Supplementary material

Metabolic rate thermal plasticity in the marine annelid *Ophryotrocha labronica* across two successive generations

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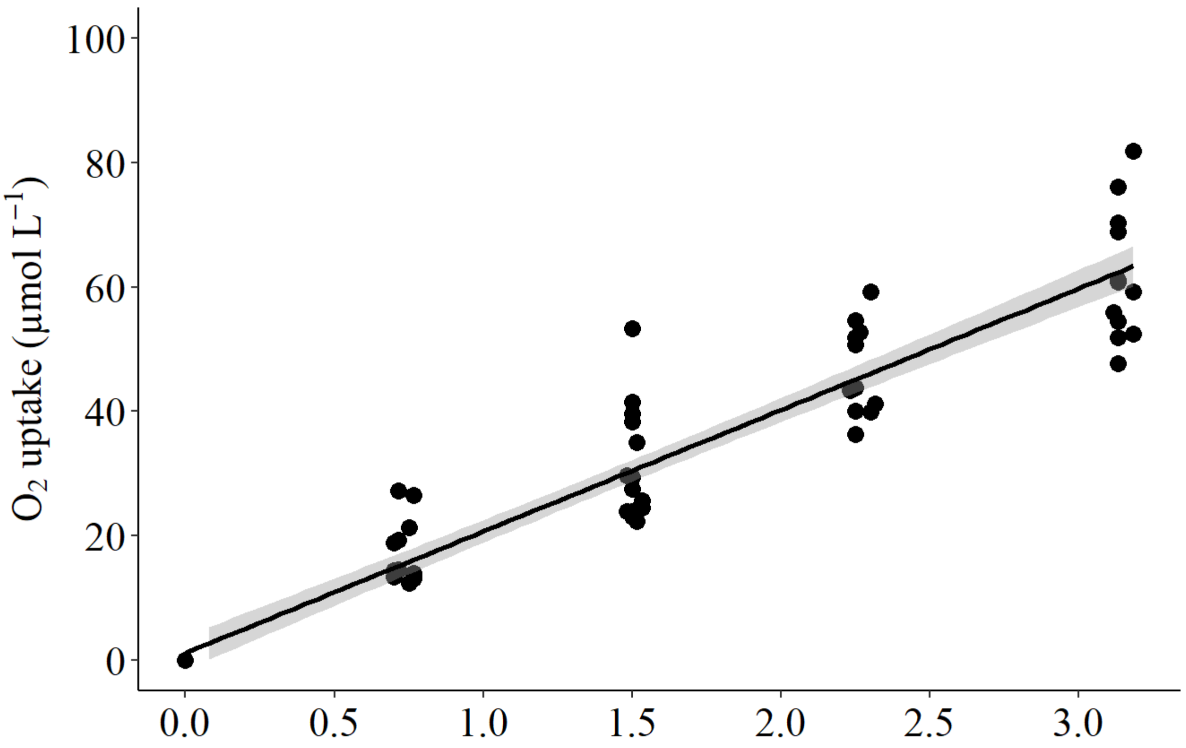
Appendix S1. Mean values and standard deviation (SD) of temperature and salinity conditions experienced by specimens of the marine annelid *Ophryotrocha labronica*, based on measurements performed every 2 d and averaged over the entire exposure time. n = sample size.

Temperature (°C)			**Salinity**		
Mean	**± SD**	**n**	**Mean**	**± SD**	**n**
20.6	1.97	84	32.4	1.3	81
24.1	0.36	95	33.2	1.9	86
26.3	0.47	114	32.4	1.8	113
29.3	0.59	156	32.7	2.3	124

Appendix S2. Linearity of the relationship between individuals' oxygen uptake and incubation time

Metabolic rates (MO_2) were quantified in our study by measuring the oxygen (O_2) uptake taken at two single moments along the incubation period. The reliability of this approach was assessed by testing the linearity of the relationship between O_2 uptake and incubation time (Marginal R^2 / Conditional R^2 = 0.91 / 0.96, df = 1, $P <$ 0.001, Fig. S1) for a subset of 18 specimens of *Ophryotrocha labronica* not used for the experiment**.** Specifically, individual O_2 uptake was measured at 24 °C every 30 min for approx. 3.5 h (between 100 and 50 % air saturation) and quantified as the difference between the O_2 concentration measured at the beginning of the trial and that measured at a specific time along the incubation. Values were corrected for the background respiration by subtracting the mean O_2 uptake measured at each round in six "blank" vials. The relationship between individual O_2 uptake and time was examined using a linear mixed model that included individual identity as random effect. Residuals were normally distributed (Shapiro test, $P > 0.05$) and homogeneity of variance was indicated by a random pattern in the residuals *versus* fitted plot.

