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# Nine years of experimental warming did not influence the thermal sensitivity of metabolic rate in the medaka fish *Oryzias latipes*

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## Abstract

1. A pressing challenge is to determine whether and how global-change drivers influence species physiology and survival. Recently, researchers have proposed the metabolic theory of ecology, defending the hypothesis of a universal thermal dependence of metabolic rate or, alternatively, the metabolic cold adaptation theory, stating that local adaptation can influence the thermal sensitivity of metabolic rate. However, the long-term (i.e. multigenerational) consequences of warming for the thermal sensitivity of metabolic rate remain largely unexplored although it determines energy use and is crucial for species response to climate change.
2. In this study, we used an evolutionary experiment with medaka fishes *Oryzias latipes* maintained for more than 12 generations at warm and cold temperatures (30 and 20°C, respectively) to address this issue. Our objective was to investigate whether thermal adaptation influences the relationship between temperature and mass-corrected metabolic rate and how this may occur.
3. In agreement with the universal thermal dependence hypothesis, we found that warming did not significantly influence the thermal sensitivity of mass-corrected metabolic rate: neither the intercept nor the slope of the temperature–metabolic rate relationship differed among fish lineages. Our small-scale laboratory experiment thus indicated that there is limited potential for evolutionary change in medaka fish metabolic rate in response to warmer temperatures.
4. Overall, we provide evidence that 9 years of experimental warming did not influence the thermal sensitivity of metabolic rate. Our results highlight the invariability of the thermal dependence of metabolic rate, which has important implications for adaptation to climate warming. This finding suggests a limited potential for metabolic adaptations in response to long-term temperature changes, which may have negative consequences for the persistence of fish populations under climate change.

Fanny Alberto-Payet and Arnaud Sentis equally contributed to this study.

  

**KEYWORDS**
ectotherms, metabolic cold adaptation theory, metabolic theory of ecology, metabolism, universal thermal dependence theory

## 1 | INTRODUCTION

Climate change is a major threat to global biodiversity, ecosystem function, and ultimately to the fate of humanity (Nelson, 2005). Forecasting these detrimental effects is an urgent challenge. Warming not only increases the speed of biochemical reactions but also influences the phenotype of species through plastic or selective processes (Boukal et al., 2019). Both the very short-term, immediate effects (mainly physiological and behavioural responses) and longer-term, lagged effects (phenotypic responses through both genetic and epigenetic inheritance) of temperature can alter the distribution of phenotypic traits and have cascading effects on communities and key ecosystem functions (Boukal et al., 2019; Sentis et al., 2015, 2017; Wang et al., 2020, 2021). A better knowledge of how short- and long-term thermal effects can modulate key physiological processes is thus important to better anticipate and manage detrimental consequences of climate change. In particular, metabolic rate is a key integrated metric quantifying the speed of biochemical reactions for resource uptake, substrate transformation, regulation, and energy allocation to fitness-related processes such as growth or reproduction (Brown et al., 2004). Consequently, changes in metabolic rate can have important implications at multiple levels of biological organisation from cells to ecosystems (Brown et al., 2004). This is especially relevant in aquatic systems, where most organisms are ectotherms and thus highly sensitive to water temperature. For instance, warming can alter the metabolic balance of freshwater ecosystems as respiration rate increases faster with temperature than photosynthesis rate, resulting in a shift towards more heterotrophic freshwater ecosystems (Yvon-Durocher et al., 2010).

