

1 **Supp. Table 1.** Summary table of the *p*-values obtained for the one-way ANOVA or Kruskal-Wallis  
2 comparisons between Rdaytime, Rnight and R24h estimated with the Max and the Most methods. SS  
3 refers to the comparison of the estimates integrated from sunrise to sunset, and PP those integrated  
4 over the production period. The *p*-values smaller than 0.05 were considered as significant and are  
5 presented in bold.

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Estimate	Integration	Oct-1	Oct-2	Oct-3	Jun-1	Jun-2	Jun-3
Rdaytime	SS	<b>6.7 x 10<sup>-6</sup></b>	<b>2.1 x 10<sup>-6</sup></b>	<b>9.4 x 10<sup>-6</sup></b>	<b>6.7 x 10<sup>-6</sup></b>	<b>1.4 x 10<sup>-4</sup></b>	<b>9.5 x 10<sup>-5</sup></b>
	PP	<b>4.9 x 10<sup>-5</sup></b>	<b>4.1 x 10<sup>-5</sup></b>	<b>1.7 x 10<sup>-4</sup></b>	<b>4.1 x 10<sup>-5</sup></b>	<b>4.0 x 10<sup>-4</sup></b>	<b>6.9 x 10<sup>-5</sup></b>
Rnight	SS	0.62	<b>0.03</b>	0.14	<b>0.03</b>	0.07	<b>0.01</b>
	PP	0.59	<b>0.03</b>	0.32	<b>0.04</b>	0.09	<b>0.01</b>
R24h	SS	<b>9.3 x 10<sup>-3</sup></b>	<b>3.7 x 10<sup>-3</sup></b>	<b>0.01</b>	<b>1.7 x 10<sup>-4</sup></b>	<b>3.1 x 10<sup>-4</sup></b>	<b>7.0 x 10<sup>-4</sup></b>
	PP	0.08	<b>0.01</b>	<b>0.02</b>	<b>2.2 x 10<sup>-3</sup></b>	<b>9.1 x 10<sup>-4</sup></b>	<b>1.1 x 10<sup>-3</sup></b>

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8 **Supp. Table 2.** Day-by-day differences between estimates obtained with the Max method and with the  
9 Most method. Comparisons were done between each value obtained with the Max method and the  
10 corresponding value obtained with the Most method (obtained for the same mesocosm and for the  
11 same day). n.a.: value not available

Mesocosm	Day	$\frac{R_{daytimeMax} - R_{daytimeMost}}{R_{daytimeMost}} * 100$ (%)	$\frac{R_{nightMax} - R_{nightMost}}{R_{nightMost}} * 100$ (%)
Jun-1	2	91.86	-10.96
Jun-1	3	255.55	-21.86
Jun-1	4	166.78	-16.94
Jun-1	5	168.50	-20.52
Jun-1	6	105.96	-11.11
Jun-1	7	127.17	-14.36
Jun-1	8	86.21	-10.58
Jun-1	9	96.33	-12.43
Jun-1	10	109.96	-15.94
Jun-1	11	48.95	7.46
Jun-1	12	81.92	-9.86
Jun-1	13	45.76	11.47
Jun-1	14	35.15	11.14
Jun-1	15	54.54	10.97
Jun-1	16	65.07	-2.31
Jun-1	17	104.72	-8.75
Oct-1	2	95.97	-5.08
Oct-1	3	267.52	-10.07
Oct-1	4	100.63	-5.80
Oct-1	5	173.96	-11.74
Oct-1	6	110.84	-6.53
Oct-1	7	149.83	-9.18

Oct-1	8	197.39	-5.91
Oct-1	9	378.59	-14.85
Oct-1	10	90.58	-6.94
Oct-1	11	107.32	-7.31
Oct-1	12	396.78	-14.76
Oct-1	13	167.79	-8.63
Oct-1	14	485.44	-5.23
Oct-1	15	212.18	-7.46
Jun-2	2	108.31	-15.83
Jun-2	3	207.13	-19.33
Jun-2	4	119.28	-11.91
Jun-2	5	218.74	-21.92
Jun-2	6	94.49	-12.20
Jun-2	7	68.23	-8.04
Jun-2	8	101.47	-16.25
Jun-2	9	66.79	-7.62
Jun-2	10	118.54	-19.20
Jun-2	11	n.a.	n.a.
Jun-2	12	59.13	-7.34
Jun-2	13	57.70	12.95
Jun-2	14	34.84	12.10
Jun-2	15	47.32	32.42
Jun-2	16	55.22	-2.09
Jun-2	17	74.94	36.07
Oct-2	2	94.88	-5.05
Oct-2	3	296.31	-10.08
Oct-2	4	105.92	-5.86
Oct-2	5	186.89	-11.86
Oct-2	6	113.24	-6.16
Oct-2	7	156.36	-9.43
Oct-2	8	436.17	-7.48
Oct-2	9	754.91	-15.35
Oct-2	10	190.30	-15.09
Oct-2	11	171.10	-12.03
Oct-2	12	437.40	-17.01
Oct-2	13	247.38	-13.45
Oct-2	14	465.52	-7.02
Oct-2	15	-12.66	0.74
Jun-3	2	109.54	-17.03
Jun-3	3	196.16	-19.31
Jun-3	4	119.06	-12.30
Jun-3	5	186.73	-23.23
Jun-3	6	129.94	-12.42
Jun-3	7	132.34	-14.68
Jun-3	8	87.04	-9.50
Jun-3	9	97.79	-14.54

