

Research Article

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

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Abstract

Marine ectotherms have evolved a range of physiological strategies to cope with temperature changes that persist across generations. For example, metabolic rates are expected to increase following an acute exposure to temperature, with potential detrimental impacts for fitness. However, they may be downregulated in the following generation if offspring experience the thermal conditions of their parents, with a resulting decrease in maintenance costs and fitness maximization. Yet, trans-generational studies on metabolic rates are few in marine ectotherms, thus limiting our ability to accurately predict longer-term implications of ocean warming on organisms’ performance, metabolic rates being the fundamental pacemaker for all biological processes. This is particularly true for small-size organisms, for which the determination of individual metabolic rates has been historically challenging, and for many groups of marine invertebrates, such as annelids, which are under-represented in physiological investigations. Here, we exposed the subtidal annelid *Ophryotrocha labronica* (body length: ~4 mm) to a thermal gradient (21, 24, 26, 29 C) and measured, for the first time in this species, the temperature dependence of metabolic rates across two generations. We found that metabolic rates were positively related to temperature, but this relationship did not differ between generations. Our study provides additional evidence for the diversity of temperature-associated physiological responses of marine ectotherms and offers a number of methodological recommendations for unveiling the mechanisms underpinning the observed trans-generational responses of metabolic rates in marine annelid species.

Introduction

Phenotypic plasticity is a ubiquitous mechanism enabling organisms to rapidly respond to environmental changes *via* modifying their phenotype without changes to their genotype (West-Eberhard, 1989). Phenotypic plasticity of physiological traits (hereafter physiological plasticity) is increasingly investigated for its key role in mediating marine ectotherms’ responses to climate-associated environmental changes, such as the increase in the mean and variation of global, regional and local ocean temperatures. Environmental temperature, in fact, has a primary importance in defining the physiological status of marine ectotherms (Pinsky *et al.*, 2019), and organismal physiology provides in turns the mechanistic link between ecological processes and their susceptibility to ocean warming (Helmuth, 2009; Somero, 2010; Godbold & Calosi, 2013; Bozinovic & P ortner, 2015). Accordingly, a greater effort should be devoted to defining the role of physiological plasticity in mitigating, or reversing, the negative effects of ocean warming on marine organisms across generations (Munday *et al.*, 2013; Calosi *et al.*, 2016).

Plasticity in metabolic rates plays a central role for the mechanistic understanding of marine ectotherms’ responses to thermal changes. Metabolic rates are the fundamental pacemaker for all biological processes and represent the overall rate of energy uptake, transformation and allocation in living systems (Brown *et al.*, 2004; Glazier, 2015). Consequently, they are among the most representative and historically used proxy for the estimation of the physiological cost of life (Brown *et al.*, 2004). Generally, metabolic rates are strongly affected by temperature variation due to the inherent temperature sensitivity of biochemical reactions that govern the pace of metabolism (Gillooly *et al.*, 2001; Brown *et al.*, 2004; Clarke, 2004; Clarke & Fraser, 2004). This ‘passive’ plasticity is commonly observed under acute temperature exposure (Gillooly *et al.*, 2001; Havird *et al.*, 2020). Under this condition, the rate of metabolic reactions, as well as the associated oxygen demand and uptake which are necessary to support cellular respiration, increase with increasing temperature before rapidly declining when temperature surpasses suboptimal levels: a response best expressed *via* thermal performance curves (Magozzi & Calosi, 2015; Schulte, 2015). The ecological importance of defining metabolic rates to help assessing species’ sensitivity to climate change has been reinforced in the last decades by the discussion around the ‘oxygen and capacity-limited

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thermal tolerance' (Pörtner, 2001). This hypothesis emphasizes the importance of oxygen delivery efficacy in setting organisms' critical temperatures, at which transition between aerobic and anaerobic metabolism occurs and within which trade-offs between reproduction, growth and feeding may happen. Accordingly, ocean warming is expected to severely affect ectotherms' metabolic rates (Dillon *et al.*, 2010), potentially resulting in reduced physiological performance and fitness (Pörtner & Farrell, 2008; Dell *et al.*, 2011). This said, marine ectotherms have evolved a range of mechanisms to cope with both extreme temperature changes (e.g. heatwaves) and long-lasting warming. If the exposure to the new thermal condition persists, organisms can adjust their metabolic rates through acclimation, an 'active' plastic adjustment that can reduce or neutralize the influence of temperature on metabolic rates *via* compensatory responses (Careau & Garland, 2012; Pettersen *et al.*, 2018). The result is a decrease in maintenance costs and re-allocation of energy for the expression of other traits affecting the vital rates of the individual, such as growth and reproduction (Steyermark, 2002; Shama *et al.*, 2014). Acclimation responses are particularly important for the resilience of marine ectotherms to ocean warming, as they allow organisms to perform efficiently over a larger thermal range (Einum *et al.*, 2019).

