Invading new climates at what cost? Ontogenetic differences in the thermal dependence of metabolic rate in an invasive amphibian

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ABSTRACT

Global warming can either promote or constrain the invasive potential of alien species. In ectotherm invaders that exhibit a complex life cycle, success is inherently dependent on the capacity of each developmental stage to cope with environmental change. This is particularly relevant for invasive anurans, which disperse on land while requiring water for reproduction. However, it remains unknown how the different life stages respond in terms of energy expenditure under different climate change scenarios. We here quantified the oxygen uptake of frogs at rest (a proxy of the standard metabolic rate) in the aquatic phase (at the tadpole and climax, i.e. during meta-morphosis, stages) and in the terrestrial phase (metamorphosed stage) at three environmental temperatures. To do so, we used marsh frogs (*Pelophylax ridibundus*), an amphibian with the largest invasive range within the palearctic realm and for which their adaptation to global warming might be key to their invasion success. Beyond an increase of metabolic rate with temperature, our data show variation in thermal adaptation across life stages and a higher metabolic cost during metamorphosis. These results suggest that the cost to shift habitat and face changes in temperature may be a constraint on the invasive potential of species with a complex life cycle which may be particularly vulnerable during metamorphosis.

1. Introduction

The successful establishment and dispersal of alien species is constrained by their capacity to overcome the conditions of their new environment (Prentis et al., 2008; Sakai et al., 2001). Alien species with a complex life cycle such as amphibians are expected to be even more challenged, because of the habitat shift they undergo throughout their life (Laudet, 2011; Lowe et al., 2021). The life cycle of invasive amphibians typically begins with an aquatic larval stage followed by a biphasic stage (i.e., aquatic and terrestrial), separated by a metamorphosis.

Metamorphosis involves massive changes in the morphology and physiology of organisms (Laudet, 2011). It is characterized by the apoptosis and resorption of larval tissues, the growth and differentiation of new adult tissues, and the remodeling of previous tissues for other functions (Dodd and Dodd, 1976). These changes are crucial, yet risky

for organisms (Huey, 1980) and therefore likely under strong selection. This may, however, impair colonization potential in such biphasic invaders (Orlofske and Hopkins, 2009; Shine, 2012). Because of its massive changes, metamorphosis induces a high metabolic cost, and unfavourable environmental conditions are expected to impact the climax stage and to result in carry-over effects which may be detrimental to metamorphosed individuals later in life (Székely et al., 2020).

Changes in environmental temperature are particularly relevant for ectotherms whose distribution often reflects their thermal tolerance and preferences (Evans et al., 2015; Ginal et al., 2021). As a consequence, the thermal variations projected due to climate change (IPCC, 2022) could have profound effects on populations (Angilletta et al., 2002; Lowe et al., 2021). Changes in temperature are known to affect amphibians (Navas et al., 2008), including invasive species at both the adult (Araspin et al., 2020; Marchetti et al., 2023; McCann et al., 2018) and larval stages (Kruger et al., 2022; Sinai et al., 2022). Successful invasive species often

show a broad thermal tolerance and a low thermal sensitivity of biological activity during both these stages (Padilla et al., 2023; Wagener et al., 2021). However, this may not be the case during metamorphosis (Laurence, 1975; Ruthsatz et al., 2020) which is critical to their establishment, dispersal, and ultimately their invasion potential.

The goal of the present study is to test the effect of temperature on resting metabolic rate at key stages (tadpole, climax, metamorphosed stages) of the life cycle of a successful amphibian invader, the marsh frog. We have chosen this species due to its wide and fast invasion of Western Europe which may have been enhanced by multiple adaptations particularly in the framework of the current global warming (Padilla et al., 2023; Dufresnes et al., 2024). Resting metabolic rate is strongly linked with the efficiency of a multitude of physiological process, such as growth, immunity, and physical performance, which are all essential to survival and the invasion success of a species. Consequently, exploring the effect of temperature on resting metabolic rate may help understand the invasive potential of a species. Resting metabolic rate is the reflection of biochemical reactions such as protein synthesis and mitochondrial activity and is variable across tissues and taxa. Because warmer temperatures increase the average kinetic energy into a system, the speed at which a chemical reaction takes place will also increase (Arrhenius, 1889). Therefore, as temperature increases, faster chemical process within the organism will require more ATP leading to an increased oxygen consumption and metabolic rate. Resting metabolic rate is therefore expected to increase with temperature at all developmental stages. For the invasive marsh frogs, we expect the sensitive climax stage to show the highest metabolic rate at each temperature and to be more sensitive to increases in temperature compared to tadpole and metamorphosed stages.

