Calculation of effective ATP/O ratio

This protocol describes the steps needed to extract and process the raw oxygen and fluorescence signals and the steps required to complete the Excel template file as done in Salin *et al.* (2016). If you have any feedbacks or questions, please don't hesitate to contact me – salin.karine@gmail.com.

1/ Calculation of the calibration curve for the fluorescence signal.

- In Datlab, select the layout showing "Amp-Raw (V)".
- Mark the 11 points of calibration: 1 before the first $MgCl_2$ addition and 10 after each of the $MgCl_2$ addition (Figure 1).

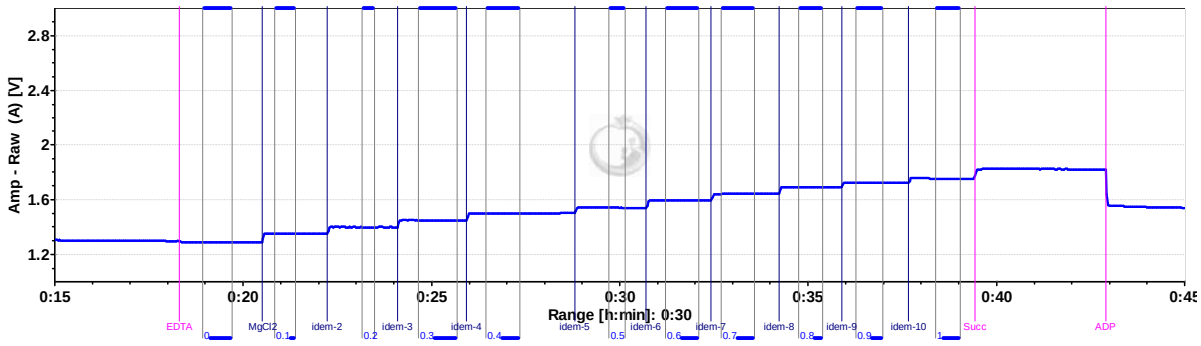


Figure 1.

- Export the data from Datlab: "Mark" > "Statistics" > Select Marks in "Amp-raw A or B" > "Copy to Clipboard".
- Past in Excel file in the sheet called "raw data Mg Calibration".
- Duplicate the "template ATP-O ratio". Name it as "*name of the sample*".
- In the Calibration curve section, copy/past-transpose (ctrl+T) the Amp(V) in cell Q2 (Figure 2).

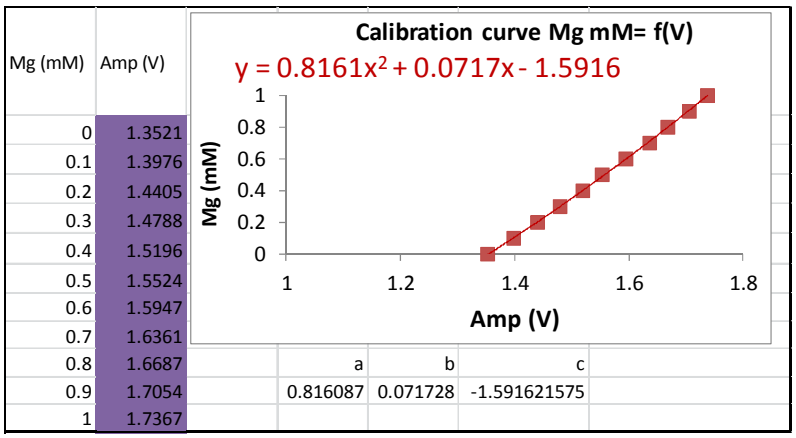


Figure 2.

- Repeat the same steps for the other chamber.

2/Extract the values for the raw oxygen and fluorescence signals for State 3 and State 4.

This will allow the calculation of the raw JO2 and raw JATP values. Data from both chambers are extracted at the same time.

