

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

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Received 29 July 2020; revised 19 October 2020; accepted for publication 20 October 2020

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 carry-over 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 location (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.

ADDITIONAL 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, e.g. 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); wider head widths (Hudson *et al.*, 2018)] and physiology [e.g. alterations in immune function (Llewellyn *et al.*, 2012; Ronce and Clobert, 2012; Brown *et al.*, 2015a; Brown *et al.*, 2015b); 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

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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. Although in some amphibians only adults disperse (Cayuela *et al.*, 2018), one or more of their earlier 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 carry-over effects [e.g. as in 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 (Sherratt *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 (Johansson *et al.*, 2010; Tejedo *et al.*, 2010; Yagi and Green, 2017).

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 tadpole 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. as in *Pelophylax* sp., and *Rana* sp. (Altwegg and Reyer, 2003; Chelgren *et al.*, 2006)]. Smith-Gill and Berven (1979) described 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. as in wood frogs, *Lithobates sylvatica* (Relyea, 2001)]. Shorter larval development usually decreases hind limb length (Gomez-Mestre and Buchholz, 2006; Tejedo *et al.*, 2010; Gomez-Mestre *et al.*, 2013) 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.*, 2017; Courant *et al.*, 2019a; Courant *et al.*, 2019b; Padilla *et al.*, 2019). Adults were found to lower resource allocation to reproduction at the periphery of the invasive range (Courant *et al.*, 2017) 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; Louppe *et al.*, 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.*, 2019b).

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 hypothesized 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-Guzmán *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 hypothesize that larval life-history traits, such as time to metamorphosis and survival, are also targets for spatial sorting (see Phillips *et al.* 2010). *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 *et al.* 2010).

To test our hypotheses, we conducted experiments in outdoor mesocosms and in laboratory microcosms. Laboratory and outdoor experiments each provide complementary and essential information (as seen in Morin, 1998). 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). However, conducting experiments in the field can result in measurements with higher variation and less precision among replicates (Morin, 1998). In contrast, the standardized experimental conditions of laboratory microcosms, such as light intensity, water volume or tadpole density reduce the environmental noise on the measured responses. Laboratory experiments have nonetheless been criticized for being less realistic than field experiments (Skelly and Kiesecker, 2001). Therefore, in response to previous criticisms of both experimental venues we used both. Over 10 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 location of the parental pond in the colonized range (core or periphery) on morphological traits related to dispersal (SVL and hind limb length), time to metamorphosis (development) and survival to metamorphosis.

MATERIAL 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 ~4500 km² (Vimercati *et al.*, 2020). This area is characterized by a high density of ponds and a dense hydrographic network (Vimercati *et al.*, 2020). The population has spread unevenly from the introduction site. There are no climatic differences between the core and current periphery of this population as they both are within the Oceanic climatic region. 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 km²) from each other to decrease microclimate dissimilarity.

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 appeared 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 euthanized 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, 2013). The development of *X. laevis* from fertilization to metamorphosis undergoes 66 Nieuwkoop and Faber (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 3 days. 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. Metamorphosis