Despite the importance of metabolic rates as predictors of marine ectotherms' capacity to withstand and respond to ongoing ocean warming (Donelson *et al.*, 2012; Magozzi & Calosi, 2015; Putnam & Gates, 2015), this physiological response has been less frequently characterized in trans-generational studies when compared with life-history traits or other proxies of metabolic adjustment, such as mitochondrial respiration (see Table 1 in Donelson *et al.*, 2018). What we know from the literature so far is that when temperature increases beyond a species' optimal condition, the consequent increase in metabolic rate is commonly accompanied by a negative impact on individual fitness (e.g. Donelson *et al.*, 2012; Shama *et al.*, 2014). However, if exposure is extended to the next generation, offspring may have the ability to take advantage of the parental exposure by reducing their metabolic rate – a mechanism known as trans-generational plasticity – with a resulting decrease in the energetic demand necessary for the maintenance of the organism (Donelson *et al.*, 2012; Shama *et al.*, 2014).

There is a limited number of studies characterizing metabolic rate plasticity across generations in small-size marine ectotherms and, more in general, in marine invertebrates, thus limiting our ability to accurately predict the longer-term implications of ocean warming on marine organisms' performance. To contribute towards filling this gap, we assessed here the thermal plasticity of metabolic rates of the interstitial marine annelid species *Ophryotrocha labronica* La Greca & Bacci, 1962 (Paxton & Åkesson, 2007; adult body size ~4 mm in length) across two successive generations. Specifically, we measured individual metabolic rates, for the first time in this species, following exposure to a gradient of constant temperatures chosen within the species' natural habitat thermal window. *Ophryotrocha labronica* is a widespread, subtidal species (Simonini *et al.*, 2009) that is emerging as a model organism for experimental investigations of multigenerational effects of global changes in marine organisms (Chakravarti *et al.*, 2016; Rodríguez-Romero *et al.*, 2016; Gibbin *et al.*, 2017a, 2017b; Jarrold *et al.*, 2019; Thibault *et al.*, 2020). Based on previous experimental observations on trans-generational metabolic rate changes (Donelson *et al.*, 2012; Shama *et al.*, 2014), we predict to observe in *O. labronica* a temperature-dependent increase of metabolic rates in the first generation of exposure, followed by their downregulation in the second generation, a pattern that would suggest the occurrence of a full or partial

Table 1. Mean metabolic rates of *Ophryotrocha labronica* expressed as oxygen uptake rates for the parental (F_0) and offspring (F_1) generations along a gradient of four different temperatures

Generation	Temperature (°C)	N (indiv.)	Mean MO_2 ($10^{-3} \mu\text{mol h}^{-1}$)	SE
F_0	21	18	4.48	0.48
F_1	21	13	4.69	1.16
F_0	24	14	5.21	0.90
F_1	24	13	6.81	1.13
F_0	26	15	7.83	0.87
F_1	26	19	5.93	0.72
F_0	29	14	5.48	1.24
F_1	29	20	7.58	0.65

SE, standard error, N, sample size.

compensatory response mediated by trans-generational plasticity.

