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3 **Abstract**

4 To evaluate the influence of temperature along salinity gradients on metabolic performance, we measured
5 respiration rate of fully acclimatized adults of invasive ctenophore *Mnemiopsis leidyi* under similar field
6 conditions representative for the Baltic Sea. Three levels of temperature (3, 8 and 17°C) and three levels of
7 salinity (12, 17 and 22‰) were considered. Oxygen consumption was temperature and not salinity dependent,
8 albeit combinations of temperature and salinity showed a significant effect on carbon specific respiration rate.
9 Q_{10} value of 3.67 indicates a strong impact of temperature on metabolic regulation. The role of combined effect
10 of temperature and salinity on over-wintering population in higher latitudes was discussed.

11

12 **Introduction**

13 Due to a global increase in abundance of gelatinous zooplankton, their future ecological role became a
14 matter of concern for marine ecologists (Jackson 2008). During the last decades, several invasion cases of
15 gelatinous carnivores (medusae of coelenterates and/or ctenophores) have been documented (e.g. Greve 1994,
16 Bolton & Graham 2004, Boersma et al. 2007, Fuentes et al. 2010). Their life history traits enable gelatinous
17 zooplankton to pass through different invasion processes and overcome several ecological barriers (Boero et al.
18 2008). Impact of gelatinous plankton and magnitude of their predatory potential became highly conspicuous
19 when the comb jelly *Mnemiopsis leidyi*, was introduced into the Black Sea, presumably via ballast water tanks,
20 leading to a massive development of this species (Shiganova 2001). *M. leidyi* is known as a generalist predator
21 due to its high clearance rate on different types of food, high reproductive potential (self-fertilizing
22 hermaphrodite, several 100 eggs/ ind day⁻¹ under optimal conditions), wide tolerance limits for salinity (2-38
23 and temperature (0-32°C) and also high tolerance for low oxygen concentration (Purcell et al. 2001). This
24 species was observed in the Baltic Sea for the first time in fall 2006 (Javidpour et al. 2006) and has established
25 its population, at least in the Western Baltic Sea (Jasper et al. 2011), albeit *M. leidyi* faces with harsh
26 environmental conditions i.e. a high spatio-temporal range of salinity and temperature in different biota of the
27 Baltic Sea.

28 Here we present first quantitative data on carbon specific respiration rate of *M. leidyi* under different
29 salinity and temperature of the Baltic region. We also provide first data on carbon unit requirement and carbon
30 turnover rates of *M. leidyi* in this area.

31 **Material & Methods**

32 *Collection of M. leidyi*- Ctenophores were collected twice from Kiel Fjord (54°19,7' N, 10°09,5' E,
33 Fig.1) by vertically towing a WP2 net from 10 m depth (500 µm mesh-size with 0.8 meter diameter) in January
34 2007. Healthy individuals were isolated immediately in a beaker at ambient temperature and salinity (T=3°C and
35 S=22). Individuals were acclimated under ambient temperature (3°C) over six hours via stepwise dilution to
36 designed salinities. *M. leidyi* is an osmo-conformer that needs a few hours to re-establish its equilibrium
37 buoyancy (Yazdani Foshtomi et al. 2007), thus acclimation time of six hours is supposed to be sufficient for
38 different salinities. Later on already acclimated individuals to salinity treatments were exposed gradually (in 1-3
39 hours intervals) to different temperature of 8° or 17° C by using a temperature controlled climatic chamber. Our
40 design for salinity and temperature combination represents the spatio-temporal range of the Western Baltic Sea.

41 Respiration experiment was started after 24 hours of acclimation process, when organisms seem to be
42 adapted to experimental conditions (active swimming and searching for food),. For experimental use, only
43 undamaged adult specimens of about the same size (17.4 ± 1.1 mm; n = 72) were selected.

44 *Oxygen consumption*- to measure respiration rate of *M. leidyi*, two individuals were transferred into a
45 270 ml Winkler bottle, filled with 0.25 µm filtered and autoclaved seawater of incubation salinity and
46 temperature. To guarantee oxygen saturation at the beginning of the experiment, seawater was aerated for ten
47 minutes prior to the experiment. The bottles were sealed air- free and placed into climatic chambers for 24 hours.
48 In the chambers, temperature was held constant within a range of +/- 0.25°C. The whole incubation period was
49 performed in darkness. Each temperature-salinity treatment consisted of four replicates plus four controls (i.e.
50 bottles without *M. leidyi*). Oxygen consumption was measured using the Winkler technique (Omori & Ikeda
51 1984). During incubation period, ctenophores were checked for vitality by observation of the regular movement
52 of the comb-rows. All 72 individuals were vital at the end of incubation. *M. leidyi* was filtered with a 1mm mesh
53 and wet weight (WW) was determined. Thereafter specimens were left to dry for 48 hours at 60°C to measure
54 dry weight (DW). Carbon and nitrogen content were determined via combustion in a gas chromatograph.

