

**Does the spatial sorting of dispersal traits affect the phenotype of the non-dispersing stages of the invasive frog *Xenopus laevis* through coupling?**

**Running title:** Spatial sorting in invasive *Xenopus laevis* tadpoles.

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**Author contributions:**

NK, JM, JS conceived and designed the study, NK performed the experiments and data acquisition, NK and JS contributed to the data analysis. NK, JM, GV, AH and JS were involved with the interpretation of data. NK drafted the manuscript and JM, GV, AH, and JS revised the manuscript and approved the final version. NK, JM, GV, AH, and JS agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work

are appropriately investigated and resolved. All authors contributed critically to the drafts and gave final approval for publication.

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#### **Ethical statement:**

This study has been carried out in compliance with ethical standards in France (Agreement Comité d'éthique des pays de la Loire APAFIS 13301). The utilisation of the frogs and the research protocols were approved by Stellenbosch University Research Ethics Committee: Animal Care and Use (ethics number: 1535).

#### **Data availability statement:**

Data will be available from the Zenodo repository. The full DOI of the data will be given as a full data citation in the reference list.

## Abstract

In amphibians, spatial sorting progressively enhances the dispersal capacities of dispersing stages in expanding populations but may enhance or limit the performance of the earlier non-dispersing stages. Phenotypic traits of non-dispersing tadpoles and metamorphs can be coupled, through carryover effects and trade-offs, or decoupled to dispersal traits in adults. We used the globally invasive amphibian, *Xenopus laevis*, to examine whether spatial sorting of adult phenotypes affects the phenotype of larval stages to metamorphosis in the core and at the periphery of an invasive population in France. We combined common garden laboratory and outdoor experiments to test the effect of parental pond position (core or periphery) on morphology, development and survival to metamorphosis and found no differences between tadpoles. After metamorphosis, the only difference observed in either of the experiments was the larger body size of metamorphs from the periphery, and then only when reared in the laboratory. Differences in metamorph size may indicate that a shift of dispersal traits occur after metamorphosis in *X. laevis*. Thus, our findings illustrate that decoupled evolution through spatial sorting can lead to changes of *X. laevis* adult phenotypes that would enhance dispersal without affecting the phenotype of tadpoles before metamorphosis.

**Keywords:** Anura, decoupling, development, dispersal, metamorph, morphology, Pipidae, survival, tadpoles.

## Introduction

The need for organisms to allocate resources and energy to survival, reproduction and dispersal generates trade-offs that constrain their anatomy, physiology and behaviour (Burton et al. 2010). In an expanding population, trade-offs may be altered at the range periphery, brought about by the evolution of dispersal traits (Phillips et al. 2010). Essentially, individuals with the best dispersal capacities, *i.e.* those that allocate more resources to dispersal, are more likely to meet and mate at the outermost sites (Travis and Dytham 2002). If these traits are heritable, this can induce generational shifts in behaviour (*e.g.* increase in boldness and exploratory behaviour, Gruber et al. 2017), morphology, (*e.g.* larger wing size, Phair et al. 2018; decreased body length, Amundsen et al. 2012) and physiology (*e.g.* alterations in immune function, Llewelyn et al. 2012, Ronce and Clobert 2012, Brown et al. 2015; decrease in standard metabolic rate, Louppe et al. 2018). Ultimately, novel phenotypes with higher dispersal capacities may emerge at the range periphery (Travis and Dytham, 2002; Simmons and Thomas 2004; Shine et al. 2011). This process is known as spatial sorting and predicts the progressive broadening of the dispersal kernel and the acceleration of population spread of a species at the periphery of the range (Travis and Dytham 2002; Phillips et al. 2008; Shine et al. 2011; Chuang and Peterson 2016; Pizzatto et al. 2017).

A more nuanced view of spatial sorting emerges for organisms with complex life cycles, like insects, fish, or amphibians, in which dispersal occurs at a particular ontogenetic stage. In many amphibians only adults disperse and reproduce (Cayuela et al. 2018). During dispersal, one or more of their life-history stages experience a range of different environmental conditions (Chuang and Peterson, 2016). The environmental pressure on traits expressed in an earlier life stage can affect the dispersal ability of a later life stage through carryover effects (*e.g.* the invasive damselfly, *Coenagrion scitulum*, Therry et al. 2014), resulting in the coupling of different traits across life-history stages (Moran 1994; Wollenberg-Valero et al. 2017). In frogs, tadpoles and adults can exhibit morphological and ecological divergence due to morphological features being controlled by independent stage-specific genes (Sherrat et al. 2017). For example, a recent study by Wollenberg-Valero et al. (2017) demonstrated that a large proportion of the genes coding for morphological traits of the tadpoles and adults of the African clawed frog, *Xenopus laevis*, are stage-specific. Thus, we might expect that morphological traits will be decoupled between *X. laevis* tadpoles and adults. However, traits can be coupled by the carry-over effects of the trade-offs experienced at an earlier stage. For instance, accelerated development in tadpoles of the common parsley frog, *Pelodytes punctatus*, is coupled with lower body mass at metamorphosis (Richter-Boix et al. 2006). This finding corroborates those of other studies on amphibians that

have described the carry-over effects of tadpole life-history to adult fitness (Tejedo et al. 2010; Johansson et al. 2010, Yagi and Green, 2018).

Morphological and developmental traits of tadpoles have been coupled to traits relevant to dispersal in adults, such as endurance and speed (e.g. Relyea 2001, Chelgren et al. 2006; Yagi and Green 2018). Tadpole snout-to-vent length (SVL) has been coupled to tadpoles stamina (e.g. American toad, *Bufo americanus*, Wassersug and Feder 1983) and metamorph size (e.g. wood frog, *Lithobates sylvatica*, Relyea 2001). In turn, larger SVL at metamorphosis is coupled with survival and endurance in adults (e.g. *Pelophylax* sp., and *Rana* sp., Altwegg and Reyer 2003; Chelgren et al. 2006). Smith-Gill and Berven (1979) also describe the importance of the correct timing of metamorphosis for metamorph SVL. The time to metamorphosis (duration of the larval period) is coupled to adult hind limb length (e.g. wood frogs, *Lithobates sylvatica*, Relyea 2001). Shorter larval development usually decreases hind limb length (Gomez-Mestre and Buchholz, 2006; Gomez-Mestre et al. 2013; Tejedo et al 2010) due to thyroid hormone (thyroxine, T4) action (Eddy and Lipner 1976, Fort et al. 2007). Finally, survival of larvae to metamorphosis is coupled to adult reproductive success (e.g. leopard frog, *Lithobates onca*, Goldstein et al. 2017). However, traits can be coded by different genes that result in adult and tadpole phenotypes developing independently, also known as decoupling (Wollenberg-Valero et al. 2017).