- In the datlab file, select layout with both Y1 as “Amp-Raw (V)”and Y2 as “O2 Concentration[nmol/ml]” (Figure 3)

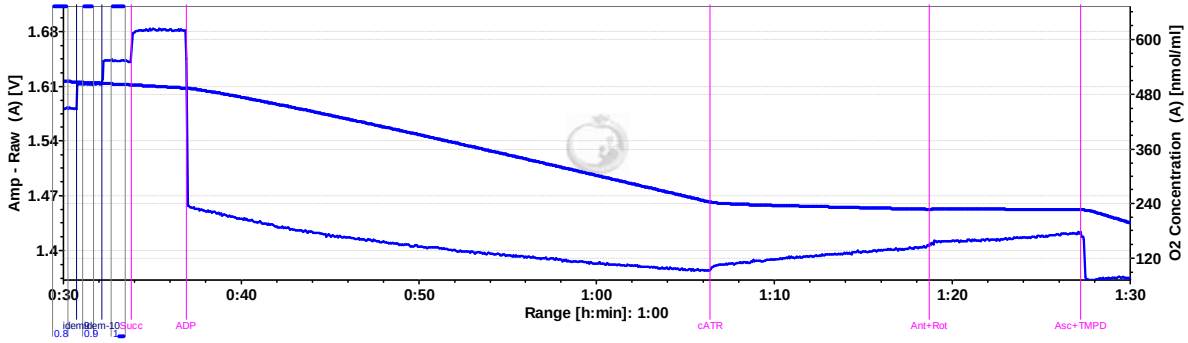
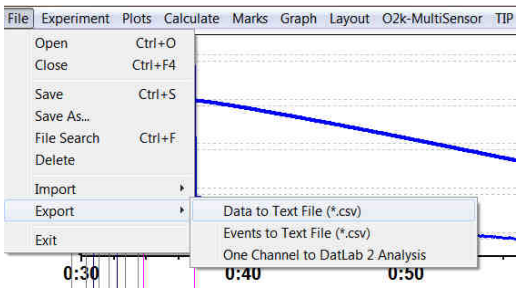


Figure 3.

- > “file”> ”export”> ”data to text file” (Figure 4)

(a)



(b)

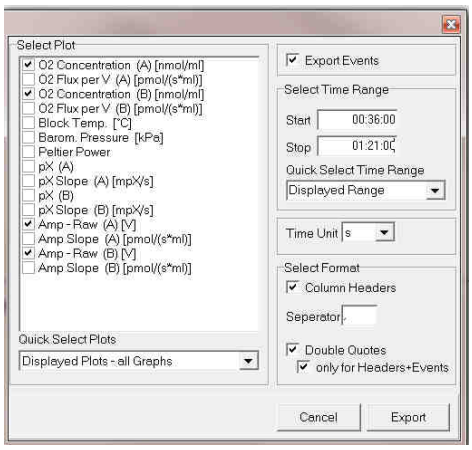


Figure 4.

- Select time range:
  - delete the time written in “Start” and “Stop” by selecting the number with the mouse and press “delete” on the keyboard.
  - introduce the “Start” time as one minute before the addition of ADP to the chamber.
  - introduce the “Stop” time where antimycin A and rotenone were added to the chamber.
- Select seconds (s) as “Time Unit”.
- Click on “Export” and “save”. This gives a csv file.

3/ Clean the raw data

- Open the csv file.
- Duplicate the sheet.
- Name them by the "*name of the sample ChA or ChB*" (Figure 5).

	Time [s]	Event Name	Chamber	Event Text	O2 Concentration (A) [nmol/ml]	O2 Concentration (B) [nmol/ml]	Amp - Raw (A) [V]	Amp - Raw (B) [V]
2	2160	O2, Pyr+Mal, Both, Left		Injected mo	495.6869	487.8157	1.6837	1.7631
3	2162				495.629	487.7285	1.6826	1.7638
4	2164				495.4843	487.6413	1.6835	1.7628
5	2166				495.4119	487.5396	1.6828	1.7624
6	2168				495.3974	487.496	1.6814	1.7619
7	2170				495.2817	487.4233	1.6824	1.7622
8	2172				495.2238	487.3216	1.6814	1.763
9	2174				495.1659	487.2198	1.6829	1.7624
10	2176				495.0501	487.1181	1.6821	1.7629
11	2178				494.9199	487.06	1.6834	1.7625
12	2180				494.8476	487.0018	1.6823	1.7635
13	2182				494.8042	486.9146	1.6826	1.7628
14	2184				494.7897	486.8855	1.6823	1.7629
15	2186				494.7173	486.7983	1.6835	1.7626
16	2188				494.6884	486.7111	1.6828	1.7636
17	2190				494.5871	486.6094	1.6816	1.7622
18	2192				494.4134	486.5222	1.6816	1.7632
19	2194				494.3122	486.4495	1.6818	1.7626
20	2196				494.2109	486.3768	1.6828	1.7629
21	2198				494.1674	486.3187	1.6825	1.763
22	2200				494.0517	486.246	1.682	1.7629
23	2202				493.9649	486.1588	1.6838	1.7618

2016-01-28 P-0 ASSAY-FISH 4 ChA | 2016-01-28 P-0 ASSAY-FISH 3 ChB

Figure 5.