The relationship between acute temperature changes and metabolic rate has been investigated for decades, dating back to Arrhenius (1889), but there is not yet a consensus on the importance of thermal adaptation in modulating metabolic rate (Angilletta & Angilletta, 2009; Somero, 2012). While the thermal sensitivity of metabolic rate appears largely conserved across the tree of life (Brown et al., 2004), previous studies, mainly on marine fishes, showed that thermal acclimation and local adaptation can modulate metabolic expression levels (Donelson et al., 2011; Kawall et al., 2002). Réveillon et al. (2019) also found repeatable inter-individual variation in the thermal sensitivity of metabolic rate in the freshwater amphipod *Gammarus fossarum*, suggesting a potential for selection on intra-specific variation in metabolic thermal sensitivity and thus evolutionary response to climate change. However, the evolutionary impact of warming on the thermal sensitivity of metabolic rate (i.e. the slope of the relationship between temperature and metabolic rate) remains largely unexplored. In a recent study, Moffett et al. (2018) tested if local adaptation to thermally contrasted geothermal springs can modulate the thermal sensitivities of field metabolic rate in the invasive mosquitofish *Gambusia affinis*. They found that the allometric and temperature dependencies of field metabolism varied in a countergradient pattern with local temperature in a way that reduces the metabolic cost of warming. They concluded that adaptation could increase population persistence under global warming. However, local adaptation can be a slow process and, in Moffett et al. (2018), fishes colonised the geothermal springs in the 1920s, which represents approximately 180 generations for adaptation to occur. It thus remains unclear if thermal adaption can be fast enough to rescue populations from rapid climatic changes, especially for organisms with long generation times such as aquatic vertebrates (Quintero & Wiens, 2013). Given the unprecedented rate of species extinctions (Sala et al., 2000), investigating if thermal adaptation can modulate the thermal sensitivity of metabolic rate is a crucial step towards a better understanding of the ecological and evolutionary consequences of global warming. This is particularly relevant for freshwater organisms, for which dispersal is often limited by human activities. In temperate rivers, fragmentation is a main driver of changes in fish biodiversity (Su et al., 2021). Moreover, river fragmentation is predicted to act jointly with climate change in promoting a considerable decrease in the probability of species persistence in the long-term because of splitting species ranges into smaller fragments resulting in isolation effects (Herrera-R et al., 2020). With limited dispersal, many freshwater species will thus have to rely on thermal adaptation if their populations are to persist in warmer lakes and rivers.

Different theories have been developed to understand the relationship between resting metabolic rate and temperature. In particular, the metabolic theory of ecology (MTE) and the theory of metabolic cold adaptation (MCA) both describe the thermal dependence of metabolic rate. The MTE (Brown et al., 2004) provides a powerful framework that links temperature $T$ (in K), body mass $M$ (in g) and metabolic rate $I$ (J/s) as follows:

$$I = b_0 M^b e^{\frac{-E}{kT}} \quad (1)$$

Where $b_0$ is a normalisation constant independent of body size and temperature, $b$ is the allometric exponent, $k$ is the Boltzmann constant ($8.62 \times 10^{-5}$ eV/K; Brown et al., 2004), and $E$ (in eV) is the slope of the relationship between temperature and metabolic rate, which indicates how fast metabolic rate increases with temperature. $E$ refers to the activation energy of metabolism based on enzymatic kinetics (Gillooly et al., 2001). The relationship between temperature and metabolic rate corresponds to Arrhenius' law predicting an exponential increase in metabolic rate with temperature.

The universal temperature dependence theory (UTD, Gillooly et al., 2001) of the MTE states that the slope of this relationship ($E$ in eqn 1) should be similar for all living organisms sharing the same type

of metabolism (e.g. photosynthesis or respiration). The activation energy for respiration *E* should therefore vary little among species or populations around an average value of 0.65 eV (Gillooly et al., 2001; Figure 1H1). Accordingly, adaptation to shifts in temperature associated with climate change could only change the intercept of the log–log relationship between mass-corrected metabolic rate and temperature but not the slope (Figure 1H2). This theory would thus imply little potential for organisms to change the thermal sensitivity (i.e. slope) of their metabolic rate in response to long-term temperature changes although they could adapt to warmer temperature through changes in the intercept. The common slope hypothesis has been recently challenged by Réveillon et al. (2019), who found repeatable inter-individual variations in the thermal sensitivity (i.e. slope) in the amphipod *G. fossarum*. Their results suggest that warmer climate may select for shallower slopes. In line with this prediction, Moffett et al. (2018) found that local adaptation to warmer temperature influenced the thermal dependency of field metabolic rate, resulting in shallower slopes for warm-adapted populations of mosquitofish.

The MCA theory (Angilletta & Angilletta, 2009; Lardies et al., 2004; Somero, 2012) predicts an increase in the metabolic rate of ectotherm populations from cold climates compared with their homologues from warmer climates (i.e. temperate or tropical). The observation that cold populations have higher metabolic rate at lower temperatures (Scholander et al., 1953) can be explained by their need to increase their energy production to develop and mature successfully at low temperatures (Lardies et al., 2004). It also suggests that, at the same ambient temperature, the metabolic rate of ectotherms from cold climates is higher than that of their counterparts from warm environments (Figure 1H2–H4). The MCA theory thus predicts a change in metabolic rate that can occur through changes of the intercept and/or the slope of the log–log temperature – mass-corrected metabolic rate relationship. In other words, species response to temperature shift can change both intercept and slope of the relationship between metabolic rate and temperature to modulate energy use according to local thermal conditions. This adaptive hypothesis has often been confirmed with interspecific (Addo-Bediako et al., 2002; Chown & Gaston, 1999; Chown et al., 1997; Torres & Somero, 1988) and intraspecific (Young, 1979) studies on both terrestrial and marine species but is less often tested in freshwater species. This adaptive pattern can be explained by genetic changes among

