compare = FALSE)
232
233 # Mass-specific metabolic rate before and after correction for background
234 # respiration (note, metabolic rate is calculated without any slope filtration)
235 QC.activity(SMR.clean, compare = TRUE)
236
237 # Extraction of target slopes for SMR
238 SMR.slope <- extract.slope(SMR.clean,
239                           method = "calcSMR.quant",
240                           p = 0.25, r2 = 0.9)
241
242 # Note, if the methods "calcSMR.quant" is used, detailed graphical
243 # description of extracted slopes will not be available
244
245 # Calculation of background respiration rate, absolute and
246 # mass-specific metabolic rate for SMR
247 SMR <- calculate.MR(SMR.slope,
248                   density = 1000,
249                   plot.BR = TRUE,
250                   plot.MR.abs = TRUE,
251                   plot.MR.mass = TRUE)
252
253 # Exporting the final dataset as a .csv file.
254 results <- export.MR(SMR, file = "results.csv", simplify = TRUE)
255
256 ##### The end #####

```

257 **File S3.** R-script demonstrating application of functions listed in alphabetical order which have
258 not been reviewed in the case studies.

```
259  
260 # Example of converting oxygen units using the function 'convert.respirometry'  
261 SMR.path = system.file("extdata/stickleback/SMR_raw.txt.xz", package = "FishResp")  
262  
263 convert.respirometry(import.file = SMR.path,  
264                     export.file = "converted_SMR_raw.txt",  
265                     n.chamber = 1, logger = "AutoResp",  
266                     from = "mg_per_l", to = "mmol_per_l",  
267                     sal = 0, atm_pres = 1013.25)  
268  
269  
270 # Example of converting oxygen units using the function 'convert.rMR'  
271 AMR.path = system.file("extdata/stickleback/AMR_raw.txt.xz", package = "FishResp")  
272  
273 convert.rMR(import.file = AMR.path,  
274            export.file = "converted_AMR_raw.txt",  
275            n.chamber = 2, logger = "AutoResp", salinity = 0,  
276            DO.units.in = "mg/L", DO.units.out = "PP",  
277            bar.press = 101.325, bar.units.in = "kpa")  
278  
279  
280 # Example of importing raw data recorded by 'Q-box Aqua' using the function 'import.meas'  
281 qbox.path = system.file("extdata/qboxaqua/qboxaqua.csv", package = "FishResp")  
282 info <- input.info(ID = "Fish_1", Mass = 2.3, Volume = 170, DO.unit = "mg/L")  
283  
284 RMR.raw <- import.meas(file = qbox.path,  
285                       info.data = info,  
286                       logger = "QboxAqua",  
287                       n.chamber = 1,  
288                       date.format = "DMY",  
289                       start.measure = "23:30:00",  
290                       stop.measure = "01:00:00",  
291                       set.date.time = "23/02/2014/23:30:22",  
292                       meas.to.wait = 200,  
293                       plot.temperature = TRUE,  
294                       plot.oxygen = TRUE)  
295  
296  
297 # Example of converting raw data from 'OxyView' (PreSens) and a summary file from  
298 # 'AquaResp' (free software) to 'FishResp' format using the function 'presens.aquaresp'  
299 presens.path.1 = system.file("extdata/presens/presens-ch1.txt", package = "FishResp")  
300 presens.path.2 = system.file("extdata/presens/presens-ch2.txt", package = "FishResp")  
301 presens.path.3 = system.file("extdata/presens/presens-ch3.txt", package = "FishResp")  
302 presens.path.4 = system.file("extdata/presens/presens-ch4.txt", package = "FishResp")
```

```

303 aquaresp.path = system.file("extdata/presens/presens-aquaresp.txt", package = "FishResp")
304
305 presens.aquaresp(presens.file = c(presens.path.1, presens.path.2,
306                               presens.path.3, presens.path.4),
307                aquaresp.file = aquaresp.path,
308                fishresp.file = "fishresp.txt",
309                date.format = "DMY",
310                n.chamber = 4,
311                wait.phase = 60,
312                measure.phase = 240)
313
314
315 # Example of converting raw data from 'Pyro Oxygen Logger' (PyroScience) and
316 # a summary file from 'AquaResp' (free software) to 'FishResp' format using
317 # the function 'pyroscience.aquaresp'
318 pyroscience.path = system.file("extdata/pyroscience/pyroscience.txt", package = "FishResp")
319 aquaresp.path = system.file("extdata/pyroscience/pyroscience-aquaresp.txt",
320                             package = "FishResp")
321
322 pyroscience.aquaresp(pyroscience.file = pyroscience.path,
323                    aquaresp.file = aquaresp.path,
324                    fishresp.file = "fishresp.txt",
325                    date.format = "MDY",
326                    n.chamber = 1,
327                    wait.phase = 120,
328                    measure.phase = 600)
329
330
331 # Example of removing poor quality data using the function 'rm.data'
332 data(AMR.clean)
333 AMR.clean.modified <- rm.data(AMR.clean,
334                             chamber = "CH3",
335                             M.phase = c("M1", "M2"))
336
337 ##### The end #####
338

```