- Delete the columns that do not correspond with the chamber being evaluated.
- Locate the rows were ADP has been added.
- Delete the extra rows above this raw such as 29 rows preceding ADP addition are kept and the raw for ADP addition is located line 30.
- Delete all rows below Ant-Rot.
- Delete columns "Event Name" "Chamber" "Event Text" (Figure 6).

	Time [s]	O2 Concentration (B) [nmol/ml]	Amp - Raw (B) [V]
2	2216	485.6501	1.7627
3	2218	485.5484	1.764
4	2220	485.5048	1.7621
5	2222	485.403	1.7617
6	2224	485.3449	1.7631
7	2226	485.2722	1.7632
8	2228	485.1996	1.7627
9	2230	485.0833	1.7625
10	2232	484.967	1.7624
11	2234	484.8943	1.7617

Figure 6.

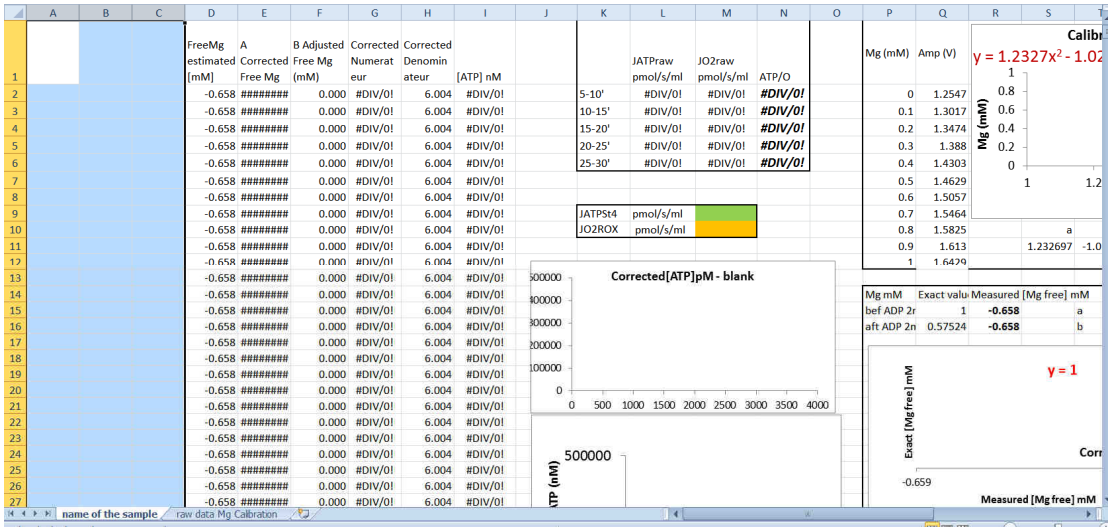
- Repeat all the steps above for the other chamber.

4/ Calculation of effective ATP/O ratio

Concentrations ADP and Mg^{2+} and K_d of ATP and ADP for free magnesium for your experimental conditions should be determined as in Chinopoulos *et al.* (2014). In the Excel sheet "template ATP-O Ratio", complete parameters for your experimental conditions in M33, M34, M35 and M36.

- Copy the three columns from the .csv file and paste them into columns A, B and C of the Excel template file (Figure 7).