Spatial sorting has been demonstrated in adults of an invasive population of *X. laevis* in France (Louppe et al. 2017, Courant et al. 2019; Padilla et al. 2019). Adults were found to lower resource allocation to reproduction at the periphery of the invasive range (Courant et al. 2017a) and increase resource allocation to physiological and morphological traits relevant to dispersal (Louppe et al., 2017; Padilla et al. 2019). The adults from the range periphery display higher endurance, lower standard metabolic rate, and longer relative hind limbs than adults from the range core (Louppe et al. 2017, 2018; Courant et al. 2019a). The population has undergone expansion for ~40 years from a single introduction point (Fouquet and Measey 2006). Dispersal occurs overland and after metamorphosis, even though all stages of this species are predominantly aquatic (Measey 2016, Courant et al. 2019).

We asked whether the spatial sorting patterns observed in adults generate variation in tadpole phenotypic traits across the invasive range. We formulated two hypotheses. Firstly, we hypothesised that morphological traits such as SVL of tadpoles and metamorphs are targets for spatial sorting at the periphery of an expanding population. We expect that metamorphs at the periphery will have larger SVL (as seen in cane toads, *Rhinella marina*, Cabrera-Guzman et al.

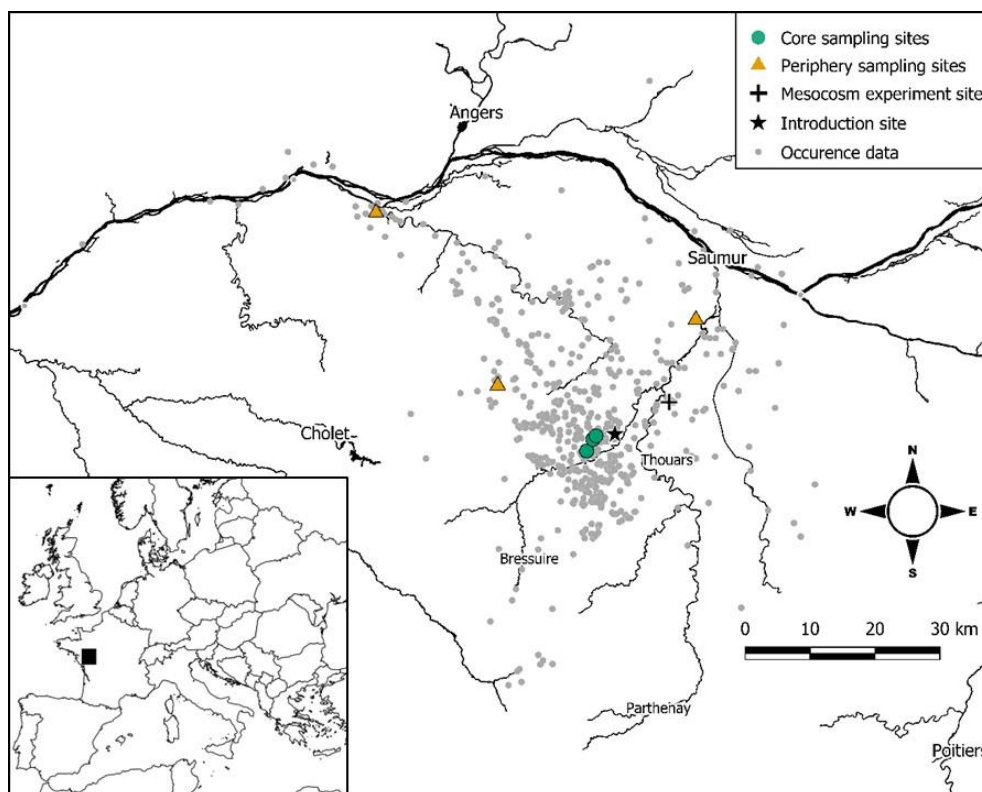
2013). A larger body size increases dispersal propensity and capacity of individuals by improving locomotor performance such as endurance during the dispersal stage (Cayuela et al. 2020). Secondly, we hypothesise that larval life-history traits, such as time to metamorphosis and survival, are also targets for spatial sorting (see Phillips 2009). *Xenopus laevis* adults have longer relative hind limbs at the periphery (Louppe et al. 2017; Padilla et al. 2019). Thus, we expect a longer larval period (i.e. postponement of the timing of metamorphosis) and longer hind limbs in metamorphs at the periphery. Lastly, we measured survival to assess whether the cost of resource re-allocation from reproduction to dispersal could be incurred by adult *X. laevis* at the periphery. We expect a mismatch in survival between the core and periphery as dispersal ability and survival in novel environments are important in shaping invasive distribution ranges. Beyond these theoretical predictions, little is known about whether spatial sorting for dispersal during range expansion impacts traits at the dispersing stage only or at all stages for species with complex life cycles. For example, larvae of the invasive cane toad (*Rhinella marina*) have been shown to have faster growth rates at the periphery of their invasive distribution than at the core (Phillips, 2009).

To test our hypotheses, we conducted experiments in outdoor mesocosms and in laboratory microcosms. Mesocosms allow for exposure to the natural variation in the local environment and a larger sample size of individuals that increases the statistical power to detect effects (Skelly and Kiesecker 2001). In contrast, the standardised experimental conditions of laboratory microcosms, such as light intensity, water volume, or density reduce the environmental noise on the measured responses. Over ten weeks, we surveyed the development of *X. laevis* tadpoles in the French invasive range, from free-swimming larvae to metamorphosis. We tested the effect of the position of the parental pond in the colonised range (core or periphery) on morphological traits related to dispersal (SVL and hind limb length), time to metamorphosis (development) and survival to metamorphosis.