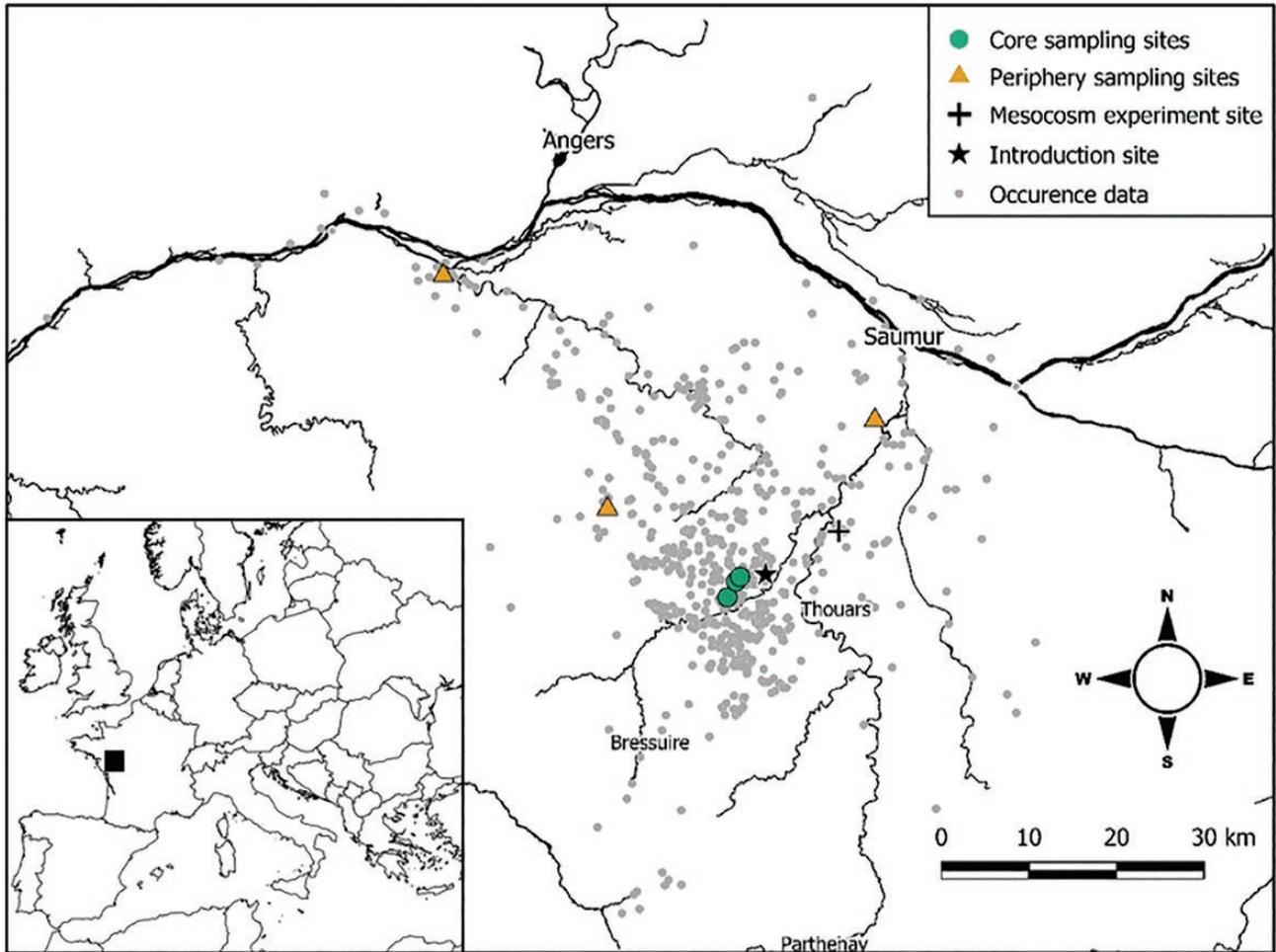


Figure 1. Current distribution of the invasive population of *X. laevis* in western France. The point of introduction (star symbol), the experimental site (plus symbol) and the six collection sites in the core (green circles) and periphery (orange triangle) used in this study are all indicated. Small grey dots indicate the occurrence data of *X. laevis* (Vimercati *et al.*, 2020).

(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 only. No wild larvae or metamorphs were collected. 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, MA, USA) were placed in mesocosms to measure variation in temperature (Supporting Information, Fig. S1). Tanks were filled with 200 L of locally available tap water and left to age for at least 1 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 standardize 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 tadpole L⁻¹). Every week after introduction, five tadpoles were captured at random with a dipnet, euthanized 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 and Faber (1994) and placed animals into stage categories according to Segerdell *et al.* (2008).

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, head width, and femur length (Fig. 2).

STATISTICAL ANALYSIS

Morphological variation

To assess the effect of location (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 in Supporting Information (Table S2; Figs S2–S4). In individuals from the microcosms, we considered only SVL and femur length of the metamorphs. Femur length was examined by using SVL as a covariate in the linear mixed models to account for correlation.

Linear mixed models [*lme4* package in R (Bates *et al.*, 2015)] were used with the above-mentioned response variables, along with location (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 selects the best transformation procedure among a set of alternatives (Peterson and Cavanaugh 2020). 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).