Before:



After:

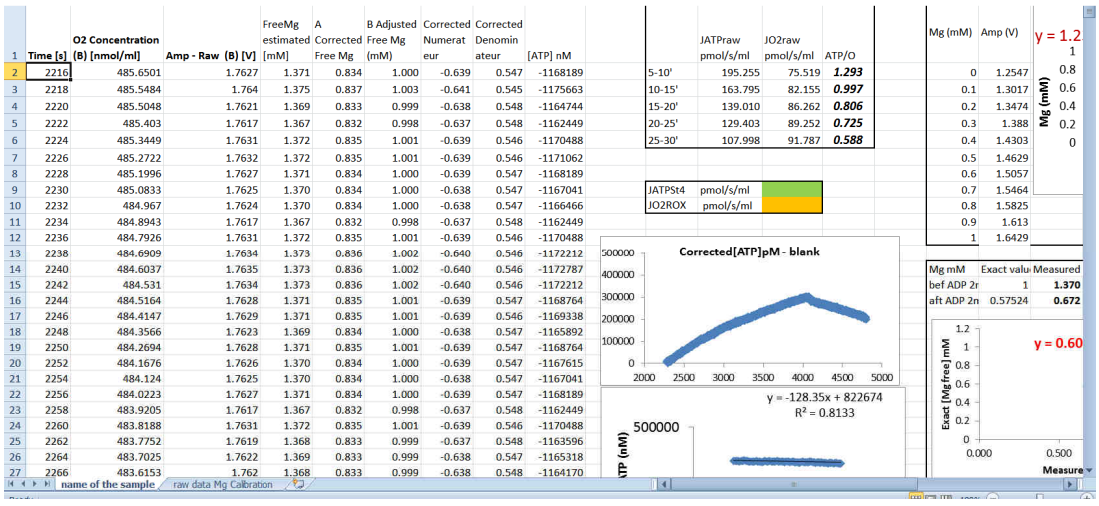


Figure 7.

- Click on the points of the graph entitled “corrected[ATP]nM - St4” to selected the data set.
- Drag the selection to the bottom of the data set (Figure 8).

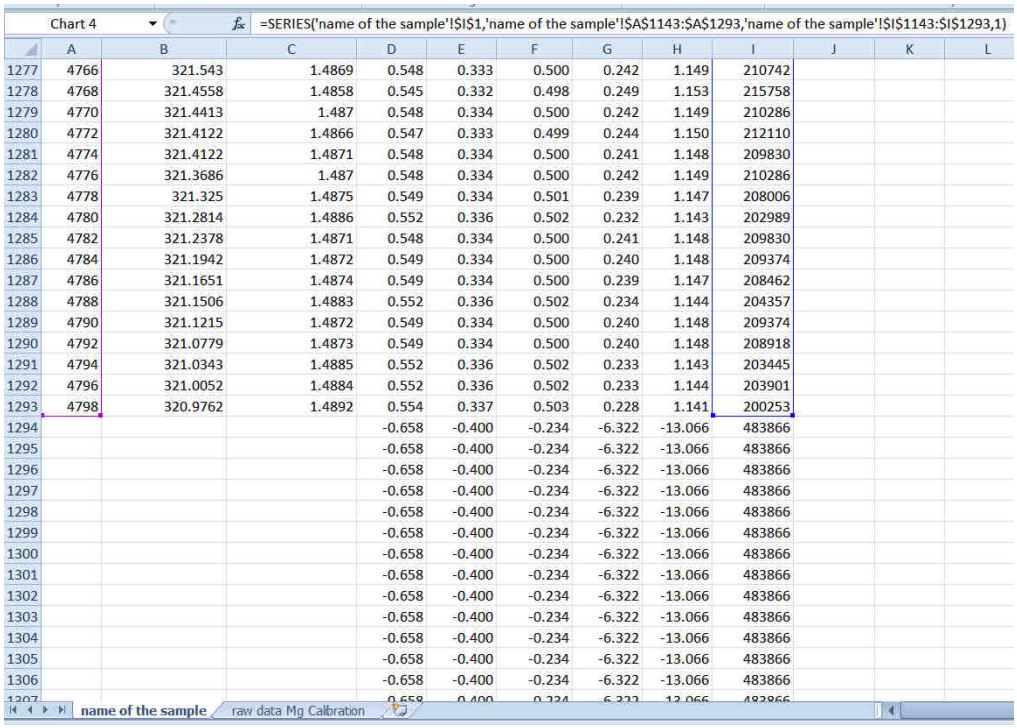


Figure 8.

- Enter the value of the slope in the cell M9 using the opposite symbol as the slope displays (Figure 9).