55 To test the effect of a 10° change in temperature on carbon specific respiration rate (Q_{10}) second experiment with
56 the same set up as mentioned above was designed. In this experiment, we applied only one salinity (S=22,
57 ambient salinity) and 3 levels of temperature including 3.5°, 10° and 20.5°C. We then combined the results with
58 our previous experiment of the same salinity in 8° and 17° C in order to obtain a five point exponential
59 regression.

60 To convert oxygen consumption of zooplankton into carbon units, a respiratory quotient of 0.97 (ICES 2000)
61 was applied to transform the carbon specific respiration rate into carbon specific carbon consumption rate (µg of
62 carbon metabolized per mg of carbon body content).

63 *Data processing*- oxygen consumption data were normalized to the milligrams of body carbon of the
64 ctenophores and to the incubation time in order to obtain carbon specific respiration rate (Schneider 1992).
65 Factorial ANOVA was used to examine main as well as interaction effects of the factors salinity and temperature
66 on respiration rate.

67 **Results**

68 *Biometric Data*- Fig. 2 provides information on the relationship between total length and WW. It can be
69 expressed with the following equation:

$$70 \quad WW=0.0029*L^{1.962} \quad (r^2_{adj}=0.855 \quad n=84; \quad p<0.01)$$

71 where L= total length including lobes in mm

72 The relationship between DW and WW showed a positive trend. To see whether this relationship is significantly
73 salinity dependent, an analysis of covariance (ANCOVA) was conducted in which the independent variable was
74 salinity, the dependent variable DW, and the covariate WW. The assumptions for ANCOVA were fully met.
75 Salinity showed a significant effect on DW/WW relationship, indicating a significant increase in DW under
76 higher salinity for the same WW ($F_{(3, 81)}=3011$, $p<0.01$, Fig.3). However, salinity shows no significant effect on
77 the individual body carbon (One- Way ANOVA, $F_{(2, 82)}=1.441$, $p=0.24$). The same is true for the body nitrogen
78 of individuals (One- Way ANOVA, $F_{(2, 82)}=1.32$, $p=0.27$). Regression analysis was applied for different levels
79 of salinity among DW/WW data (Table 1). S. 1 provides a comparison between different studies.

80 *Oxygen consumption, salinity effect*- Because WW and DW of individuals of *M. leidy* co-vary with salinity,
81 oxygen consumption data were normalized to milligrams of body carbon of the ctenophores and to the
82 incubation time in order to obtain carbon specific respiration rate (i.e. $\mu\text{mol O}_2$ per mg C $\text{ind}^{-1} \text{h}^{-1}$). Both
83 Temperature, salinity and the combined effect of them were significant (see table xxx)

84 *Q₁₀*- For the temperature range of 3.5° to 20.5°C in salinity of 22 (Fig.5) we found the exponential regression
85 equation of

$$86 \quad \text{Respiration } (\mu\text{mol O}_2/\text{mg Carbon ind}^{-1} \text{h}^{-1}) = 0.0298 e^{0.13T} \quad (r^2_{adj}=0.8; \quad p<0.01)$$

87 Whereby we have calculated Q_{10} of

$$88 \quad Q_{10}=e^{0.13*10}=3.67$$

89 *Energetic requirements* – Based on the ICES Zooplankton Methodology Manual of 2000, a respiratory quotient
90 of 0.97 is assumed to convert oxygen consumption of zooplankton into carbon units (ICES 2000). This allows a
91 transformation of the carbon specific respiration rate into carbon specific carbon consumption rate (μg of carbon
92 metabolized per mg of carbon body content). For the exponential function of respiration rate at salinity of 22 this
93 yields:

94 $0.0298e^{0.13*T} * 0.97 * 12 = 0.35e^{0.13*T}$ ($\mu\text{g Carbon/mg body Carbon ind}^{-1} \text{h}^{-1}$)

95 This means that at 20° C and salinity of 22, an adult *M. leidyi* turns over 11.2 % of its body carbon per day and
96 therefore its whole body carbon every 214 hours (~9 days), working at basal metabolism. Table 3 provides
97 values of turnover rates under different temperatures based on Q_{10} .