## MATERIALS AND METHODS

### Study site:

The population of *X. laevis* is thought to have been introduced in the 1980s in western France (Fouquet and Measey 2006). The invasive range now covers ~4000 km<sup>2</sup> (JS unpublished data). This area is characterised by a high density of ponds and a dense hydrographic network (Vimercati et al. 2019). The population has spread unevenly from the introduction site. We sampled six ponds (Fig. 1). Three ponds were located at the range core. To ensure sampling of the true core, sampling sites were situated close to the introduction point: site 1 (47°00'38.2"N 0°21'29.2"W; distance from the introduction site (dis) = 5 km), site 2 (47°01'33.9"N 0°20'40.8"W; dis = 3.4 km), and site 3 (47°01'39.5"N 0°20'39.8"W, dis = 3.4 km). Three sites were close to the estimated range periphery: site 4 (47°11'02.6"N 0°07'51.2"W; dis = 21.2 km), site 5 (47°20'38.5"N 0°45'49.0"W; dis = 49.4 km), and site 6 (47°06'13.1"N 0°31'50.4"W, dis = 19.2 km). All sites were located within a relatively small spatial area (~690 km<sup>2</sup>) from each other to decrease microclimate dissimilarity.



**Figure 1:** Current distribution of the invasive population of *Xenopus laevis* in western France. Indicated are the point of introduction, the experimental site and six collection sites used in this study. Small grey dots indicate the occurrence data of *X. laevis* (Vimercati et al. 2019).

## **Adult collection, care, and breeding:**

Four breeding pairs of *X. laevis* were collected at each site. To initiate breeding, individuals were injected with the human chorionic gonadotrophin hormone (HCG). The Amphibian Metamorphosis Assay (AMA) and the *Xenopus* Metamorphosis Assay (XEMA) recommend using HCG to induce breeding in *X. laevis*. Males and females were kept separate and were primed with 50 international units (IU) of HCG shortly after collection. Males were considered sexually mature when the nuptial pads on the forelimbs appear black and females were considered sexually mature when the cloaca was red and swollen. Sexually mature females were injected for a second time with 500 IU and males with 250 IU on the third day (dosages as prescribed by Wlizla et al. 2018). The male and female of a breeding pair were kept separately and only joined on the third day in a plastic aquarium (7-10 L). A plastic mesh was inserted underneath the frogs so the laid eggs could fall through the mesh and not be eaten or damaged by the parents. Frogs were removed from the aquaria the following morning. Eggs were left to hatch in their aquaria maintained at 22°C and well aerated until they developed into free-swimming tadpoles (stage 45). Adults were euthanised at the conclusion of the experiment.

## **Staging**

The larval development of *X. laevis* is well studied and widely used as a model in developmental biology (Nieuwkoop and Faber 1994; Segerdell et al. 2008; Segerdell et al. 2013). The development of *X. laevis* from fertilisation to metamorphosis undergoes 66 NF stages (Nieuwkoop and Faber 1994) grouped into nine stage categories according to an anatomical ontology (Segerdell et al. 2008). The embryonic development (NF stage 1–44, cleavage to tailbud stage category) is rapid and takes place within the first three days. The post-embryonic development within the pre- and prometamorphosis stage categories (NF stage 45–57) includes limb bud development and toe differentiation. Overall body size increases during the pre- and prometamorphosis stage categories and decreases during the climax stage category (NF stage 58–66) during which tail resorption occurs. Thus, for mesocosms the dataset was divided according to stage categories (Segerdell et al. 2008). Due to the linear increase in body size, the pre- and prometamorphosis stage categories (NF stage 45–57) will collectively be referred to as ‘larvae’. These were analysed separately from the climax stage category (NF stage 58–65) where body size decreases. Metamorphs (NF stage 66) were analysed separately as the tail has been completely

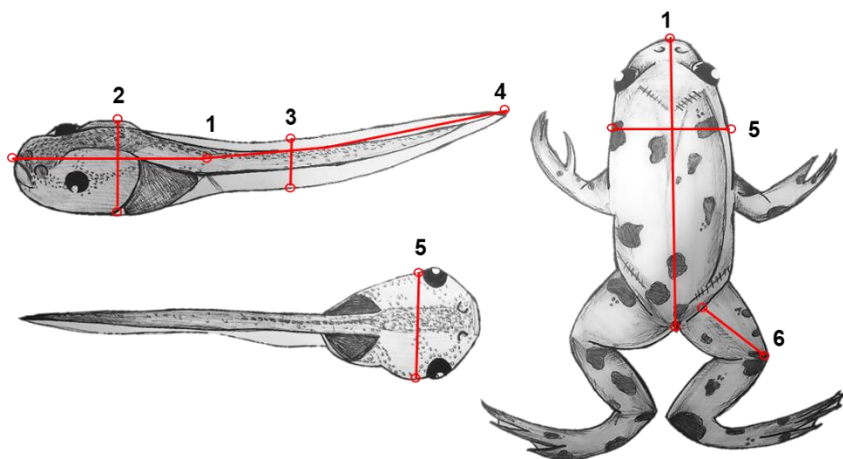


resorbed and data were compared with those from the laboratory microcosms. For microcosms only metamorphs (NF stage 66) were analysed.

## Experiments

We conducted parallel common garden studies in outdoor mesocosms and microcosms in the laboratory. Four egg clutches from all six sites ( $n = 24$ ) were split and allocated to both the mesocosm and microcosm experiment.

