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Do vulnerable life-history stages of *Xenopus laevis* reduce its invasion potential ?

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THESE de DOCTORAT DE L'UNIVERSITE DE LYON
opérée au sein de
l'Université Claude Bernard Lyon 1

Ecole Doctorale N° ED 341
Évolution, Écosystèmes, Microbiologie, Modélisation
(E2M2)

Spécialité de doctorat :
Ecologie, Biologie évolutive et biologie des populations

Thèse en cotutelle

Soutenue publiquement le 09/03/2020, par :
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***Xenopus laevis* reduce its invasion**
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Devant le jury composé de :

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Do vulnerable life-history stages of *Xenopus laevis* reduce its invasion potential?

by

Natasha Kruger

Dissertation presented for the degree of Doctor of Philosophy in the Faculty of Science at Stellenbosch University. This dissertation has also been presented at Université Claude Bernard Lyon 1 in terms of a joint-/double-degree agreement.

Supervisors: Prof. John Measey

Dr. Jean Secondi

Co-supervisor: Prof. Anthony Herrel.

December 2020

Declaration.

By submitting this thesis/dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights, and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

This dissertation includes one article published in *Aquatic invasions* titled “Anti-predator strategies of the invasive African clawed frog, *Xenopus laevis*, to native and invasive predators in western France”. It also includes one article submitted and under review (Chapter 3). Two papers (Chapter 2 and 4) are yet to be submitted for publication. The development and writing of the papers (published and unpublished) were the principal responsibility of myself.

Natasha Kruger

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Abstract.

Introduced populations must overcome several barriers to become invasive in a novel environment where they may experience new ecological conditions. Phenotypic plasticity and local adaptation are two mechanisms that can assist introduced populations to overcome these barriers. The African clawed frog, *Xenopus laevis* (Daudin, 1802) is native to southern Africa but is invasive on four different continents. This study aimed to understand four major aspects of the African clawed frogs' invasion in western France.

Firstly, I assessed for the level of phenotypic plasticity and local adaptation of *X. laevis* tadpoles from the two contrasting rainfall regions in the native range, as this could provide information on the level of phenotypic plasticity in the French invasive range. The winter rainfall region is characterised by colder temperature and a winter rainfall pattern whereas the summer rainfall region is characterised by warmer temperatures and a summer rainfall pattern. I performed a reciprocal exchange experiment using outdoor mesocosms. I measured body size, timing of metamorphosis, and survival of tadpoles (NF stage 45 - 66). I found that both phenotypic plasticity and local adaptation determined the tadpole phenotype in the winter and summer rainfall regions. I also identified a survival cost in individuals translocated to the other region. However, the cost was lower for winter rainfall tadpoles. Thus, the interaction between phenotypic plasticity and local adaptation likely explains the persistence of this species in contrasting environments.

Secondly, in amphibians, tadpole phenotypic traits can be coupled or decoupled to dispersal traits in adults. Spatial sorting has been observed in adults of *X. laevis* in western France. Thus, I tested whether spatial sorting altered the morphology and life-history traits of tadpoles due to the coupling of traits between stages. I conducted common garden experiments in laboratory microcosms and outdoor mesocosms. I compared body size, timing of

metamorphosis and survival between tadpoles from the core and from the periphery of the invasive range but found no effects of the position in the invasive range on tadpole phenotype. The only difference in both the outdoor and laboratory experiments was the larger body size of post-metamorphic individuals at the periphery. These findings support the hypothesis of decoupling between adult traits and tadpole phenotypic traits. Decoupling can allow each stage of the complex life cycle to respond independently to environmental conditions.

Thirdly, I assessed whether the degree of phenotypic plasticity changed between the core and the periphery during the expansion process. I tested this hypothesis for invasive *X. laevis* in western France. I measured the critical thermal limits and modelling the thermal performance curves of the burst swimming performance (velocity, acceleration, and sinuosity) of core and periphery tadpoles reared at three developmental temperatures (low, intermediate, and high). I found that both the position in the distribution (core/ periphery) and the developmental temperature was significant in determining burst swimming performance. However, no differences were identified between the performance of core and periphery tadpoles reared in the same developmental temperature. Tadpoles reared at the high temperature acclimated their performance to warmer temperatures, however, tadpoles displayed limited acclimation to cooler temperatures.

Finally, I tested whether tadpoles identified and responded to novel predators in their invasive range. In western France, *X. laevis* can encounter novel aquatic predators. Some may be related to predators found in the native range, but others belong to different taxonomic groups. I tested whether naïve tadpoles from the invasive French population exhibited anti-predator response to local predators, and whether the response depended on the degree of relatedness with predators in the native range. I exposed naïve lab-reared tadpoles to a non-predatory water snail, *Planorbarius corneus*, a native predatory beetle, *Dytiscus dimidiatus*, and an invasive predatory crayfish, *Procambarus clarkii*. I found that tadpoles innately reduced

their activity when exposed to beetle and crayfish olfactory cues, but not to snail cue. Reducing activity usually decreases the probability of being detected by predators. This demonstrates that invasive tadpoles respond to known and novel predators regardless of the evolutionary history of the prey-predator interaction.

This work focused entirely on tadpoles. It demonstrates the high phenotypic plasticity of larval development in the native range, which may provide the necessary variation for the colonisation of new areas, provided no evidence of coupling between stages, which allow each stage to respond its own constraints, and showed the recognition of local predators, which is expected to enhance survival. Overall, this thesis describes mechanisms whereby *X. laevis* tadpoles can contribute to the invasion success of the species in France, and globally.

Résumé.

Les populations introduites doivent surmonter plusieurs obstacles pour devenir envahissantes dans un nouvel environnement où elles peuvent subir de nouvelles conditions écologiques. La plasticité phénotypique et l'adaptation locale sont deux mécanismes qui peuvent aider les populations introduites à surmonter ces obstacles. Le Xénope lisse, *Xenopus laevis* (Daudin, 1802) est originaire d'Afrique australe et envahissante sur quatre continents. Cette étude visait à comprendre quatre aspects majeurs de l'invasion du Xénope lisse dans l'ouest de la France.

Premièrement, j'ai évalué le niveau de plasticité phénotypique et d'adaptation locale des têtards de *X. laevis* des deux régions pluviométriques de l'aire de répartition d'origine, car cette connaissance pourrait fournir des informations sur le niveau de plasticité phénotypique au sein de l'aire colonisée en France. La région des précipitations hivernales est caractérisée par des températures plus froides et un régime pluviométrique hivernal tandis que la région des précipitations estivales se caractérise par des températures plus chaudes et un régime pluviométrique estival. J'ai effectué une expérience d'échange réciproque en utilisant des mésocosmes en milieu extérieur. J'ai mesuré la taille du corps, le moment de la métamorphose et la survie des têtards (stade NF 45 - 66). J'ai trouvé que la plasticité phénotypique et l'adaptation locale déterminaient le phénotype du têtard dans les régions deux régions pluviométriques (précipitations hivernales ou estivales). J'ai également identifié un coût à la survie chez les individus transférés dans l'autre région climatique. Cependant, le coût était plus faible pour les têtards de la région à précipitations hivernales. Ainsi, l'interaction entre plasticité phénotypique et adaptation locale explique probablement la persistance de cette espèce dans des environnements contrastés.

Deuxièmement, chez les amphibiens, les caractères phénotypiques des têtards peuvent être couplés ou découplés des caractères liés à la dispersion chez les adultes. Un processus de « spatial sorting » (tri spatial des phénotypes) a été observé chez des adultes de *X. laevis* dans l'ouest de la France. J'ai ainsi testé si ce processus modifiait la morphologie et les traits d'histoire de vie des têtards en raison d'un couplage traits entre les stades. J'ai mené des expériences en environnement commun dans des microcosmes au laboratoire et des mésocosmes en milieu extérieur. J'ai comparé la taille du corps, le moment de la métamorphose et la survie entre les têtards du centre et de la périphérie de la zone colonisée, mais je n'ai trouvé aucun effet de la position dans la zone colonisée sur le phénotype du têtard. La seule différence détectée dans les deux expériences réalisées en mésocosme et en laboratoire était la plus grande taille corporelle des individus post-métamorphiques à la périphérie. Ces résultats soutiennent l'hypothèse d'un découplage entre les traits adultes et les traits phénotypiques têtards. Le découplage peut permettre à chaque étape du cycle de vie complexe de répondre indépendamment aux conditions environnementales.

Troisièmement, j'ai évalué si le degré de plasticité phénotypique avait changé entre le noyau et la périphérie pendant le processus d'expansion. J'ai testé cette hypothèse pour la population invasive de *X. laevis* dans l'ouest de la France. J'ai mesuré les limites thermiques critiques et modélisé les courbes de performances thermiques pour le comportement de déclenchement de la nage (vitesse, accélération et sinuosité) des têtards du centre et de la périphérie ayant été élevés à trois températures de développement (basse, intermédiaire et élevée). J'ai trouvé que la position dans la distribution (centre / périphérie) et la température de développement avaient des effets significatifs. Cependant, aucune différence n'a été détectée entre les performances de nage des têtards du centre et de la périphérie s'étant développés à la même température. Les têtards élevés à la température élevée se sont acclimatés à des

températures plus chaudes. En revanche, les têtards ont montré une capacité d'acclimatation limitée à des températures basses.

Enfin, j'ai testé si les têtards identifiaient et répondaient à de nouveaux prédateurs dans leur zone colonisée. Dans l'ouest de la France, *X. laevis* peut rencontrer de nouveaux prédateurs aquatiques. Certains peuvent être apparentés à des prédateurs de l'aire de répartition d'origine, mais d'autres appartiennent à des groupes taxonomiques différents. J'ai testé si les têtards naïfs de la population invasive française présentaient une réponse anti-prédatrice aux prédateurs locaux et si la réponse dépendait du degré de parenté avec les prédateurs de l'aire d'origine. J'ai exposé des têtards naïfs élevés en laboratoire à un gastéropode aquatique non-prédateur, *Planorbarius corneus*, un coléoptère prédateur indigène, *Dytiscus dimidiatus*, et une écrevisse prédatrice invasive, *Procambarus clarkii*. J'ai trouvé que les têtards réduisaient naturellement leur activité lorsqu'ils étaient exposés à des signaux olfactifs du coléoptère et de l'écrevisse, mais pas à des signaux du gastéropode. La réduction de l'activité diminue généralement la probabilité d'être détecté par les prédateurs. Cela démontre que les têtards de populations invasives répondent à des prédateurs connus et nouveaux indépendamment de l'histoire évolutive de l'interaction proie-prédateur.

Ce travail s'est concentré entièrement sur les têtards. Il démontre la plasticité phénotypique élevée du développement larvaire dans l'aire d'origine, ce qui peut fournir la variation nécessaire pour la colonisation de nouvelles zones, n'a fourni aucune preuve de couplage entre les stades, ce qui permet à chaque stade de répondre à ses propres contraintes, et a montré la reconnaissance de prédateurs locaux, ce qui devrait améliorer la survie. Globalement, cette thèse décrit les mécanismes par lesquels les têtards de *X. laevis* peuvent contribuer au succès de l'invasion de l'espèce en France et dans le monde.

Opsomming.

Ingevoerde bevolkingsgroepe moet verskeie hindernisse oorkom om 'n indringerspesie te raak in 'n nuwe omgewing waar hulle nuwe ekologiese toestande kan ervaar. Fenotipiese plastisiteit en plaaslike aanpassing is twee meganismes wat ingevoerde bevolkings kan help om hierdie hindernisse te oorkom. Die algemene platanna, *Xenopus laevis* (Daudin, 1802) is inheems aan suider Afrika, maar is indringend op vier verskillende kontinente. Hierdie studie se doelwit was om vier belangrike aspekte van die indringing van die platanna in die weste van Frankryk te verstaan.

Eerste, het ek die vlak van fenotipiese plastisiteit en die plaaslike aanpassing van *X. laevis* paddavissies van die twee kontrasterende reënvalstreke in die inheemse reeks beoordeel, aangesien dit inligting kon verskaf oor die vlak van fenotipiese plastisiteit in die Franse indringerreeks. Die winterreënvalstreek word gekenmerk deur kouer temperatuur en 'n winterreënvalpatroon, terwyl die somerreënvalstreek gekenmerk word deur warmer temperature en 'n somerreënvalpatroon. Ek het 'n wederkerige uitruileksperiment met behulp van buitelugmesokosms uitgevoer. Ek het liggaamsgrootte, tydsberekening van metamorfose en oorlewing van paddavissies gemeet (NF stadium 45 - 66). Ek het gevind dat beide fenotipiese plastisiteit en plaaslike aanpassing die paddavis-fenotipe in die winter- en somerreënvalgebiede bepaal. Ek het ook 'n oorlewingskoste by individue geïdentifiseer wat na die ander klimaatstreek oorgeplaas is. Die koste vir die winterreënval-paddavissies was laer. Dus, die interaksie tussen fenotipiese plastisiteit en plaaslike aanpassing verklaar waarskynlik die volharding van hierdie spesie in kontrasterende omgewings.

Tweedens, by amfibieë, kan die paddavis-fenotipiese eienskappe gekoppel of ontkoppel word aan verspreidingseienskappe by volwassenes. Ruimtelike sortering is waargeneem by volwassenes van *X. laevis* in die weste van Frankryk. Dus het ek getoets of

hierdie proses die morfologie en lewensgeskiedenis kenmerke van paddavissies verander het as gevolg van die koppeling van eienskappe tussen stadiums. Ek het algemene tuineksperimente uitgevoer in laboratorium mikrokosms en mesokosms buite. Ek het liggaamsgrootte, tydsberekening van metamorfose en oorlewing tussen paddavissies uit die binnekern en vanaf die periferie van die indringende reeks vergelyk, maar het geen effek gevind van die posisie in die indringende reeks op die paddavis-fenotipe nie. Die enigste verskil in beide eksperimente was die groter liggaamsgrootte van post-metamorfiese individue aan die periferie. Hierdie bevindings ondersteun die hipotese van ontkoppeling tussen eienskappe van volwassenes en fenotipiese eienskappe van die paddavisse. Ontkoppeling kan elke fase van die komplekse lewensiklus onafhanklik reageer op omgewingstoestande.

Derdens, het ek beoordeel of die mate van fenotipiese plastisiteit gedurende die uitbreidingsproses tussen die kern en die periferie verander het. Ek het hierdie hipotese vir indringende *X. laevis* in die weste van Frankryk getoets. Ek het die kritieke termiese grense gemeet en die termiese prestasiekurwes van die bars-swemprestasie (snelheid, versnelling en sinuositeit) van kern- en perifere paddavissies gemeet by drie ontwikkelingstemperature (laag, tussen en hoog). Ek het gevind dat beide die posisie in die verspreiding (kern / periferie) en die ontwikkelingstemperatuur beduidend was. Geen verskille is egter geïdentifiseer tussen die werkverrigting van kern- en perifere paddavissies wat in dieselfde ontwikkelingstemperatuur geteel is nie. Paddavissies wat by die hoë temperatuur grootgemaak is, het hul termiese optimum tot die warmer temperatuur geskuif, maar die paddavissies het beperkte akklimasie tot koeler temperature getoon.

Uiteindelik het ek getoets of paddavissies op nuwe roofdiere in hul indringende reeks kan identifiseer en daarop reageer. In die weste van Frankryk kan *X. laevis* nuwe roofdiere in die water teëkom. Sommige is moontlik verwant aan roofdiere wat in die inheemse reeks voorkom, maar ander behoort tot verskillende taksonomiese groepe. Ek het naïewe

laboratorium-grootgemaakte paddavissies blootgestel aan 'n nie-roofdiere waterslak, *Planorbarius corneus*, 'n inheemse roofkewer, *Dytiscus dimidiatus* en 'n indringende roofkreef, *Procambarus clarkii*. Ek het gevind dat paddavissies hul aktiwiteit verminder as hulle blootgestel word aan kewers en krewes se olifaktoriese leidrade, maar nie aan die waterslak nie. Die vermindering van aktiwiteit verminder gewoonlik die waarskynlikheid dat dit deur roofdiere opgespoor kan word. Dit demonstreer dat indringende paddavissies reageer op bekende en nuwe roofdiere, ongeag die evolusionêre geskiedenis van die prooi-roofdiere-interaksie.

Hierdie werk het geheel en al op die paddavissies gefokus. Dit demonstreer die hoë fenotipiese plastisiteit van die larfontwikkeling in die inheemse omvang, wat moontlik die nodige variasie vir die kolonisasie van nuwe gebiede kan verskaf, mits geen bewyse bestaan van die koppeling tussen stadiums nie, waardeur elke stadium sy eie onafhanklik kan reageer op omgewingstoestande, en die erkenning van plaaslike roofdiere, wat na verwagting die oorlewing sal verhoog. In die geheel word hierdie tesis meganismes beskryf waardeur *X. laevis* paddavissies kan bydra tot die sukses van die spesie in Frankryk en wêreldwyd.

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Chapter 1: General introduction.

1.1. Biological invasions.

Invasive species are taxa with independent populations that spread in novel locations (Blackburn et al., 2011). To become invasive introduced individuals must overcome several barriers, a ‘filter’ that can result in populations with altered phenotypes (Richardson et al., 2000). The ‘filters’ can be present during the stages of the invasion process: (1) transport, (2) introduction, (3) establishment, and (4) spread.

Transport and introduction- Biological invasions can occur when species are transported beyond their natural distribution to a novel range usually by anthropogenic activity (Blackburn et al., 2011). Species can be introduced into a novel environment either accidentally or deliberately (see Richardson et al., 2000; Pimentel, 2002; Blackburn et al., 2011). A population genetic bottleneck might occur during the introduction due to the small number of initial propagules, thus decreasing genetic diversity (Barret & Kohn, 1991; Sakai et al., 2001). Multiple introductions can cause intraspecific admixture that increase genetic variations, and are often suggested to promote invasiveness (Sakai et al., 2001). Increased genetic variation can contribute to invasion success by decreasing the effects of bottlenecks, and thus, enhances evolution of fitness-related traits.

Establishment- An introduced population that becomes self-sustaining is considered to be established (Blackburn et al., 2011). The characteristics of both the introduced species and the novel environment can play a role in the success of establishment (Duncan et al., 1999). Species that display life-history characteristics such as a faster generation period and high fecundity may have increased probability for establishment success (Sakai et al., 2001). However, the adaptive potential during the establishment is equally as important as the degree of pre-adaptation (Roy, 1990; Sakai et al., 2001). Phenotypic plasticity can also contribute to

the colonisation of an introduced species (e.g. Manfredini et al., 2019). Different environmental conditions in the novel environment can induce changes in the individual's phenotype (morphology or physiology). For instance, introduced species can experience biological resistance when the native predators are able to consume the invader (e.g. Skein et al. 2020). This can result in the development of novel anti-predator responses of the invader that can assist in the successful establishment of the species (Sakai et al., 2001). Phenotypic plasticity increases the fitness of individuals without adaptive genetic differentiation from the source (Price et al., 2003; Fox et al., 2019).