98 **Discussion**

99 *Biometric Data-* For length /WW measurement in this study, we have used a size range of 0.5-30 mm,
100 because *M. leidyi* population of the western Baltic is dominated by small sized individuals of 20 ± 0.3 mm in
101 average (Javidpour *et al.* 2008). Mutlu (2009) underlined that length-WW of *M. leidyi* is highly dependent to
102 spatio-temporal size composition of the species, though the regression equation we have gained in this study can
103 be used for parameterization of the Baltic population. In this study lobes were included for biomass estimation
104 that varies with nutrition conditions (Reeve and Walter 1978). Despite, the slop calculated from our data is as the
105 same as one calculated for native population (Kremer and Nixon 1976).

106 *Oxygen consumption – salinity effects-*The basal metabolic rate is the energy required by an organism to
107 maintain vital functions, without growing, digesting, reproducing or any other activities that demand additional
108 energy. Oxygen consumption rate is considered as a good parameter to approximate whole organism metabolic
109 rate of a heterotrophic organism, because heterotrophs mainly gain their energy by oxidizing carbon compounds
110 with oxygen to carbon dioxide and water (Brown, 2004). Thus, in case a change in one parameter requires a
111 higher oxygen consumption rate and if this change does not come along with a raise in energy (food) supply, this
112 new condition leads to lose of energy reservoir. This means that an organism will turn over its body carbon faster
113 and, therefore, has less chance to withstand a period of starvation. For *M. leidyi*, the optimum salinity at a given
114 temperature, should therefore be the one that provokes least oxygen consumption. Experimental data from the
115 current study suggests that respiration response to temperature might be salinity dependent. At higher
116 temperatures the higher salinity leads to highest respiration, however at colder condition it is the lower salinity
117 which leads to lower respiration (Fig. 4 and Table 3). This finding might partly explain the distribution pattern of
118 *M. leidyi* within its native and invasive ecosystems. Purcell *et al.* (2001) report that at low winter temperatures in
119 Chesapeake Bay waters, ctenophores were only found in higher salinities (> 6) while in summer they could be
120 found in waters with a salinity of 2 (Purcell *et al.*, 2001). Furthermore, local populations of *M. leidyi* in winter
121 are found only in the deepest parts of Kiel Fjord, where salinity is somewhat higher (2-4 units) than at surface
122 (Javidpour *et al.* 2008), albeit in summer most individuals are found in surface waters of lower salinity. On the
123 other hand, there are evidences that a seasonal vertical migration of *M. leidyi* to deeper depths is an indication of
124 a seasonal refuge against rough surface conditions (Kremer and Nixon 1976). We have to confess that *in vitro*

125 measurements of respiration under manipulated conditions and the fact that individuals might still be stressed
126 while using them for experiment, should not be interpreted as actual oxygen consumption rate of *M. leidyi*,
127 however we believe that the pattern of changes in respiration rate among different salinity gradients has to be
128 take into account, as our experimental preparation was tried to be as constant as possible. Moreover, salinity
129 dependent reproduction of *M. leidyi* has been just recently reported which might be also due to metabolic
130 response (Jasper et al. 2011). Thus, we believe further field based measurements combined with experimental
131 one is needed to justify our assumption.

132 *Oxygen consumption – temperature effects and Q_{10} .* The Q_{10} established in our experiment was within
133 the expected range for gelatinous plankton (Svetichny et al., 2004). Kremer found a Q_{10} of 3.67 for *M. leidyi* in
134 its native habitat for a temperature range of 10.3 to 24.5 °C (Kremer, 1977). Carbon specific respiration rate
135 measured in current study was about 30% higher than that measured by Kremer (see Table 1). The difference is
136 not very pronounced and could be due to differences in experimental conditions, like feeding status of the
137 ctenophores or smaller sizes of individuals we have used. Another factor which can affect our results is
138 nutritional states of *M. leidyi* that can change the respiration rate by a factor of 1.7 to 2 folds higher at a given
139 temperature (Kremer 1982, Abolmasova 2002). Overall, the temperature dependant response of oxygen
140 consumption in *M. leidyi* of the Kiel Fjord is very similar to that of *M. leidyi* in its native habitat (Kremer 1977).