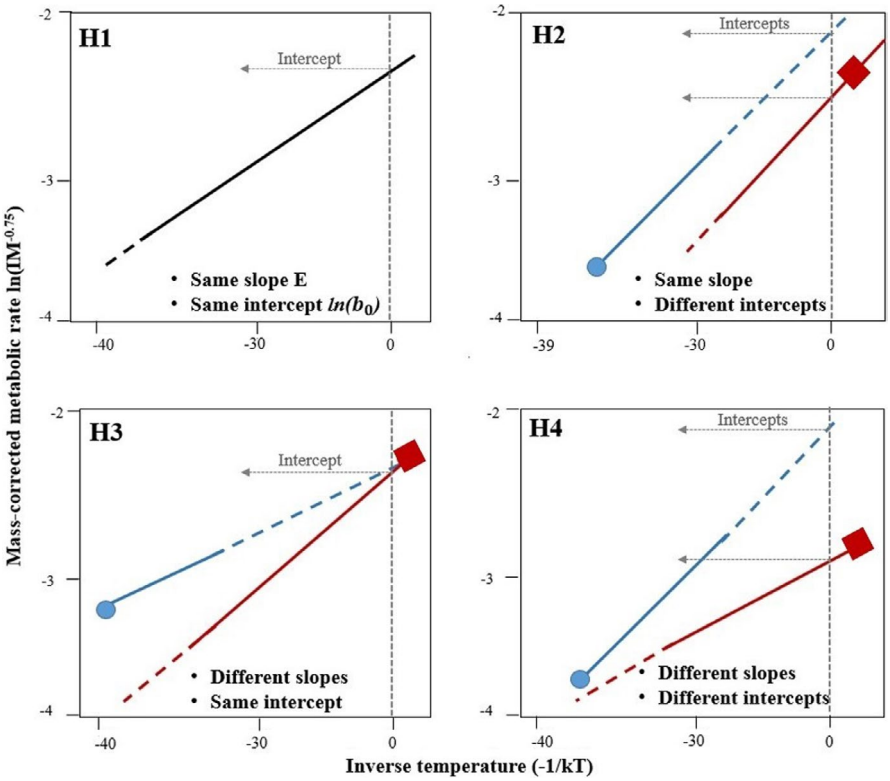


**FIGURE 1** Relationship between mass-corrected metabolic rate $\ln(I.M^{-0.75})$ in watts $g^{-0.75}$, and temperature (−1/kT) measured in K, according to the universal temperature dependence (UTD) and metabolic cold adaptation (MCA) theories for populations living in cold (in blue) or warm (in red) environments. Under the UTD, the metabolic rate of the two populations is a function of temperature, with a similar slope and intercept (H1), or with a similar slope and different intercepts (H2). According to the MCA, ectotherms living at cold temperatures (blue circles and lines) have higher metabolic rates than their counterparts living at high temperatures (red squares and lines). This can occur through three different scenarios: with a similar slope but different intercepts (H2), with the same intercept but different slopes (H3), or with different values of both intercepts and slopes (H4). The circles or squares represent the metabolic rate for each population at its local thermal condition (circles for cold conditions and squares for warm conditions)

populations or by the transmission of intragenerational changes over generations through epigenetic mechanisms (Danchin et al., 2011). For instance, phenotypic responses to environmental cues can be epigenetically inherited across generations in mice (Dias & Ressler, 2014), or in the damselfish *Acanthochromis polyacanthus* to mediate the impact of warming (Donelson et al., 2011). However, in opposition to the MCA, other studies did not find an increase in the metabolic rate of ectotherms from cold environments compared with their more temperate counterparts (Lee & Baust, 1982; Nylund, 1991; Steffensen, 2002). Because of the absence of a clear general pattern, the MCA theory is still one of the most controversial in physiological ecology (Lardies et al., 2004). It also remains unclear which of the two theories (UTD or MCA) best explains the links between local adaptation, temperature, and metabolic rate.