**Outdoor mesocosm experiment** - We used 400 L plastic tanks as mesocosms (1.22 m diameter, 0.56 m deep). The experimental site was located in a rural open habitat within 10 m from a pond where *X. laevis* breed and reach high density (47°04'22.8"N; 0°11'20.4"W). Twenty-four mesocosms were set-up in a Latin square design, one clutch was assigned to one mesocosm. In addition, eight temperature data loggers (HOBO K8 ® Temperature/Alarm (Waterproof) Data Logger - UA-001-08, Onset Computer, Bourne, Mass, USA) were placed in mesocosms to measure variation in temperature (Fig. S1). Tanks were filled with 200 L of locally available tap water and left to age for at least one month prior to the experiment. Each mesocosm was partially shaded with a plastic sheet and covered with metal mesh above the tank to avoid predation, to standardise light intensity in each tank and to avoid overheating. Tadpoles were fed Frog Brittle ® for tadpoles (NASCO, Fort Atkinson, WI) throughout the rearing period. The standard NASCO instructions for food (2 g per 37.85 L) once per week was followed and surplus food was avoided to uphold water quality. In each mesocosm, we introduced individuals from a single clutch with an initial density of 200 free swimming larvae (NF stage 45) ( $1 \text{ tadpole} \cdot \text{L}^{-1}$ ). Every week after introduction, five tadpoles were captured at random with a dipnet, euthanised by an overdose of tricaine methanesulfonate (ms-222) and preserved in 70% ethanol. We measured full length, SVL, body depth, tail depth, head width, and femur length (Fig. 2), staged individuals according to Nieuwkoop & Faber (1994) and placed animals into stage categories according to Segerdell et al. (2008).



**Figure 2:** Morphometric measurements taken of *Xenopus laevis* tadpoles (left) and metamorphs (right) at different stages during the mesocosm and microcosm experiments. 1- Snout-to-vent length (SVL); 2- Maximum head/body depth; 3- Maximum tail depth; 4- Tail length; 5- Head width; 6- Femur length (Illustrated by NK).

**Microcosm laboratory experiment-** We conducted a laboratory experiment to measure the growth of tadpoles under constant conditions. The temperature was kept at 22 °C, and the photoperiod was 12 h (OECD, 2007). Initially, 20 tadpoles were collected from each clutch and placed individually into containers with 0.8 L of aged tap water. Containers were randomly arranged on shelves, by assignment from randomly generated numbers. The feeding regime was kept the same as in the outdoor mesocosms. Because of the low sample size, tadpoles were not removed during the experiment but kept until metamorph (NF stage 66) when size and survival were measured. Each metamorph was photographed to measure SVL and hind limb length (Fig. 2).

## Statistical analysis

**Morphological variation-** To assess the effect of position (core vs periphery) on the morphology of each stage (NF stages 45–66), we used SVL and relative femur length (femur length/SVL) as response variables for larvae, climax individuals and metamorphs from the mesocosms. We also analysed the first component of a PCA carried out on the following traits (SVL, head width, body depth, larva tail length, larva tail depth). The details of the procedure and

results are provided as supplementary information (Table S2, Fig. S2, S3, and S4). In individuals from the microcosms, we considered only SVL and femur length of the metamorphs.

Generalised linear mixed models (*lme4* package in R; Bates et al., 2015) were used with the above-mentioned response variables, along with position (core/periphery) and NF stage as fixed effects. Clutch nested within collection site was considered as a random effect. All variables were tested for normality with the *bestNormalize* package that select the best transformation procedure among a set of alternatives (Peterson 2019). Consequently, the best transformation, was the Ordered Quantile (ORQ) transformation. The ORQ transformation function is a rank-based procedure whereby the values are mapped to their percentile, which is then mapped to the percentile of the normal distribution. This transformation results in a uniform distribution provided there are no ties present in the data. Finally, SVL, metamorph SVL, and metamorph relative femur length was plotted using *ggplot2* (Wickham 2009).

**Survival in mesocosms and microcosms-** We removed 50 individuals from the mesocosms owing to our weekly sampling for the morphological study whereas no individuals were removed from the microcosms. Thus, the number of surviving individuals at the end of the experiment was tallied out of 150 for mesocosms and out 20 for microcosms. We modelled the probability of surviving until the end of the experiment in mesocosms and microcosms using generalised binomial mixed models with fate (1 = survived, 0 = died) as response variable, position (core/periphery) as fixed effects and clutch nested within collection site as a random effect. To analyse the dynamics of mortality events during development more thoroughly, we recorded individually the date of death (if applicable) in microcosms. This was not possible in mesocosms. We carried out a Cox proportional hazards analysis using the *coxme* (Therneau 2019a) and *survival* (Therneau 2019b) packages in R with position (core/periphery) as fixed effect and clutch nested within collection site as random effect.