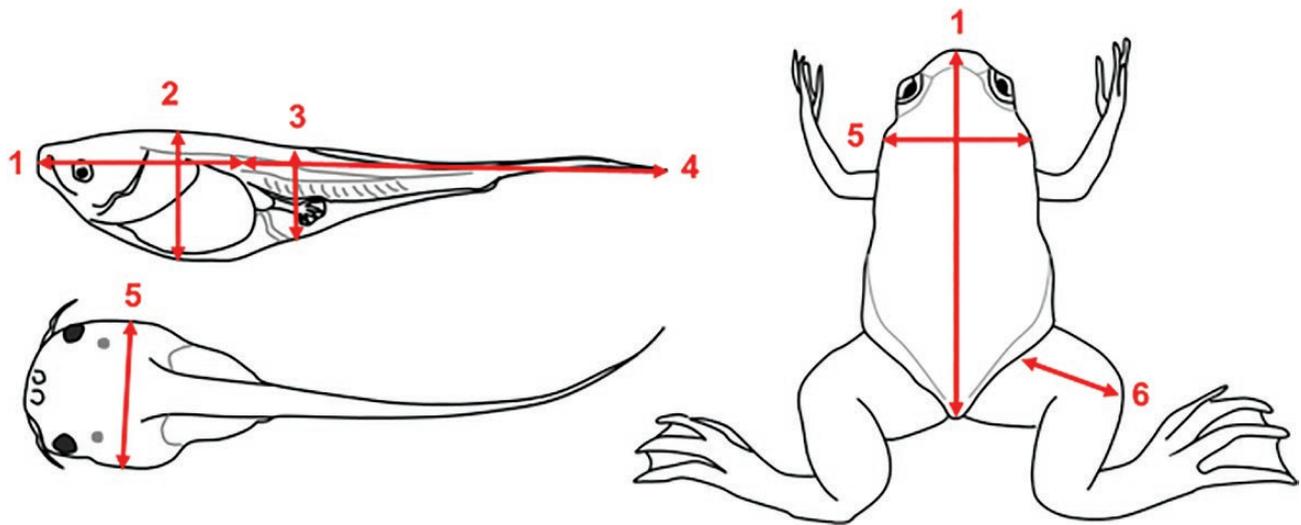


Figure 2. Morphometric measurements taken of *X. laevis* tadpoles (left) and metamorphs (right) at different stages during the mesocosm and microcosm experiments: (1) snout-to-vent length (SVL); (2) maximum head/body depth; (3) maximum tail depth; (4) tail length; (5) head width; (6) femur length (illustrated by N.K.).

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 generalized binomial mixed models with fate (1 = survived, 0 = died) as a response variable, location (core/periphery) as a fixed effect and clutch nested within collection site as a random effect. To analyse the dynamics of mortality events during development more thoroughly, we individually recorded 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 location (core/periphery) as a fixed effect and clutch nested within collection site as a 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. Location (core/periphery) was treated as a fixed effect and clutch nested within collection site as a random effect.

For all analyses, we selected the best fitting model using the corrected Akaike information criterion (AICc) according to parsimony (Burnham and Anderson, 2002). To account for model selection uncertainty between the top models ($\Delta\text{AICc} < 2$), multi-model inference (model averaging) techniques were carried out using the *MuMIn* (Barton, 2020) package in R (Burnham and Anderson, 2002). Model coefficients were subsequently averaged across the set of top models and the resulting averaged coefficients were used for predictions. If the top model was equivalent ($\Delta\text{AICc} < 2$) to the null model, the null model was not rejected. Model diagnostics were carried out using the *DHARMA* package (Hartig, 2019). All analyses were carried out using the statistical software R v.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). No significant

differences in SVL between core and periphery tadpoles reared in the mesocosms were found. Stage was retained as the most important predictor among all competing models. This was true for both tadpoles and climax individuals (Fig. 3; Table 1; Supporting Information, 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 (± 1.08 mm SD; $N = 34$) vs. a mean SVL of 11.94 mm (± 1.08 mm SD; $N = 38$) at the core. The model with only location as a fixed effect was chosen as the most suitable (Table 1, GLMM, $\Delta\text{AIC} = 3.12$). In contrast, no differences in SVL (Fig. 4A) were detected and the null model was chosen as the best model for metamorphs reared in mesocosms (Table 1, GLMM, $\Delta\text{AIC} = 1.3$). Similar results were observed for body size (PC1) (Supporting Information, Appendix S1, Table S3). No differences were detected in femur length between core and periphery individuals reared in mesocosms ($\Delta\text{AIC} = 7.5$) and microcosms (Table 1, GLMM, $\Delta\text{AIC} = 8.2$) as the model with only SVL was chosen as the best fit model (Table 1).