Jun-3	10	108.25	-14.36
Jun-3	11	57.35	1.72
Jun-3	12	77.13	-11.17
Jun-3	13	48.71	-0.54
Jun-3	14	55.88	-0.19
Jun-3	15	38.21	19.86
Jun-3	16	59.91	-3.77
Jun-3	17	39.47	16.09
Oct-3	2	65.22	-4.18
Oct-3	3	140.75	-6.97
Oct-3	4	206.28	-9.65
Oct-3	5	205.15	-12.17
Oct-3	6	101.39	-5.93
Oct-3	7	469.64	-19.02
Oct-3	8	457.29	-8.84
Oct-3	9	230.56	-9.68
Oct-3	10	218.97	-13.64
Oct-3	11	104.12	-7.29
Oct-3	12	352.56	-14.90
Oct-3	13	149.94	-8.42
Oct-3	14	n.a.	-3.64
Oct-3	15	463.95	-8.07

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13 **Supp. Table 3.** Average duration of the Positive NCP period and of the periods of light-enhanced  
 14 respiration in hour.

Mesocosm	Positive NCP period	From the start of the Negative NCP period to the maximal respiration	From the sunset to the maximal respiration
Oct-1	10.22	5.65	5.22
Oct-2	10.15	5.37	4.81
Oct-3	10.29	5.28	4.82
Jun-1	11.13	5.61	2.16
Jun-2	11.14	5.67	1.94
Jun-3	11.44	5.41	2.10

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16 **Supp. Table 4.** Mean GPP estimates with standard deviations ( $\text{gO}_2 \text{ m}^{-3} \text{ d}^{-1}$ ) obtained with the Max and  
 17 the Most methods and integrated over the production period (PP) or from sunrise to sunset (SS).

Mesocosm	GPP-Max-PP ( $\text{gO}_2 \text{ m}^{-3} \text{ d}^{-1}$ )	GPP-Most-PP ( $\text{gO}_2 \text{ m}^{-3} \text{ d}^{-1}$ )	GPP-Max-SS ( $\text{gO}_2 \text{ m}^{-3} \text{ d}^{-1}$ )	GPP-Most-SS ( $\text{gO}_2 \text{ m}^{-3} \text{ d}^{-1}$ )
Oct-1	$0.36 \pm 0.11$	$0.25 \pm 0.09$	$0.38 \pm 0.08$	$0.26 \pm 0.07$
Oct-2	$0.36 \pm 0.14$	$0.26 \pm 0.13$	$0.38 \pm 0.09$	$0.26 \pm 0.10$
Oct-3	$0.42 \pm 0.18$	$0.28 \pm 0.13$	$0.42 \pm 0.13$	$0.29 \pm 0.10$
Jun-1	$0.54 \pm 0.19$	$0.41 \pm 0.17$	$0.72 \pm 0.19$	$0.54 \pm 0.18$
Jun-2	$0.51 \pm 0.21$	$0.38 \pm 0.19$	$0.68 \pm 0.24$	$0.51 \pm 0.22$
Jun-3	$0.58 \pm 0.21$	$0.43 \pm 0.18$	$0.77 \pm 0.25$	$0.58 \pm 0.23$

18

19 Appendix 1.

20 ***Sensor deployment duration and sampling frequency***

21 In the present study, the data used was obtained with a frequency of 1 measurement every minute.  
22 This measurement frequency or sampling frequency, which is the time period between two  
23 consecutive data acquisitions (Staeher et al. 2010a), has to be chosen wisely. Useful information from  
24 the DO data can be missed if the sampling frequency is too slow, leading to differences in metabolic  
25 estimates. On the other hand, a rapid sampling frequency might result in the generation of an extensive  
26 dataset, which is not necessary if it does not provide additional information. A sampling frequency of  
27 30 min has been estimated to be sufficient to provide reliable daily metabolic estimates in field  
28 observations of lakes (Staeher et al. 2010a). In the same study, the required duration of sensor  
29 deployment to obtain powerful metabolic estimates (i.e., within 20% of the mean with a certainty of  
30 80%) was calculated for various sampling frequencies (from 10 min to 4 hours).