In this study, we conducted an evolutionary experiment over 9 years to investigate if and how warming could change the relationship between resting metabolic rate and temperature. In particular, our objective was to test: (1) the UTD hypothesis that the intercept of the relationship between metabolic rate and temperature can change but that the slope should be conserved with an activation energy of 0.65 eV (Figure 1, H1 or H2); and (2) the MCA hypothesis that populations living in warmer conditions would have a lower resting metabolic rate than populations from cooler temperatures (Figure 1, H2, H3, or H4). To test these hypotheses, we measured respiration rate, a proxy for metabolic rate, of two different experimental populations of medaka fishes *Oryzias latipes* that have been maintained for more than 10 generations (corresponding to 9 years) at two different water temperatures (30°C and 20°C, referred as warm and cold, respectively). The resting metabolic rate of each fish was measured along a thermal gradient from 15°C to 35°C. For each population, we then estimated the activation energy $E$ (i.e. slope) and the normalisation constant $b_0$ (i.e. intercept) of the relationship between temperature and resting metabolic rate to test the following hypotheses (Figure 1): H1: neither the intercepts nor the slopes change with temperature between the two populations; H2: only the intercepts change; H3: only the slopes change; and H4: the slopes and the intercepts change with temperature between cold and warm populations. The MCA allows for both the intercept and/or the slope to change (hypotheses H2, H3, or H4) whereas, with the UTD, only the intercept could change (H1 or H2). Therefore, both the UTD and the MCA can explain the second hypothesis where the slopes are similar, but the intercepts differ among populations from warm and cold environment. In other words, changes in the intercept are consistent with both UTD and MCA and changes in the slope of the temperature dependence are only consistent with MCA hypothesis. As (1) changes in metabolic rate can have important implications at multiple levels of biological organisation (Brown et al., 2004) and (2) climate change is a gradual process affecting populations over successive generations, our study helps to better understand the ecological consequences of climate warming by investigating if adaptation to warming can change the relationship between metabolic rate and temperature.

## 2 | MATERIALS AND METHODS

### 2.1 | Biological model

The Japanese medaka fish *Oryzias latipes* is a small (20–40 mm, 250–500 mg), egg-laying freshwater fish native to Asia which is found in Japan, Korea, and eastern China. It is a eurythermal fish that can live in temperatures ranging from 0 to 40°C (Leaf et al., 2011; Shima & Mitani, 2004) with a thermal optimum at 25°C (Dhillon & Fox, 2007). It has a short generation time of 2–3 months and can reach sexual maturity within only 10–12 weeks at 27°C (Hirshfield, 1980).

In this study, we used medaka fishes from an evolutionary experiment that started in August 2011 with a starting population of 76 individual medakas (46 females and 30 males) belonging to the CAB strain from Carolina Biological Supply Company (Burlington, NC, U.S.A.) and reared by AMAGEN© (Gif-sur-Yvette, France) and WatchFrog© (Evry, France). Since 2011, fishes were maintained at the RECOVER Laboratory (Aix-en-Provence, France) over generations at two different temperatures, cold (20°C ± 0.63 *SD*) and warm (30°C ± 0.52). At each evolutionary temperature, the fishes were distributed over five aquaria (25 × 40 × 20 cm) with a density of *c*. 20 individuals per aquaria. Fishes were fed with dry food (TetraMin©) twice a day ad libitum.

At each fish generation, the generation ($n + 1$) was started with egg clutches laid by females around the optimum fecundity period (i.e. when the number of laying females and the average number of eggs per female per day were at their maximum) and then placed in nursery tanks. Newborn fishes were then distributed over the five aquaria to compose the new generation. The optimum fecundity period differed between the two experimental populations, being reached at an age of *c*. 200 and 250 days for the warm and cold lineages, respectively. In a previous study using the same experimental lineages, we showed that the optimum fecundity period shifted over time with a diminution of the difference between warm and cold lineages as warm lineages evolved later maturation and at a larger size (Loisel et al., 2019). Moreover, using data on fish abundance from age 30 to 350 days, we estimated a mean mortality rate of 0.6 and 2.3 fish per day in the cold and warm lineages, respectively. Mortality rate was thus 3.8 times higher in the warm evolutionary treatment, which should lead to stronger selective pressure in the warm treatment compared to the cold one.

After 9 years of experimental evolution, we measured the resting metabolic rate of fishes from the 15$^{th}$ and 12$^{th}$ generations from the warm and cold populations, respectively. It was logistically not possible to use fishes from the same generation at the two evolutionary temperatures as generation time is reduced in the warm population compared to the cold one. We used 24 mature females (12 from each temperature regime) with an average age of 174 days (*SD* 18 days). Each of the two fish groups was named with its evolutionary temperature: T20 and T30 for fishes from the cold and warm temperature, respectively. Prior to the respiration measurement, fishes were isolated and fasted for 24 hr at their respective

evolutionary temperature (i.e. 20°C or 30°C). We weighed each fish (in g) before starting the experiments using a micro-balance (Sartorius©, Göttingen, Germany).