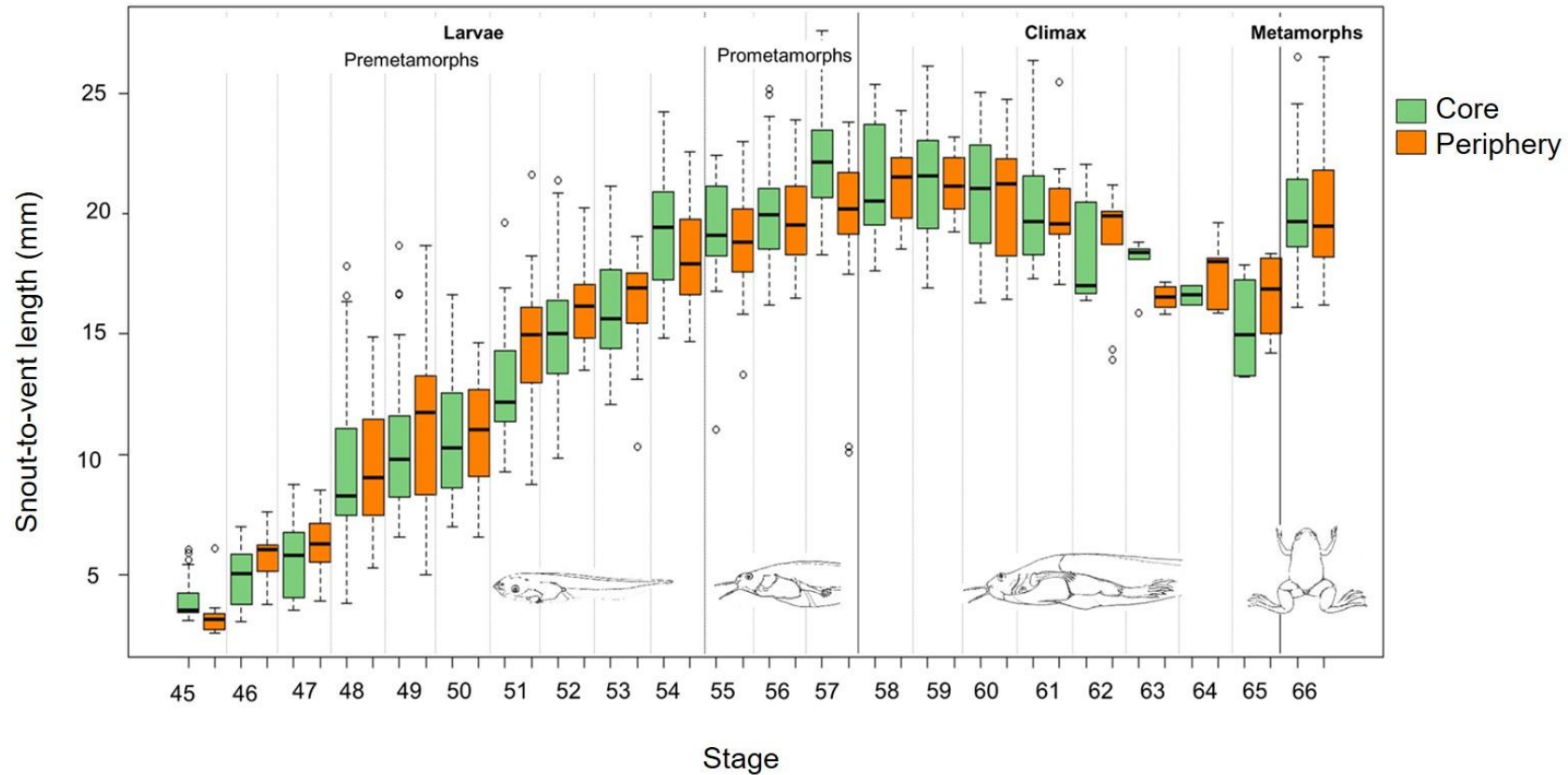
**Time of metamorphosis-** To determine whether phenology of development differed between the core and periphery of the invasive range, we conducted a Cox proportional hazards analysis on transition between stage categories using the *coxme* and *survival* packages in R. The week when transition occurred from larva-to-climax was considered as the response variable. Position (core/periphery) was treated as a fixed effect and clutch nested within collection site as random effects.

281           For all analyses, we selected the best fitting model using the corrected Akaike information  
282 criterion (AICc) according to parsimony (Burnham and Anderson 2002). To account for model  
283 selection uncertainty between the top models ( $\Delta\text{AICc} < 2$ ) multi-model inference (model  
284 averaging) techniques were carried out using the *MuMin* (Barton, 2020) package in R (Burnham  
285 and Anderson, 2002). Model coefficients were subsequently averaged across the set of top models  
286 and the resulting averaged coefficients were used for predictions. If the top model was equal  
287 ( $\Delta\text{AICc} < 2$ ) to the null model, the null model was not rejected. Model diagnostics were carried  
288 out using the *DHARMA* package (Hartig, 2019). All analyses were carried out using the statistical  
289 software R 3.4.1 (R Core Team, 2018).