2. Materials and methods

2.1. Metamorphosed marsh frogs

We used invasive marsh frogs which have been introduced to southern France from different localities, particularly the Balkans, at the end of twentieth century and which colonized most of our study area over the last two decades (Dufresnes et al., 2017; Denoël et al., 2022). A previous study has revealed a broad thermal tolerance, a high thermal preference, and a performance-specific thermal sensitivity at the adult stage (Padilla et al., 2023). Here, metamorphosed frogs ($n = 29$; i.e. terrestrial juveniles and adults) were caught in August 2020 by hand or with a dip net from the border of typical artificial ponds called "Lavogne" in the Larzac region (43°46′N - 3°21′E to 43°53′N - 3°30′′E; Hérault, France). Previous molecular analysis confirmed the taxonomic assignment and the invasive status of the marsh frogs in the study area (i.e. *Pelophylax ridibundus* sensu lato; previously named *Rana ridibunda*) (Dufresnes et al., 2017) whereas previous dietary studies in the study area showed their predation on local amphibians and invertebrates (Pille et al., 2021, 2023). All individuals were PIT-tagged (Biolog-ID, 134.2 KHz; Agrident reader) allowing individual identification (Donnelly et al., 1994). The tag was inserted dorsally under the skin (Christy, 1996). Frogs were brought inside large coolers filled with 3 cm of water to the Function and Evolution (FUNEVOL) laboratory at the Muséum national d'Histoire naturelle of Paris (France). The frogs were maintained singly in 5 L tanks (21.4 x 36.5 × 16.5 cm; water depth: 5 cm) with holes in the lid. Tanks were inclined by placing a 10 cm wood block under one of the sides creating a dry area that occupied one third of the tank. Water in the tanks was renewed weekly. Two adult crickets (*Acheta domestica*) were provided twice weekly. Night and day periods were set to 12 h each. The room temperature was set at 24 °C (Padilla et al., 2023) which is a typical temperature in the field during frog activity (pers. obs.) and close to the temperature where they engage in amplexus in captivity (Michaels and Försäter, 2017).

2.2. Tadpoles

Two adult frogs from the same pond (43°47′N - 3°29′′E) which were not used for other purposes and which had visible sexual traits (nuptial pads and vocal sacs in males; high body mass relatively to size in females) were placed together in a 140 L plastic tank (80 x 60 × 46 cm; water depth: 15 cm). A shelter (Turtle Hut, ZooMed®, 16.5 x 17 × 7 cm), artificial vegetation (Cashuarina, ZooMed®, 56 cm) and the same conditions as for metamorphosed frogs were provided. After two days, eggs were laid in the artificial vegetation. The two adult's frogs were then moved to individual boxes. The tank was filled with tap water and water was cleaned every two weeks. Powdered fry food (sera®, Micron Nature) composed of zooplankton (18% krill) and phytoplankton (51% spirulina) was provided *ad libitum*. After 14 days, most tadpoles had reached stage 25 (i.e. corresponding to the total disappearance of external gills and the presence of a fully developed spiracle (Gosner, 1960) were switched to 5 L tanks (21.4 × 36.5 × 16.5 cm; water depth: 14 cm), with five individuals per tank. It took three months after fertilization for the first tadpoles to reach metamorphosis. The climax was defined at stage 42, when metamorphosis begun and was identified by the abrupt emergence of the internally developed forelimbs (Gosner, 1960; Zechini et al., 2015).