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 ± 26.41 SD) and the periphery (100/150 tadpoles ± 22.80 SD) (Fig. 5). Moreover, no other model gave a better fit than the null model (Table 1, binomial GLMM, $\Delta\text{AIC} = 0.3$). 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 ± 1.41 SD) and individuals from the periphery (6.5/20 tadpoles ± 3.26 SD). Once again, the null model was the best model (Table 1, binomial GLMM, $\Delta\text{AIC} = 0.03$). 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, $\Delta\text{AIC} = 0.16$).

No difference in the time of metamorphosis was observed between individuals from the core and the periphery (Cox proportional hazard, Fig. 6, Table 1). The difference in the AICc of the null-only model and the corresponding model that included only location was < 2 for the transitions from larvae to climax ($\Delta\text{AIC} = 0.003$).

DISCUSSION

Spatial sorting theory predicts enhanced dispersal of individuals at the periphery of an expanding population. Furthermore, it suggests that trade-offs

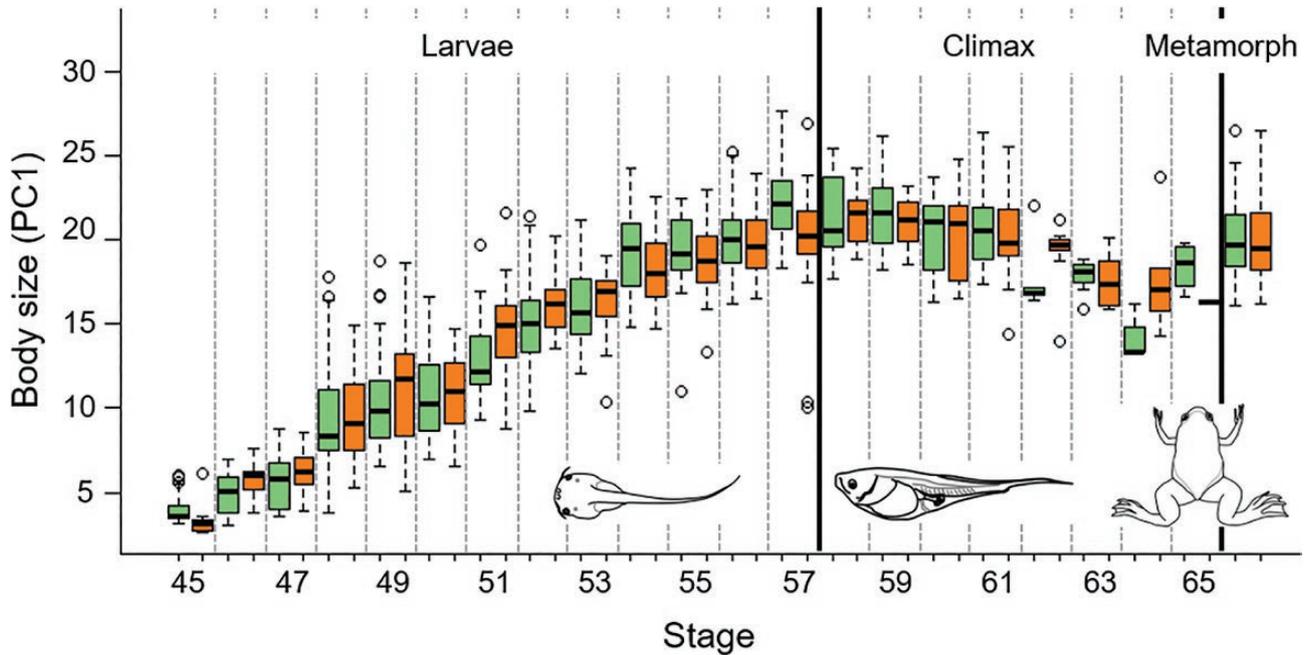


Figure 3. Snout-to-vent length variation during larval development in core (green boxplot) and periphery (orange boxplot) sites of the invasive range colonized by *X. laevis* in western France, as measured from individuals reared in the outdoor mesocosms experiment. In the boxplot, the lowest boundary indicates the 25th percentile, a black line within the box indicates the median, and the highest boundary indicates the 75th percentile. Whiskers above and below the box indicate the 10th and 90th percentiles. Points above and below the whiskers indicate outliers above and below the 10th and 90th percentiles.