Materials and methods

Specimen collection and maintenance

Ophryotrocha labronica is a gonochoric species that colonizes coastal environments enriched in organic matter (Simonini *et al.*, 2009, 2010). Females lay eggs in tubular masses that are externally fertilized by males and cared for by at least one parent until they hatch (Paxton & Åkesson, 2007). In this study, we used a laboratory strain descended from ~60 individuals collected in the port of Gela, Italy (37°04'32.52"N 14°14'13.34"E), following the protocol described by Prevedelli *et al.* (2005). Prior to the experiment, the collected specimens were kept for four generations at 24°C, a temperature found within the natural thermal range (14–30°C) experienced by this species and considered optimal for laboratory rearing (Prevedelli & Simonini, 2001; Massamba-N'Siala *et al.*, 2011, 2012). This exposure aimed to reduce the influence of the thermal history of the experimental individuals on their responses to the experimental temperatures. Conditions of salinity (34‰) and photoperiod regime (12 h:12 h dark:light) were maintained constant throughout the experiment (Massamba-N'Siala *et al.*, 2011, 2012). Artificial seawater was prepared by mixing distilled water (type II) with artificial sea salt (Instant Ocean™, Blacksburg, VA, USA).

Experimental design

The F_0 generation of our experiment was composed of 60 reproductive pairs randomly taken from the cultures at the fourth generation of exposure to the reference conditions and formed before first reproduction occurred. Pairs were equally and randomly assigned to one of four experimental temperatures: 21, 24, 26 and 29°C. Each pair was isolated in one well of a six-well flat bottom culture plate (Tissue Culture Plates, VWR International, Radnor, PA, USA). After the first egg mass was laid, hatchlings (the F_1 generation) were transferred to a new plate immediately after being released from the egg mass and kept in the same conditions as their parents until they produced their first egg mass. Metabolic rates were measured individually on 13–20 specimens *per* generation *per* temperature treatment, and employing a similar number of individuals between males and females, immediately after the first reproductive event: ~28 days post-hatch at 21°C, 20 days at 24°C, and 10–15 days at 26 and 29°C. For all measurements, selected females bore no visible eggs in the

coelom, thus avoiding confounding effects associated with energy allocation toward reproduction (Ellis *et al.*, 2017).

The experimental temperatures were chosen within the thermal range experienced by the studied species at the collection site, and comprised the optimal range for survival, growth and reproduction (Prevedelli & Simonini, 2001). Constant temperature, salinity and photoperiod conditions were recreated inside environmental climatic chambers (MLR-352H-PA, Panasonic Healthcare Co. Ltd, Tokyo, Japan). Initial exposure was achieved by progressively increasing/decreasing temperature by a rate of 1°C h^{-1} from the rearing temperature (24°C) (Massamba-N'Siala *et al.*, 2012). Specimens were fed weekly *ad libitum* with minced spinach at a frequency and quantity that allowed all the spinach to be eaten, avoiding the accumulation of leftovers, the proliferation of bacteria or the accumulation of undesired compounds. Preliminary trials showed that water changes carried out every 2 days maintained stable salinity conditions and oxygen levels >70%. Temperature and salinity values were also measured every 2 days throughout the experiment with a high accuracy J/K input thermocouple thermometer (type K, HH802U, $\pm 0.1^{\circ}\text{C}$, Saint-Eustache, QC, Canada) and a portable refractometer (DD H₂Ocean, ± 1.0 , MOPS aquarium supplies, Hamilton, ON, Canada), respectively. Mean environmental values are reported in Supplementary Appendix S1.

Determination of metabolic rates

Metabolic rates (MO_2) were determined by using routine oxygen uptake rates as a proxy (Ege & Krogh, 1914), specifically allowing the specimens to move freely within the vials without any physical constraints causing stressful conditions. Individual MO_2 measurements were obtained by miniaturizing a technique based on the optical detection of molecular oxygen (Peck & Moyano, 2016), already used on larger-sized organisms (Marsh & Manahan, 1999; Papkovsky & Dmitriev, 2013; Noisette *et al.*, 2016). Individuals were transferred to a glass bowl containing filtered seawater to remove food and faecal particles, thus reducing microbial contamination and therefore background respiration. Seawater was filtered through Whatman® glass microfibre filters (grade GF/F 0.7 μm , GE Healthcare, Chicago, IL, USA). Specimens were individually transferred to modified borosilicate glass vials (volume $0.44 \text{ ml} \pm 0.004$) with push-in airtight glass caps (Natural SepCap, Thermo Scientific, Waltham, MA, USA). Each vial was then submerged and maintained at the tested temperature over the whole incubation period inside a temperature-controlled shaking water bath (VWR International) to prevent any form of water stratification around and in the vials. The size of each individual was measured after each trial by counting the number of chaetigers, i.e. the metameric segments bearing bristles (Massamba-N'Siala *et al.*, 2011). For each experimental run, 3–5 vials containing only filtered seawater (no individuals inside) were prepared following the same procedure described above. These vials were used as 'blanks' to determine background microbial respiration, whose average for each run was subtracted from associated individual MO_2 measurements to obtain more accurate estimates for annelids' oxygen uptake rates.