Population spread- After successful establishment, colonising species may spread into locations away from the introduction point (Sakai et al., 2001). The continued spread of the population often occurs because of selection on dispersal-relevant phenotypic changes at the periphery of the population (Phillips et al., 2008; Shine et al., 2011). Individuals with the highest dispersal ability can accumulate at the periphery of the distribution, and subsequently meet and mate with each other. This is known as spatial sorting (Shine et al., 2011). The mating of individuals with high dispersal abilities at the distribution periphery is predicted to produce offspring with even higher dispersal abilities than the previous generation (Phillips et al., 2008, Perkins et al., 2016). Ultimately, this can result in evolution of a novel phenotype that is selected for dispersal at the distribution periphery.

Effective population size at the periphery may be reduced during expansion and can cause successive bottlenecks regardless of spatial sorting. The lowered genetic diversity due to bottlenecks of the periphery individuals can be problematic as inbreeding may limit population growth and persistence (Newman & Pilson, 1997; Nieminen et al., 2001). Gene flow from the core can increase genetic diversity to the periphery of the population distribution (Holt & Gomulkiewicz, 1997). Gene flow from the core of the distribution range can limit the ability of periphery individuals to adapt to the local conditions at the periphery and dampen the effects

of spatial sorting (e.g. Kirkpatrick & Barton, 1997). Thus, in an invasive population continuously expanding, an increase in plasticity is expected at the periphery of the distribution (Chevin & Lande, 2011). The rate of spread may then depend on the pattern and extent of gene flow from the core to the periphery (Sakai et al., 2001, Chevin & Lande, 2011). However, several costs have been associated with plasticity, such as maintenance costs (De Witt et al., 1998). The costs associated with plasticity suggest that in an unchanged environment, plasticity is too costly to maintain, and will erode (Price et al., 2003). A reduction of plasticity may occur and be compensated by the evolution of more specialised and less costly phenotypes (Price et al., 2003). The evolution of specialization of established species can be rapid (e.g. Ellstrand & Schierenbeck, 2000; Quinn et al., 2000). Local adaptation usually takes place during a lag time, where established species can become specialised to the new habitat (Sakai et al., 2001). Studying the ‘natural experiments’ that are biological invasions can assist in elucidating how the invader evolves in response to the novel environment (biotic and abiotic conditions) (Richardson et al., 2000). Invasive ectotherms are especially sensitive to thermal variation; thus, it is interesting to study their spread in the novel environment (Li et al., 2013). Ectotherms such as amphibians are particularly interesting because of their complex life cycles. For instance, during the spread phase, dispersal is enhanced for the adults due to spatial sorting, but how does this influence the tadpole stage?

1.2. Amphibian invasions.

Invasive amphibians are well studied (van Wilgen et al., 2018). Amphibians can be introduced into new environments via the nursery trade or cargo stowaways, but one major pathway is the pet trade and release (Kraus, 2009; Mohanty & Measey, 2019). Invasive amphibians are often characterised by generalist traits that can assist in successful establishment in the novel environment such as phenotypic plasticity and the ability to rapidly

undergo evolutionary change (Bomford et al., 2009; Tingley et al., 2012; Allen et al., 2017). A neglected yet important characteristic of invasive amphibians is the presence of a free-living larval stage. In anurans, tadpoles of anuran species can modify their shape, and behaviour when exposed to certain pressures such as the presence of a predator (Van Buskirk & McCollum, 2000; Relyea, 2001a). Food quality and availability, competition, temperature, and the presence of predators, are all important factors that can induce different phenotypes in tadpoles (Kupferberg et al., 1994; Kupferberg, 1997; Relyea, 2003; Walsh et al., 2008; Nunes et al., 2014; San Sebastian et al., 2015). Carryover effects of tadpole development can influence adult survival and fitness, for instance, larger body size at metamorphosis increases post-metamorphic survival, fecundity in females and induces earlier maturity (Smith, 1987; Tejedo, 1992; Altwegg & Reyer, 2003; Cabrera-Guzman et al., 2013). Thus, adjustments of tadpole phenotypes can affect adult fitness during the invasion process and should be considered when studying invasion dynamics of amphibians.

1.3. The African clawed frog, *Xenopus laevis* (Daudin, 1802).

The African clawed frog, *Xenopus laevis* is native to southern Africa (Furman et al., 2015). Within the native range, *X. laevis* has a wide distribution from South Africa to Malawi (Measey, 2004; Furman et al., 2015). It occurs in natural, but also artificial waterbodies and occurs in different rainfall regions such as the southern part of the country which is characterised by rainfall in the winter and the northern part of the country which is characterised by rainfall in the summer (Measey, 2004). Mitochondrial and nuclear DNA and phenotypic differentiation is observed for *X. laevis* throughout its native range (Du Preez et al., 2009; Furman et al., 2015; De Busschere et al., 2016). Furman et al. (2015) described four distinct phylogeographic lineages based on the mitochondrial and nuclear DNA of *X. laevis*

within southern Africa: (1) south-western Cape clade, (2) Beaufort West clade, (3) Niewoudtville clade, and (4) northern South Africa clade. This indicates that native *X. laevis* in southern Africa are genetically diverse. Males from the winter rainfall region are larger and heavier than summer rainfall males displaying phenotypic differentiation between the two rainfall regions (Du Preez et al., 2009).

Xenopus laevis is a model organism for molecular and developmental studies (Gurdon & Hopwood, 2000). This has resulted in the extensive use of *X. laevis* in laboratories across the world (Measey et al., 2012). Thousands of live *X. laevis* from the south-western Cape clade were exported globally (Van Sittert & Measey, 2016). Exports were followed with reports of invasive populations present in Europe, including France (Fouquet, 2001; Fouquet & Measey, 2006), Portugal (Rebelo et al., 2010), and Italy (Lillo et al., 2005). The invasive populations in Italy and Portugal originate from the south-western Cape clade in South Africa, whereas the invasive population in western France originates from the south-western Cape clade and the northern South Africa clade (De Busschere et al., 2016). Thus, western France was invaded by *X. laevis* from two distinct phylogeographic lineages followed by admixture during the colonisation phase.

The invasive population of *X. laevis* in western France was introduced at a site near a breeding facility where *X. laevis* was reared from the 1950s until the 1980s (Fouquet and Measey, 2006). It is speculated that individuals may have been released when this facility closed in the 1980s (Measey et al., 2012). In addition to the high genetic diversity (and the preceding admixture of phylogeographic lineages in the breeding facility) in western France several pre-adapted characteristics of *X. laevis* could have contributed to the successful establishment in this novel region (Rödder et al., 2016). *Xenopus laevis* adults are principally aquatic and can migrate overland which enhances their capacity to rapidly colonise new

breeding habitats around a source habitat (Measey et al. 2016, De Villiers & Measey, 2017). The adults also have a high tolerance to saltwater and anoxic conditions (Jokumsen & Weber, 1980). The development of *Xenopus laevis* tadpoles from embryos to metamorphs have been well described and studied (Nieuwkoop & Faber, 1994; Segerdell et al., 2008). Tadpoles of *X. laevis* are pelagic suspension filter feeders (Viertel, 1992). The tadpoles are free-swimming within the first couple of days after hatching (Nieuwkoop & Faber, 1994).

In the invasive range of *X. laevis* in western France, macroinvertebrates, fish, adult *X. laevis* can prey on native anuran tadpoles. Tadpoles of some species can respond differently to predator type for instance, when exposed to fish, tadpoles have been found to school rather than hide (Wassersug & Hessler, 1971). Another response of some anuran tadpoles is to reduce the activity in the presence of macroinvertebrate predators (Relyea, 2001b). However, no studies have tested the presence of anti-predator response of *X. laevis* tadpoles from an invasive population to local predators.

Spatial sorting during the spread phase has been well studied for the adults of *X. laevis* in western France (Louppe et al., 2017; Courant et al., 2017a; Courant et al., 2019a, 2019b; Padilla et al., 2019). The invasive distribution of *X. laevis* in France now covers an area of ~5000 km² near the city of Saumur (Vimercati et al., 2019). Thus, they have been rapidly expanding for ~40 years from a single known introduction point. Adults were found to lower resource allocation to reproduction at the periphery of the invasive distribution (Courant et al., 2017a) and increase resource allocation to dispersal relevant anatomy (Padilla et al., 2019). Due to the differential resource allocation, the adults from the core and the periphery display differential dispersal abilities (i.e. higher endurance at the periphery) and dispersal relevant morphology (i.e. longer relative hind limbs at the periphery) (Louppe et al., 2017; Courant et al., 2019a). However, no previous studies have investigated the effects of spatial sorting on the

other life-history stages of *X. laevis* in western France. The reduced investment of resources to reproduction at the periphery and the carryover effects between life-history stages can potentially limit or enhance the rate of expansion of *X. laevis* adults.

The invasive *X. laevis* population in western France has higher genetic diversity than in Portugal, and Italy (De Busschere et al., 2016), it has rapidly expanded its range from a single introduction point (Vimercati et al., 2019), and has differentiated morphology and physiology between core and periphery (Louppe et al., 2017; Courant et al., 2019a; Padilla et al., 2019). Additionally, the realized niche occupied by *X. laevis* in Europe is partially different from the realized niche occupied in its native range (Rödder et al., 2016). This makes for an interesting situation in which to study the evolution of developmental phenotypic plasticity within the invaded range.

1.4. Project aims.

This study aims to understand four major aspects of the African clawed frogs' invasion into western France. Firstly, I assessed the level of phenotypic plasticity vs. local adaptation of *X. laevis* tadpoles from two different rainfall regions of the native range, as this could provide information on the phenotypic plasticity of invasive *X. laevis* in France that originate from both regions. Secondly, I investigated whether spatial sorting present in the adults of *X. laevis*, the dispersing stage, affects the non-dispersing life stages. Thirdly, I assessed whether the degree of phenotypic plasticity changed between the core and the periphery during the expansion process. Finally, I tested whether invasive *X. laevis* tadpoles can identify and respond to novel predators in the invasive range. Collectively, these studies expand the findings of the EU Biodiversa project INVAXEN (the Invasive biology of *Xenopus laevis* in Europe: Ecology, Impact, and predictive models) and will contribute to the EU Life CROAA project (Control

and Strategies of Alien Amphibians) and by addressing knowledge gaps on how the pre-metamorphosis life stages limit or enhance the invasion success of *X. laevis* during expansion. This study uses multiple approaches such as outdoor mesocosm experiments, laboratory microcosm experiments and laboratory performance experiments to address these knowledge gaps. Finally, this thesis will bring insights into the invasion process of all stages of an invader with a complex life cycle.

Chapter 2: There's no place like home: The phenotypic plasticity of translocated *Xenopus laevis* tadpoles from two contrasting climatic regimes comes at a cost.

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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, LDP, AH and JS were involved with the interpretation of data. NK drafted the chapter.

2.1. Abstract.

The phenotypic variations between populations often correlate with climatic variables as a result of local adaptation or phenotypic plasticity. The modification of traits due to phenotypic plasticity does not result in genetic change, whereas, if an organism is locally adapted genetic differentiation has taken place. Phenotypic plasticity is costly but favoured in heterogeneous environments, whereas local adaptation is less costly in more homogeneous environments. Amphibians are especially sensitive to environmental variations during all stages of their complex life cycle. Importantly, the environmental variations experienced during the development of tadpoles can influence the fitness of adults. Thus, it is of interest to assess how amphibians with broad distribution ranges cope with large-scale spatial heterogeneity of the environment. We tested whether a broadly distributed amphibian tadpoles' phenotype is determined by acute environmental context (phenotypic plasticity) or population origin (local adaptation). In ectotherms, a common finding is that phenotypic differentiation between populations is generally caused by a combination of phenotypic plasticity and local adaptation. The African clawed frog, *Xenopus laevis*, naturally experiences two contrasting climatic regimes in South Africa: the summer and the winter rainfall regions. We performed a reciprocal exchange experiment using outdoor mesocosms to test our hypothesis that both would influence the phenotypic variations between the two populations of *X. laevis*. We measured body size, timing of metamorphosis, and survival. We found that, corresponding to our hypotheses, the experimental venue and population origin explained the variation in body size and timing of metamorphosis. We also found that the phenotypic response to translocation came at a survival cost as survival significantly decreased in translocated tadpoles than when reared in their environment of origin. For translocated winter rainfall tadpoles, the survival rates did not differ from the non-translocated summer rainfall tadpoles, indicating that even though survival is lower than the local winter rainfall tadpole survival, the cost of survival

might be less. High levels of phenotypic plasticity likely explain the persistence of this species in the geographical range encompassing contrasting environments.

2.2. Introduction.

In many species, environmental conditions can differ remarkably across their distribution. The local environment can influence diet composition, predator-prey dynamics (Van Beest et al., 2013), and reproduction (Komers & Brotherton, 1997; Spritzer et al., 2005). The environmental variations across the species' distribution change the genotypic and phenotypic characteristics of populations (Phillimore et al., 2010). Several possible mechanisms can drive phenotypic variation. Modifications can arise through phenotypic plasticity (Price et al., 2003) and genetic differentiation (Sultan & Spencer, 2002) or their interaction. Firstly, phenotypic plasticity results in the expression of plastic traits but does not result in genetic change (Williams, 1966). Phenotypic plasticity has many ecological benefits such as producing an environmentally matched phenotype (Scheiner, 1993). However, several potential costs and limits of phenotypic plasticity have been identified such as maintenance costs (De Witt et al., 1998). Secondly, phenotypic variation can arise through genetic differentiation when the cost of plasticity is high (Sultan & Spencer, 2002). In a constant environment, where conditions are not met to maintain a costly plastic phenotype, plasticity can be lost and specialization can evolve (Kawecki & Ebert, 2004; Hereford, 2009; Blanquart et al., 2013). Phenotypic plasticity is most likely to occur when the environmental variability among habitats is high and the fitness cost of maintenance is low (Scheiner, 1993). Thirdly, a common finding is that phenotypic differentiation between populations is generally the result from the combination of phenotypic plasticity and genetic differentiation (Phillimore et al., 2010; Chevin & Lande, 2011).

Ectothermic animals such as amphibians provide a good model for studies of local adaptation as they are especially sensitive to thermal variations (Li et al., 2013). Amphibians have complex life cycles, where the adults and the tadpoles may respond differently to the same environmental variables (Smith-Gill & Berven, 1979; Perotti et al., 2018). Thermal and rainfall

regimes are powerful selective forces in amphibian populations and can influence all ontogenic stages (Blaustein et al., 2010). For adults, breeding activity is strongly dependent on the phenology of rainfall and temperature (Bertoluci & Rodrigues, 2002). Rainfall regime and temperature can also affect the life-history of tadpole stages (Newman, 1989). The larval stage of amphibians is especially interesting as the temperature experienced by growing tadpoles influences size and mass at metamorphosis as well as adult fitness (Berven & Smith-Gill, 1983; Semlitsch et al., 1988, Atkinson, 1996; Walsh et al., 2008; Blaustein et al., 2010; Gomez-Mestre et al., 2010). Smith-Gill & Berven (1979) describe the importance of correct timing of metamorphosis during amphibian development. Exposure to higher temperatures shortens the time to metamorphosis in many species (Morand et al., 1997; Buchholz & Hayes, 2000). The thermal environment in the embryonic stage can also affect survival (Blaustein et al., 2010). Larval survival to metamorphosis is a major contributor to the reproductive success of adults (Goldstein et al., 2017). The local environmental conditions of amphibians drive the evolution of traits that can ultimately result in plastic or genetic modifications of the phenotype (Angilletta, 2009; Bozinovic et al., 2011).

Southern Africa is characterised by two main rainfall regions, winter and summer (Chase & Meadows, 2007). The winter rainfall region is generally characterised by colder weather (16 – 26 °C in the summer and 4 – 18 °C in the winter) and a winter rainfall pattern. The summer rainfall region is a larger area and is characterised by warmer weather (15 – 33 °C in the summer and 4 – 25 °C in the winter) and rainfall predominantly in summer (South African Weather Service, 2020). These two regions are separated by the Cape Fold Mountains and the Great Escarpment. These contrasting regimes are known to separate native amphibian fauna (Poynton & Broadley, 1978; Schreiner et al., 2013). However, some species like the African clawed frog, *Xenopus laevis*, straddle both rainfall regions (Measey, 2004, Matthews et al., 2016). Different phylogeographic lineages occur in these regions, the south-western Cape

clade in the winter rainfall region, and the northern South Africa clade in the summer rainfall region (Furman et al., 2015). In the native distribution, *X. laevis* display reproductive differentiation as they generally breed at the commencement of the rains, thus at different times in the summer and winter rainfall areas (Berk, 1938; Kalk, 1960). Additionally, *X. laevis* adults also display population differentiation in morphology between the different regions (Du Preez et al., 2009). However, no studies have investigated whether populations from each climatic region exhibit local adaptation to their local environment or whether phenotypic plasticity allowed the species to colonise its range without further genetic changes.

In this study, we investigated whether *X. laevis* populations from the summer and winter rainfall regions display local adaptation or phenotypic plasticity or a combination of both using a reciprocal exchange experiment focusing on larval survival, timing of metamorphic climax and body size. We reared larval *X. laevis* from the summer- and winter rainfall regions from free-swimming tadpoles to metamorphs in mesocosms in each climatic region. They were reared at their place of origin (home) and at the novel region (away) Thus, we tested whether population origin (local adaptation) or experimental venue (phenotypic plasticity) or both influences the phenotype of *X. laevis* tadpoles. We hypothesized that both would influence the phenotypic variations between the two populations of *X. laevis*. Thus, we specifically predict that (1) population origin and experimental venue explains the variation between *X. laevis* tadpoles. (2) Due to the cost of phenotypic plasticity we predicted that the survival of the translocated tadpoles would be the lowest.

2.3. Materials and methods.

2.3.1. Sampling sites.

For this study, Stellenbosch, Western Cape province, is chosen as a study area in the winter rainfall region and Potchefstroom, North-West province, is chosen in the summer rainfall region (Fig. 2.1). These sites are separated by approximately 1 300 km and are representative of each climatic region. Within these study areas, two sites (ponds) separated by 10-40 km were sampled: summer rainfall region: site 1 ($26^{\circ}40'54.6''\text{S}$, $27^{\circ}05'38.4''\text{E}$; elevation = 1355 m); site 2 ($26^{\circ}45'19.8''\text{S}$, $27^{\circ}03'38.2''\text{E}$; elevation = 1347 m); winter rainfall region: site 3 ($33^{\circ}57'46.8''\text{S}$, $18^{\circ}55'33.6''\text{E}$; elevation = 241 m); and site 4 ($33^{\circ}49'01.2''\text{S}$, $18^{\circ}52'58.8''\text{E}$; elevation = 196 m).