2.3. Standard metabolic rate

We used oxygen consumption at rest on fasted animals (24 h) as a proxy of standard metabolic rate. For tadpoles and climax stages, metabolic rate was quantified using an intermittent respirometry system (Loligo® Systems, Complete minichamber system, #SY20000) set inside an incubator (Aqualytic-Liebherr, TC 256 G/256L/2–40 °C) to obtain the desired water temperature. Before each trial, individuals were weighed using a digital scale (Ohaus, Brooklyn, NY, USA; precision 0.1 mg). Each individual was transferred using a plastic tube filled with water and placed inside a respirometry chamber of 270 ml, diameter of 1.85 cm and a length of 6 cm filled with tap water and oxygenated with an air bubbler. Oxygen consumption was measured through the chamber by a fiber optic probe reading a sensor foil on the inside wall of the chamber. Probe calibration was done each week for 0% oxygen using a 10% mixture of Na_2SO_3 and a bath of tap water hydrated for 20 min with a water bubbler. The temperature of the bath was continuously recorded, and a temperature compensation was done automatically by the software during recording. The trial consists of a flushing cycle of 180 s followed by a delay phase of 30 s and a closed cycle of 180 s. Recordings were done continuously from 5 p.m. to 10 a.m. and the mean oxygen consumption rate (MO_2) from each cycle (162 cycles for each individuals) was used. To account for background microbial respiration, we measured the empty chambers before and after the trial for 3 h. Background respiration corrections were done on the MO_2 values using the slope of its drift and then by the subtraction of the mean MO_2 in empty chambers after measurements from the mean MO_2 of the tadpoles. The system and chambers were cleaned after each trial using a chemical agent (Virkon®, Antec International, A DuPont Company).

The standard metabolic rate in metamorphosed frogs was measured using a flow through respirometer. Records were performed at least 60 h after animals were fed. To avoid a bias by the time of recording (morning/afternoon) animals were haphazardly selected for testing. Before each measurement, the frogs were patted dry and weighed. Eight respirometric chambers of 700 ml, diameter of 25.5 cm and a length of 7 cm were set inside an incubator (Aqualytic-Liebherr, TC 256 G/256L/2–40 °C) to briefly acclimate and maintain the animal to the desired air temperature. A direct sampling method (Lighton, 2008) was used by pushing air with a pump (PP2 dual channel field pump, Sable Systems International) to a mass flow meter (FB8 flowbar-8 mass flow meter system, Sable Systems International) and then directly into the chambers. The first chamber was left empty for baselining. Flow was then transmitted to a multiplexer (RM8 respirometry flow multiplexer, Sable

Systems International) and into a gas analyser (FMS field Metabolic System, Sable Systems International) allowing a sequential recording of each chamber. A week of preliminary measurements were performed which allowed animals to become familiar with the chambers and to optimise the set-up. A flow rate of 150 ml/min or 250 ml/min for larger animals (mass >25 g) resulted in CO_2 concentrations between 0.05 and 0.2 ml/min which we considered as a good trade-off between distinguishing gas exchange and avoiding hypercapnia (i.e., too much CO_2 in the blood). We set the relative humidity to 80% using a controller of water vapor content of the air stream (DG-4 Dew Point generator, Sable Systems International), and at the end of trials, animals were always wet. The digital recording started with 2 min of baselining, 5 min of recording for each chamber and ended with 3 min of baseline. Recordings were done for three and a half hours to allow the incubator and animals to reach the desired body temperature. O_2 and CO_2 received the same corrections (Lighton, 2008): (i) a mathematical scrubbing transformation, (ii) a drift correction using baseline measurements, (iii) a division by 100 to get fractional concentrations. Because O_2 was analysed last, we corrected its lag time response so that its decrease corresponded to the CO_2 increase. Because incurrent flow was known we could substitute excurrent flow and calculate the oxygen absorbed ($\dot{V}O_2$) and the carbon dioxide produced ($\dot{V}CO_2$) in ml/min by the animal using these equations:

$$\dot{V}O_2 = \frac{FRi[(FiO_2 - FeO_2) - FeO_2(FeCO_2 - FiCO_2)]}{1 - FeO_2}$$

$$\dot{V}CO_2 = \frac{FRi[(FeCO_2 - FiCO_2) - FeCO_2(FiO_2 - FeO_2)]}{1 - FeCO_2}$$

Where FR denotes flow rate, FeO_2 and $FeCO_2$ are the fractional content of each gas. The lower case "i" or "e" respectively refer to which mass flow was measured, incurrent or excurrent. Data were automatically processed using the macros in the ExpeData 1.9.27 software. We also used a macro to extract the VO_2 mean lowest 90 s section pre-sampled from a 180 s stable recording for each individual. VCO_2 mean was thereafter extracted from the same section. After acquisition, animals were weighed, and then were put back inside their home terrarium with two crickets, which were eaten quickly.