## 2.2 | Respiration rate measurement

We measured respiration rate using an intermittent flow respirometry system (Svendsen et al., 2016). Four Plexiglass cylindrical chambers (20 mm in diameter × 64 mm in length, 18 ml in volume) were used as respirometry chambers that were immersed in a water bath. The bath temperature was maintained with a TK150 water chiller (TECP S.R.L., Ravenna, Italy) or a ETH 300 heater (HYDOR©, Bassano del Grappa, Italy) depending on the experimental water temperature. Each respirometry chamber was connected to a peristaltic pump (Watson Marlow©, Wilmington, MA, U.S.A.) that circulated water throughout the system and through oxygen probes with a flow rate of 10.0 ml/min. Two other MC 450 water pumps (Newa Tecno Industria S.R.L., Loreggia, Italy) were used to restore oxygen content in the chamber by pumping oxygenated water from the bath to the chambers. Oxygen concentration was measured each second using a multi-channel fibre-optic oxygen meter (FSO2-x, FirestingO2, Pyroscience, Aachen, Germany) coupled to an external temperature probe (PT100, FirestingO2, Pyroscience) and connected to four flow-through cells with integrated oxygen and temperature sensors (TOFTC2, FirestingO2, Pyroscience). Maximal aeration was ensured by an air stone bubbling during the whole experiment.

Oxygen concentration was measured inside chambers during successions of open- and closed-water circulation phases. During the open phases (duration: 19 min), fully oxygenated water was flushed into the chambers with a pump as described above. During the closed phases (duration: 18 min), water was circulated throughout the system using the peristaltic pump. Oxygen consumption rate inside all the chambers was determined during the closed phases, after fitting a linear model to estimate the oxygen depletion slopes for each closed phase. Preliminary experiments were conducted to determine the durations of open and closed phases, verifying that the oxygen did not reach stressful concentrations for the fish. During the experiment, the whole respiratory system was covered with an opaque cardboard sheet to keep fishes unstressed.

Each fish was tested at each experimental temperature (15°C, 20°C, 25°C, 30°C, and 35°C) after an acclimation time of 4 hr, during which temperature was progressively increased or decreased to reset the test temperature. A given fish was always placed into the same respirometry chamber (chamber 1, 2, or 3). Chamber 4 was always left empty to control for background oxygen depletion. For each temperature, all fishes were tested within a single week. The order of the tested experimental temperatures was chosen randomly; it started with 20°C on the first week followed by 30°C, 15°C, 35°C and 25°C. Fishes were maintained at their evolutionary temperature (i.e. 20°C or 30°C) for 1 week in between two measurements. Because of logistic constraints, half of the fishes were tested during daytime (from 09.00 to 17.00) and the other half during night-time (from 18.00 to 08.00). Fishes from both evolutionary temperatures were first randomly assigned to a given time and day for the first experimental temperature and this assignment was conserved for the other experimental temperatures. Overall, a given fish was always tested in the same chamber on the same day of the week and at the same time of the day. Only the temperature changed across weeks. Fishes can be stressed when they are introduced in the respirometry device which often inflates respiration rates (Snyder et al., 2016). We thus used respiration data for each closed phases recorded between 4 and 6 hr after the start of the measurements to obtain respirometry measurements when fishes were calm and oxygen rates were stable corresponding to resting metabolic rate. The time range between 4 and 6 hr was determined based on a former experiment and visual inspection of the oxygen consumption rates curves. Analyses on the full-time range yielded the same qualitative results (analyses not shown). Overall, for each of the 24 medaka fishes, we obtained three or four estimates of respiration rate at each of the five experimental temperatures yielding a total of 394 respiration rate estimates.