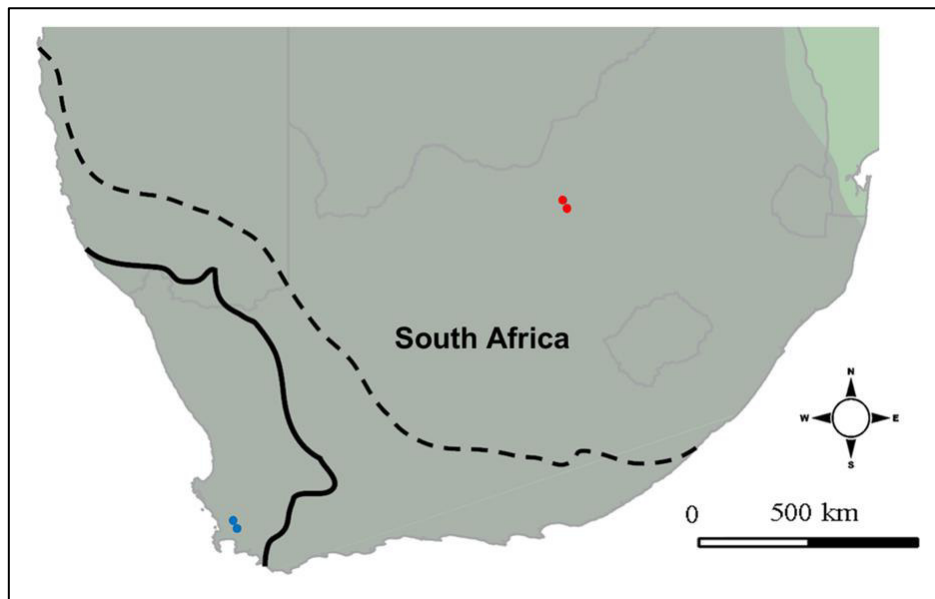


Figure 2.1: Locality of the sampling sites in the summer rainfall region (red dots) and in the winter rainfall region (blue dots). Grey area indicates the current distribution of *Xenopus laevis* in southern Africa. The area beneath the solid line illustrates the current winter rainfall region and the area to the right of the dashed line illustrates the current summer rainfall region. The area between the dashed and solid line illustrates the region where rainfall occurs throughout the year. Rainfall zones as indicated by Chase and Meadows (2007).

2.3.2. Adult collection, care, and breeding.

Two to three breeding pairs of adult *X. laevis* were collected at each site and transported to the experimental site in each region. The Amphibian Metamorphosis Assay (AMA) and the *Xenopus* Metamorphosis Assay (XEMA) recommend using human chorionic gonadotrophin hormone (HCG) to induce breeding in *X. laevis*. Adults were inspected to determine whether hormonal priming is necessary. To determine the female reproductive states the cloaca was examined. Everson (2006) described three classes of cloacal folds: (1) Swollen and red cloaca indicates readiness for spawning and no priming was necessary. (2) Minimal swelling but no red colouring of the cloaca indicated that hormonal priming was necessary. (3) No swelling or red colouring of cloaca indicated that hormonal priming was necessary. To determine the reproductive state of males the forearm was inspected for the presence of black nuptial pads. If the nuptial pads were hardly visible or grey males were primed with hormones. Both males and females were primed with the 50 international units (IU) HCG to ensure that adults are in the correct reproductive state. On the third day, males were injected with 250 IU HCG and females were injected with 500 IU HCG (dosages as prescribed by Wlizla et al., 2018). Injection of hormones took place shortly after collection. Individuals were kept separate until the third day and placed in a plastic aquarium (7 - 10 L). To prevent predation and damage of the eggs, a plastic mesh was placed in the bottom of the tank. Frogs were removed from the aquaria the following morning. Schultz and Dawson (2003) and OECD (2008) recommend that the preferred temperature for rearing *X. laevis* tadpoles is between 18 - 24 °C. The daily average temperature in the winter and summer rainfall region falls within the a 21 °C and 24 °C margin. Therefore, the breeding and subsequent holding temperature of eggs were chosen to be 22 °C. We do not expect that breeding and holding tadpoles in this temperature will favour tadpoles from one population more than the other. Adults were kept at 22 °C to ensure that when eggs

were laid, they are all in the same temperature. Eggs were left to hatch in their aquaria maintained at 22 °C and well aerated until they developed into free-swimming tadpoles.

2.3.3. Staging.

The development of *X. laevis* from fertilisation to metamorphosis undergoes 66 Nieuwkoop and Faber (NF) stages (Nieuwkoop & Faber, 1994) grouped into nine stage categories according to anatomical ontology (Segerdell et al., 2008). The post-embryonic development (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 metamorphic climax stage category (NF stage 58 - 66) during which tail resorption occurs. Thus, the dataset was divided according to Segerdell et al.'s (2008) pre-defined stage categories. Individuals from the pre- and prometamorphosis stage categories (NF stage 45 - 57) are collectively referred to as 'larvae'. These were analysed separately from the metamorphic climax stage category (NF stage 58 - 66) where body size decreases.

2.3.4. Experiment.

The experiment in the summer rainfall region was conducted from 02/11/2017 to 10/01/2018 and the experiment in the winter rainfall region was conducted from 19/11/2018 to 26/01/2019, each for a period of 10 weeks. Tadpoles originating from the summer rainfall region were reared in the summer rainfall region (SR_{Home}) or reared in the winter rainfall region (SR_{Away}). Tadpoles originating from the winter rainfall region were reared in the winter rainfall region (WR_{Home}) and the summer rainfall region (WR_{Away}). We used 650 L plastic tanks as mesocosms (1.08 m x 1.28 m x 6.45 m). The experimental sites were located in rural open habitats (Potchefstroom: 26°40'27.9"S, 27°06'23.7"E; Stellenbosch: 33°56'30.0"S, 18°51'54.2"E). Ten mesocosms were randomly arranged by assignment from randomly generated numbers. In addition, temperature data loggers (HOBO K8 ® Temperature/Alarm

(Waterproof) Data Logger - UA-001-08, Onset Computer, Bourne, Mass, USA) were randomly placed in mesocosms as well as in sampling ponds to determine variation in temperature. The temperature loggers were attached to a floating piece of polystyrene and suspended 15 cm under the water to avoid temperature inversion. We calculated the coefficient of variation to determine the measure of dispersion of data points around the mean to assess the daily temperature variation. We added 200 L of tap water aged for at least one month to the tanks prior to the experiment. Each mesocosm was covered with 20% shade mesh to avoid predation, to standardise light intensity in each tank, and avoid overheating. Tadpoles were fed Sera-Micron (1 g) per mesocosm per week; surplus food was avoided to maintain water quality. In each mesocosms, 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, euthanised by overdosing with 300 mg/L to 500 mg/L of tricaine methanesulfonate (ms-222) buffered with NaHCO_3 , and preserved in 70% ethanol (EtOH). We measured full length, snout-to-vent length (SVL), body depth, tail depth, head width, and femur length (Fig. 2.2), and staged individuals according to Nieuwkoop & Faber (1994) and placed staged animals in stage categories according to Segerdell et al. (2008).

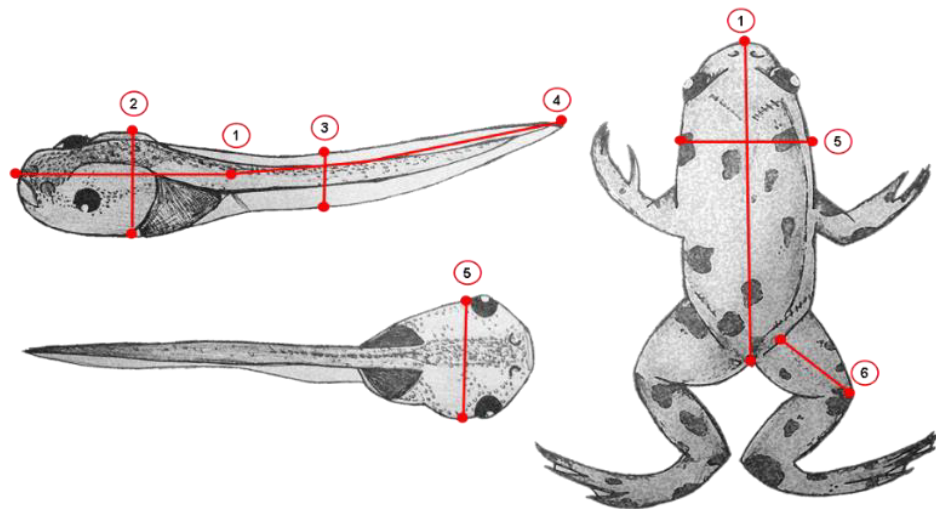


Figure 2.2: Morphometric measurements taken of *Xenopus laevis* tadpoles (left) and metamorphs (right) at different stages during the mesocosm and microcosm experiments. 1- Snout-to-vent length (SVL); 2- Maximum head/body depth; 3- Maximum tail depth; 4- Tail length; 5- Head width; 6- Femur length.

2.3.5. Statistical analyses:

Morphological variation- We could only measure the morphology of larval (NF stage 45 - 57) individuals as the sample size for climax individuals (NF stage 58 - 65) was too small after the 10-week experimental period. To assess the effect of experimental venue (summer- or winter rainfall region) and population origin (summer-or winter rainfall) on larval morphology we performed a PCA using SVL, head width, maximum body depth, tail length, and tail depth. The first principal component accounted for 94% of variation in larvae (Table S2.1). All loadings were positive for larvae. We retained only the first PCA axis in our analysis that represent a global measurement of body size.

Using the *lme4* R package (Bates et al., 2015), linear mixed models were utilized with body size (PC1) as response variable, and experimental venue, population origin and NF stage

(discrete variable) as fixed effects. Clutch nested within collection site was considered as a random effect. All variables (measurements for PC1) were normalized with the *bestNormalize* R package (Peterson, 2019). The best transformation informed by *bestNormalize* was Ordered Quantile transformation. PC1 was plotted using *ggplot2* R package (Wickham, 2009).

Timing of metamorphosis - To determine the timing of metamorphosis we measured the transition events from larvae to metamorphic climax individuals between the experimental venues and the origins of native *X. laevis*. We conducted a cox proportional hazards analysis on transition between stage categories using the *coxme* R package (Therneau, 2019a) and *survival* R package (Therneau, 2019b) packages. The weeks when transition occurred between larvae-to-climax were considered as the response variable, and experimental venue and population origin as fixed effect and clutch nested within collection site as random effects.

Variation in survival - We removed 50 individuals from the mesocosms owing to our weekly sampling for the morphological study. Thus, the number of surviving individuals at the end of the experiment was tallied out of 150. We modelled the probability of surviving until the end of the experiment in mesocosms using generalised binomial mixed models with fate (1 = survived, 0 = died) as response variable, experimental venue, and population origin as fixed effects and clutch nested within collection site as a random effect.

For all analyses, we selected the best fitting model using corrected Akaike information criterion (AICc) according to parsimony. We considered models with a difference in AICc less than 2 to be equal and selected the model with the least predictors (Symonds & Moussalli, 2011). The chosen model was then used to calculate the least square means of interactions using the *lsmeans* R package (Lenth, 2016). Model diagnostics were carried out using the

DHARMA *R* package (Hartig, 2019). All analyses were done using the statistical software R 3.4.1 (R Core Team, 2018).

2.4. Results.

2.4.1. Variation in water temperature:

The summer rainfall region had the highest average maximum temperature ($25.82\text{ }^{\circ}\text{C} \pm 2.28$ SD) compared to the winter rainfall region ($21.42\text{ }^{\circ}\text{C} \pm 2.05$) within the 10 weeks of the experiment. The average daily temperature for the summer rainfall region was $22.37\text{ }^{\circ}\text{C} \pm 1.69$ SD and $20.50\text{ }^{\circ}\text{C} \pm 2.78$ at the winter rainfall region. The winter rainfall region had the lowest average minimum temperature ($19.48\text{ }^{\circ}\text{C} \pm 3.60$) compared to the winter rainfall region ($19.84\text{ }^{\circ}\text{C} \pm 1.26$). Thus, a larger daily variation of temperature from the mean was observed in the summer rainfall region (8.95%) than the winter rainfall region (3.16%) (Fig. 2.3). There was a drop in the temperature at 09h00 for the summer rainfall region presumably due to a shadow of a tree overshadowing all mesocosms. This drop in temperature was present in all mesocosms.

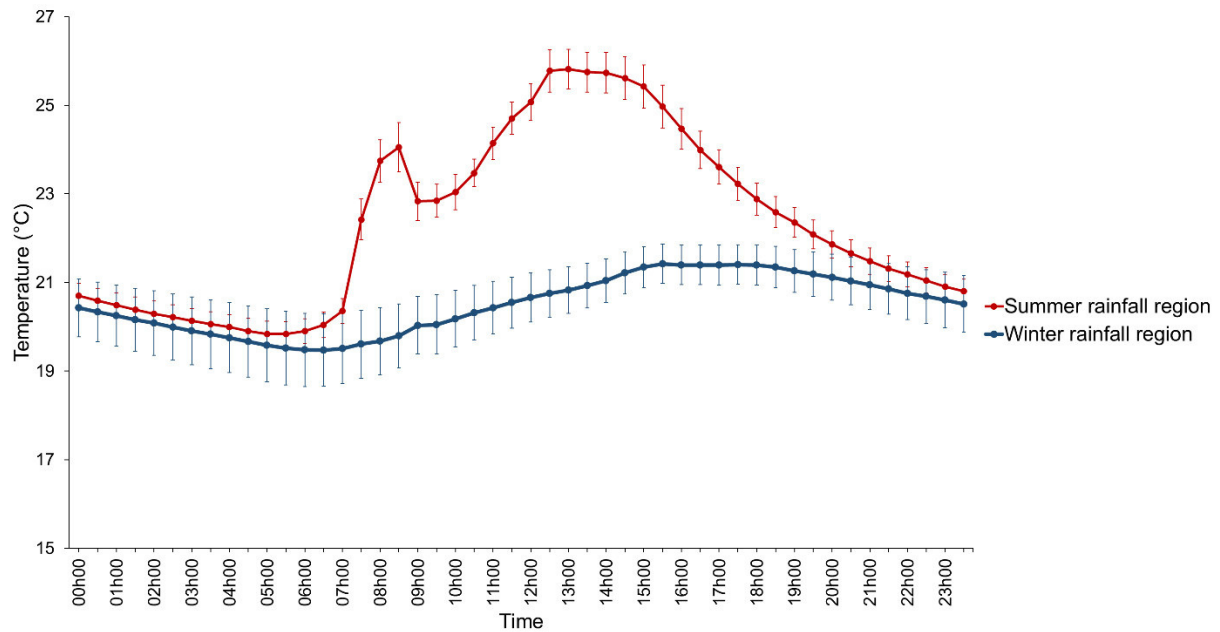


Figure 2.3: Average daily water temperature variation and standard errors of all days for a period of ten weeks in the summer rainfall regions (solid red line) and the winter rainfall region (solid blue line) measured every 30 minutes.

2.4.2. Variation in morphology:

We found that both experimental venue and population origin in interaction with stage explained the variation of body size (PC1) (Fig. 2.4., Table 2.1, GLMM, $\Delta AICc = 2.58$). All tadpoles reared in the summer rainfall experimental venue were significantly larger ($p < 0.05$) at the later stages (NF stages 49 - 57) than all tadpoles reared at the winter rainfall experimental venue (Fig. 2.4 c-d). All tadpoles (SR_{Home} and SR_{Away}) originating from the summer rainfall region were significantly larger at NF stages 55 - 56 ($p < 0.05$) than the tadpoles originating from the winter rainfall region.

Table 2.1: Model selection of top models ($\Delta\text{AICc} < 2$) for each response variable: larval body size (PC1), timing of metamorphosis and survival of *Xenopus laevis* larvae. Predictors in the models were NF stages within stage category (Stage), Population origin and Experimental venue. 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	Fixed effects	LogLik	k	AICc	ΔAICc	Weight
Body size (PC1)	Experimental venue x Stage + Population origin x Stage	-850.70	42	1790.1	0	0.78
Timing of metamorphosis	Experimental venue x Population origin	-217.24	11	457.6	0	0.45
	Experimental venue + Population origin	-217.46	11	458.6	0.96	0.28
	Experimental venue	-217.514	11	458.7	1.09	0.261
Survival	Experimental venue x Population origin	-1296.14	6	2604.3	0	1

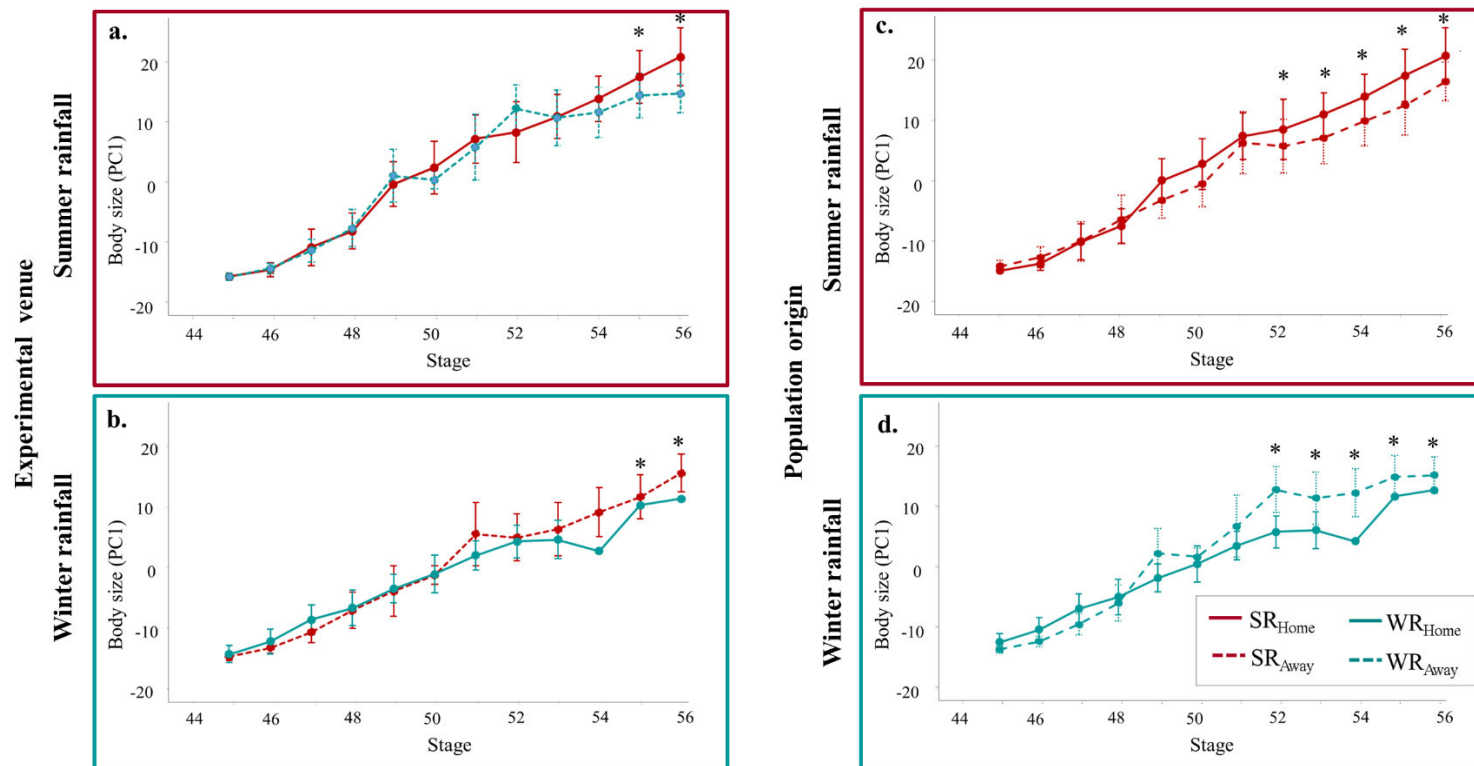


Figure 2.4: Body size (PC1) variation and standard error of *Xenopus laevis* during the larval development. a.) Pairwise comparison of the summer rainfall home individuals (SR_{Home}: solid red line) and the winter rainfall away individuals (WR_{Away}: dashed blue line) raised in the summer rainfall region. b.) Pairwise comparison of winter rainfall home individuals (WR_{Home}: solid blue line) and summer rainfall away individuals (SR_{Away}: dashed red line) raised in the winter rainfall region. c.) Pairwise comparison of SR_{Home} and SR_{Away} originating from the summer rainfall region. d.) Pairwise comparison of WR_{Home} and WR_{Away} originating from the winter rainfall region. Asterisks indicate a significant difference at $p = 0.05$.