Measurements for tadpoles, climax, and metamorphosed stages were tested in a random order at three temperatures (13, 23, 30 °C) that describe a wide range of temperature within the limits that has been observed for adults (Padilla et al., 2023). VO_2 was transformed in ml per hour for all stages. Oxygen consumption measurements were performed in accordance with all guidelines for the use of animals in research by the Association for the Study of Animal Behaviour (ASAB) and the Animal Behaviour Society (ABS). All animals quickly consumed the food provided after measurements indicating their well-being.

2.4. Statistical analysis

To avoid correlation effects between body mass and developmental stage but still accounting for allometric effects, we used VO_2 divided by body mass. A linear mixed model was run on VO_2/mass as our response variable, developmental stage and temperature as fixed factors, and individual identity as random effect to account for repeated measurements across the three temperatures. Conditional R-squared variance was extracted using the "rsquaredGLMM" function from the MuMIn packages (Barton, 2023). Within groups comparisons were performed using the "contrast" function from the emmeans package (Lenth, 2023) and we reported p-values. Validation was performed by visualising the residuals which had a homogeneous, normal distribution and by plotting the response variable on fitted values which resulted in a linear relation. All analyses were done using R version 4.3.1 (R Core Team, 2023).

3. Results

The mixed model explained 85.58% of the conditional variance in oxygen consumption where 8.44% of the variation was explained by individual repeated measurements (random effect). Developmental stage ($F_{2, 164} = 59.49$, $p < 0.001$), temperature ($F_{2, 164} = 34.31$, $p < 0.001$), and the interaction between developmental stage and temperature ($F_{5, 164} = 232.39$, $p < 0.001$) had a significant effect on oxygen consumption relatively to the mass of each individual (VO_2 ml h^{-1} g^{-1}) (Figs. 1 and 2).

Post-hoc contrasts of estimated marginals means revealed that almost all but two pairwise comparisons were significantly different from one another ($p < 0.001$; Fig. 2). Within tadpoles, the one tested at 23 °C did not show significantly different oxygen consumption from the one tested at 30 °C ($p = 0.32$; Fig. 2). Within a 13 °C test temperature, tadpoles did not show a significantly different oxygen consumption from metamorphosed stages ($p = 0.95$; Fig. 2).

4. Discussion

The present work supports the hypothesis that organisms with a complex life cycle can show different biological responses to changes in their thermal environment throughout their life cycle. More specifically, it shows that organism at the climax stage (i.e. during the peak of metamorphosis) have the greatest energetic cost of all stages tested. Metabolic rate increases with temperature but with variation across life stages, particularly at the highest temperatures. In contrast to previous observations on physical performance of metamorphosed amphibians, the temperature where metabolic rate is at its lowest differs from the preferred temperature and the optimal performance temperature (Padilla et al., 2023). The higher preferred and optimal temperature induce increased energetic demands on standard metabolic rate which may limit the energy available to other biological process. Still, thermoregulatory behaviours in both metamorphosed individuals, such as basking or diving into the pond, and in earlier stages, such as vertical movements across the water column, may mitigate these effects.