141 Our results accentuate the importance of how to display respiration rate of ctenophores, considering the
142 salinity dependence of WW/DW relationship. *M. leidyi* is an osmo-conformer over a wide range of salinities.
143 While the water is evaporated to measure dry weight the salt is retained together with organic matter. If
144 respiration rates are related to dry weight, they are not only related to organism biomass, but also to salt, which is
145 non-respiring matter. There is no agreement to apply a unit for weight specific respiration rate that sometimes is
146 expressed in $\mu\text{g O}_2/\text{mgWW h}^{-1}$ (Svetichny et al. 2004), $\text{ml O}_2/\text{gDW h}^{-1}$ (Abolmasova 2002) and in $\mu\text{g-atoms}$
147 $\text{O}_2/\text{gDW h}^{-1}$ (Kremer, 1977). If all relationships of the biometric data have been measured and indicated in
148 publications, it would be possible to convert one rate into another, but if that point has been missed, respiration
149 data might be incomparable (Schneider, 1992). Therefore, the use of dry weight related respiration rate for
150 comparison of metabolism is misleading. We strongly recommend the use of carbon specific metabolic rate to
151 make measurements comparable between two studies.

152 *Energetic requirements-* winter conditions in severe environments of high latitudes might be an obvious
153 bottleneck for the establishment of planktonic populations. Overwintering populations of *M. leidyi* are composed
154 mainly by adults that sink to the deeper layer where salinity and temperature are both higher. In 2010, the Kiel
155 Fjord faced a long period of frost compared to the last five years, after the first observation of this species in the

156 western Baltic Sea. Even during extreme cold winter conditions, in which water temperature was in average 1-2°
157 C (already with ice clumps on the surfaces), we could find relatively high density (10 ind m⁻³ in average) of *M.*
158 *leidyi* in the deep part of the Kiel Fjord (Javidpour unpublished data). Carbon turnover rate of more than 80 days
159 calculated for 3°C might explain an overwinter strategy of *M. leidyi* (Table 3). It shows that adult individuals are
160 able to survive the entire winter without feeding and by working at basal metabolism. The values of carbon
161 turnover rate presented here are quite comparable with those of native population (Kremer 1977). It is still not
162 clear whether *M. leidyi* could feed on the benthic food sources during winter; albeit it was found that adults
163 reduce their size and shrink to larval size due to starvation (Reeve & Walter 1978).

164 **Legends**

165 Table 1- Relation of wet weight (WW), Nitrogen (N) and Carbon (C) in relation to dry weight (DW) for the three
166 different salinities.

167 Table 2 – Comparison of bioconversions obtained in this study with previous studies (DW = dry weight in g;
168 WW = wet weight in g; C%DW = carbon as percentage of dry weight; N%DW = nitrogen as percentage of dry
169 weight).

170 Table 3- Values of carbon specific respiration rate of *M. leidyi* for different temperature and salinities.

171 Table 4- Carbon turnover rate (days) for different temperature calculated based on carbon specific respiration
172 rate.

173 Fig. 1 - Western Baltic and the Kiel Fjord

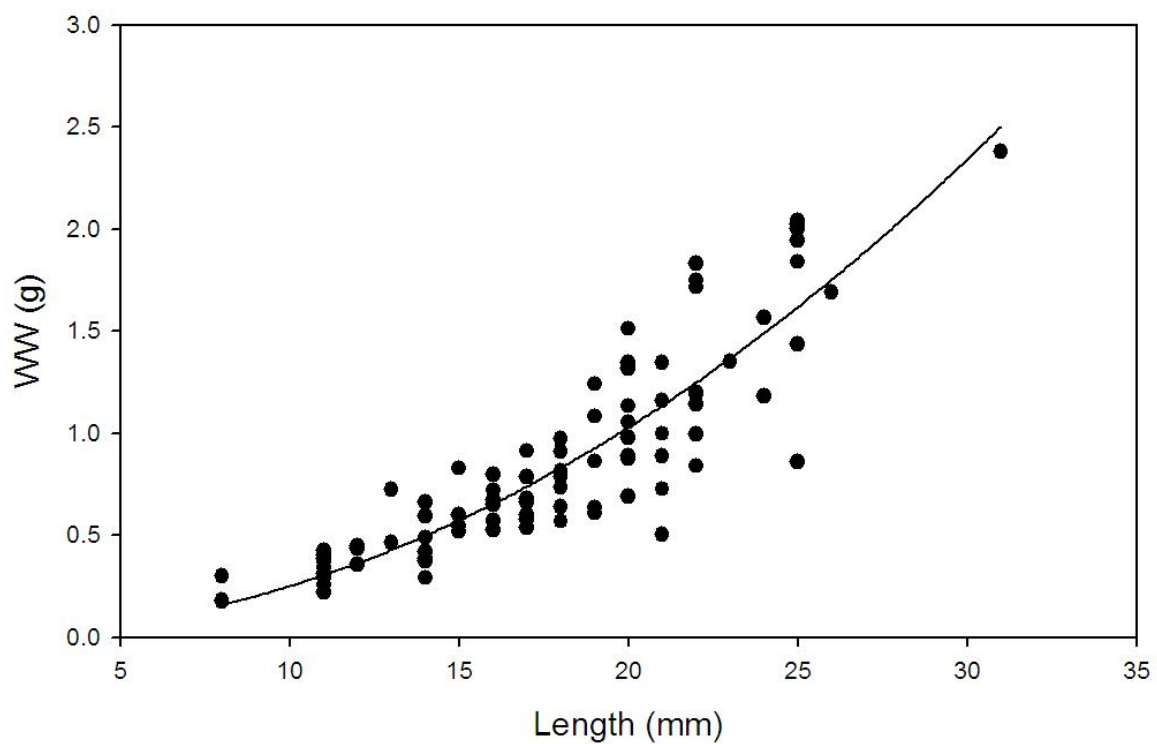
174 Fig. 2- Relationship between wet weight (in grams) and total length (in millimetres) of *M. leidyi* under natural
175 salinity of 22.