Each incubation lasted no more than 2.5 h and was halted when oxygen levels reached 70% saturation in the vials to avoid exposing specimens to hypoxic conditions. Oxygen measurements were taken at the beginning and at the end of the incubation period using a non-invasive fibre-optical system (FIBOX 4, PreSens, Regensburg, Germany) consisting of an external optical fibre probe and oxygen reactive dots, which were glued to the inner wall of each vial. Temperature was monitored continuously using a thermocouple (type K, HH802U, $\pm 0.1^{\circ}\text{C}$, Saint-Eustache, QC, Canada) mounted on a digital thermometer (HH802U,

Omega Eng. Inc.) and it was maintained at the designated experimental condition throughout the incubation.

Individual MO_2 ($\mu\text{mol h}^{-1}$) were calculated as the difference in oxygen concentration $[\text{O}_2]$ between the beginning and the end of the incubation using the following equation (1)

$$\text{MO}_2 = \frac{\Delta[\text{O}_2] \times V}{\Delta t} \quad (1)$$

where ΔO_2 ($\mu\text{mol O}_2 \text{ l}^{-1}$) is the difference between initial and final $[\text{O}_2]$, V (L) is the volume of the vial, and Δt (h) is the incubation time.

To assess the reliability of the MO_2 measurements taken at two single moments along the incubation period, we evaluated the linearity of the relationship between the annelids' oxygen consumption and incubation time (marginal R^2 /Conditional $R^2 = 0.91/0.96$, $\text{df} = 1$, $P < 0.001$) for a subset of 18 specimens of *O. labronica* not used for our experiment (see Supplementary Appendix S2 and Figure S1 for more details). Mean MO_2 values obtained from this pre-trial were comparable with those obtained from other temperate marine annelids at similar temperatures, providing a further validation of our data (see Supplementary Appendix S3 and Figure S2).

Statistical analyses

To investigate the temperature-dependence of metabolic rates over two successive generations in *O. labronica*, we fitted a multiple linear model with individual MO_2 as the dependent variables and the terms 'Generation' (categorical), 'Temperature' (continuous), and their interaction, as explanatory variables. The additive effect of the term 'Sex' (categorical) was included in the models to account for the effect of physiological differences between females and males (Ellis *et al.*, 2017). Finally, 'Body size' was included as covariate in all models for MO_2 to control for the effect of size on metabolic rates (Clarke & Fraser, 2004).

Statistical model selection was performed by removing progressively non-significant interactions (Generations \times Temperature) or predictive variables (Generations, Temperature, Sex) from the full model and comparing the Akaike Information Criterion (AIC) of the different models, following the procedure of Burnham & Anderson (2002). Briefly, models were considered different when their AIC differed more than two AIC units (delta AIC), and the lowest AIC indicated the best-fit model/models.

For all models, residuals were normally distributed and met the assumption of homogeneity of variance ($P > 0.05$), tested by Shapiro and Bartlett's tests, respectively. All statistical analyses were performed using the R software, version 4.0.0 (R Core Team, 2013).

Results

Mean MO_2 (\pm SE) ranged between $4.48 \pm 0.48 \cdot 10^{-3}$ and $7.83 \cdot 10^{-3} \pm 0.87 \cdot 10^{-3} \mu\text{mol O}_2 \text{ h}^{-1}$ at 21 and 26°C , respectively, both measured in the F0 (Table 1). Metabolic rates significantly increased with temperature (maximum t -value = 2.90, $P = 0.004$; Figure 1), as indicated by the most parsimonious models explaining the observed variation in MO_2 (maximum $F_{2,123} = 7.89$, $P = 0.001$, adjusted- $R^2 = 0.1$; Table 2a–c). In addition, body size had a significant positive relationship with MO_2 (maximum t -value = 2.93, $P = 0.004$). No significant effect of the interaction between the terms 'Generation' and 'Temperature' was found, and mean MO_2 did not differ between sexes (Supplementary Appendix S4).