4.1. Not extreme enough

Our results show that temperatures increasing from 13 to 23 °C significantly increase the metabolic rate of all stages. The same increase was observed in individuals at climax and metamorphosed stages from 23 to 30 °C. Similarly, most previous studies highlighted this positive correlation (Kern et al., 2015; Ruthsatz et al., 2020). However, from 23 to 30 °C only a trend of lower amplitude, but not significant, was observed in tadpole stage. This result calls for more research to further explain these variations. As the temperature at the bottom of the studied ponds and in the air reach respectively a maximum of 21.4 °C and a maximum of 33.2 °C (Padilla et al., 2023), warmer temperatures likely occurring during the next century (IPCC, 2022) may increase the metabolic rate of all stages and therefore induce a cost in terms of energy available for other biological activities (Steyermark, 2002). Indeed, in the main area currently invaded by marsh frogs in southern France, annual mean warming on land is expected to increase in the range of 0.9–5.6 °C by the end of the century (Ali et al., 2022). Yet, as metamorphosed marsh frogs can sustain a high performance at high temperatures with a preferred temperature at 21.8 °C (Padilla et al., 2023), it is likely that they can support this metabolic cost during key behaviours such as dispersal or predator escape. Variation is also expected to occur within each life stage and calls for more research at multiple time points during development. This would be particularly relevant at the different steps of metamorphosis and during the biphasic or terrestrial life, i.e. before and after maturity.
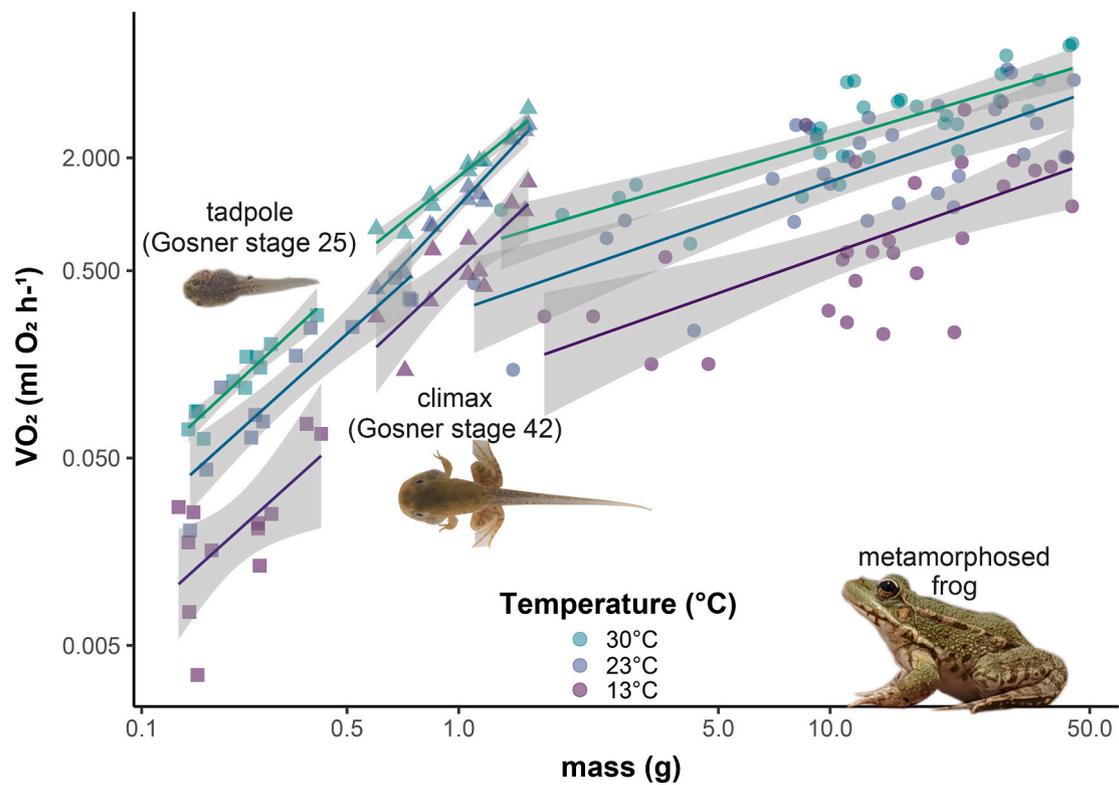
Fig. 1. Allometric effect of body size on oxygen consumption for three developmental stages and three temperatures in invasive marsh frogs (*Pelophylax ridinbundus*). Logarithm scales were used for each axis. Each square stand for an individual tadpole, triangles for climax stage (i.e. metamorphosing individuals), and circles for metamorphosed stages. The lines represent the linear regressions of oxygen consumption on body mass and the grey shade the confidence interval. The violet color stands for a 13 °C test temperature, blue for 23 °C, and green for 30 °C. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
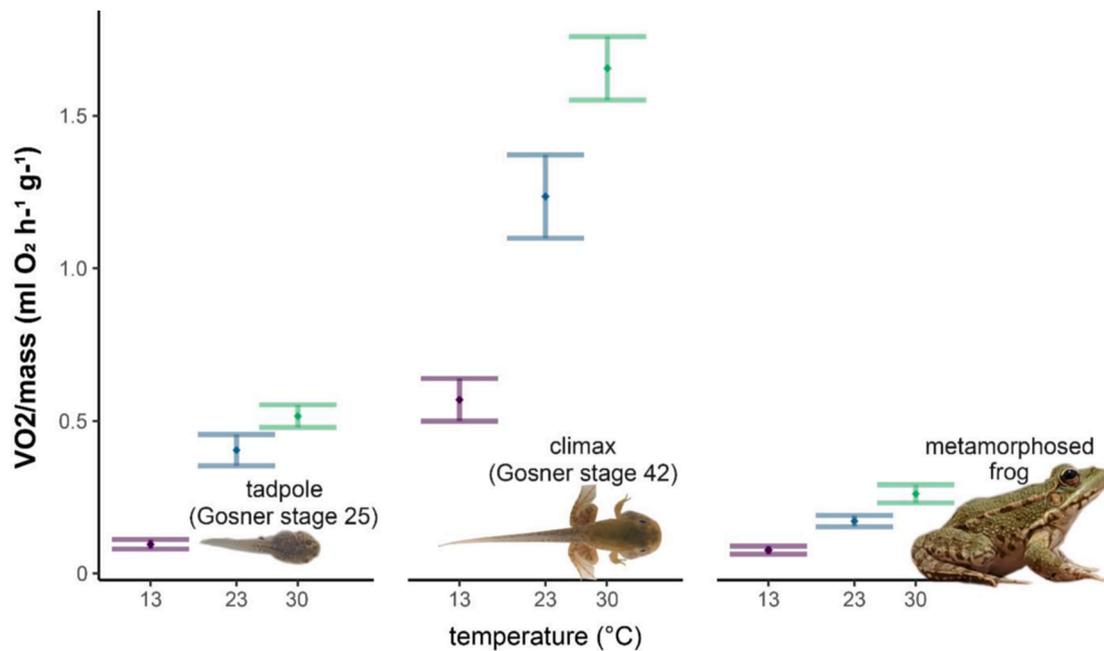