Table 1. Model selection summary of top models ($\Delta\text{AICc} < 2$) 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 (location). 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	ΔAICc	W_i
Larval SVL (mesocosm)	Stage	520.3	16	1072.5	.0	0.6
	Location \times stage	507.1	29	1072.6	1.0	0.4
Climax SVL (mesocosm)	Location \times 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
	Location	125.1	5	261.4	1.3	0.1
Metamorph SVL (microcosm)	Location	108.2	5	227.2	.0	0.8
Femur length (mesocosm)	SVL	68.7	5	-126.2	.0	1.0
Femur length (microcosm)	SVL	98.8	5	-186.7	.0	1.0
Survival (mesocosm)	Null	-275.2	3	556.4	.0	0.5
	Location	-274.1	4	556.4	0.3	0.5
Survival (microcosm)	Null	-275.2	3	556.2	.0	0.5
	Location	274.2	4	556.3	0.1	0.5
Larvae to climax (mesocosm)	Location	-822.3	13	1659.0	.0	0.5
	Null	-822.0	1	1658.9	0.1	0.5
Weekly survival (microcosm)	Null	-1458.5	18	2954.8	.0	0.5
	Location	-1458.4	18	2955.0	0.2	0.4

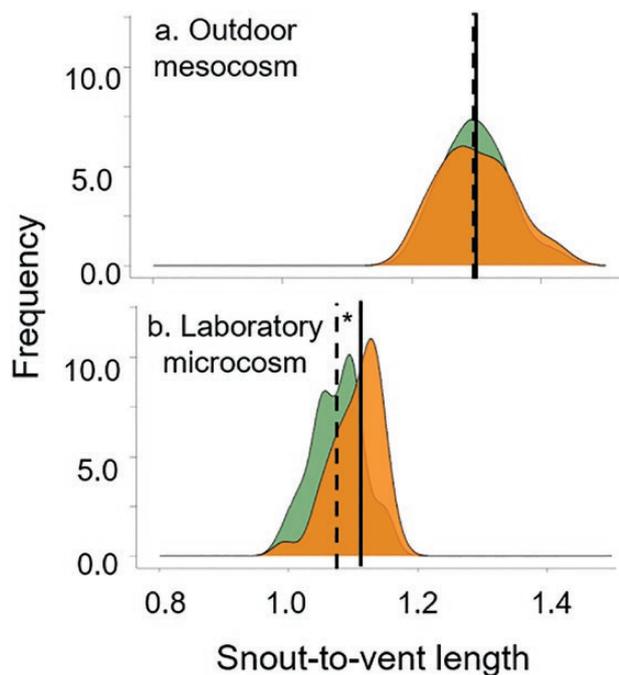


Figure 4. Frequency of snout-to-vent length (SVL) from the core (green shaded) and periphery (orange shaded) metamorphs. Mean SVL of metamorphs from the core (dashed black vertical line) and periphery (solid black vertical line) reared in: (a) outdoor mesocosms and (b) laboratory microcosms. Asterisks indicate significant differences ($P < 0.05$).

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 *X. laevis* in western France, spatial sorting has enhanced some phenotypic traits that promote dispersal capacity in adults (Loupe *et al.*, 2017; Loupe *et al.*, 2018; Courant *et al.*, 2019a; 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. The differences between the core and the periphery identified in our microcosm study might display a trade-off effect from resource reallocation in adults at the periphery. Adults have been found to allocate more resources to dispersal and less resources to reproduction (Courant *et al.*, 2017). Another 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