## 2.3 | Statistical analyses

We tested for differences in fish body mass between evolutionary temperatures using a Welch two-sample *t*-test due to unequal variances between fish populations. To test our four hypotheses (see Introduction and Figure 1), we analysed the effects of experimental temperature and evolutionary temperature on the logarithm of the mass-corrected resting metabolic rate (i.e. $\log(I.M^{-b})$) using a linear mixed model with inverse experimental temperature (1/kT), evolutionary temperature (T20 and T30), and their interaction as fixed effects. The model also included the experimental period (day or night) and its interaction with inverse experimental temperature as fixed factors to investigate whether the thermal dependency of metabolic rate differs between day and night. Fish ID nested within experimental chamber, fish ID nested within experimental temperature, and fish ID nested within day of the week (e.g. Monday) were included in the model as random effects to account for repeated measurement and for the fact that each fish was tested in a given chamber at a given day of the week. The most parsimonious model was determined by sequential deletion of the least significant explanatory parameters or interaction terms from the full model. Parameter significance was evaluated using Kenward–Roger *F*-tests from the analysis of deviance (Zuur et al., 2009). The final model included only parameters with significant *p*-values ($<0.05$). The *b* value was evaluated by analysing the effects of inverse experimental temperature (1/kT), fish body mass (M), evolutionary temperature, and the interaction between body mass and evolutionary temperature on the logarithm of the metabolic rate with a linear mixed model including the same random structure as described above. We found no significant interactions between fish body mass and evolutionary temperature indicating that *b* was not significantly influenced by evolutionary temperature. The estimated value of *b* was 0.45

(±0.9 *SE*), which is lower than the theoretical value from the metabolic theory of ecology (i.e. 0.75). To test the robustness of our results we computed the analyses above by correcting metabolic rate using either our estimated *b* value (i.e. 0.45) or the theoretical value (i.e. 0.75). Both mass corrections yielded similar qualitative and quantitative results except that the intercept of the relationship between the mass-corrected resting metabolic rate and inverse experimental temperature was significantly lower with our estimated allometric exponent (estimated value ± *SE*: −3.49 ± 0.19) than with the theoretical allometric exponent (estimated value ± *SE*: −2.89 ± 0.19). Below, we present results for mass-corrected metabolic rate using the theoretical allometric exponent (0.75) as our objective was to test for the UTD, which concerns only the slope of the relationship between temperature and mass-corrected metabolic rate (i.e. *E* in eqn 1) and not the allometric exponent. The linear mixed models were implemented using the *lmer* function from the R package *lme4* (Bates et al., 2015). To test the assumptions of linear mixed models, we plotted residuals against fitted values to identify violation of homogeneity and verified normality using quantile–quantile plots (Zuur et al., 2009).

## 3 | RESULTS

We found no significant body mass differences between fishes from the cold and the warm populations ($t = 0.31$, $df = 17.96$, $p = 0.76$, Figure 2). Mass-corrected metabolic rate was not significantly influenced by the statistical interaction between experimental temperature and evolutionary temperature ($F_{1,20.96} = 1.40$, $n = 394$, $p = 0.250$), nor by the main effect of evolutionary temperature ($F_{1,15.99} = 0.54$, $n = 394$, $p = 0.473$). Moreover, mass-corrected metabolic rate was not significantly influenced by the day period (day or night; $F_{1,15.99} = 1.90$, $n = 394$, $p = 0.186$) or by the interactions between the day period and temperature ($F_{1,21.00} = 0.40$, $n = 394$, $p = 0.535$). The most parsimonious model was a model where mass-corrected metabolic rate increased exponentially with experimental temperature ($F_{1,22.96} = 249.19$, $n = 394$, $p < 0.0001$; Figure 3) as predicted by the MTE. We found an activation energy of 0.67 ± 0.04 eV (mean ± *SE*), which fits within the range of values predicted by the UTD hypothesis (i.e. between 0.60 and 0.70 eV, Brown et al., 2004). These results indicate that evolutionary temperature had no significant effect on the intercept or the slope of the relationship between mass-corrected metabolic rate and temperature (Figure 3).

## 4 | DISCUSSION

Warmer temperatures lead to higher metabolic rates and thus energy demand, which can strongly limit population persistence in the absence of adaptation (Archer et al., 2019; Vucic-Pestic et al., 2011). This is particularly relevant to freshwater organisms, which are threatened by both climate change and fragmentation and must thus adapt to their local environment, as dispersal is limited (Herrera-R et al., 2020; Su et al., 2021). According to the UTD hypothesis (Gillooly et al., 2001), the slope of the thermal dependence of metabolic rate should vary little among and within species. The MCA hypothesis predicts that populations from a warmer climate should have a lower metabolic rate compared to populations from cooler climates. Under the MCA hypothesis, local thermal conditions thus influence the slope and/or the intercept of the relationship between temperature and metabolic rate. As the UTD only concerns the slope of the thermal dependence of metabolic rate, changes in the intercept are consistent with both UTD and MCA and changes in the slope of the temperature dependence are only consistent with MCA hypothesis. These two hypotheses have important implications for the impact of climate change on organisms and their populations, as metabolism provides the energy needed to maintain their functions, development, and