31 As said before, in the present investigation, data were collected with a sampling frequency of 1min. To  
32 verify whether the duration of the experiments used in this work is sufficient to get powerful  
33 estimations of NCP using the new method described, the same power analysis was conducted with  
34 sampling frequency ranging from 1 to 60 min, using data from the Oct-1 mesocosm.

35 The Oct-1 data were collected with a 1-min SP (SP1) by the sensors. Data for SPs of 5, 10, 15, 30 and  
36 60 min (SP5, SP10, SP15, SP30, SP60) were then obtained from the initial SP1 data. Then, the  
37 instantaneous and daily metabolic parameters were calculated with these 5 datasets. The results of the  
38 power analysis of the number of days that would be required for the Oct experiment with the tested  
39 sampling frequencies are presented in Supp. Table 5. The results showed that the 15 days of  
40 acquisition of data of Oct-1 obtained with a sampling frequency of 1 min greatly surpassed 0.8 days,  
41 which is the minimum required number of days to have powerful estimates of NCP in this case.

42 **Supp. Table 5.** Required duration of sensor deployment (in days) to obtain powerful NCP estimates  
43 (i.e. within 20% of the mean with a certainty of 80%) for the Oct-1 data at various sampling  
44 frequencies ranging from 1 to 60 min.

Sampling frequencies (minutes)	Required deployment time (days)
1	0.80
5	4.21
10	9.37
15	14.51
30	30.07
60	55.17

45

46 On a more general note, this power analysis highlights the fact that sampling frequency must be  
 47 considered when designing a mesocosm experiment and when using automated sensors that can  
 48 perform high-frequency measurements.

49 Appendix 2.

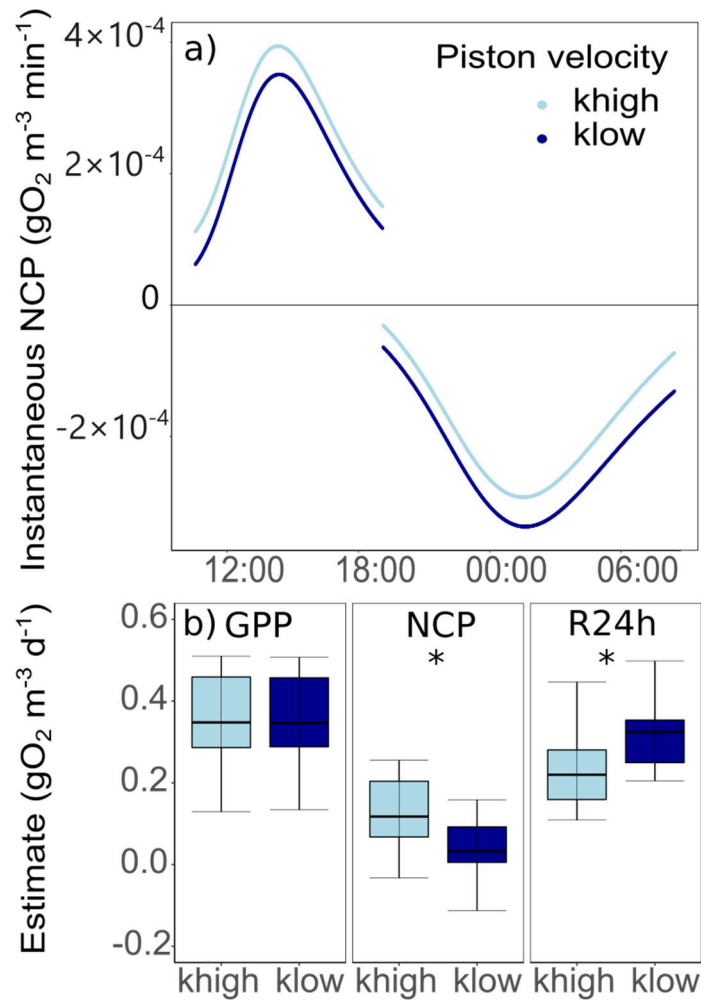
50 ***Sensitivity analyses of the method to the air-water exchange coefficient value***

51 The rate at which oxygen is exchanged with the atmosphere over time can be approximated as being  
 52 proportional to the oxygen deficit (e.g., the difference between DO and the oxygen saturation level)  
 53 (Cox 2003). The piston velocity coefficient  $k$ , a proportional constant, is needed to estimate the  
 54 transfer of oxygen between the atmosphere and the water surface. This parameter is related to surface  
 55 turbulence, internal mixing, water viscosity and temperature. In the field,  $k$  can be calculated with the  
 56 wind speed at 10 m above the water surface, but in enclosed mesocosms that are covered by a dome  
 57 and therefore not directly subject to wind-induced turbulence, this calculation is not possible.