Discussion

Our results demonstrate that individual metabolic rates have a positive relationship with temperature in the marine annelid

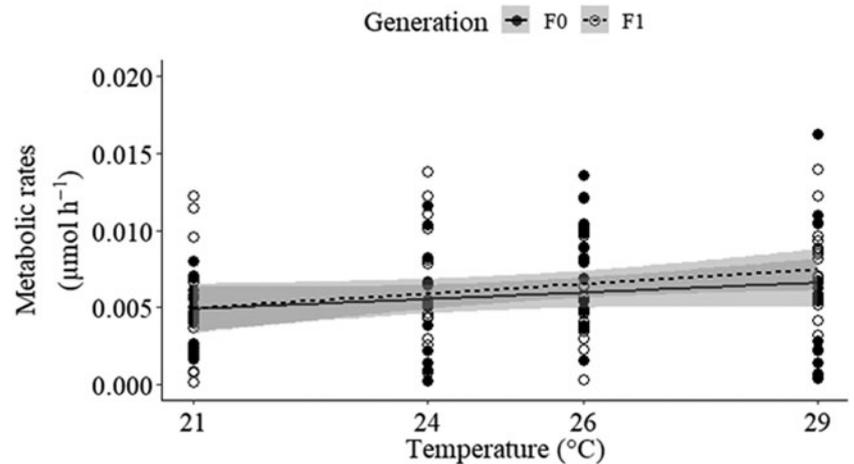


Fig. 1. Relationship between metabolic rates (MO_2), measured as oxygen uptake rates, and seawater temperature in the annelid *O. labronica* across two generations of exposure to a thermal gradient. Solid and empty circles represent individual MO_2 measurements for the F_0 and F_1 , respectively. The black continuous and dotted lines represent the regression lines for the F_0 and F_1 , respectively, and the grey shaded areas represent their 95% confidence interval.

Table 2. Results of the best-fitted linear regression models investigating the relationship between metabolic rates (MO_2) and temperature (continuous variable) across two successive generations in *O. labronica*, controlling for the effect of sex and body size

Coefficient	Estimate	DF	<i>t</i> -value	<i>P</i> -value	Model summary	AIC	dAIC
(a) $MO_2 \sim \text{Body size} + \text{Sex} + \text{Temperature}$							
Intercept	-0.01	1;126	-1.51	0.13	$F_{3,122} = 5.97$	-1071.2	0
Body size	0.0003	1;126	1.93	0.06	$R^2 = 0.11$		
Sex	-0.001	1;126	-1.41	0.16	$P = 0.001$		
Temperature	0.0003	1;126	2.87	0.005			
(b) $MO_2 \sim \text{Body size} + \text{Temperature}$							
Intercept	-0.01	1;126	-2.3	0.02	$F_{2,123} = 7.89$	-1071.1	0.05
Body size	0.0001	1;126	2.93	0.004	$R^2 = 0.10$		
Temperature	0.0003	1;126	2.9	0.005	$P = 0.001$		
(c) $MO_2 \sim \text{Body size} + \text{Generation} + \text{Temperature}$							
Intercept	-0.01	1;126	-2.26	0.03	$F_{3,122} = 3.64$	-1069.2	1.97
Body size	0.0005	1;126	2.82	0.006	$R^2 = 0.10$		
Generation	-0.00005	1;126	-0.08	0.93	$P = 0.002$		
Temperature	0.0003	1;126	2.86	0.005			

Values of delta AIC (dAIC) are provided relative to the most parsimonious model (a). Results for models with $dAIC \geq 2$ are shown in Appendix S4. DF, Degrees of Freedom (numerator; denominator); R^2 , adjusted R-squares.