2.4.3. Variation in time to metamorphosis.

The model including only the interaction between population origin and experimental venue had the best fit. However, two other models fell within 2 AICc units (Table 2.1). According to parsimony, the simplest model including experimental venue as the only predictor was chosen as the best model (cox proportional hazard). We found that SR_{Home} and WR_{Away} at the summer rainfall experimental venue had a significantly higher number of individuals transitioning from larvae to climax in ten weeks than SR_{Away} and WR_{Home} at the winter rainfall experimental venue (Fig. 2.5). SR_{Away} individuals had the fastest transitioning with climax individuals ($n = 2$) emerging in week 7. WR_{Home} had the slowest transition with only one climax individual emerging in week 10.

2.4.4. Variation in survival.

The model including the interaction between population origin and experimental venue was by far the best (Fig. 2.6, Table 2.1, GLMM, $\Delta AIC = 146.35$). The SR_{Home} tadpole survival (26/150 tadpoles ± 15.24 SD) was significantly lower than the WR_{Home} (64/150 tadpoles ± 40.03 SD) tadpole survival. Secondly, for both populations, survival was significantly lower for translocated individuals (SR_{Away} : 9/150 tadpoles, ± 11.72 SD; WR_{Away} : 29/150 tadpoles, ± 8.41 SD). Thirdly, Survival between SR_{Home} and WR_{Away} tadpoles reared at the summer rainfall experimental venue did not significantly differ (Fig. 2.6).

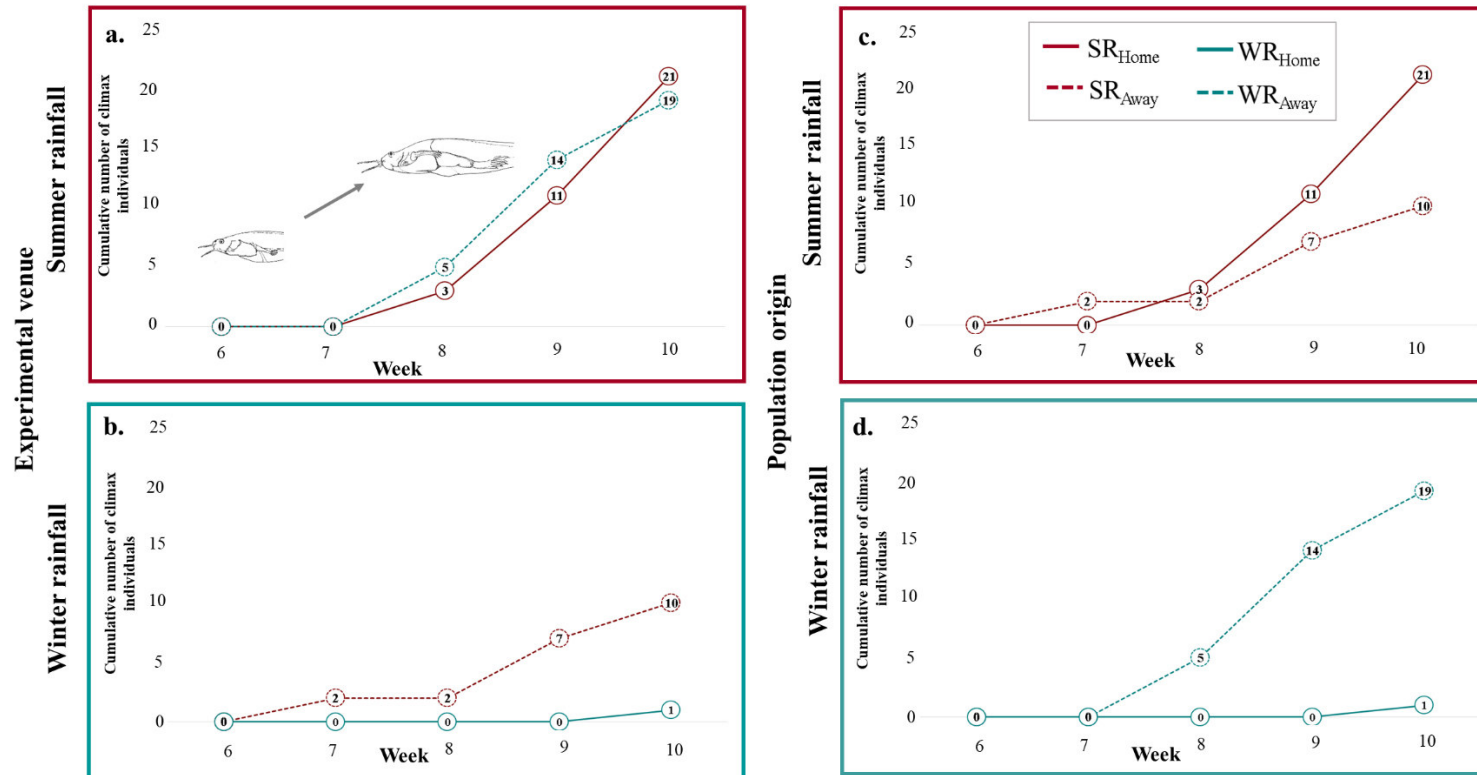


Figure 2.5: Cumulative number of *Xenopus laevis* individuals that transitioned between larvae and climax per week in all mesocosms. a.) Pairwise comparison of summer rainfall home individuals (SR_{Home}: solid red line) and the winter rainfall away individuals (WR_{Away}: dashed blue line) raised in the summer rainfall region. b.) Pairwise comparison of winter rainfall home individuals (WR_{Home}: solid blue line) and the summer rainfall away individuals (SR_{Away}: dashed red line) in the winter rainfall region. c.) Pairwise comparison of SR_{Home} and SR_{Away} tadpoles originating from the summer rainfall region. d.) Pairwise comparison of WR_{Home} and WR_{Away} tadpoles originating from the winter rainfall region.

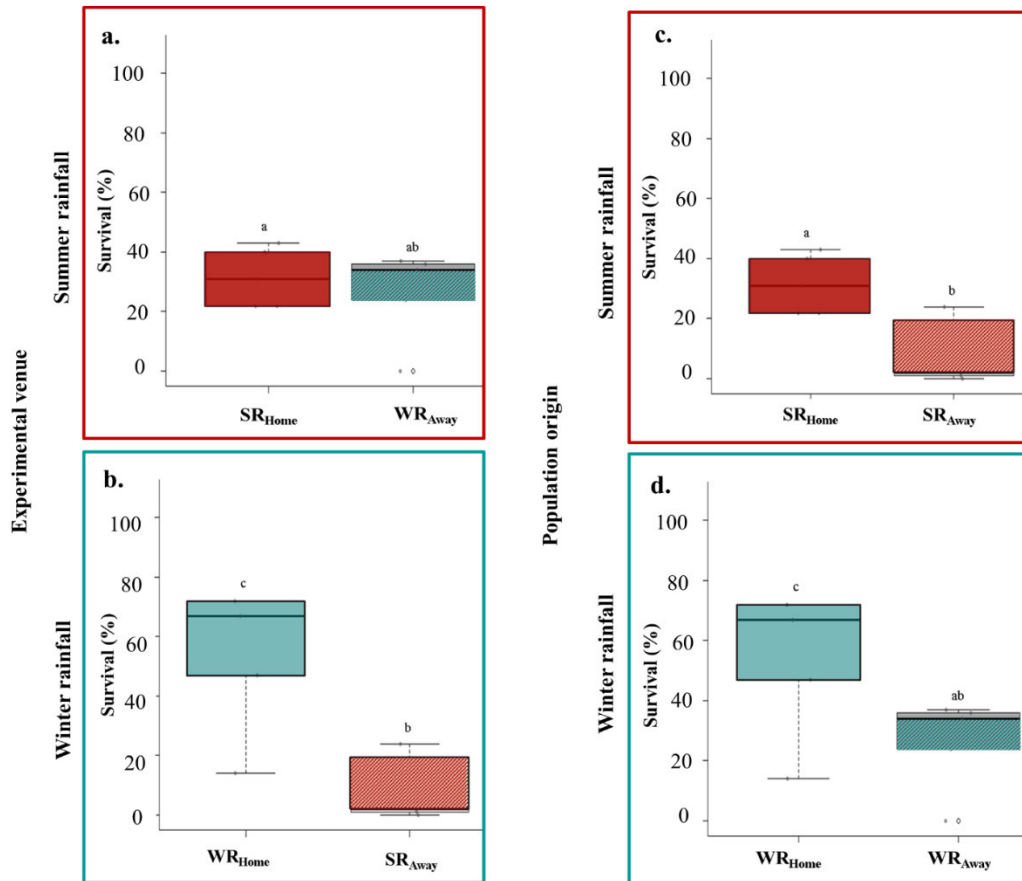


Figure 2.6: Survival of *Xenopus laevis* after ten weeks. a.) Pairwise comparison of summer rainfall home individuals (SR_{Home}: solid red fill) and the winter rainfall away individuals (WR_{Away}: dashed blue fill) raised in the summer rainfall region. b.) Pairwise comparison of winter rainfall home individuals (WR_{Home}: solid red fill) and the summer rainfall away individuals (SR_{Away}: dashed red fill) in the winter rainfall region. c.) Pairwise comparison of SR_{Home} and SR_{Away} tadpoles originating from the summer rainfall region. d.) Pairwise comparison of WR_{Home} and WR_{Away} tadpoles originating from the winter rainfall region. Survival significantly decreased between individuals of the same origin. 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 indicates the 10th and 90th percentile. Points above and below the whiskers represent outliers above and below the 10th and 90th percentile. Boxplots sharing a letter are not significantly different ($p > 0.05$) according to post hoc pairwise comparisons.

2.5. Discussion.

Our results suggest that body size and survival variation between *X. laevis* tadpoles from the summer and the winter rainfall region are determined by a combination of both phenotypic plasticity and local adaptation. However, variation in the timing of metamorphosis between summer and winter rainfall tadpoles is determined by phenotypic plasticity. The interaction between phenotypic plasticity and local adaptation is expected to result from the unpredictability of the environment of selection (De Jong, 1999). Population origin had an effect where tadpoles originating from the summer rainfall region were significantly larger at the last two stages before metamorphosis. However, a stronger trend was observed for experimental venue where all tadpoles reared in the summer rainfall experimental venue were larger in the last six stages of development, before metamorphosis, than all tadpoles reared in the winter rainfall experimental venue. Thus, local climate rather than evolutionary history can influence tadpole body size. Temperature can significantly affect the body size of tadpoles (e.g. Morand et al., 1997). The amount of daily temperature fluctuation also has an effect on tadpole morphology (Arrighi et al., 2013). Larger variation of daily temperature fluctuations has been linked to increased growth in the Australian marsh frog, *Limnodynastes peronii* (Niehaus et al., 2006). We recorded a higher daily temperature variation in the summer rainfall region. Thus, suggesting that the larger body size of tadpoles reared at the summer rainfall region can be due to the higher variation in temperature fluctuations.

The experimental venue accounted for variation in the timing of metamorphosis. This suggests that timing of metamorphosis of *X. laevis* is phenotypically plastic. We found that all tadpoles reared in the summer rainfall experimental venue started metamorphosing earlier (in larger numbers) than the tadpoles reared in the winter rainfall experimental venue. We recorded the highest maximum temperature in the summer rainfall region and the lowest minimum temperature in the winter rainfall region during the experiment. Timing of metamorphosis increases with an

increase in temperature (e.g. Loman, 2001). Thus, possibly explaining the discrepancy between the winter and the summer rainfall experimental venues. Tadpoles of *X. laevis* transition to metamorphic climax around day 41 after hatching (Nieuwkoop & Faber, 1994). However, we found that among the local tadpoles in the winter rainfall region only one reached metamorphic climax within the 10 weeks, thus, about twice the duration of the expected time to metamorphose. However, a slower developmental rate can result in larger body sizes at metamorphosis, which reduces the risk of predation (Berven & Smith-Gill, 1983).

The translocated winter rainfall tadpoles timing to metamorphosis and the number of individuals that metamorphosed did not differ from the summer rainfall tadpoles in the summer rainfall experimental venue. However, a significantly lower number of summer rainfall tadpoles metamorphosed when translocated but it was still higher than for the winter rainfall local tadpoles. We hypothesise that the differences in reaction norms is due to the evolution and maintenance of plasticity in each region. The local adaptation hypothesis states that the local population would outperform the translocated population (Williams, 1966). This, illustrating the possible advantage for slower development in the winter rainfall region. In the winter rainfall region, breeding of adults occur at the commencement of the rain in early winter when temperatures are cooler (Berk, 1938; Kalk, 1960). Previous studies have indicated that decreased temperature can decrease the developmental rate of tadpoles (e.g. Morand et al., 1997). It is possible that the translocated summer rainfall tadpoles were developmentally and genetically constrained to reduce time to metamorphosis to such an extent that survival was reduced. Nevertheless, translocated summer rainfall tadpoles managed to decrease the stage transitioning process to significantly differ from local summer rainfall tadpoles.

We found that both summer and winter rainfall tadpoles incurred a decrease in survival when translocated. Our results are congruent with previous studies that also observed that the benefit of phenotypic plasticity comes to the cost of higher tadpole mortality (Amburgey et al.,

2016). Summer rainfall tadpoles matched the body size but only managed to partially ‘match’ the timing of metamorphosis of local tadpoles in the winter rainfall experimental venue, additionally their survival was the lowest. Thus, the cost of acclimation was the highest for this group. Translocated winter tadpoles ‘matched’ the body size and timing of metamorphosis of the local summer rainfall tadpoles. However, the decrease in survival also illustrated a cost of plasticity. Thus, the reaction norms differ between the two regions.

The plasticity of the species at the larval stage can explain population persistence in the heterogeneous native distribution, as well as its capacity to colonize new areas with novel environmental conditions (Rödder et al., 2016). The high plasticity and relatively low survival cost of winter rainfall tadpoles can possibly explain their global invasion success. When introduced in a new area, a rapid sorting of individuals may take place that will keep individuals with the higher plasticity due to their relatively higher survival rate. This process can, within a few generations, massively impact the allele-pool of the invasive population. The mitochondrial DNA of all the invasive populations of *X. laevis* globally correspond to the phylogeographic lineage found in the winter rainfall region (Wang et al., 2019), with the exception of the invasive population in western France, where haplotypes from the winter rainfall and summer rainfall phylogeographic lineages have been detected (De Busschere et al., 2016). Nonetheless, we found tadpoles from both climatic regions display phenotypic plasticity with regards to body size and timing of metamorphosis. The adaptive potential, through plasticity, of an introduced population is important during the initial colonisation period and ultimately the establishment in the novel environment (Roy, 1990; Sakai et al., 2001). Thus, the initial ability of *X. laevis* to cope with the novel environment through plasticity could contribute to explaining the invasion success of introduced populations on several continents.

Chapter 3: 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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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 submitted for review.

This chapter has been submitted to the *Journal of Animal Ecology* for review for publication.

3.1. Abstract.

Spatial sorting progressively enhances the dispersal capacities of expanding populations. This selective process may enhance or limit the performance of the earlier non-dispersing stages which are usually subjected to different constraints in species with complex life cycles. Such a coupling between stages on dispersal traits has been little investigated despite its interest for understanding the dynamics of expanding population, especially in the context of invasion biology. In amphibians, phenotypic traits of non-dispersing tadpoles and metamorphs can be coupled, through carryover effects and trade-offs, or decoupled to traits relevant to dispersal in adults. We used the globally invasive and model amphibian, *Xenopus laevis*, as a study system to examine whether spatial sorting affects the phenotype of larval stages to metamorphosis in the core and at the periphery of an invasive population in France. Spatial sorting resulting in distinct phenotypes of adults has already been described from this population. We combined common garden laboratory and outdoor experiments to test the effect of position of parental pond in the colonised range (core or periphery) on morphological traits, development and survival to metamorphosis. We found no changes in morphology, development or survival of 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 individually in microcosms. Changes in adult phenotypes at the periphery are not reflected in the tadpole phenotype of *X. laevis*. This could be due to the decoupling of traits between adults and tadpoles that allows for each stage of a complex life cycle to respond independently to selective forces. Additionally, differences in metamorph size may indicate that a shift of dispersal traits occur after metamorphosis in *X. laevis*. The phenotypic traits of invasive *X. laevis* tadpoles appear to be conserved and are not influenced by a change in the dispersal traits of adults. 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.