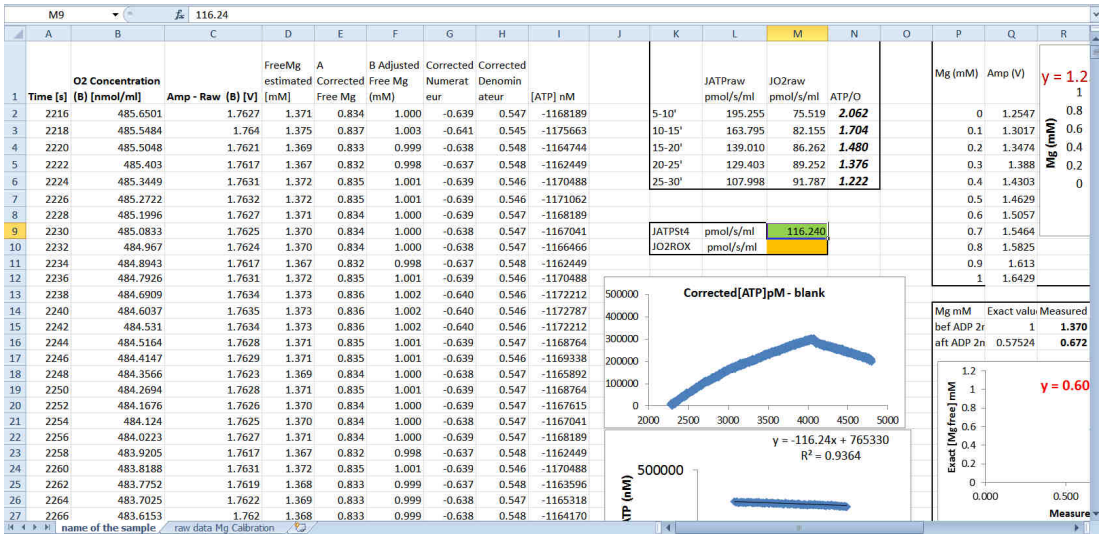
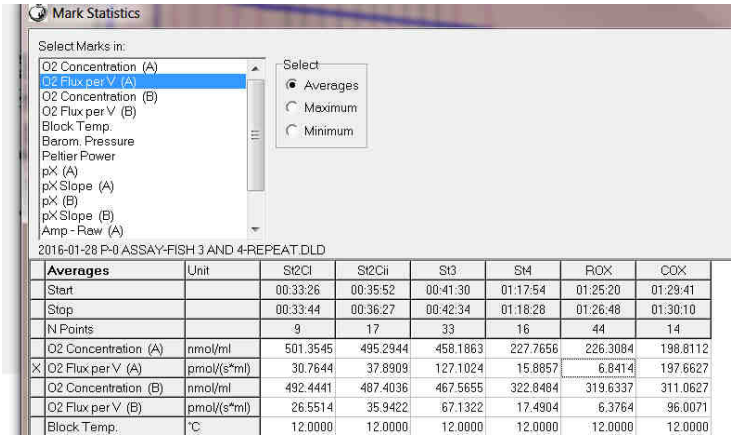


Figure 9.

- Introduce the value for JO_2-ROX (in “pmol/s.ml”) obtained in Datlab into cell M10:
  - In Datlab: set the Mark.

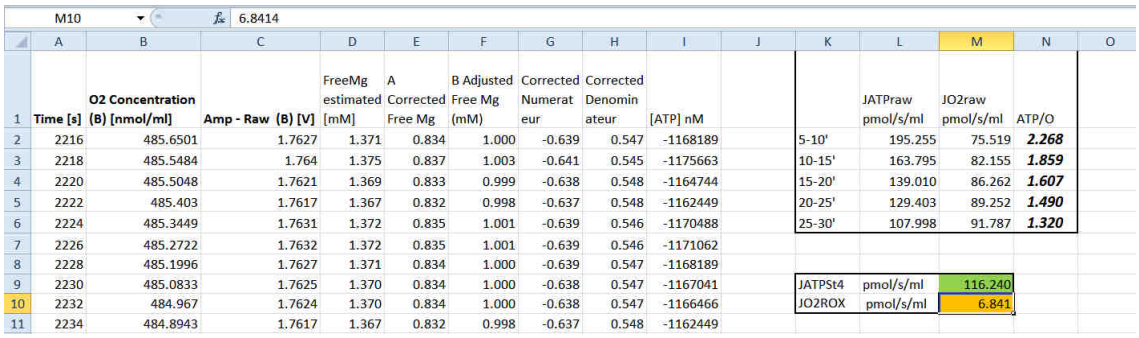
- Go to “Marks”>“Statistics” (Figure 10).
- Click on the value > Ctrl c.