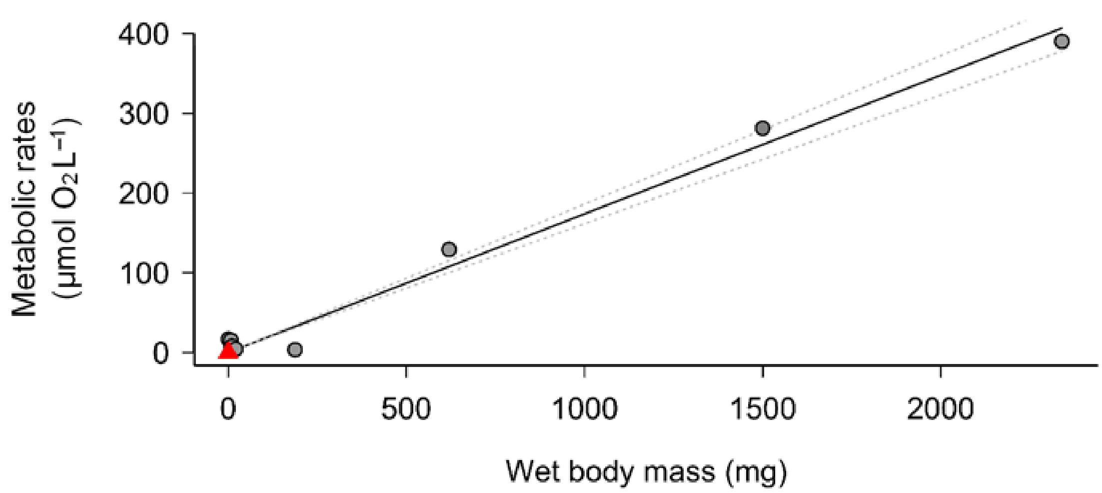
Fig. S1. Relationship between O_2 uptake and incubation time in individuals of *Ophryotrocha labronica* exposed to 24 °C (n = 18). Solid, black circles represent [O_2] measurements performed in a single vial, the black continuous line represents the regression line, and 95 % CI are indicated by the grey area.



Appendix S3. Validation of the metabolic rate measurements

Validation of the data on metabolic rates (MO_2) of *Ophryotrocha labronica* was achieved by comparing our measurements, obtained at 24 °C on 18 specimens used for assessing the linearity of the relationship between oxygen concentration and incubation time (see for details Appendix S2), with those found in the literature from other marine annelid species from temperate regions. Only species exposed to 24 or 25 °C were selected for further analyses: *Alitta succinea* (mass 187.5 mg, Sturdivant et al. 2015), *Amphiglena mediterranea* (mass 0.483 mg, Calosi et al. 2013a), *Lysidice collaris* (mass 10.9 mg, Calosi et al. 2013a), *Lysidice ninetta* (mass 7.8 mg, Calosi et al. 2013a), *Neanthes japonica* (mass 620.0 – 2340.0 mg, (Liu et al. 2009)), *Platynereis dumerilii* (mass 21.3 mg, Calosi et al. 2013a), and *Polyophtalmus pictus* (mass 7.2 mg, Calosi et al. 2013a). The relationship between MO_2 and wet body mass was assessed using a linear regression model and its 95 % CI, initially excluding our data. The intercept of the relationship was forced to 0, as no oxygen is consumed in the absence of biomass. We considered our data validated if it was included within the 95% CI around the regression curve. According to our results, MO_2 increased with wet body mass across species, following a linear trend ($R^2 = 0.99$, $F_{1, 8} = 766$, $p < 0.001$), and the predicted MO_2 value for an annelid species averaging the size of *O. labronica* (0.5 ± 0.05 mg [mean ± SE], Carignan, Beaudet and Debacker unpublished data) was predicted to be 0.008 µmol O_2 h^{-1} (CI = [0.007, 0.009]). The mean MO_2 value measured for *O. labronica* at 24 °C, 0.009 µmol O_2 h^{-1}, laid within this interval of confidence, thus validating the reliability of our measurements (Fig. S2).