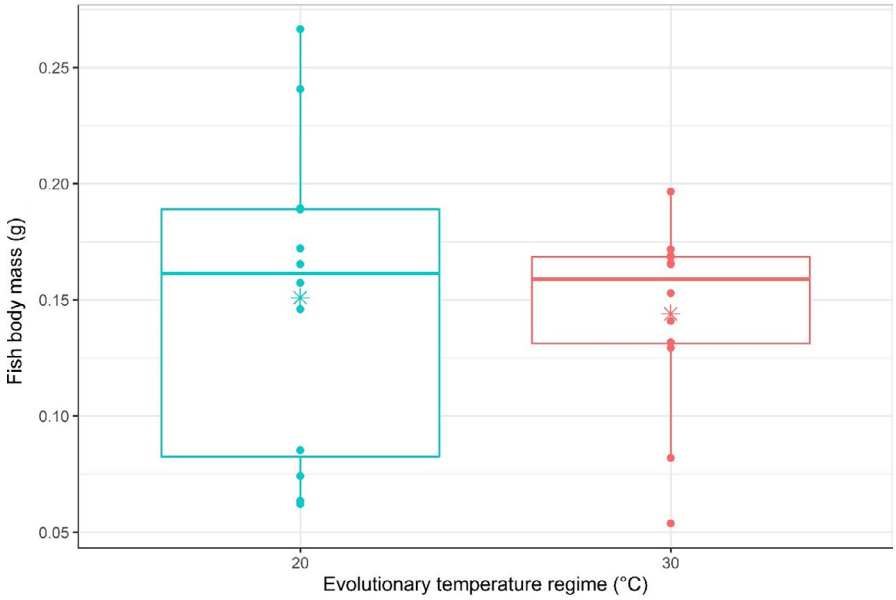


FIGURE 2 Body mass (g) of medaka fishes from the two evolutionary temperature regimes: 20°C (in blue) or 30°C (in red). Boxplot showing the median, first and third quantiles and the mean (star)

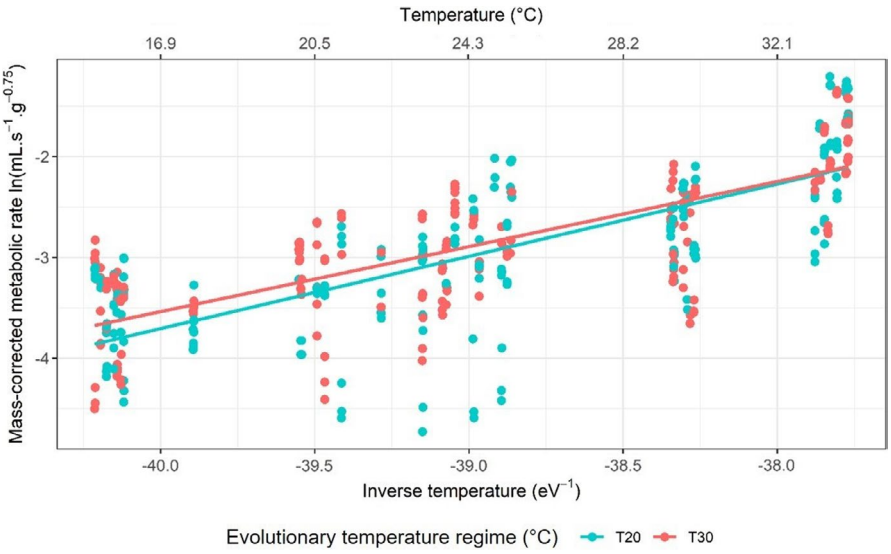


**FIGURE 3** Relationship between mass-corrected metabolic rate and inverse temperature for medakas from the two evolutionary temperature regimes 20°C (in blue) and 30°C (in red)

reproduction. However, the UTD and MCA hypotheses remain largely untested, especially in vertebrate freshwater species such as fishes (but see Moffett et al., 2018). In this study, we tested these alternative predictions by comparing the thermal dependence of mass-corrected metabolic rate of two populations of medaka fishes (*Oryzias latipes*) after more than 10 generations under cold and warm conditions.