## Results

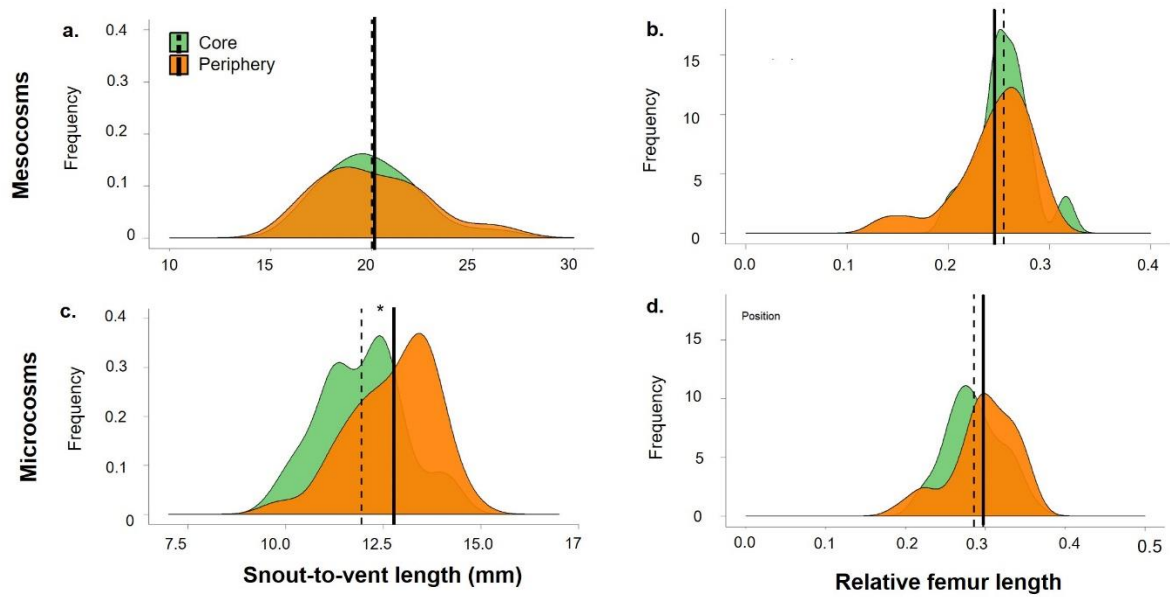
### Variation in morphological traits

Overall, we observed high levels of variation in SVL within each specific stage (Fig. 3) and no significant difference in SVL between core and periphery in individuals reared in the mesocosms. This was true for both tadpoles and climax individuals (Fig. 3, Table 1, table S1). Conversely, metamorphs reared in the microcosms displayed a significant difference in SVL between core and periphery. Individuals were larger at the periphery with a mean SVL of 12.76 mm ( $\pm 1.08$  mm SD;  $n = 34$ ), versus a mean SVL of 11.94 mm (Fig. 4c,  $\pm 1.08$  mm SD;  $n = 38$ ) at the core. The model with only position as a fixed effect was chosen as the most suitable (Table 1, GLMM,  $\Delta AIC = 3.12$ ). In contrast, no differences in SVL (Fig. 4a) and relative femur length were detected and the null model was chosen as the best model for metamorphs reared in mesocosms (Fig. 4b, Table 1, GLMM,  $\Delta AIC = 1.3$ , and  $\Delta AIC = 1.72$ , respectively). No differences were detected in relative femur length between core and periphery individuals reared in microcosms (Fig 4d, Table 1, GLMM,  $\Delta AIC = 8.5$ ). Similar results were observed for body size (PC1) (Appendix S1).



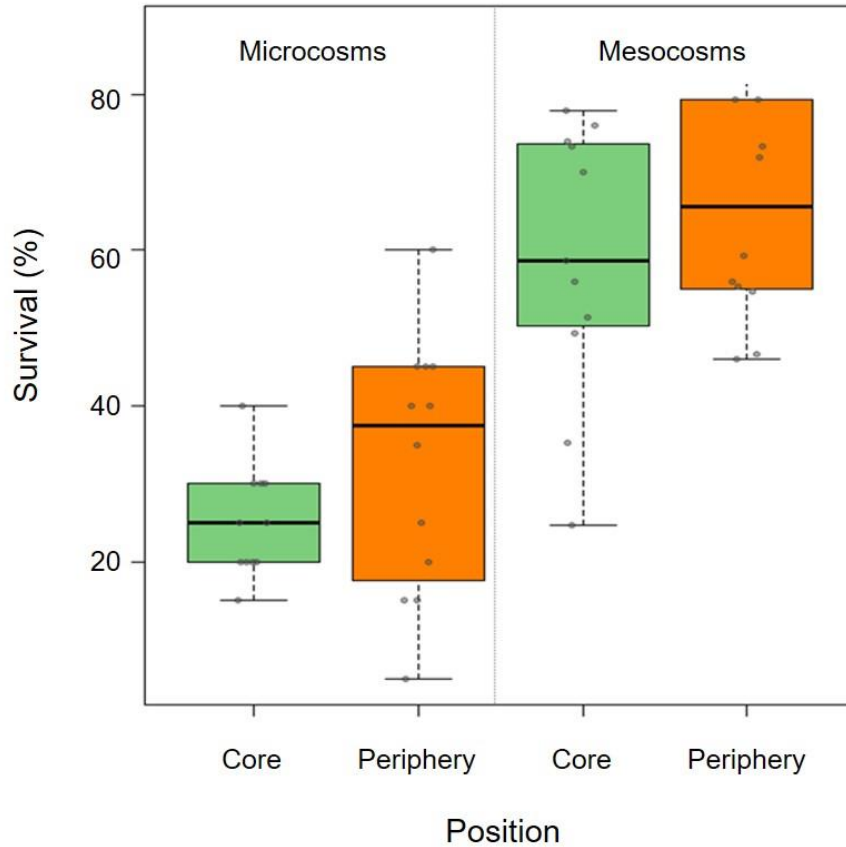
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306 **Figure 3:** Snout-to-vent length variation during the larval development in core and periphery sites of the invasive range colonised by *Xenopus*  
 307 *laevis* in Western France, as measured from individuals raised in the outdoor mesocosms experiment. In the boxplot, the lowest boundary  
 308 indicates the 25<sup>th</sup> percentile, a black line within the box indicates the median, and the highest boundary indicates the 75<sup>th</sup> percentile. Whiskers  
 309 above and below the box indicates the 10<sup>th</sup> and 90<sup>th</sup> percentile. Points above and below the whiskers indicate outliers above and below the  
 310 10<sup>th</sup> and 90<sup>th</sup>



**Figure 4:** Frequency of snout-to-vent length (SVL) and relative femur length of metamorphs: a. SVL between core (green shaded) and periphery (orange shaded) metamorphs reared in outdoor mesocosms. Dotted line indicates the mean SVL or relative femur length of metamorphs from the core and solid line indicates the mean SVL or relative femur length of metamorphs from the periphery reared in the outdoor mesocosms. b. Femur length relative to SVL between core and periphery metamorphs raised in outdoor mesocosms. c. SVL between core and periphery metamorphs raised in laboratory microcosms. A significant shift in SVL is observed for individuals from the periphery raised in the laboratory microcosms. d. Relative femur length between metamorphs from core and periphery sites raised in laboratory microcosms. Variation in survival and development