Ophryotrocha labronica, but the strength and shape of this relationship does not change significantly across generations. The importance of habitat temperature in shaping metabolic rates is well documented across a variety of taxonomic groups (Fry & Hart, 1948; Brown *et al.*, 2004; Clarke & Fraser, 2004). Indeed, the strongest evidence for the temperature-dependence of metabolic rates comes from studies on marine ectotherms (Clarke & Fraser, 2004). For example, temperature was found to account for more than 90% of variation in metabolic rates measured as resting oxygen uptake in 43 species of marine copepods collected across a latitudinal gradient (Ikeda, 1985; Ikeda *et al.*, 2001). Similarly, a meta-analysis revealed that metabolic rates, again measured as resting oxygen uptake in both invertebrates (molluscs, echinoderms, cnidarians and crustaceans) and fish, increased with increasing temperature in most marine species investigated (Lefevre, 2016). The positive relationship between temperature and metabolic rates is assumed to approximate an exponential shape following an acute thermal exposure, a time frame during which the control of metabolic pathways is passively shaped by thermodynamic principles (Gillooly *et al.*, 2001; Brown *et al.*, 2004). In some marine ectotherms, metabolic rates double or even triple following a rapid 10°C increase in temperature (Q_{10}

> 2, Ikeda *et al.*, 2001; Castellani *et al.*, 2005; Scheffler *et al.*, 2019). In our study, the temperature–metabolic rates relationship in the first generation of exposure is not as strong as we expected ($Q_{10} = 1.3$, calculated according to Semsar-Kazerouni & Verberk, 2018), suggesting that an acclimation response may have already occurred in the individuals exposed to the experimental temperature conditions when metabolic rates were measured. In fish, for example, acclimation does not completely remove the effect of temperature on metabolic rates, leaving post-acclimation Q_{10} values between 1.0 and 2.0 (Jutfelt, 2020), a result that supports our hypothesis. Indeed, measurements of metabolic rates at the F_0 were taken between 2–4 weeks after the acute temperature exposure, a time period sufficient for many marine ectotherms to reduce the thermal sensitivity of metabolic rates through acclimation (Marshall *et al.*, 2003; Scheffler *et al.*, 2019), and likely more so for small-size (i.e. small surface to volume ratios), short-generation time species as *O. labronica*. For example, in the supratidal copepod *Tigriopus californicus* Baker, 1912, a small-size species colonizing splash pools, metabolic rates significantly increased with temperature when measured within 6 h immediately after the acute exposure to an elevated temperature, but they were unaffected by this thermal change just after 48 h of

chronic exposure (Scheffler *et al.*, 2019). The capacity for within-generational acclimation *via* the weakening or disappearance of the thermal dependence of metabolic rates, known as ‘*metabolic temperature compensation*’ (Bullock, 1955; Precht, 1958; Somero, 1969) allows an increase in energy efficiency by minimizing maintenance costs and maximizing energy allocation to other functions, such as survival, growth and reproduction, under varying temperatures (Robinson & Davison, 2008; Angilletta, 2009). As such, the rapid activation of reversible compensatory responses is considered an adaptive response to temperature variation in several marine species colonizing thermal fluctuating environments, such as intertidal and subtidal zones, or shallow waters (Le Moullac *et al.*, 2007; Schaefer & Walters, 2010; Healy & Schulte, 2012; White *et al.*, 2012). In the opossum shrimp *Gastrosaccus brevifissura* Tattersall, 1952, for example, metabolic rates increased with an acute increase in temperature, but were unaffected after seven days of acclimation (Marshall *et al.*, 2003). Similarly, the eastern oyster *Crassostrea virginica* Gmelin, 1791, and the hard-shell clams *Mercenaria* Linnaeus, 1758, showed a lack of temperature-dependence of aerobic metabolic rates already after respectively 2 weeks and after 8–15 weeks of exposure to a 5°C increase in temperature (Matoo *et al.*, 2013). Finally, in the supratidal copepod *T. californicus*, among three populations tested, one did not increase its metabolic rates even few hours immediately after the temperature change, suggesting an even faster compensatory response to temperature increase (Scheffler *et al.*, 2019). Rapid within-generational adjustments of metabolic rates are plausibly adaptive also in *O. labronica*, a subtidal species commonly found in temperate shallow waters (Prevedelli & Simonini, 2003; Massamba-N’Siala *et al.*, 2011).