Our results provide good evidence for hypothesis H1 (invariance in both the intercept and slope of the relationship between temperature and metabolic rate). We found: (1) that metabolic rate increases exponentially with temperature; (2) an estimated activation energy of 0.67 eV, close to the UTD predicted value of 0.65 eV; and (3) no significant differences of slope or intercept between cold and warm fish lineages. This implies that metabolic rate increases with temperature at the same pace and with a similar expression level for both warm and cold medaka fish lineages. These results are consistent with the UTD, and incompatible with the MCA hypothesis. As predicted by the UTD, our results indicate that the relationship between temperature and metabolic rate varies little among populations and that 12–15 generations of experimental evolution did not lead to significant differences in this relationship.

Our findings contrast with a previous study by Moffett et al. (2018) showing that the field metabolic rate of mosquitofish varies according to local thermal conditions and with other studies showing that local adaptation modulates metabolic expression levels in marine fishes and invertebrates (Kawall et al., 2002; Somero, 2012). In these local adaptation studies, populations have evolved for hundreds of generations under their local thermal conditions, which certainly increases the likelihood of adaptive evolution. In contrast, in our study, experimental evolution lasted only 12–15 generations (9 years), which may not be long enough for thermal adaptation to significantly influence the relationship between metabolic rate and temperature. Assessing the genetic divergence between our fish lineages would have been useful to identify the occurrence of adaptive genetic responses in our experimental system. The evolutionary temperatures used in our study fall within the thermal range of the medaka fish (Leaf et al., 2011; Shima & Mitani, 2004). It is thus possible that the selection pressure was not strong enough to result in rapid adaptive evolution, even if mortality rate was 3.8 times higher at the warmer temperature in our evolutionary experiment.

Beside adaptive evolution, thermal acclimation can modulate phenotypic traits, including metabolic rate (Angilletta & Angilletta, 2009). Previous studies reported that acclimation can influence maximum metabolic rate (i.e. when animals are active) and aerobic scope (i.e. the difference between maximum and resting metabolic rates; Donelson et al., 2011; Nyboer & Chapman, 2017). For instance, Nyboer and Chapman (2017) compared the metabolic performance of the Nile perch after 3 weeks of thermal acclimation. They found that the resting metabolic rate of acclimated and non-acclimated perch did not differ but that acclimated perch had lower maximum metabolic rate and aerobic scope compared to non-acclimated individuals. These previous studies suggest that field or maximum metabolic rate are more flexible and prone to evolution than resting metabolic rate, which is probably more constrained (but see Pilakouta et al., 2020; Sandblom et al., 2016). This would explain why the thermal dependency of metabolic rate is well conserved across taxonomic groups (Brown et al., 2004), whereas field and maximum metabolic rates appear to be more variable and less conserved across and within taxa (Glazier, 2014; Moffett et al., 2018). We may thus expect to observe stronger adaptive responses in more flexible traits such as maximum metabolic rate, growth rate or resource acquisition than in more physiologically constrained traits such as resting metabolic rate. In line with this expectation, we showed in a previous study using the same experimental lineages that the growth curve and optimum fecundity period differed between the cold and warm fish linages (Loisel et al., 2019). The warm fish lineage evolved towards a *slow* strategy in which fishes tended to mature later and at a larger size with an increased egg load (Loisel et al., 2019). It is thus surprising that, despite potential evolution in other fitness-related traits, resting metabolic rate did not evolve in our study.

## 5 | CONCLUSION

After 9 years of evolutionary experiment, we found that fish lineages from warm and cold temperatures did not significantly differ in their metabolic rates. The metabolic response to elevated water temperature was independent of the evolutionary temperature, indicating that the thermal sensitivity of metabolic rate is well conserved as predicted by the UTD. Our finding suggests that the thermal dependency of metabolic rate is strongly constrained, which would limit the evolutionary potential of this species. If the fish populations cannot adjust their metabolic rate in response to long-term temperature changes, then these thermal changes may have negative impacts on fish populations, especially when the metabolic demand exceeds energy acquisition through feeding interactions (Sentis et al., 2017). This phenomenon may be exacerbated in freshwater environments where oxygen availability significantly decreases with warmer temperature and thus limits metabolic scope further.

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## CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

## AUTHOR CONTRIBUTIONS

A. Sentis, M. Daufresne, and F. Alberto-Payet conceived and designed the experiments. F. Alberto-Payet and R. Lassus performed laboratory experiments and statistical analyses with the assistance of A. Sentis and A. Isla. F. Alberto-Payet and A. Sentis wrote the first draft of the manuscript and all authors contributed substantially to revisions.

## DATA AVAILABILITY STATEMENT

All datasets detailed in the present manuscript will be submitted to DRYAD online repository once the paper is accepted for publication.

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