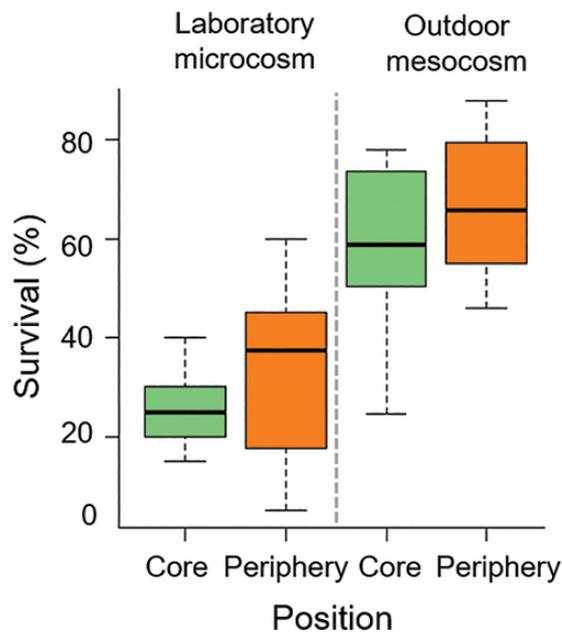


Figure 5. Survival of individuals from the core and the periphery in the microcosms and the mesocosms. In the boxplot, the lowest boundary indicates the 25th percentile, a black line within the box indicates the median, and the highest boundary indicates the 75th percentile. Whiskers above and below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

(Smith, 1987; Tejado, 1992; Altwegg and Reyer, 2003; Cabrera-Guzmán *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, this finding was not corroborated by the outdoor mesocosm experiment, which may be due to increased natural variation in field experiments (Morin, 1998).

Although the finding from our laboratory microcosm experiment is consistent with our first prediction, we found no other evidence for morphological differences for tadpoles in our outdoor mesocosm and laboratory microcosm experiments. Tadpoles and metamorphs in outdoor mesocosms displayed a large variation, as previously seen in outdoor mesocosm experiments (Morin, 1998), in SVL and relative femur length within each stage and no effect of location could be detected. The same pattern was observed when using a multivariate approach of body size, instead of a single response variable approach which strengthens our findings. The discrepancy between the laboratory microcosms and outdoor mesocosms can be due to experimental effects (Brown *et al.*, 2006). Mesocosm experiments are more likely to reflect the realistic variation and measurements. The differences detected by the laboratory experiment

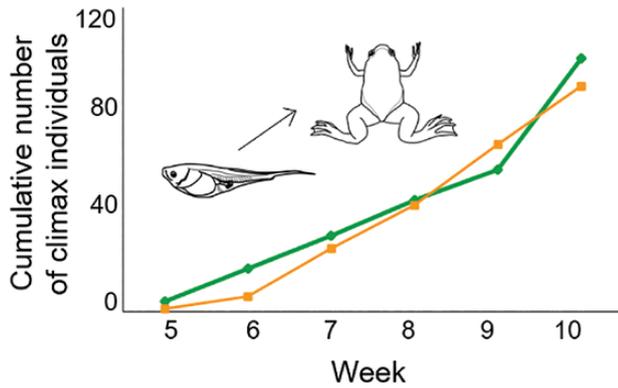


Figure 6. Cumulative number of *X. laevis* individuals that transitioned from larvae to climax per week in all mesocosms for core (green line) and periphery (orange line) sites.

might not be reflecting the real scenario in the field (Morin, 1998; Skelly and Kiesecker, 2001, Mikó *et al.*, 2015). Thus, different experimental designs 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. We found an opposite effect in our experiment with *X. laevis* metamorphs being smaller in the laboratory microcosms, with tadpoles from the periphery being larger than from the core.

Density and intraspecific interactions of tadpole aggregations are known to affect the size of tadpoles (Dash and Hota, 1980; Kehr *et al.*, 2014). Unmeasured variables in the mesocosm experiment such as density and temperature throughout the trial period may have varied, whereas, in the laboratory the effect of density and temperature were controlled. Therefore, the results may reflect tadpole size when variables such as temperature and density are controlled for. Typically, tadpoles reduce growth rates at high densities; however, Gouchie *et al.* (2008) found that *X. laevis* tadpoles at relatively higher densities did not have a significant reduction in growth rates. Contrastingly, when confronted with a simulation of conspecifics (their reflection in a mirror), *X. laevis* tadpoles displayed decreased growth rates possibly due to increased activity (Gouchie *et al.*, 2008). Nonetheless, *X. laevis* tadpoles have shown to aggregate in natural waterbodies (Wassersug and Hessler, 1971; Wassersug *et al.*, 1981), which in addition to other benefits can form thermal sinks and can elevate body temperature more than when individuals are isolated (e.g. Guilford, 1988). Furthermore, Katz *et al.* (1981) suggested that the growth potential of *Xenopus* tadpoles can increase when they aggregate in the water column. The aggregative behaviour of tadpoles can, therefore, enhance tadpole