Fig. S2. Relationship between metabolic rates (MO_2) and wet body mass in temperate marine annelids exposed to 24–25 °C. The continuous black line represents the linear regression curve, the area delimited by the dashed lines its 95% CI, and the red triangle the mean MO_2 experimentally measured at 24 °C in our study for a subset of individuals of *Ophryotrocha labronica* (n = 18).



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Liu Y, Xian W and Sun S (2009) Metabolism of polychaete *Neanthes japonica* Izuka: relations to temperature, salinity and body weight. *Chinese Journal of Oceanology and Limnology* **27(2)**, 356–364.

Sturdivant SK, Perchik M, Brill RW and Bushnell PG (2015) Metabolic responses of the Nereid polychaete, *Alitta succinea*, to hypoxia at two different temperatures. *Journal of Experimental Marine Biology and Ecology* **473**, 161–168.

Appendix S4. Summary of the results of the linear regression models with dAIC ≥ 2 investigating the relationship between metabolic rates (MO_2) and Temperature (continuous variable) across two successive generations in *Ophryotrocha labronica*, controlling for the effect of sex and body size. Values of delta AIC (dAIC) are provided relative to the most parsimonious model (Table 2a in the main manuscript). Predictors with a significant relationship with MO_2 are highlighted with bold text. DF = Degrees of Freedom (numerator; denominator). R^2 = adjusted R–squares.

a) MO_2 ~ Body size + Sex + Generation + Temperature

Coefficient	Estimate	DF	*t*–value	*P*–value	Model summary	AIC	dAIC
Intercept	–0.006	1;126	–1.41	0.16	$F_{4,121}$ = 5.22	–1069.1	2.05
Body size	0.0003	1;126	1.76	0.08	R^2 = 0.10		
Sex	–0.001	1;126	–1.42	0.16	*P*–value = 0.002		
Generation	0.0001	1;126	0.18	0.86			
Temperature	**0.0003**	**1;126**	**2.79**	**0.006**			

b) MO_2 ~ Body size + Generation * Temperature

Coefficient	Estimate	DF	*t*–value	*P*–value	Model summary	AIC	dAIC
Intercept	–0.01	1;126	–1.52	0.13	$F_{4,121}$ = 4.04	–1067.7	3.44
Body size	**0.001**	**1;126**	**2.87**	**0.005**	R^2 = 0.09		
Generation	–0.004	1;126	–0.74	0.46	*P*–value = 0.004		
Temperature	0.0002	1;126	1.51	0.13			
Generation * Temperature	0.0001	1;126	0.73	0.46			

c) MO_2 ~ Body size + Sex + Generation * Temperature

Coefficient	Estimate	DF	*t*–value	*P*–value	Model summary	AIC	dAIC
Intercept	–0.004	1;126	–0.89	0.37	$F_{5,120}$ = 3.64	–1067.7	3.41
Body size	0.0003	1;126	1.81	0.07	R^2 = 0.10		
Sex	–0.001	1;126	–1.4	0.16	*P*–value = 0.004		
Generation	–0.004	1;126	–0.68	0.5			
Temperature	0.0002	1;126	1.48	0.14			
Generation * Temperature	0.0001	1;126	0.7	0.48			

d) MO_2 ~ Body size + Sex + Generation

Coefficient	Estimate	DF	*t*–value	*P*–value	Model summary	AIC	dAIC
Intercept	0.002	1;126	0.78	0.44	$F_{3,122}$ = 3.17	–1063.4	7.76
Body size	0.0003	1;126	1.37	0.17	R^2 = 0.05		
Sex	–0.001	1;126	–1.54	0.13	*P*–value = 0.03		
Generation	0.0004	1;126	0.63	0.53			

e) MO_2 ~ Body size + Generation

Coefficient	Estimate	DF	*t*–value	*P*–value	Model summary	AIC	dAIC
Intercept	–0.0003	1;126	–0.11	0.91	$F_{2,123}$ = 3.54	–1063	8.17
Body size	**0.0004**	**1;126**	**2.44**	**0.02**	R^2 = 0.04		
Generation	0.0002	1;126	0.37	0.71	*P*–value = 0.03		

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