176 Fig.3- Relation of wet weight (WW) and dry weight (DW) for the three different salinities tested.

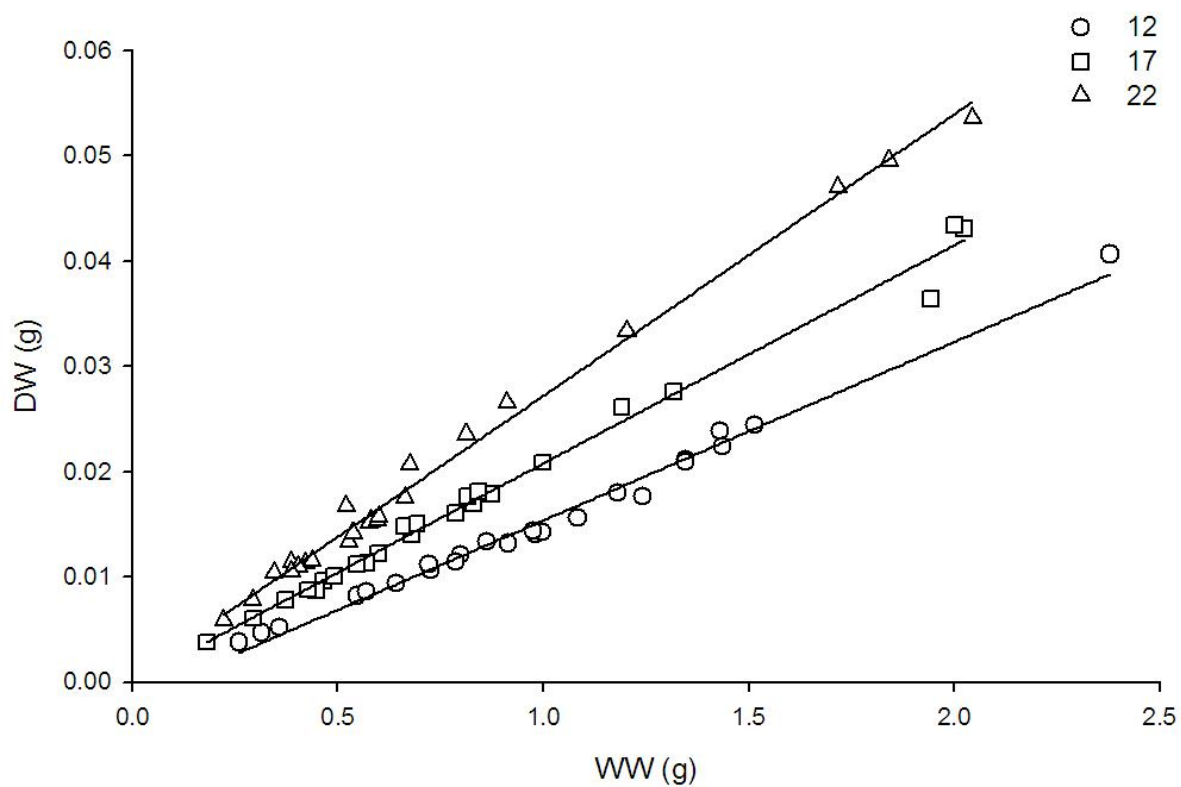
177 Fig. 4 - Carbon specific respiration rate ($\mu\text{molO}_2/\text{mgCarbon h}^{-1}$) of *M. leidyi* under different temperatures and
178 salinities.