fitness in the mesocosms (Hokit and Blaustein, 1997). Furthermore, 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 reared in the laboratory experiment may be spurious or only last for a limited period. It has been observed that at the periphery of expanding populations density can be lower than at the core (Phillips *et al.*, 2010; Shine *et al.*, 2011). This has not been demonstrated for *X. laevis* populations and should be prioritized for future study. 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 is 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; Sherratt *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). Although a few studies have focussed on differences in physiology between core and periphery populations of adult *X. laevis* (Louppe *et al.*, 2018; Padilla *et al.*, 2020), no studies have investigated potential differences in tadpole

physiology. Studies centred on physiological difference may be worthwhile to better determine the impacts of spatial sorting. However, 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 (Bonett 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 canalization (Levis and Pfennig, 2019). Canalization 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 colonization 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 ago), it is unknown whether the strength/intensity of decoupling changes over time in an expanding population, and then enhances or moderates the effects of spatial sorting on dispersal. However, future studies on additional taxa are necessary to be able to generalize these findings. 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.

ACKNOWLEDGEMENTS

The authors would like to thank the Editor and anonymous reviewers for their contribution to improve the manuscript. N.K. and J.M. would like to thank the DSI-NRF Centre of Excellence for Invasion Biology (South Africa). N.K. would like to acknowledge the Ambassade de France en Afrique du Sud (France). This study was part of the project Life Control strategies Of Alien invasive Amphibians (CROAA)—LIFE15 NAT/FR/000864 funded by the Life program of the European Commission. We thank the Communauté de Communes du Thouarsais and Communauté de Communes du Bressuirais for allowing access to sampling sites and for their technical assistance. We would like to acknowledge Guénoél Le Peutrec and Fanny Raux for their field and laboratory assistance. We declare that we know of no conflicts of interest associated with this publication.

N.K., J.M. and J.S. conceived and designed the study, N.K. performed the experiments and data acquisition, N.K. and J.S. contributed to the data analysis. N.K., J.M., G.V., A.H. and J.S. were involved with the interpretation of data. N.K. drafted the manuscript and J.M., G.V., A.H. and J.S. revised the manuscript and approved the final version. N.K., J.M., G.V., A.H. and J.S. 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.

ETHICAL STATEMENT

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

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Testing the effects of position and stage on tadpole and metamorph morphology as described for principal component analysis.

Figure S1. Averaged daily temperature profile in outdoor mesocosms (solid black line) with standard error from the average (standard error bars) for a period of 10 weeks in 2018.

Figure S2. Body size (PC1) variation during the larval development of larvae (NF stages 45–57) from the core (green) and the periphery (orange) of the invasive range of *X. laevis* in western France as measured from individuals raised in outdoor mesocosms.

Figure S3. Body size (PC1) variation during the larval development of climax individuals (NF stages 58–65) from the core and the periphery of the invasive range of *X. laevis* in western France as measured from individuals raised in outdoor mesocosms.

Figure S4. Body size (PC1) variation of metamorphs (NF stage 66) from the core and the periphery of the invasive range of *X. laevis* in western France as measured from individuals raised in an outdoor mesocosm.

Table S1. Full model-averaged parameter coefficients, estimates and standard error for GLMM (tadpole and climax individuals' snout-to-vent length).

Table S2. Results of principal component (PC) analyses of the morphology of *X. laevis* larvae, climax individuals and metamorphs.

Table S3. Model selection summary for body size (PC1) of larvae, body size (PC1) of climax individuals, and body size (PC1) of metamorphs reared in the mesocosms.

SHARED DATA

The data for this study have been archived in the Zenodo data repository (doi:[10.5281/zenodo.4071234](https://doi.org/10.5281/zenodo.4071234)).