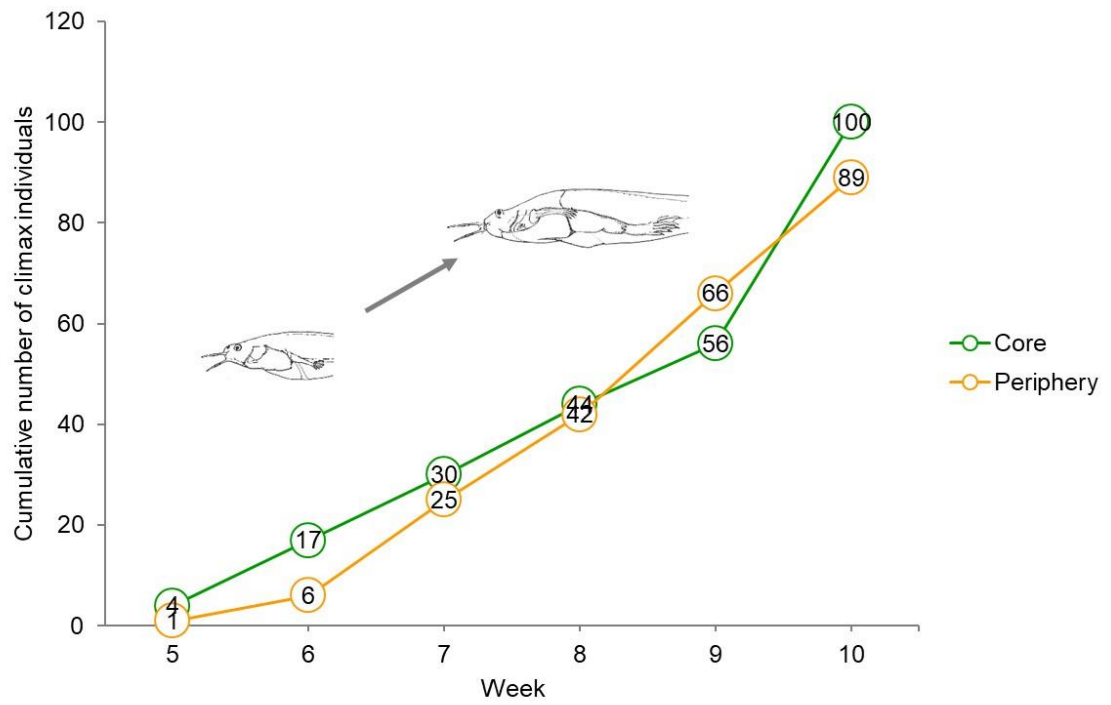
We observed no difference in the proportion of surviving tadpoles in the mesocosms between individuals from the core (88/150 tadpoles  $\pm$  26.41 SD) and the periphery (100/150 tadpoles  $\pm$  22.80 SD) (Fig. 5). Moreover, no other model gave a better fit than the null model (Table 1, binomial GLMM,  $\Delta$ AIC = 0.3). A similar outcome was observed for the microcosms with no difference in the proportion of surviving tadpoles between the individuals from the core (5/20 tadpoles  $\pm$  1.41 SD) and individuals from the periphery (6.5/20 tadpoles  $\pm$  3.26 SD). Once again, the null model was the best model (Table 1, binomial GLMM,  $\Delta$ AIC = 0.03). Similarly, we observed no difference in mortality events between core and periphery individuals in microcosms, with the null model again emerging as the best model (Table 1, cox GLMM,  $\Delta$ AIC = 0.16).



**Figure 5:** Survival of individuals from the core and the periphery in the microcosms and the mesocosms. In the boxplot, the lowest boundary indicates the 25<sup>th</sup> percentile, a black line within the box indicates the median, and the highest boundary indicates the 75<sup>th</sup> percentile. Whiskers above and below the box indicates the 10<sup>th</sup> and 90<sup>th</sup> percentile. Points above and below and the whiskers indicate outliers above and below the 10<sup>th</sup> and 90<sup>th</sup> percentile.

No difference in the time of metamorphosis was observed between individuals from the core and the periphery (Fig 6, Cox proportional hazard, Table 2). The difference in the AICc of the null only model and the corresponding model that included only position was <2 for the transitions from larvae to climax ( $\Delta AIC = 0.003$ ).





**Figure 6:** Cumulative number of *Xenopus laevis* individuals that transitioned from larvae to climax per week in all mesocosms for core and periphery sites.

**Table 1:** Model selection summary of top models ( $\Delta AICc < 2$ ) for each response variable: snout-to-vent length (SVL), relative femur length, overall survival and week of transition between larvae and climax stage category of individuals reared in the mesocosms and microcosms. Predictors in the models were NF stages within stage category (Stage) and core or periphery (Position). Models are ranked by AICc weight ( $W_i$ ), where higher weighted models have more support. K indicates the number of model parameters and logLik the log-likelihood of the model.

Response	Parameters	logLik	K	AICc	$\Delta AICc$	$W_i$
Larval SVL (mesocosm)	Stage	520.3	16	1072.5	0	0.6
	Position x Stage	507.1	29	1072.6	1.0	0.4
Climax SVL (mesocosm)	Position x Stage	325.9	19	695.3	0	0.5
	Stage	336.1	11	696.0	0.7	0.3
Metamorph SVL (mesocosm)	Null	125.6	4	260.1	0	0.2
	Position	125.1	5	261.4	1.3	0.1
Metamorph SVL (microcosm)	Position	108.2	5	227.2	0	0.8
Relative femur length (mesocosm)	Null	74.6	4	157.9	0	0.6
Relative femur length (microcosm)	Null	130.3	4	252	0	0.9
Survival (mesocosm)	Null	-275.2	3	556.4	0	0.5
	Position	-274.1	4	556.4	0.3	0.5
Survival (microcosm)	Null	-275.2	3	556.2	0	0.5
	Position	274.2	4	556.3	0.1	0.5
Larvae to climax (mesocosm)	Position	-822.3	13	1659	0	0.5
	Null	-822.	1	1658.9	0.1	0.5
Weekly survival (microcosm)	Null	-1458.5	18	2954.8	0	0.5
	Position	-1458.4	18	2955.0	0.2	0.4

## Discussion

Spatial sorting theory predicts enhanced dispersal of individuals at the periphery of an expanding population. Furthermore, it suggests that trade-offs with other functions are progressively shifted to allocate more resources to morphological, behavioural and physiological traits favouring dispersal (Travis and Dytham, 2002; Simmons and Thomas 2004; Shine et al. 2011). In the invasive population of *Xenopus laevis* in western France, spatial sorting has enhanced some phenotypic traits that promote dispersal capacity in adults (Louppe et al. 2017; Louppe et al. 2018; Courant et al. 2019; Padilla et al. 2019). In turn, this may affect the phenotypic traits of the non-dispersing stages. In accordance with our predictions, we found that the metamorphs in our laboratory microcosms had larger SVL at the range periphery than at the core. Differences between the core and the periphery identified in our microcosm study can display a trade-off effect from resource re-allocation in adults at the periphery. Adults have been found to allocate more resources to dispersal and less resources to reproduction (Courant et al. 2017). An alternative explanation might be that modifications in tadpole life-history result in higher dispersal abilities in adults. For example, in addition to advantages relevant to dispersal, a larger size at metamorphosis may hold other advantages for adults such as higher fecundity in females, an increase in fitness and an earlier onset of sexual maturity (Smith 1987; Tejedo 1992; Altwegg and Reyer 2003; Cabrera-Guzman et al. 2013). Therefore, body size (snout-to-vent length) is a potential target for spatial sorting in adults at the periphery and can be of interest in future studies. However, adults from the periphery have not previously been found to display larger SVL (Louppe et al. 2017) or a faster growth rate (Courant et al. 2019b). Conversely, males from the periphery were smaller (Louppe et al. 2017; Courant et al. 2019a). Thus, the larger size of peripheral individuals at metamorphosis may only last for a limited period.