Fig. 2. Thermal dependency of the mass specific metabolic rate among developmental stages of invasive marsh frogs (*Pelophylax ridibundus*). Standard errors are represented by error bars and the dots represent the mean. The violet color stands for a 13 °C test temperature, blue for 23 °C, and green for 30 °C. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

4.2. The energetic cost of metamorphosis

As hypothesised, during climax, individuals exhibit a significantly higher energetic cost of body maintenance at each temperature compared to either the tadpole or metamorphosed stages. The numerous physiological and biochemical processes associated with metamorphosis from an aquatic larval into a metamorphosed biphasic (aquatic/terrestrial) state likely do lead to an increase in the metabolic rate at climax (Hulbert and Else, 2004; Kim, 2008). These changes, primarily triggered by thyroid (Gudernatsch, 1912; Magnus-Levy, 1895) and glucocorticoid hormones (Kirschman et al., 2017; Shewade et al., 2020), require a synchronized apoptosis of the larval structures followed by the growth and differentiation of adult cells (Furlow and Neff, 2006; Shi et al., 2001), such as the reduction of the gills followed by the development of the lungs (Vitt and Caldwell, 2013) or the whole restructuration of the immune system to avoid autoimmune responses of tadpole lymphocytes to antigens of new metamorphosed frog's tissues (Rollins-Smith, 1998; Robert and Ohta 2009). Beyond the immediate (Huey, 1980; Wassersug and Sperry, 1977) and carry over effects later in life (Beck and Congdon, 2000; Moore and Martin, 2019), a high metabolic cost may also restrict the energy available for other biological activities and decrease the survival and fitness of climax stages. We can expect this effect to be exacerbated in amphibians with a slower larval development, such as *P. ridibundus* or other ranids (Orlofske and Hopkins, 2009; Pandian and Marian, 1985) in contrast to rapid developing species such as bufonids (Beck and Congdon, 2003; Cabrera-Guzmán et al., 2013).

4.3. Conclusion

The overall low sensitivity of invasive species to changes in environmental temperature likely makes them more resilient to future climate change (Kelley, 2014; Zerebecki and Sorte, 2011). The fact that invasive amphibians are facing a global warming during both their aquatic and biphasic life stages as well as during metamorphosis may challenge their invasion success as this increases their metabolic rates. The present study is, to our knowledge, the first to quantify how changes in temperature throughout the three major stages (tadpole, climax, and metamorphosed stage) of the life cycle of an amphibian invader impact the metabolic cost of body maintenance. Using mechanistic species distribution modelling combining the knowledge on this invasive species (see also Padilla et al., 2023) may provide a better estimation of the distribution changes under predicted climate warming scenarios.

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CRediT authorship contribution statement

Pablo Padilla: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Anthony Herrel:** Conceptualization, Methodology, Writing – review & editing, Supervision. **Mathieu Denoël:** Conceptualization, Methodology, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare no competing interests.

Data availability

Data are available on figshare DOI: 10.6084/m9.figshare.25029176.

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