3.2. 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 evolution in dispersal traits. Essentially, individuals with the best dispersal capacities (*i.e.* those that allocate more resources to life-history traits related to dispersal), are more likely to meet and mate at the outermost sites (Travis & Dytham, 2002). If these traits are genetically determined, it can induce 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 & 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 & Dytham, 2002; Simmons & 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 & Dytham 2002; Phillips et al. 2008; Shine et al. 2011; Chuang & 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 ontogenic stage. In many amphibians only the adults disperse and reproduce (Cayuela et al., 2018). During dispersal, one or more of the life-history stages of amphibians experience a range of different environmental conditions (Chuang & Peterson, 2016). The environmental pressure on traits experienced in an earlier life stage can affect the dispersal ability of a later life stage (*e.g.* the invasive damselfly, *Coenagrion scitulum*, Therry et al., 2014), resulting to 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 & 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, 2001a, Chelgren et al., 2006; Yagi & Green, 2018). Tadpole snout-to-vent length (SVL) has been coupled to stamina (e.g. American toad, *Anaxyrus americanus*, Wassersug & Feder, 1983) and metamorph size (e.g. wood frog, *Lithobates sylvatica*, Relyea, 2001a). In turn, larger SVL at metamorphosis is coupled with survival and endurance in adults (e.g. *Pelophylax* sp., Altwegg & Reyer, 2003; and *Rana* sp., Chelgren et al., 2006). Smith-Gill and Berven (1979) also describe the importance of the correct timing of metamorphosis for metamorph SVL. The timing of metamorphosis and the duration of the larval period is coupled to adult hind limb length (e.g. wood frogs, *Lithobates sylvatica*, Relyea, 2001a). Shorter larval development usually decreases hind limb length (Gomez-Mestre & Buchholz, 2006; Gomez-Mestre et al., 2013; Tejedo et al, 2010) due to thyroid hormone (thyroxine, T4) action (Eddy & 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., 2019a; 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 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 & Measey, 2006). Dispersal occurs overland and after metamorphosis, even though all stages of this species are principally aquatic (Measey et al., 2016, Courant et al., 2019a).

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 hypothesise 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 timing 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, not much 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 & 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), timing of metamorphosis (development) and survival to metamorphosis.

3.3. Materials and methods.

3.3.1. Study site.

The population of *X. laevis* is thought to have been introduced in the early 1980s in western France (Fouquet & Measey, 2006). The invasive range now covers ~ 4000 km² (JS unpublished data, Vimercati et al., 2019) The population has spread unevenly from the introduction site. We sampled six ponds (Fig. 3.1). 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.

3.3.2. 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*. The reproductive states of adults were inspected to determine whether individuals should be hormonally primed. Males were considered ready for spawning when the nuptial pads on the forelimbs appear black. Females were considered ready for spawning when the cloaca was red and swollen. Males and females not ready for spawning were kept separate and were primed with 50 IU of HCG shortly after collection. 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 by an overdose of tricaine methanesulfonate (ms-222) at the conclusion of the experiment.

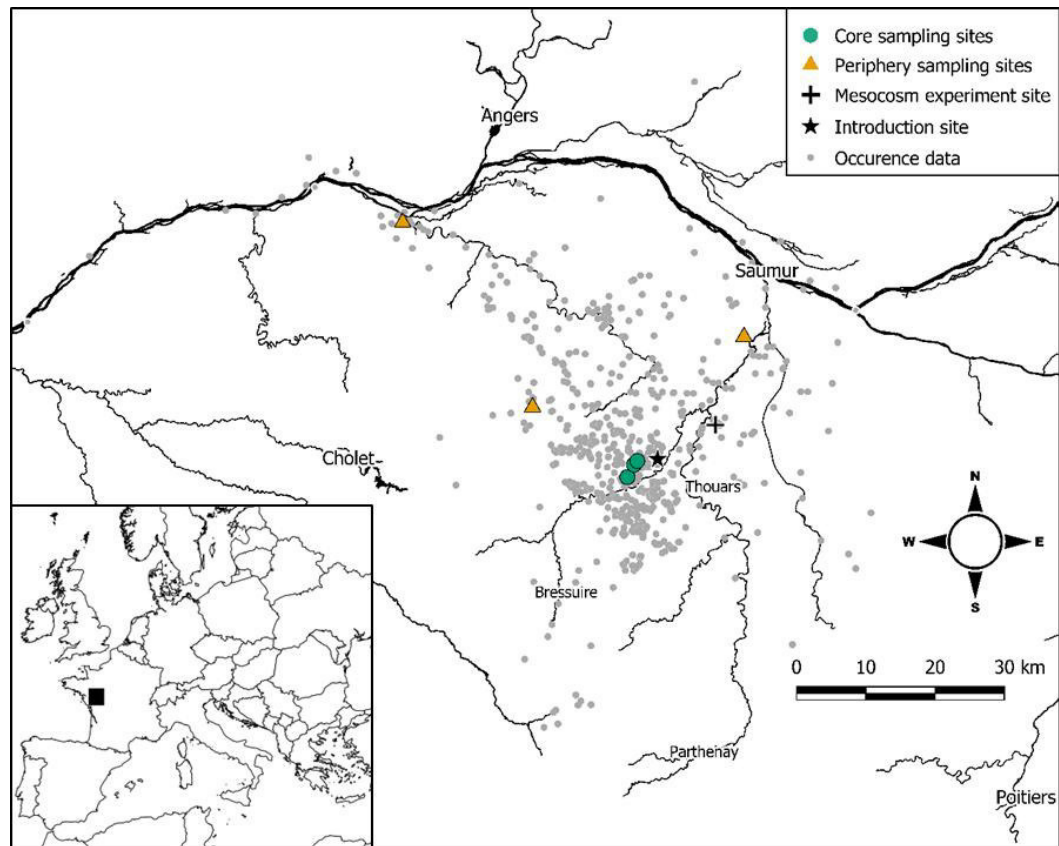


Figure 3.1: Current distribution of the invasive population of *Xenopus laevis* in western France. Indicated are the point of introduction, the experimental site and six collection sites used in this study. Small grey dots indicate the occurrence data of *X. laevis* (Vimercati et al., 2019).

3.3.3. Staging.

The larval development of *X. laevis* is well studied and widely used as a model in developmental biology (Nieuwkoop & Faber 1994; Segerdell et al., 2008; Segerdell et al., 2013). The development of *X. laevis* from fertilisation to metamorphosis undergoes 66 NF stages (Nieuwkoop & 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.

3.3.4. Experiments.

We conducted parallel common garden studies in outdoor mesocosms and microcosms in the laboratory. Four egg clutches from each of the 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. 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 determine variation in temperature (Fig. S3.1). The temperature loggers were attached to a floating piece of polystyrene and suspended 15 cm under the water to avoid temperature inversion. Tanks were filled up 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 tadpole.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 (Chapter 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, 2008). Initially, 20 tadpoles were collected from each clutch (4 clutches per site) 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.

3.3.5. 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 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 S3.2, Fig. S3.2, S3.3, and S3.4). 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 (Peterson, 2019). Consequently, the best transformation, as pointed out by *bestNormalize*, 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* *R* package (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.

Timing 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.

For all analyses, we selected the best fitting model using the corrected Akaike information criterion (AICc) according to parsimony (Burnham & 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 (Table S3.1). If the top model was equal ($\Delta\text{AICc} < 2$) 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 3.4.1 (R Core Team, 2018).

3.4. Results.

3.4.1. Variation in morphological traits.

Overall, we observed high levels of variation in SVL within each specific stage (Fig. 3.3) 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. 3.3, Table S3.1). 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; $n = 34$), versus a mean SVL of 11.94 mm (± 1.08 mm SD; $n = 38$; Fig. 3b,) at the core. The model with only position as a fixed effect was chosen as the most suitable (Table 3.1 GLMM, $\Delta\text{AICc} = 3.12$). In contrast, no differences in relative femur length were detected and the null model was chosen as the best model for metamorphs reared in both mesocosms (Fig. 3.3b, Table 3.1, GLMM, $\Delta\text{AICc} = 1.72$) and microcosms (Fig 3.3d, Table 3.1, GLMM, $\Delta\text{AICc} = 8.5$). No differences were detected in relative femur length between core and periphery individuals reared in microcosms (Fig 3.4d, Table 3.1, GLMM, $\Delta\text{AIC} = 8.5$). Similar results were observed for body size (PC1) (Appendix 3.1).

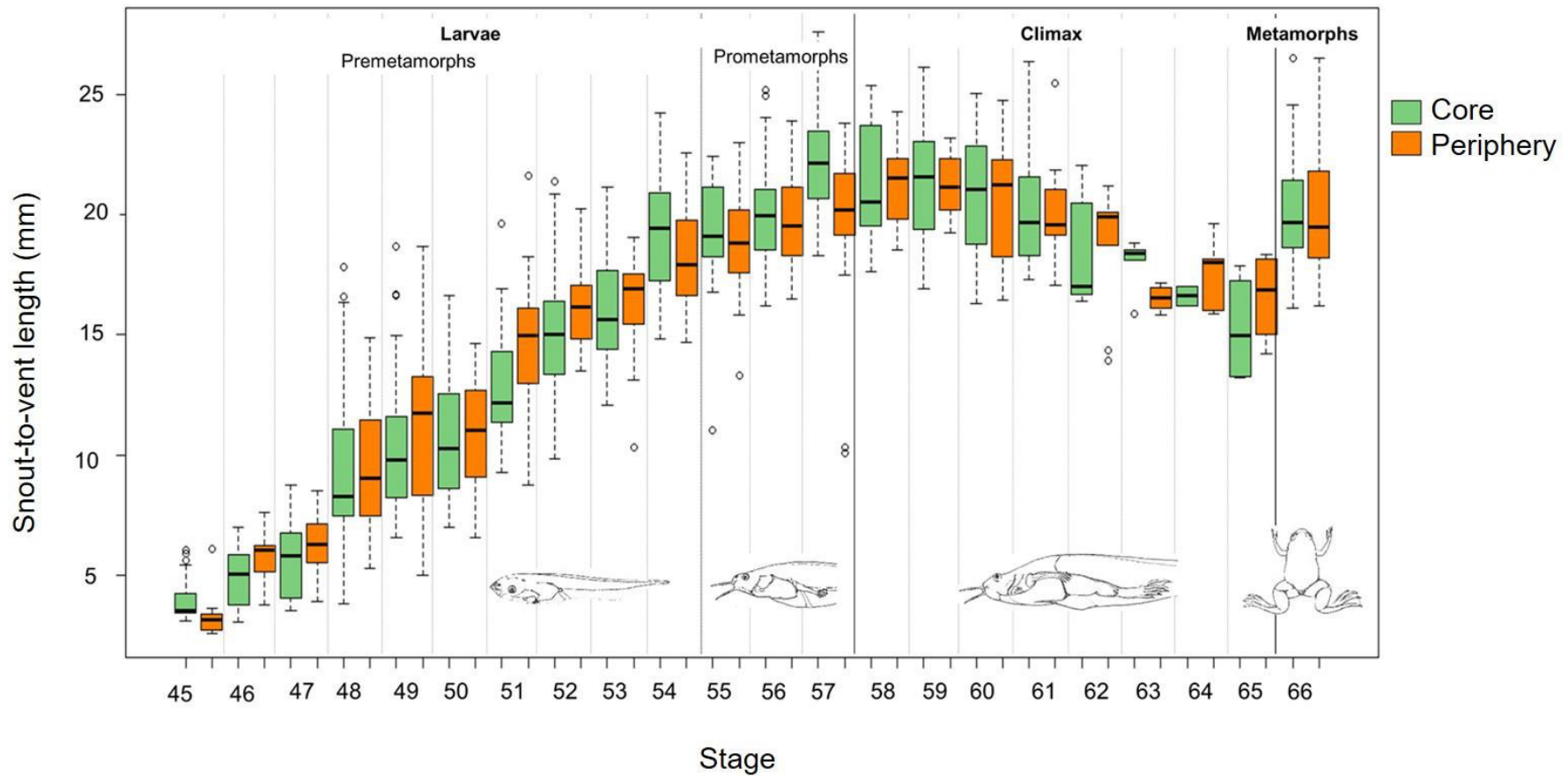


Figure 3.2: Snout-to-vent length variation during the larval development in core and periphery sites of the invasive range colonised by *Xenopus laevis* in Western France, as measured from individuals raised in the outdoor mesocosms experiment. In the boxplot, the lowest boundary indicates the 25th percentile, a black line within the box indicates the median, and the highest boundary indicates the 75th percentile. Whiskers above and below the box indicates the 10th and 90th percentile. Points above and below and the whiskers represent outliers above and below the 10th and 90th percentile

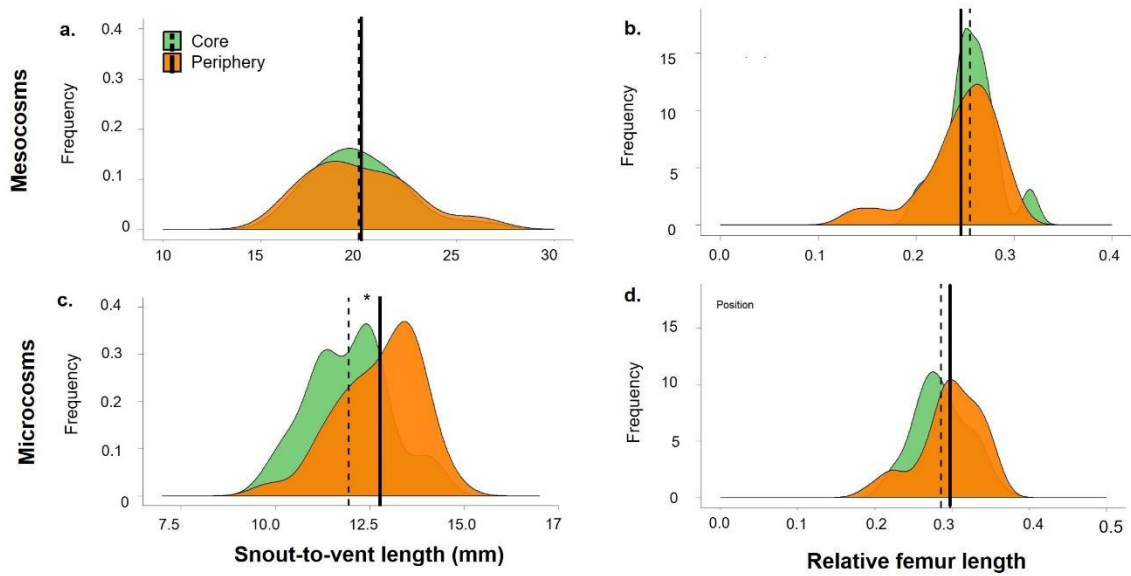


Figure 3.3: Frequency of snout-to-vent length (SVL) and relative femur length of metamorphs: a. SVL between core (green shaded) and periphery (orange shaded) metamorphs reared in outdoor mesocosms. Dotted line indicates the mean SVL or relative femur length of metamorphs from the core and solid line 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.

Table 3.1: 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

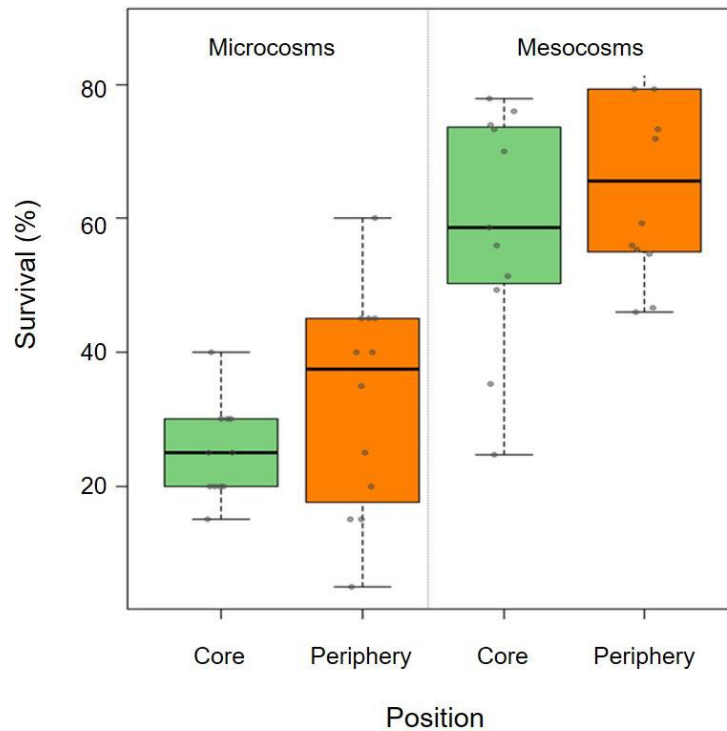


Figure 3.4: Survival of individuals from the core and the periphery in the microcosms and the mesocosms. In the boxplot, the lowest boundary indicates the 25th percentile, a black line within the box indicates the median, and the highest boundary indicates the 75th percentile. Whiskers above and below the box indicates the 10th and 90th percentile. Points above and below the whiskers indicate outliers above and below the 10th and 90th percentile.

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; $n = 11$ clutches) and the periphery (100/150 tadpoles \pm 22.80 SD; $n = 12$ clutches) (Fig. 3.4). Moreover, only the null model was identified as suitable (Table 3.1, binomial GLMM, $\Delta\text{AICc} = 0.3$). 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; $n = 11$ clutches) and individuals from the periphery (6.5/20 tadpoles \pm 3.26 SD; $n = 12$ clutches). Once again, the null model was the best model (Table 3.1, binomial GLMM, $\Delta\text{AICc} = 0.03$). 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 3.1, cox GLMM, $\Delta AICc = 0.16$)

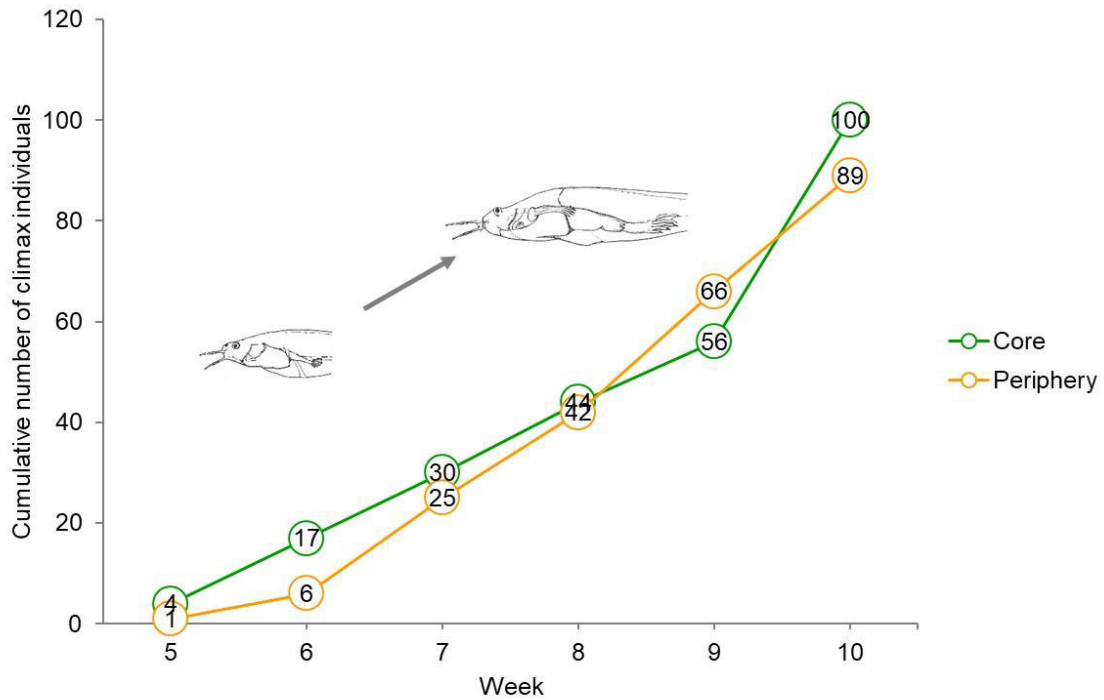


Figure 3.5: Cumulative number of *Xenopus laevis* individuals that transitioned from larvae to climax per week in all mesocosms for core and periphery sites.