While the finding from our microcosm experiment is consistent with our first prediction, we found no other evidence for differences in our outdoor mesocosm and laboratory microcosm experiments. Tadpoles and metamorphs in outdoor mesocosms displayed a large variation in SVL and relative femur length within each stage and no effect of position in the range could be detected. The same pattern was observed when using a multivariate approach of body size instead of single response variable approach which strengthens our findings. This may indicate that density constrains the enhancement of tadpole traits at the periphery, or that microcosm results are not indicative of what is actually happening in the field. The discrepancy

between the laboratory microcosms and outdoor mesocosms can also be due to experimental effects (Brown et al. 2006). Both laboratory and mesocosm experiments can be advantageous. In laboratory-based experiments conditions are standardised, whereas outdoor mesocosms more closely mimic some aspects of natural conditions, such as fluctuating temperatures, food availability and community interactions (Mikó et al. 2015). Thus, they can deliver different results (Skelly, 2002). Mikó et al. (2015) found that agile frog (*Rana dalmatina*) metamorphs are larger in laboratory microcosms than in outdoor mesocosms. In our microcosms, tadpoles were reared individually, whereas in the mesocosms they were reared with siblings. Density and intraspecific interactions are known to affect the size of tadpoles (Dash and Hota, 1980; Kehr et al. 2014). Importantly, it has been observed that at the periphery of expanding populations density can be lower than at the core (Phillips et al. 2010b; Shine et al. 2011). This has not been demonstrated for *X. laevis* populations and should be prioritised for a future study; therefore, the initial density was the same for all clutches in our mesocosms. However, should *X. laevis* density indeed be lower at the periphery, periphery tadpoles should experience less developmental constraints due to lower conspecific density and grow larger than core tadpoles.

Our results do not support the second prediction that spatial sorting would affect tadpole survival and time to metamorphosis. In both experiments, we found no differences in tadpole survival probability between the core and the periphery of the invasive range, in spite of the fact that adults have a higher survival probability at the periphery (Courant et al. 2017). Likewise, we found no differences in development (time to metamorphosis) between core and periphery in the mesocosm experiment. Tadpole survival depends on many abiotic (e.g. hydroperiod, Amburgey et al. 2012) and biotic (e.g. predators and competition, Relyea and Hoverman 2003) factors. Thus, it is possible that the absence of a phenotypic shift in *X. laevis* tadpoles are due to developmental and environmental constraints (Fink 1982, Moran 1994). At metamorphosis, climax individuals make use of their hind limbs to swim and catch food (Combes et al. 2004; Handrigan and Wassersug 2007), whereas adults additionally use hind limbs for overland movements (Handrigan and Wassersug 2007; Padilla et al. 2019). Thus, the strength of the selective force on hind limb length may be reduced in juveniles.

This discrepancy in spatial phenotypic variation between adults and tadpoles in response to range expansion can be due to the decoupling of traits (Moran 1994; Sherrat et al. 2017). Decoupling describes the process by which variation occurs in a trait at a particular stage of the life cycle without affecting the expression of this trait at another stage. In

*X. laevis* only a small fraction of the genes that account for morphology are expressed (1.6%) in both tadpoles and adults (Wollenberg-Valero et al. 2017). The similarity observed between core and periphery populations may simply reflect the fact that, from a functional perspective, tadpoles are constrained to keep their morphological features and developmental rate constant. This can indicate that in *X. laevis* each life stage can experience a unique set of constraints, also seen in salamanders (Bonnet and Blair, 2017). Our results may also indicate that the genetic regulation causing the hind limb to grow longer does not occur until after metamorphosis. In that case, the trade-offs between dispersal and reproduction or metabolism are only expressed at the dispersing stage of ontogeny. However, the degree of decoupling across stages is trait-dependent and some traits can be less plastic than others due to canalisation (Levis and Pfennig, 2019). Canalisation describes the development of a fixed phenotype in response to environmental variation if plasticity becomes too costly (Debat and David, 2001; Levis and Pfennig, 2019). Decoupled evolution through spatial sorting can lead to modifications of adult *X. laevis* phenotypes that would promote dispersal without affecting the phenotype of the tadpole stage. This study provides evidence for the decoupling of dispersal traits in *X. laevis* adults from tadpole morphology (SVL), developmental rate and survival. However, the larger SVL of metamorphs at the periphery suggests that metamorph morphology is influenced by spatial sorting and possibly coupled to adult fitness.

Spatial sorting is predicted to occur in expanding populations. Because species with complex life cycles experience stage-specific selection pressures, the decoupling of traits between stages can be enhanced during the colonisation process, especially when novel environmental conditions are encountered, as expected for invasive populations. Our study highlights that invasive *X. laevis* tadpole traits can be conserved and are not necessarily influenced by the change of dispersal traits in adults, possibly due to decoupling. Due to the fact that the introduction of *X. laevis* in France is fairly recent (~40 years), it is unknown whether the strength/intensity of decoupling changes over time in an expanding population, and then enhance or moderate the effects of spatial sorting on dispersal. Currently, many species experience changes in distribution ranges as a result of translocation or climate change (Chuang and Peterson, 2016). Investigating spatial sorting and the coupling or decoupling of traits across life stages in expanding populations of species with complex life cycles, either native or invasive, may help us to better understand how constraints at the non-dispersing stage may contribute to the success or failure of expansion.

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