179 Fig. 5 - Respiration rate in $\mu\text{molO}_2/\text{mgCarbon h}^{-1}$ for the five temperature treatments with exponentially fit
180 (salinity = 22)

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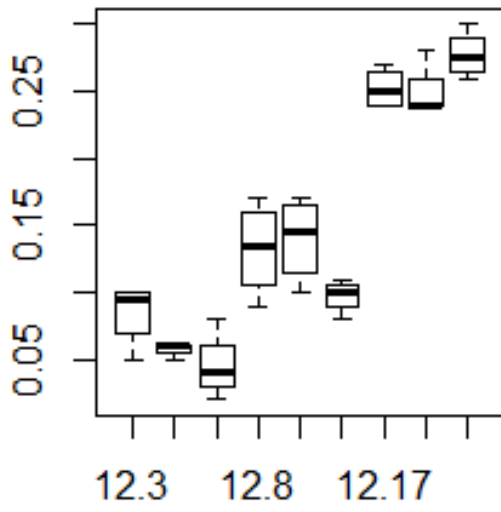
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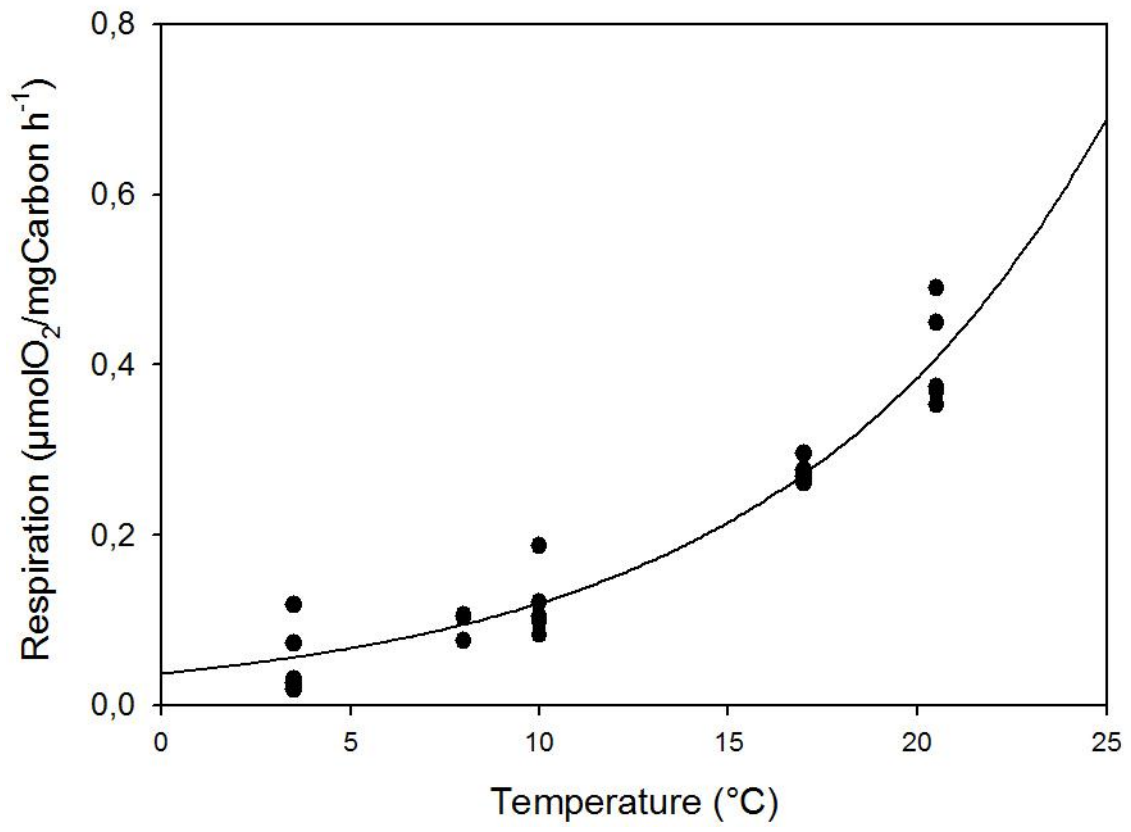
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