No difference in the timing of metamorphosis was observed between individuals from the core and the periphery (Fig 3.5, Cox proportional hazard, Table 3.1). The difference in the AICc of the intercept model and the corresponding model that included only position was < 2 for the transitions from larvae to climax ($\Delta AICc = 0.003$).

3.5. 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 & Dytham, 2002; Simmons & Thomas 2004; Shine et al., 2011). In the invasive population of *Xenopus laevis* in western France, spatial sorting has enhanced those adult phenotypic traits that promote dispersal capacity in adults (Louppe et al., 2017; Louppe et al., 2018; Courant et al., 2019a). 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., 2017a). 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 & Reyer 2003; Cabrera-Guzman et al., 2013). Therefore, body size (snout-to-vent length) is a potential target for spatial sorting 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.

In France, no studies have specifically identified and studied the predators of *X. laevis* tadpoles and metamorphs. Kruger et al. (2019) found that invasive crayfish (*Procambarus clarkii*) and a native diving beetle (*Dytiscus dimidiatus*) can potentially predate on *X. laevis* tadpoles. The tadpoles of the invasive population in France display an anti-predator response by decreasing their activity in the presence of both predators (Kruger et al., 2019). However, predators may also induce a change in morphology in addition to behaviour (Relyea, 2003). Changes in morphology, such as deeper tail fins, shorter bodies, and narrower mouths may indicate that the adaptations may increase anti-predator response of those tadpoles. Courant et al. (2018) describes a higher density of nektonic macroinvertebrates that can predate on tadpoles and metamorphs at the periphery of the range than at the core. Thus, displaying an uneven distribution of predators within the population which we hypothesise may induce the larger size in metamorphs at the periphery. There is currently no evidence for this hypothesis and can be of interest for future study.

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 using a 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 the fluctuating temperatures in outdoor mesocosms better represent the natural conditions, food

availability and community interactions (Mikó et al., 2015). As a result, 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 the 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 & 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., 2010; Shine et al., 2011). This has not been demonstrated for *X. laevis* populations and should be prioritised for a future study. 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 timing of 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., 2017c). Likewise, we found no differences in development (timing of 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 & Hoverman, 2003) factors. It is also possible that the lack 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 & Wassersug, 2007), whereas adults additionally use hind limbs for overland movements (Handrigan & 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 (Bonett & 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 & 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 & 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 change 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 & 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.

Chapter 4: The effect of range expansion on the burst swimming performance of invasive *Xenopus laevis*.

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Author contributions:

NK, JM, AH, JS conceived and designed the study, NK performed the experiments and data acquisition, NK, JS and AH contributed to the data analysis. NK, JM, AH and JS were involved with the interpretation of data. NK drafted the chapter.

4.1. Abstract.

In heterogeneous environments, phenotypically plastic individuals can modify their phenotypic traits to affect their fitness. Invasive species often exhibit a high level of phenotypic plasticity. However, within the invaded range the individuals at the periphery are presumed to have higher phenotypic plasticity than at the core due to the canalisation at the introduction point. Measuring the thermal performance of individuals at the core and at the periphery of the range of an invasive population can help address whether plasticity of burst swimming speed performance evolved in the invasive range. Performance such as locomotion is commonly measured in ectotherms. Additionally, the thermal conditions experienced during development of ectotherms can influence the physiology and thus the locomotor performance of the organism. Ectotherms with complex life cycles, such as amphibians, can respond differently to environmental pressures at each stage. We aimed to elucidate whether phenotypic plasticity evolved in the tadpoles of the invasive African clawed frog, *Xenopus laevis*, in western France. We compared the acclimation ability of burst swimming performance and critical thermal limits of invasive *X. laevis* tadpoles at the core and the periphery of their distribution. We hypothesised that phenotypic plasticity is higher at the periphery than at the core. We predicted that when exposed to different developmental temperatures, tadpoles from the periphery will acclimate their maximum performance to the relevant temperature. Subsequently, we predicted that when exposed to different developmental temperatures, core tadpoles will have a limited ability to acclimate their maximum performance to the developmental temperatures. We tested our hypothesis by measuring three parameters of burst swimming performance (velocity, acceleration, and sinuosity) of core and periphery tadpoles reared at three developmental temperatures (17 °C, 22 °C, and 27 °C) at each of five test temperatures (5 °C, 10 °C, 20 °C, 30 °C, and 35 °C). Performance measures were used to model thermal performance curves. Additionally, we measured the critical thermal minimum and maximum to bound the limits of

the performance curve. Contrary to our hypothesis, we could not find differences in the plastic response of core and periphery tadpoles reared at three developmental temperatures. Additionally, we found no differences in CT_{min} and CT_{max} between core and periphery and developmental temperatures. All tadpoles reared at 27 °C exhibited a shift of the thermal optimum to warmer temperatures. Interestingly, our results show that the acclimation response of *X. laevis* tadpoles reared at 17 °C is in the same direction, i.e. also shifted to warmer temperatures. Thus, invasive *X. laevis* tadpoles from the core and the periphery have the ability to acclimate their burst swimming performance to warmer but not colder temperatures.

4.2. Introduction.

Phenotypically plastic organisms can exhibit different phenotypes in diverse environmental conditions and keep or enhance their fitness in response to the challenges experienced in a novel environment (West-Eberhard, 2003). Invaders in the novel environment can encounter unfamiliar challenges such as novel predators, unknown resources, and novel climatic conditions (Simmons & Thomas, 2004; Myles-Gonzalez et al., 2015). Thus, not surprisingly, invasive populations have higher levels of phenotypic plasticity (Davidson et al., 2011). Phenotypic plasticity can increase the fitness of individuals without adaptive genetic differentiation from the genetic pool of the source population (Price et al., 2003). Phenotypic plasticity of invaders has been mostly studied in plants (Matesanz et al., 2010) often within the context of differences between native and invasive species (Davidson et al., 2011).

Few studies have investigated the evolution of phenotypic intraspecific plasticity within an invaded range (e.g. Ducatez et al., 2016). During the invasion process, an increase of expansion of the population occurs due to the increased dispersal ability of individuals at the periphery of the distribution (Shine et al., 2011). At the periphery, individuals with the highest dispersal ability will meet and mate and their offspring are expected to have a higher dispersal ability than the previous generations if dispersal relevant traits are heritable, this is known as spatial sorting (Phillips et al., 2008). Few theoretical studies have investigated the evolution of phenotypic plasticity within an invasive population that is known to have undergone spatial sorting (Chevin & Lande, 2011; Lande, 2015; Ducatez et al., 2016). These studies, however, have contradicting predictions whether phenotypic plasticity will increase or decrease at the periphery. For instance, the successive colonisation events may increase the effects of genetic bottlenecks and thus reduce population genetic diversity at the periphery and eventually the expansion of the invasive range (Ellstrand & Elam, 1993; Newman & Pilson, 1997; Nieminen et al., 2001). Additionally, the higher dispersal ability at the periphery may impose metabolic

trade-offs that can be transmitted to the next generation. This is expected to decrease the phenotypic plasticity of individuals at the periphery (Ducatez et al. 2016) However, gene flow from the core which is presumed to be more abundant to the less abundant periphery of the population can be maladaptive (Kirkpatrick & Barton, 1997). This can create a phenotype at the periphery which deviates from the mean phenotype at the core (Kirkpatrick & Barton, 1997). Thus, in a population that is continuously spreading, an increase in plasticity is expected at the edge of the range (Chevin & Lande, 2011). A transient increase in plasticity is expected in colonising populations when colonising new habitats during range expansion (Lande, 2015). The correct prediction will depend on the dynamics of the population, however, in both cases this can lead to the differentiation of phenotypes between the core and the periphery and has been previously found in ectotherms (Schlichting & Pigliucci, 1998; Chevin & Lande, 2011; Ducatez et al., 2016).

Measuring the performance of individuals at the core and at the periphery of the range of an invasive population can help address whether the reaction norm evolved in a long-term invasive population. Locomotion, growth, development and survivorship of an organism are measures of performance (Chevin et al., 2010). Locomotion is essential for foraging and escaping predators and is commonly measured in ectotherms (Huey & Kingsolver, 1989). Additionally, the thermal conditions experienced during development of ectotherms can influence the physiology and thus the locomotor performance of the organism (Seebacher & Grigaltchik, 2014). Temperature affects the musculoskeletal performance of ectotherms and is usually described by a thermal performance curve (Huey & Stevenson, 1979). Angilletta (2009) describes a general model that can be applied to thermal performance curves and proposes to measure: (1) maximum performance (P_{max}) at the peak of the thermal performance curve, (2) temperature at the maximum performance (T_{opt}), (3) critical thermal minimum

(CT_{min}) and maximum (CT_{max}) where locomotor performance drops to biologically non-relevant levels.

The thermal performance curve is not fixed and can shift between populations. For instance, a vertical shift (a shift in P_{max}) in the thermal performance curve indicates changes in the level of performance, and a horizontal shift (a shift in T_{opt}) indicates a higher performance in warmer or cooler temperatures (Huey & Kingsolver, 1989). The phenotypically plastic horizontal shift in a thermal performance curve of a specific population to match the current thermal conditions is known as thermal acclimation (Little & Seebacher, 2016). Acclimation may act as a buffer against environmental change (Wilson & Franklin, 2002). During acclimation, ectotherms modify physiological pathways to shift their thermal performance curves to optimise performance in otherwise restrictive environments (Little & Seebacher, 2016). Simply put, acclimation enables organisms to avoid the possibly lethal or damaging consequences of thermal extremes (Brattstrom & Lawrence, 1962). Thus, in variable environmental conditions, there would be a strong selection for individuals that can maintain their locomotor performance via thermal acclimation.

In ectotherms with complex life cycles, some stages have a greater ability to acclimate locomotor performance to thermal change. For example, in amphibians, performance of primarily terrestrial adults showed limited plasticity to recurrent temperature change (Wilson et al., 2000), whereas, the fully aquatic tadpole stages can acclimate their locomotor performance (Wilson et al., 2000; Laugen et al., 2003). For tadpoles, maximum burst velocity (U_{max}) and maximum acceleration (A_{max}) are commonly used as a measure of locomotor performance (e.g. Seebacher & Grigaltchik, 2014). These parameters describe the burst swimming escape of tadpoles that is used to evade an attack of a predator (Wassersug, 1989; Wilson et al., 2000). However, these parameters alone might not be effective if the tadpole is disorientated and swimming in circles, which may happen at temperature extremes. Thus, it is

important for the escape response to be as linear as possible (Van Buskirk & McCollum, 2000). Predator escape is especially important for smaller (younger) tadpoles as they are more vulnerable to predators than larger tadpoles, as larger prey may exceed predator limitations of especially invertebrate predators (Werner & Gilliam 1984; Brose et al., 2005; Asquith & Vonesh, 2012).

The morphology and locomotor performance of the African clawed frog, *Xenopus laevis*, adult and tadpole stage has been found to be phenotypically plastic (Chapter 2, Wilson et al., 2000, Padilla et al., 2019). Wilson et al. (2000) found that *X. laevis* tadpoles were faster at colder temperatures when reared in cold temperatures than tadpoles reared in warm temperatures. *Xenopus laevis*, is invasive across four continents, including western France (Measey et al., 2012). This population is unique as it is the only one where mitochondrial lineages from two rainfall regimes (winter and summer rainfall) in the native range of *X. laevis* in southern Africa have been detected (De Busschere et al., 2016).

The climatic niches occupied by *X. laevis* in southern Africa partially differ from the niche occupied in France (Rödder et al., 2017). The invasive region in France is characterised by an Oceanic climate, whereas the climate in its native region is variable from Mediterranean in the Western Cape, South Africa, to Tropical in Malawi (Measey et al., 2012; Rödder et al., 2017). Within the native range, *X. laevis* is distributed in the winter rainfall region that is generally characterised by a winter rainfall pattern and colder weather (16 - 26 °C in the summer and 4 - 18 °C in the winter) and the summer rainfall region which is characterized by summer rainfall and warmer weather (15 - 33 °C in the summer and 4 - 25 °C in the winter) (South African Weather Service, 2020). In the summer rainfall region, breeding takes place from September to mid-March from spring to summer (Everson, 2006). Whereas, in the winter rainfall region breeding takes place from July to September from winter to the onset of spring (Kalk, 1960). The adults can breed up to twice a year when the conditions are favorable (Wood,

1965; Tinsley et al. 1996). The invasive range in western France, is characterized by colder weather (9.5 - 25.3 °C in the summer and 2.5 - 19.4 °C in the winter, (Beaucouzé weather station) and rainfall throughout the year. In France adults were found to breed from April to June from spring to the onset of summer (Courant et al., 2017a). Tadpoles emerge within a couple of days of fertilisation, therefore, the breeding season can also be seen as the larval period. Thus, tadpoles are present at different seasons and temperatures in the different native regions and the invasive region.

The population in western France is interesting as it has been expanding from a single known introduction point in the early 1980s and now covers an area of ~ 4000 km² (JS unpublished data, Vimercati et al., 2019). Thus, the core has been colonised for at least 40 years, colonisation at the periphery is ongoing. The enhancement of dispersal traits of adults at the periphery is not coupled to tadpole phenotypes which allows tadpoles to respond to the environment independently (Chapter 3), therefore, we do not expect adult trade-offs to decrease phenotypic plasticity at the periphery as hypothesised by Ducatez et al. (2016). According to Chevin & Lande (2011) the increase in genetic flow can limit the ability of periphery individuals to become locally adapted. Therefore, we hypothesise that phenotypic plasticity is higher at the periphery. We predict that there will be a differentiation in ability to acclimate maximum performance between the core and the periphery when reared in different developmental temperatures; periphery individuals displaying better acclimation capacity than core individuals. Regarding thermal physiological limits it has been reported that for ectotherms the CT_{max} is more conserved than CT_{min} (Clusella-Trullas & Chown, 2014). Thus, we predict that the successful acclimation to lower/higher temperatures will be supported by a shift in CT_{min} to cooler or warmer temperatures.

4.3. Materials and methods.

4.3.1. Study sites.

Xenopus laevis was introduced in western France approximately 40 years ago and now covers a distribution area of ~5000 km². A high density of ponds creates a dense hydrographic network in this area (Vimercati et al., 2019). We selected two sampling ponds at the core: 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 two sampling sites from the estimated periphery: site 3 (47°11'02.6"N, 0°07'51.2"W; dis = 21.2 km) and site 4 (47°06'13.1"N, 0°31'50.4"W, dis = 19.2 km) (Fig. 4.1).

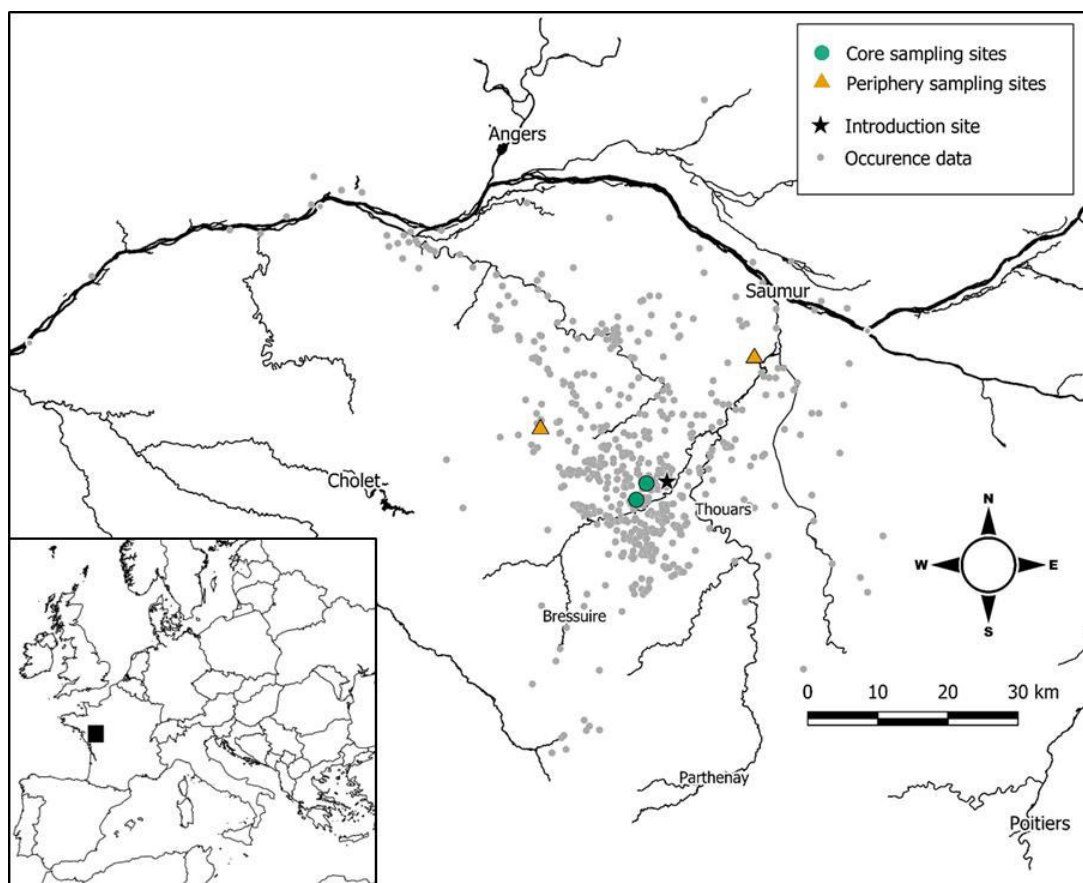


Figure 4.1: Current distribution of the invasive population of *Xenopus laevis* in western France. Indicated are the point of introduction, and four sites used in this study. Small grey dots indicate the occurrence data of *X. laevis* (Vimercati et al., 2019).