58 However, despite the lack of direct wind effects,  $k$  cannot be considered equal to 0 in mesocosms  
 59 because of the water mass movement in the mesocosm caused by the external waves surrounding the  
 60 mesocosm and because of the mixing induced by the pump. An analysis of the sensitivity of the  
 61 calculation method to the value of  $k$  was performed in Staehr et al. (2010), with a  $k$  value of  $0.4 \text{ m d}^{-1}$   
 62 ( $0.017 \text{ m h}^{-1}$ ). However, this analysis has never been done for an enclosed mesocosm situation, in  
 63 which estimating  $k$  can be very challenging.

64 Therefore, in the present investigation, metabolic parameters estimated with low and high piston  
65 velocity values were compared. More precisely, the instantaneous NCP and the daily metabolic  
66 parameters (Eq. 6 & Eq. 10-Eq. 12) obtained for the Oct-1 mesocosm with 2 piston velocity values  
67 from Alcaraz et al. (2001) were compared. The choice to compare the dataset obtained in October is  
68 justified because the experiment was carried out under similar temperature and salinity conditions as  
69 in Alcatraz et al. (2001). Thus, a low piston velocity value was used ( $k_{low} = 0.00936 \text{ m h}^{-1}$ ) that  
70 corresponded to the lowest value obtained under nonnull turbulent conditions, as we considered that  
71 turbulence would not be zero in the mesocosms due to the external waves and pumping effects, and a  
72 high piston velocity value was also tested ( $k_{high} = 0.02322 \text{ m h}^{-1}$ ).

73 The instantaneous metabolism seemed to be affected by the value of  $k$  used for its calculation (Supp  
74 Fig. 1a). For example, the instantaneous NCP was the highest using  $k_{high}$  during the Positive NCP  
75 period. On the other hand, the instantaneous NCP was closer to 0 using  $k_{high}$  during the Negative  
76 NCP period (Supp. Fig. 1a). The daily GPP values obtained with  $k_{high}$  and with  $k_{low}$  were not  
77 significantly different (Supp. Fig. 1b) (ANOVA,  $p = 0.98$ ), while the R24h value obtained with  $k_{high}$   
78 was significantly higher than that obtained using  $k_{low}$  (ANOVA,  $p = 0.02^*$ ). Consequently, the daily  
79 NCP, which is the difference between GPP and R24h, was significantly greater when  $k_{high}$  was used  
80 (ANOVA,  $p = 0.01^*$ ).



81

82 **Supp. Figure 1.** (a) Instantaneous positive and negative NCP on day 3 (C1-Oct) calculated with khigh  
 83 = 0.02322 m.h-1 (light blue) and klow = 0.009326 m h-1 (dark blue). (b) Daily estimates of GPP, NCP  
 84 and R calculated with khigh (dark blue) and klow (light blue). The levels of significance from  
 85 ANOVA are indicated with \* (p < 0.05 \*, p < 0.01 \*\*, p < 0.001 \*\*\*). For each box, the lower  
 86 quartile, median, and upper quartile values are displayed with horizontal lines. Whiskers show the  
 87 range of the data, from the minimum to the maximum.

88

89 The instantaneous NCP value during Positive and Negative NCP periods was increased and decreased,  
 90 respectively, by a greater k value. This result explains why the daily GPP was not significantly  
 91 affected, as it is estimated using data from both the Positive and Negative NCP periods. The effects of  
 92 the piston velocity value on production were counterbalanced by its effects on daytime respiration,  
 93 resulting in an almost unaffected GPP estimate. However, R24h is calculated using only the

94 instantaneous NCP from the Negative NCP period. Hence, the magnitude of  $k$  affects R24h estimates  
95 in the same way that it affects instantaneous NCP estimates during Negative NCP periods.

96 To conclude, the present analysis highlights the fact that the method using high-frequency data to  
97 estimate metabolic parameter is sensitive to the air-water exchange coefficient value. This work  
98 underlines the need for a reliable estimation of the air-water exchange coefficient to obtain precise  
99 metabolic parameters. The dependence of the free-water method on an accurate estimation of the air-  
100 water exchange coefficient is one of the weaknesses of this method, and future research should focus  
101 on this topic. However, the aim of mesocosm experiments is to assess the effects of one or several  
102 simulated disturbance(s) on the studied system and therefore to compare control mesocosms with  
103 mesocosm(s) in which the disturbance(s) was applied. Hence, even if the air-water exchange is under-  
104 or overestimated, it will be estimated in the same way in all mesocosms. The comparison between the  
105 control and the other mesocosms will thus not be affected by the uncertainty related to the air-water  
106 exchange coefficient as long as the same coefficient is applied for all mesocosms and all mesocosms  
107 experience similar environmental conditions.

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