The level of metabolic rate acclimation achieved in the F_0 is maintained unchanged after one additional generation of exposure to the same thermal conditions. Our results diverge from previous findings reporting the occurrence of adaptive trans-generational responses to temperature increase mediated by metabolic compensation *via* trans-generational plasticity (Donelson *et al.*, 2012; Miller *et al.*, 2012; Shama *et al.*, 2014). In the tropical damselfish *Acanthochromis polyacanthus*, individuals exposed for two generations to elevated temperatures (+1.5 and +3.0°C) showed a reduction in resting metabolic rates compared with the parental generation at the highest temperature (Donelson *et al.*, 2012). Similarly, metabolic compensation mediated by the maternal environment was observed in marine sticklebacks (Shama *et al.*, 2014). In this latest case, the optimization of the metabolic performance at the warmest condition was associated with the production of larger offspring than those produced by mothers exposed to cooler temperatures (Shama *et al.*, 2014). Following this line of evidence, we may suppose that the activation of trans-generational plastic responses may not be always necessary to cope with thermal changes. Similar conclusions were drawn from a trans-generational experiment with the water flea *Daphnia pulex* Leydig, 1860, where the beneficial effect of metabolic rate adjustment activated in the first generation of exposure to new temperature conditions extended across generations and was sufficient to maximize fitness in the new thermal environment (Kielland *et al.*, 2017). Altogether, the literature on the role of metabolic rate plasticity in mediating organismal thermal responses, to which our study contributes, highlights that there is a range of different phenotypic responses to thermal variation in marine ectotherms, and points to the need for further experiments on a wider array of taxa to reach a more accurate understanding of the mechanisms that will allow species to cope with ongoing climate changes.

Our study also offers a basis for future methodological and technical improvements for the investigation of trans-generational changes in metabolic rates in this small-size, annelid species. First, a higher level of replication per treatment and a continuous

monitoring of oxygen levels during the incubation period may counterbalance the high inter-individual variation of metabolic rates that we observed in our study. We found in fact a 5-fold to an 83-fold increase between minimum and maximum individual metabolic rates at 21°C in the F_0 and F_1 , respectively. There was no clear pattern in the magnitude of inter-individual variation, being 54 and 18 at 24°C, 8 and 37 at 26°C and 38 and 6 at 29°C in the parental and offspring generation, respectively. The behavioural habits of these interstitial errant annelid species, coupled with the methodological approach used – i.e. the measurement of individual routine oxygen uptake as proxy for metabolic rates – could have been responsible for this irregular variation. In particular, the transfer of the experimental individuals into a new vial for the metabolic rate measurements, with no food or substrate to hide in, could have been stressful. These annelids can respond to disturbance by either strongly swimming in the water, slowly crawling along the vial walls, or staying immobile in the vial’s bottom fold (Massamba-N’Siala, *pers. obs.*), a variety of behaviours that can have significant different implications for individual metabolic rates. Secondly, the implementation of full factorial designs where offspring from the same brood are assigned to different treatments, while recording any selective mortality, can help to more accurately characterize the mechanisms involved in cross-generational responses of metabolic rates, and distinguish between the contribution of genetic changes and non-genetic responses such as within- and trans-generational plasticity (Kielland *et al.*, 2017; Donelson *et al.*, 2018). In fact, we cannot discard the possibility that the different genotype composition within each treatment or the potential effect of different levels of selective mortality between treatments or across generations, which we did not record, may have favoured rapid evolutionary changes in metabolic rates (Kielland *et al.*, 2017; Norin & Metcalfe, 2019). The use of iso-female lines could help minimize the contribution of genetic variation in determining patterns of trans-generational changes in a sexually reproducing species as *O. labronica*.

In conclusion, our results provide additional evidence for the diversity of climate-associated physiological responses of marine ectotherms and suggest that the capacity, or need, for trans-generational adjustment of metabolic rates is not ubiquitous but context-dependent. Further experiments may unveil, for example, whether the level of heat stress caused by the acclimation temperature plays a role in activating trans-generational changes in metabolic rates, these changes having been often observed after exposure to suboptimal thermal conditions (e.g. Donelson *et al.*, 2012). Finally, our study contributes to the understanding of annelids’ physiological thermal plasticity, a phylum characterized by great biodiversity, particularly in the marine environment, but still under-represented in eco-physiological investigations and global change studies.

Supplementary material. The supplementary material for this article can be found at <https://doi.org/10.1017/S0025315422000303>

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Conflict of interest. The authors declare none.

Ethical standards. All applicable institutional and/or national guidelines for the care and use of animals were followed [Canadian Council on Animal Care].

Data availability. The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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