4.3.2. Adult collection, care and breeding.

One breeding pair was collected at each site. Adults were kept at 22 °C before breeding and exposed to a 12 h photoperiod. The reproductive state of individuals determined whether they would receive hormonal priming. Adults not in the correct reproductive state were primed before breeding. Males and females were primed with 50 international units (IU) of human chorionic gonadotrophin hormone (HCG) shortly after collection. On the third day, females were injected with 500 IU and males with 250 IU (Wlizla et al., 2018). The breeding pair were kept separate and only joined on the third day in a plastic aquarium (7 - 10 L) fitted with a plastic mesh so the laid eggs fell through the mesh would not be eaten or damaged. Frogs were removed from the aquaria the following morning. Eggs were left to hatch in their aquaria maintained at 22 °C and subsequently moved after hatching into acclimation tanks. Adults were euthanised by an overdose of tricaine methanesulfonate (ms-222) after breeding was completed.

4.3.3. Experiment.

An adapted version of the protocol to measure activity of tadpoles in response to acute temperature as designed by Wilson et al. (2000) was implemented. The experimental protocol consisted of two phases. Firstly, lab-reared *X. laevis* tadpoles underwent an acclimation period at three temperatures, and secondly the burst swimming performance of tadpoles were measured when exposed to five acute temperatures.

Acclimation period- The recommended temperature to keep *X. laevis* is 22 °C (OECD, 2008). Thus, we chose this as the intermediate temperature and added 17 °C as a low temperature and 27 °C as a high temperature. After hatching, free-swimming larvae were

placed in the three acclimation groups until they reached the pre-metamorphosing stage category (NF stage 45-57) and were exposed to a 12:12 LD photoperiod. Temperature in the high and intermediate acclimation tanks were controlled by Tempo 50 heaters (50 Watt, Wat Sea, E2evolution) and the low temperature was maintained by air conditioning (temperature profiles Fig. S4.1). Tadpoles were reared at a high density of 15 tadpoles per litre, to slow developmental rates (Tejedo & Reques, 1994). Pre-metamorphic tadpoles were tested over a period of 30 days, thus, we noted the age of the individuals at the date of testing. Tadpoles were fed Frog Brittle ® for tadpoles (NASCO, Fort Atkinson, WI) throughout the rearing period.. Following the exposure period, individuals (n = 10) from each site at each acclimation temperature were selected for burst swimming speed performance at five temperatures between 5 °C and 35 °C.

Performance test- Before each trial, tadpoles were acclimated to test temperatures by changing the tank water temperature at a rate of 0.2 °C.min⁻¹. Tests were randomised by assigning random numbers to each test. Burst swimming speed performance was recorded by filming at least five initial burst swimming responses for one individual at a specific test temperature: 5 °C, 10 °C, 20 °C, 30 °C, and 35 °C. Each individual was only tested once at one test temperature and euthanised by an overdose of tricaine methanesulfonate (ms-222). Tadpoles were touched with a fine wire at the tip of their tail and the initial burst swimming sequence was recorded with a camera (Olympus TG-4) at 120 frames.s⁻¹. Trials were performed in a 420 mm x 80 mm x 170 mm clear plastic tank filled with 1 L aged tap water. A mirror at an angle of 45° was attached to the side of the tank, to visualise movements in the lateral plane. Water circulated through the custom-built copper pipes at the bottom of the tank, covered with a false bottom, and a water bath (Julabo F12, 4.5 L, max flow = 15 L/min, pressure = 0.35 bar) maintained the desired water temperature.

Video analysis- High-speed videos were recorded from two different views (the bottom of the tank and the image in the mirror). All burst swimming sequences were visually checked and only sequences perpendicular to the recording camera were selected. The X and Y coordinates from the high-speed video recordings were extracted by the motion analysis software: Tracker (Open Source Physics, USA, Brown, 2009). Coordinates were exported to Excel (Microsoft, Redmond, WA) and the displacement (cm) of the individual was calculated. Displacement data were filtered using a fourth order zero-phase shift low-pass Butterworth filter (Winter, 2004) with a 12 Hz filter frequency. The cut-off frequency was determined as a 10th of the recording frame rate. From the filtered data, maximum velocity (U_{\max}) measured as distance travelled over time, maximum acceleration (A_{\max}) which is the rate of change in velocity per unit of time, and sinuosity index were extracted. The sinuosity index is determined by calculating the ratio between total distance travelled and the Euclidean distance between the start and end of the travelled path. If the displacement of the tadpole was linear the sinuosity index would be equal to one.

4.3.4. Testing critical thermal limits.

Tadpoles ($n = 8$) from each acclimation group were tested for critical thermal minimum (CT_{\min}) and critical thermal maximum (CT_{\max}). Tests were performed in small circular containers (100 mL) filled with aged tap water suspended inside a water bath. At the onset of the test, the temperature of the water bath was set to the acclimation temperature (17 °C, 22 °C, and 27 °C) the tadpole developed in. The temperature of the water in the container was considered the temperature of the tadpole due to their small body size (Lutterschmidt & Hutchison, 1997). Prior to temperature change, tadpoles were left to acclimate to the experimental setup. Temperature was changed at a constant cooling/heating rate of 0.25

$^{\circ}\text{C}\cdot\text{min}^{-1}$ (Gutiérrez-Pesquera et al., 2016). The test was terminated when the tadpole was unable to right itself after the observer (NK) touched its tail and turned it upside down. The minimal and maximal temperatures at which this event occurred were recorded with a thermocouple (to the 0.01 $^{\circ}\text{C}$) as the CT_{min} or CT_{max} , respectively.

4.3.5. Statistical analysis.

The values of U_{max} and A_{max} were \log_{10} -transformed to meet assumptions of normality and homoscedasticity. The sinuosity index and \log_{10} -transformed U_{max} , A_{max} were used as response variables to measure the burst swimming speed performance of tadpoles.

Values of the response variables were used to create thermal performance curves, with CT_{min} and CT_{max} bounding the thermal performance curves. A polynomial function of test temperature was used to account for non-linearity observed in the data. It is important to select the correct polynomial degree (David et al., 1997; Izem & Kingsolver, 2005; Gvoždík & Van Damme, 2008). We selected the appropriate polynomial degree by comparing models fitted with different polynomial degrees starting with a global model including all polynomial degrees. A third-degree polynomial effect of test temperature, ‘poly(test temperature,3)’, was selected for U_{max} ($\Delta\text{AICc} = 2.07$), A_{max} ($\Delta\text{AICc} = 2.19$), and sinuosity index ($\Delta\text{AICc} = 8.88$). A third-degree polynomial corresponding to a sigmoid decreasing curve is a better fit to the data for a thermal performance curve than lower degree polynomials (David et al., 1997; Katzenberger et al., 2014). Thermal performance curves were plotted using *ggplot2* R package (Wickham, 2009). From the plotted thermal performance curves, we extracted the estimated maximum performance values (\pm SD) for each response variable and T_{opt} the temperature at which the maximum performance was reached.

Using the *lme4* R package (Bates et al., 2015) generalised linear mixed models were used with U_{\max} , A_{\max} , and sinuosity index as response variables, poly(test temperature,3), position (core/periphery), developmental temperature (17 °C, 22 °C, and 27 °C), and the interaction of position and developmental temperature were considered as fixed effects. Collection site (within position) and age were considered as a random effect.

For all analyses, we selected the best fitting model using the corrected Akaike information criterion (AICc). We considered models with a difference in AIC less than 2 to be equal and selected the simpler model (Burnham & Anderson, 2002). If the specified model was equal to the null model, the null model cannot be rejected, and was chosen as the best fitting one. Model diagnostics were carried out using the *DHARMA* R package (Hartig, 2019). The chosen model was then used to calculate the least square means of interactions using the *lsmeans* R package (Lenth, 2016). To determine significant differences between core and periphery pairwise comparisons we used Bonferroni corrected p-values. All analyses were carried out using the statistical software R 3.4.1 (R Core Team, 2018).

4.4. Results.

Position interacting with developmental temperature explained the variance of U_{\max} and A_{\max} (Table 4.1). For sinuosity the model with test temperature only was chosen as the best fit model.

We observed no significant differences (according to the Bonferroni corrected $p = 0.006$) of U_{\max} between core and periphery tadpoles reared at 17 °C ($p = 0.379$), 22 °C ($p = 0.373$) and 27°C ($p = 0.006$) (Table 4.2, Fig. 4.2). Similarly, we observed no significant differences of A_{\max} between core and periphery reared at 17 °C ($p = 0.239$), 22 °C ($p = 0.805$) and 27 °C ($p = 0.663$) (Table 4.2).

The interaction between position and developmental temperature determined the variation between performance measures of tadpoles reared at different temperatures from the same position. For U_{\max} , tadpoles from the core reared at 17 °C were significantly faster than tadpoles raised at 22 °C ($p = 7.07e-05$) but not for tadpoles reared at 27 °C ($p = 0.033$) (Table 4.2). Core tadpoles reared at 22 °C also significantly differed from tadpoles reared at 27 °C ($p = 1.98e-08$) (Table 4.2). The U_{\max} of periphery tadpoles reared at 17 °C significantly differed from tadpoles reared at 22 °C ($p = 0.0005$) and 27 °C ($p = 0.0001$). Tadpoles reared at 22 °C did not differ from tadpoles reared at 27 °C ($p = 0.523$) (Table 4.2).

For A_{\max} , core tadpoles reared at 17 °C did not significantly differ from tadpoles reared at 22 °C ($p = 0.036$) but did significantly differ from tadpoles reared at 27 °C ($p = 0.0003$). Core tadpoles reared at 27 °C significantly differed from tadpoles reared at 22 °C ($p = 7.95e-08$) (Table 4.2, Fig. 4.3). Significant differences of A_{\max} were observed for tadpoles from the periphery reared at 17 °C compared to 22 °C ($p = 0.0001$), and 27 °C ($p = 0.0001$). Periphery tadpoles reared at 22 °C did not differ from tadpoles reared at 27 °C ($p = 0.855$) (Table 4.2).

We observed horizontal shifts of T_{opt} for both core and periphery tadpoles reared at three developmental temperatures (Fig. 4.2, Table 4.3). Regardless of position, tadpoles reared at 17 °C displayed a shift toward warmer temperatures with higher T_{opt} (Fig 4.2 A, D, G). Tadpoles reared at 22 °C displayed a shift towards cooler temperatures (Fig 4.2 B, E, H). We also observed a shift of T_{opt} towards warmer temperatures of all tadpoles reared at 27 °C having higher T_{opt} (Fig. 4.2 C, F, I) than tadpoles reared at 22 °C.

Critical thermal limits (CT_{\min} and CT_{\max})-

The null model was chosen as the best fit model for CT_{\min} (GLMM, $\Delta\text{AICc} = 3.09$, Table 4.1). Thus, we detected no effect of position, developmental temperature and their interaction on the two response variables (Table 4.4). For CT_{\max} , the model including

developmental temperature was the best fit model. However, the null model fell within 2 AICc units (Table 4.1). According to parsimony, the null model was chosen as the best model. Thus, neither position nor developmental temperature influences CT_{max} (Table 4.4).

Table 4.1: Model selection of top models ($\Delta AICc < 2$) for each response variable: maximum velocity, maximum acceleration, sinuosity, CT_{min} and CT_{max} . Predictors in the models were the third degree polynomial of test temperature (poly(test temperature, 3), core or periphery (Position), developmental temperature and age. 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 variables	Model Parameters	k	logLik	AICc	$\Delta AICc$	weight
Maximum Velocity	poly(Test temperature,3) + Position x Developmental temperature	12	-10.53	45.6	0	0.89
Maximum acceleration	poly(Test temperature,3) + Position x Developmental temperature	12	-3309.60	6643.7	0	1
Sinuosity	poly(Test temperature,3)	7	22.78	-31.4	0	0.92
CT_{min}	Null	3	-21.88	50.61	0	0.82
CT_{max}	Developmental temperature	5	-26.12	64.54	0	0.73
	Null	3	-29.82	66.50	1.96	0.27

Table 4.2: Least square means pairwise comparison of the top linear mixed model: maximum velocity ($\log_{10}(U_{\max})$), maximum acceleration ($\log_{10}(A_{\max})$), and sinuosity index between core (C) and periphery (P) tadpoles reared at three developmental temperatures (17 °C, 22 °C, 27 °C). Asterisks indicate significant differences of Bonferroni corrected p-value (< 0.0055) of the performance parameter between core and periphery. Indicated in the table is the model estimate (Est), residual standard error (Std. Error), the degrees of freedom (df), the t-value, the lower and the upper confidence intervals (CI), and the p-value ($\text{Pr}(>|t|)$)

	Est	Std. Error	df	t-value	Lower CI	Upper CI	Pr(> t)	
U_{max}								
C:17 - P:17	-0.071	0.069	3.290	-1.014	-0.282	0.141	0.379	
C:22 - P:22	-0.072	0.069	3.179	-1.035	-0.285	-0.0142	0.373	
C:27 - P:27	0.188	0.007	3.559	2.630	-0.002	0.396	0.006	
C:17 - C:22	0.150	0.037	274.663	4.036	0.077	0.223	7.07E-05	*
C:17 - C:27	-0.084	0.039	153.443	-2.148	-0.161	-0.007	0.033	
C:22 - C:27	-0.234	0.0394	147.635	-5.938	-0.312	-0.156	1.98E-08	*
P:17 - P:22	0.149	0.041	77.424	3.652	0.0679	0.231	0.0005	*
P:17 - P:27	0.174	0.004	69.711	4.123	0.009	0.259	0.0001	*
P:22 - P:27	-0.002	0.0039	151.381 5	0.640	-0.052	0.102	0.523	
A_{max}								
C:17 - P:17	- 30.125	20.67	3.091	-1.457	-94.832	0.346	0.239	
C:22 - P:22	-5.53	20.455	2.966	-0.270	-71.054	59..992	0.805	

C:27 - P:27	56.745	21.093	3.340	2.690	-6.680	120.171	0.663	
C:17 - C:22	21.211	10.083	335.002	2.104	1.38	0.410	0.036	
C:17 - C:27	- 39.108	10.716	198.296	-3.650	-60.240	-17.977	0.0003	*
C:22 - C:27	- 60.319	10.815	197.552	-5.578	-81.647	--38.993	7.95E- 08	*
P:17 - P:22	45.805	11.421	91.597	4.011	23.121	68.489	0.0001	*
P:17 - P:27	47.762	11.838	87.396	4.035	24.234	71.290	0.0001	*
P:22 - P:27	1.957	10.727	179.127	0.182	-19.211	23.124	0.855	

Table 4.3: Maximum performance values for maximum velocity ($\log_{10}(U_{\max})$), maximum acceleration ($\log_{10}(A_{\max})$), and sinuosity index with stand deviations (SD) at thermal optimum (T_{opt}) between core and periphery tadpoles reared at three developmental temperatures.

Developmental temperature (°C)	Core		Periphery	
	$U_{\max} \pm \text{SD}$	T_{opt} (°C)	$U_{\max} \pm \text{SD}$	T_{opt} (°C)
17 °C	0.96 ± 0.11	23.95	1.10 ± 0.11	21.51
22 °C	0.79 ± 0.11	19.84	0.97 ± 0.10	19.43
27 °C	1.15 ± 0.11	23.52	0.98 ± 0.10	24.39
	$A_{\max} \pm \text{SD}$	T_{opt} (°C)	$A_{\max} \pm \text{SD}$	T_{opt} (°C)
17 °C	2.31 ± 0.23	22.69	2.52 ± 0.23	21.43
22 °C	2.15 ± 0.24	18.61	2.35 ± 0.22	19.43
27 °C	2.58 ± 0.22	23.52	2.38 ± 0.22	23.52
	Sinuosity	T_{opt} (°C)	Sinuosity	T_{opt} (°C)
17 °C	1.09 ± 0.09	23.53	1.07 ± 0.09	21.85
22 °C	1.09 ± 0.10	22.31	1.03 ± 0.10	19.43
27 °C	1.09 ± 0.09	23.52	1.10 ± 0.09	23.96

Table 4.4: The critical thermal maximum (CT_{\max}) and minimum (CT_{\min}) of core and periphery tadpoles reared at three developmental temperatures.

Developmental temperature (°C)	Core		Periphery	
	$CT_{\min} \pm \text{SD}$	$CT_{\max} \pm \text{SD}$	$CT_{\min} \pm \text{SD}$	$CT_{\max} \pm \text{SD}$
17 °C	3.73 ± 0.58	37.00 ± 0.51	3.80 ± 0.43	36.98 ± 0.73
22 °C	3.25 ± 0.34	38.35 ± 0.10	3.60 ± 0.43	38.05 ± 0.10
27 °C	4.20 ± 0.28	36.00 ± 0.23	4.00 ± 37	35.85 ± 0.62

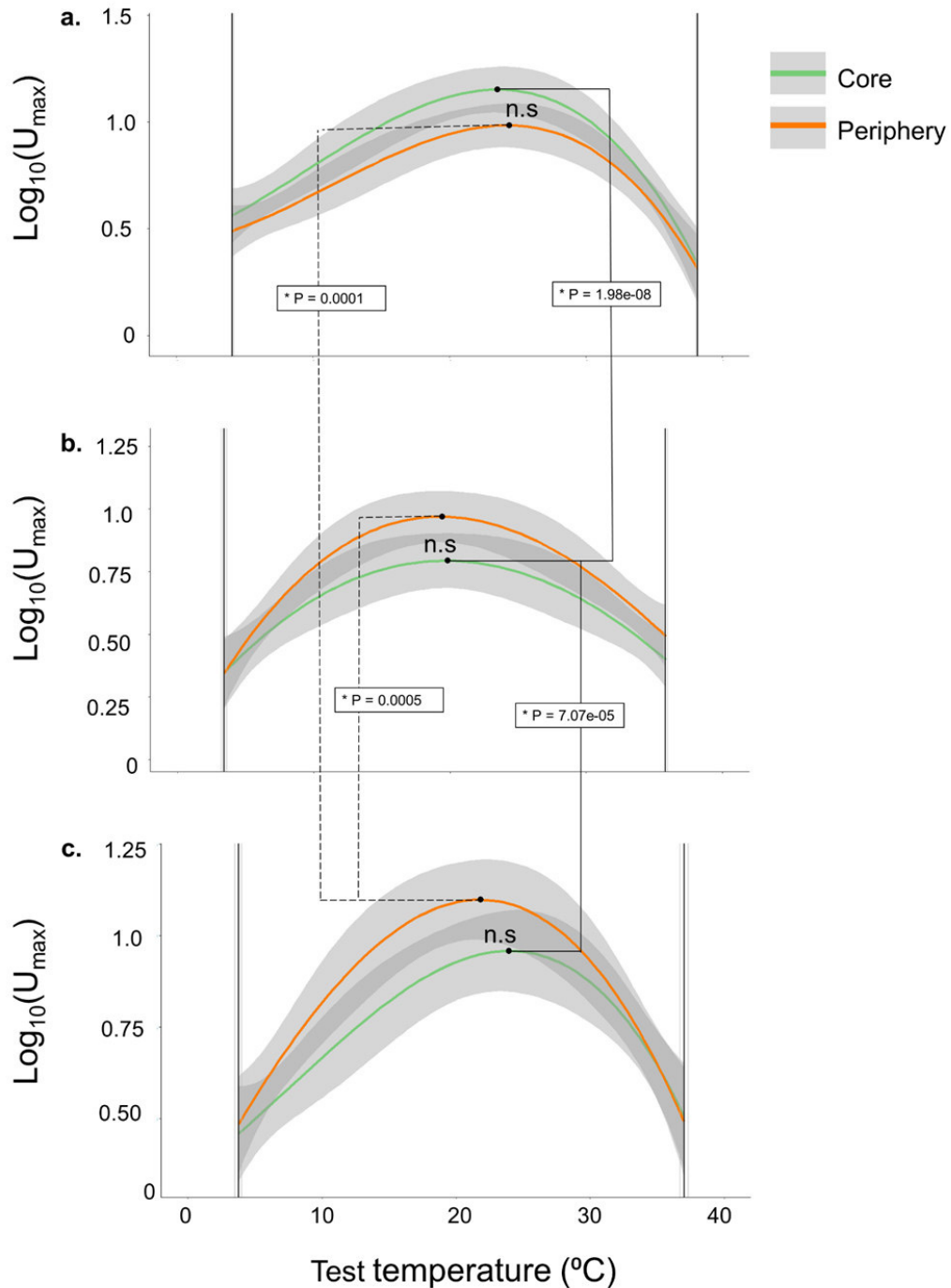


Figure 4.2: Thermal performance curves (TPC) of maximum velocity (U_{max}) of core (green line) and periphery (orange line) tadpoles reared at three developmental temperatures (17 °C, 22 °C, and 27 °C). Indicated as black points on the curve is thermal optimum (T_{opt}) at maximum performance (P_{max}). Critical thermal minimum (CT_{min}); and critical thermal maximum (CT_{max}) are indicated as grey lines. a.) TPC of maximum velocity [$\log_{10}(U_{max})$] of tadpoles reared at 27 °C, b.) TPC of maximum velocity [$\log_{10}(U_{max})$] of tadpoles reared at 22 °C, and c.) TPC of maximum velocity [$\log_{10}(U_{max})$] of tadpoles reared at 17 °C. Grey envelope represents 95% confidence interval. Overarching black dashed (periphery) and solid (core) lines between plots and asterisks indicate significant differences.