Averages	Unit	St2CI	St2CIi	St3	St4	ROX	COX
Start		00:33:26	00:35:52	00:41:30	01:17:54	01:25:20	01:29:41
Stop		00:33:44	00:36:27	00:42:34	01:18:28	01:26:48	01:30:10
N Points		9	17	33	16	44	14
O2 Concentration (A)	nmol/ml	501.3545	495.2944	458.1863	227.7656	226.3084	198.8112
O2 Flux per V (A)	pmol/(s*ml)	30.7644	37.8909	127.1024	15.8857	6.8414	197.6627
O2 Concentration (B)	nmol/ml	492.4441	487.4036	467.5655	322.8484	319.6337	311.0627
O2 Flux per V (B)	pmol/(s*ml)	26.5514	35.9422	67.1322	17.4904	6.3764	96.0071
Block Temp.	°C	12.0000	12.0000	12.0000	12.0000	12.0000	12.0000

Figure 10.

- In excel, Ctrl v in M10 (Figure 11).



M10 | 6.8414

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	Time [s]	O2 Concentration (B) [nmol/ml]	Amp - Raw (B) [V]	FreeMg estimated [mM]	A Corrected Free Mg	B Adjusted Free Mg (mM)	Corrected Numerateur	Corrected Denominateur	[ATP] nM			JATPraw pmol/s/ml	JO2raw pmol/s/ml	ATP/O	
2	2216	485.6501	1.7627	1.371	0.834	1.000	-0.639	0.547	-1168189		5-10'	195.255	75.519	2.268	
3	2218	485.5484	1.764	1.375	0.837	1.003	-0.641	0.545	-1175663		10-15'	163.795	82.155	1.859	
4	2220	485.5048	1.7621	1.369	0.833	0.999	-0.638	0.548	-1164744		15-20'	139.010	86.262	1.607	
5	2222	485.403	1.7617	1.367	0.832	0.998	-0.637	0.548	-1162449		20-25'	129.403	89.252	1.490	
6	2224	485.3449	1.7631	1.372	0.835	1.001	-0.639	0.546	-1170488		25-30'	107.998	91.787	1.320	
7	2226	485.2722	1.7632	1.372	0.835	1.001	-0.639	0.546	-1171062						
8	2228	485.1996	1.7627	1.371	0.834	1.000	-0.639	0.547	-1168189						
9	2230	485.0833	1.7625	1.370	0.834	1.000	-0.638	0.547	-1167041		JATPSt4	pmol/s/ml	116.240		
10	2232	484.967	1.7624	1.370	0.834	1.000	-0.638	0.547	-1166466		JO2ROX	pmol/s/ml	6.841		
11	2234	484.8943	1.7617	1.367	0.832	0.998	-0.637	0.548	-1162449						

Figure 11.

→ In the ATP/O column (N2 to N6), the *J*ATP and JO_2 are automatically corrected for rates of non-mitochondrial ATP hydrolyse ($JATP_{St3} = JATP_{raw} - JATP_{St4}$) and respiration ($JO_{2\text{-}St3} = JO_{2\text{-}raw} - JO_{2\text{-}ROX}$) respectively; the ATP/O ratio is calculated as the ratio of $JATP_{St3}$ to 2-fold $JO_{2\text{-}St3}$.

Chinopoulos, C, Kiss, G, Kawamata, H, Starkov, AA (2014) Chapter seventeen - **Measurement of ADP-ATP exchange in relation to mitochondrial transmembrane potential and oxygen consumption**. In 'Methods in enzymology.' (Eds G Lorenzo, K Guido.) Vol. 542 pp. 333-348. Elsevier academic press inc, San Diego).(http://dx.doi.org/10.1016/B978-0-12-416618-9.00017-0)

Salin, K, Villasevil, EM, Auer, SK, Anderson, GJ, Selman, C, Metcalfe, NB, Chinopoulos, C (2016) **Simultaneous measurement of mitochondrial respiration and ATP production in tissue homogenates and calculation of effective P/O ratios**. *Physiological Reports* **4**, e13007. (http://dx.doi.org/10.14814/phy2.13007)