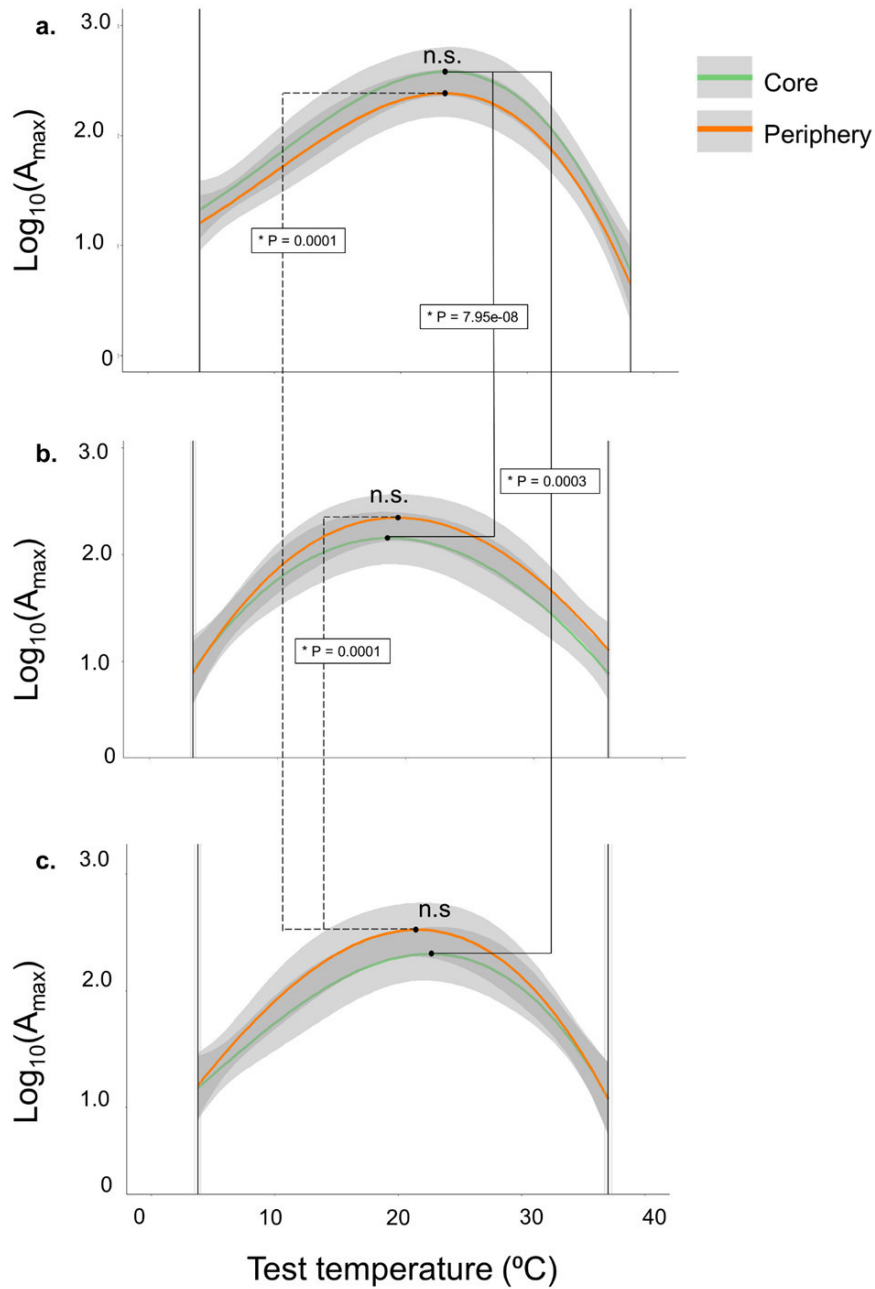


Figure 4.3: Thermal performance curves (TPC) of maximum acceleration (U_{\max}) of core (green line) and periphery (orange line) tadpoles reared at three developmental temperatures (17 °C, 22 °C, and 27 °C). Indicated as black points on the curve is thermal optimum (T_{opt}) at maximum performance (P_{max}). Critical thermal minimum (CT_{min}); and critical thermal maximum (CT_{max}) are indicated as grey lines. a.) TPC of maximum velocity [$\log_{10}(A_{\text{max}})$] of tadpoles reared at 27 °C, b.) TPC of maximum velocity [$\log_{10}(A_{\text{max}})$] of tadpoles reared at 22 °C, and c.) TPC of maximum velocity [$\log_{10}(A_{\text{max}})$] of tadpoles reared at 17 °C. Grey envelope represent 95% confidence interval. Overarching black dashed (periphery) and solid (core) lines between plots and asterisks indicate significant differences.

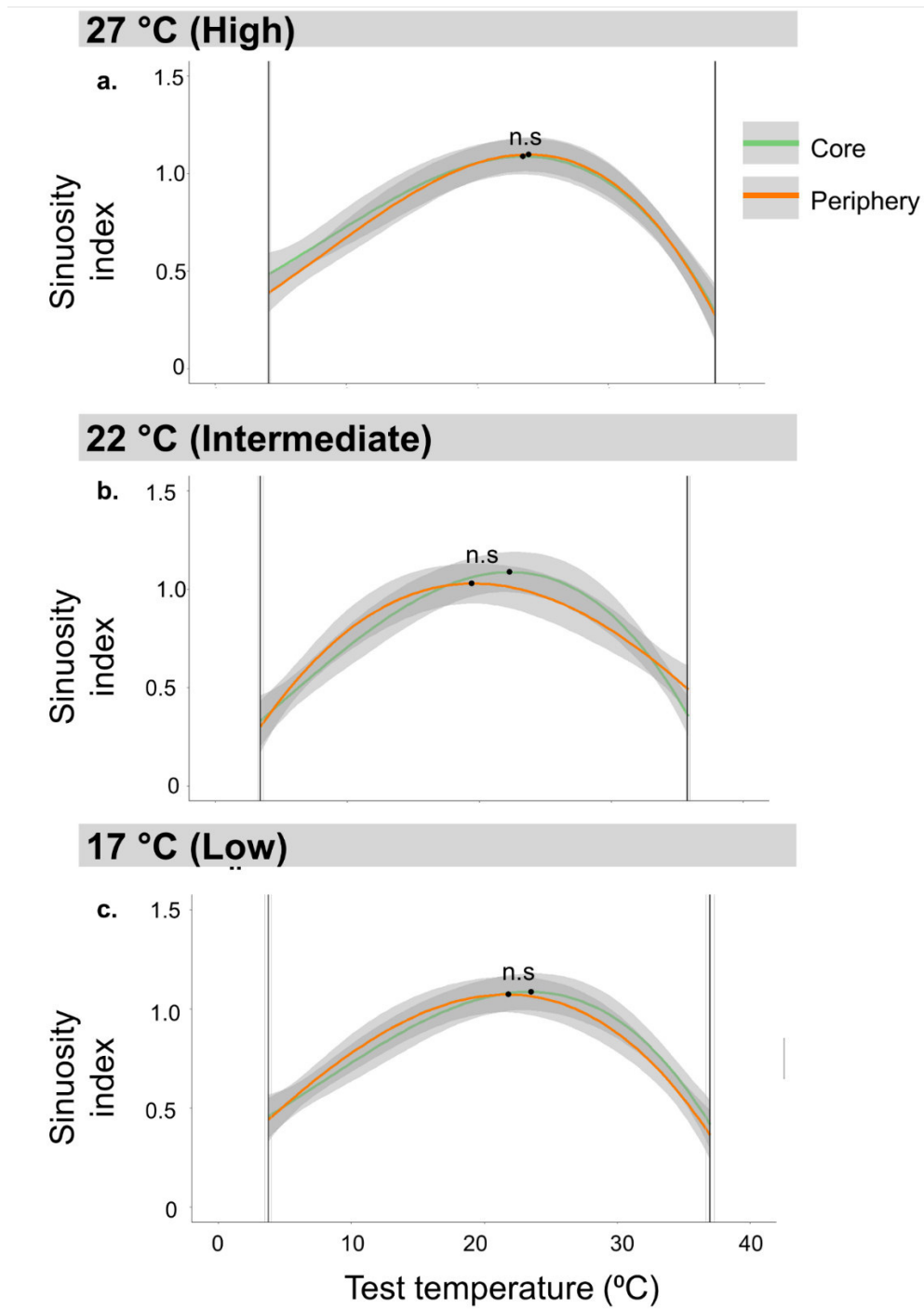


Figure 4.4: Thermal performance curves (TPC) of sinuosity of core (*green line*) and periphery (*orange line*) tadpoles reared at three developmental temperatures (17 °C, 22 °C, and 27 °C). Indicated as black points on the curve is thermal optimum (T_{opt}) at maximum performance (P_{max}). Critical thermal minimum (CT_{min}); and critical thermal maximum (CT_{max}) are indicated as grey lines. a.) TPC of sinuosity of tadpoles reared at 27 °C, b.) TPC of sinuosity of tadpoles reared at 22 °C, and c.) TPC of sinuosity of tadpoles reared at 17 °C. *Grey envelope* represent 95% confidence interval.

4.5. Discussion.

Our hypothesis that phenotypic plasticity is higher at the periphery and a lower at the core is not supported by our results. No differences could be detected between the performance curves (including CT_{\min} and CT_{\max}) of core and periphery tadpoles reared in different developmental temperatures. Development temperature interacted with position for all performance measurements. The thermal performance curves of maximum velocity of tadpoles reared at the low, intermediate and high temperature did not significantly differ between the core and periphery. However, periphery individuals were 19% faster than core tadpoles when reared at the intermediate temperature, although not significantly different. To confirm this trend future studies should increase the sample sites as the small sample size in this study possibly resulted in this trend as not being significant. Previously, differences in locomotor performance of invasive *X. laevis* adults from the core and periphery have been reported (Louppe et al., 2017; Courant et al., 2019a). Due to spatial sorting adults from the periphery have longer limbs and increased dispersal ability at the periphery (Louppe et al., 2017; Courant et al., 2019a; Padilla et al., 2019). The changes in adult phenotype can affect the phenotype of the offspring, through differentially invested resources and maternal effects (epigenetic inheritance) (Kaplan & Phillips, 2006).

In the invasive range of *X. laevis* in western France, a higher density of nektonic macroinvertebrates was found at the periphery of the range than the core (Courant et al., 2018). Nektonic macroinvertebrates such as *Dytiscus* sp. are common predators of *X. laevis* tadpoles (Kruger et al., 2019). The adults of *X. laevis* are also major predators of *Xenopus* larvae (Vogt et al., 2017, Thorp et al., 2018). At the core of the distribution, where macroinvertebrate density is low, the adults are expected to be the main predators. Thus, predator type and densities differ between the core and the periphery, which may explain the observed trend. Thermal acclimation can also affect the interactions between predators and prey. For instance,

Grigaltchik et al. (2012) found that predatory bass were more resilient than their mosquitofish prey at colder temperatures. Discrepancies between predator and prey acclimation may be due to physiological and evolutionary differences (Bremer & Moyes, 2011). Temperature can modify the trophic interactions by affecting predator-prey dynamics (Winder & Schindler, 2004). Thus, predator acclimation needs to be considered when assessing the efficacy of the anti-predator response of *X. laevis* tadpoles at different temperatures. Future studies can assess whether invasive *X. laevis* tadpoles at the periphery, benefit from increased performance at intermediate temperatures, particularly if their predators are unable to acclimate performance to a similar extent. Indicating that the discrepancy between core and periphery can be due to the differential responses of both the tadpoles and the predators.

The developmental temperature affected the maximum velocity and acceleration of core tadpoles and periphery tadpoles. At the core and the periphery tadpoles reared at the intermediate temperature were the slower than compared to tadpoles reared at the high and low temperature. The low performance of tadpoles at the intermediate temperature can possibly be the cause of the strong interaction effect observed in the best-fit model. Currently, we cannot elucidate why performance would be lower at the intermediate (preferred) temperature, but these results indicate that tadpoles are faster when reared at the extreme temperatures. Differences could have emerged due to fluctuating temperatures in the developmental chambers, however, the variation from the mean was relevantly low (Fig. S4.1).

No difference of sinuosity was observed between core and periphery tadpoles reared at different developmental temperatures. Our results show that the linear displacement of all tadpoles decreased before the critical thermal limits are reached: This indicates that both core and periphery tadpoles alike will become more disorientated at the thermal limits. We observed that especially at the lower test temperatures tadpoles tend to swim in circles and even upside down.

All tadpoles reared in the high temperature adjusted their thermal optimum to warmer temperatures. Overall, the burst swimming performance of both core and periphery tadpoles reared at the high temperature had an average T_{opt} of 14% and 19% greater than tadpoles that were reared at the intermediate temperature. The shift of T_{opt} can improve the fitness of both core and periphery tadpoles at warmer temperatures, when reared at the high temperature. This finding corresponds to the temperatures reported in the native range, where temperatures are higher than western France. However, this shift of the thermal optimum to warmer temperatures was not accompanied by a shift in CT_{max} and CT_{min} . This suggests that the limits remained unchanged and are possibly conserved. In western France, breeding of *X. laevis* was observed between April and June late spring at the onset of summer (Courant et al., 2017c). The specific stage of tadpoles that we tested emerged within a week after fertilisation (Nieuwkoop & Faber, 1994). Thus, the phenotypically plastic response of the thermal optimum to warmer temperatures of *X. laevis* tadpoles at this early stage, can influence their ability to persist in warmer temperatures.

When tadpoles were reared at the low temperature, the shift of maximum performance was to warmer temperatures rather than to cooler temperatures. This indicates that invasive *X. laevis* tadpoles have limited ability to acclimate to cooler temperatures. Similarly, Seebacher & Franklin (2011) found that the adults of invasive cane toads, *Rhinella marina*, acclimated to warmer temperatures, but had a limited capacity to acclimate to colder temperatures. Additionally, when tadpoles were reared at the intermediate temperature, the shift was in the opposite direction (to cooler temperatures), than would be expected (intermediate response). Currently, we cannot explain this shift of T_{opt} of tadpoles reared at a low temperature in the same direction as tadpoles reared in high temperature, however, this can be due to the fluctuation of temperature in the developmental tanks, as the temperature variation from the mean for the low temperature group was the highest (1.05%).

Finally, climate change may facilitate the north- and eastward expansion of *X. laevis* in areas previously deemed unsuitable as temperature will keep on rising on the European continent (Ihlow et al., 2016). The ability to acclimate to warmer temperatures can facilitate the spread of *X. laevis* into Mediterranean and warmer climates. Species distribution models revealed that large areas can potentially be colonized on several continents (Measey et al., 2012; Ihlow et al., 2016). In this study when reared at a low and a high temperature the shift in the thermal optimum of tadpoles is in the direction of warmer temperatures. Thus, the plastic response of *X. laevis* tadpoles might limit the expansion of *X. laevis* towards continental climates with colder temperatures. This is supported by Rödger et al. (2017) where the modelled potential distribution of *X. laevis* derived from current niche data did not predict colonisation of *X. laevis* distributing into the continental climate regions.

In conclusion, currently no differentiation in plasticity of thermal acclimation for three performance parameters between the core and the periphery has taken place within the invasive population of *X. laevis* in western France. The invasion, however, is relatively recent (~ 40 years), and it is unknown whether this phenomenon may still occur for this population. Ducatez et al. (2016) found that, within ~ 80 years of colonisation in Australia, invasive *R. marina* tadpoles exhibit differential phenotypes between the core and the periphery in response to different larval densities. However, they found that periphery tadpole phenotypes were less plastic than core tadpoles, contrary to our hypothesis. This can be due to inbreeding at the periphery due to spatial sorting and drift in sites recently colonised at the periphery (Ducatez et al., 2016). This suggests that the erosion of plasticity within the invaded range will depend on the specific dynamics of the invasion. Additionally, we found that invasive *X. laevis* tadpoles display phenotypic plasticity both at the core and the periphery. Finally, the ability of invasive tadpoles to acclimate to warmer temperatures may facilitate its spread into warmer climates.

Chapter 5: Anti-predator strategies of the invasive African clawed frog, *Xenopus laevis*, to native and invasive predators in western France.

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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, AH and JS were involved with the interpretation of data. NK drafted the manuscript and JM, AH, and JS revised the manuscript and approved the final version.

Citation:

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5.1. Abstract.

When species are translocated to a novel environment, individuals become exposed to new predators against which they may not express very efficient defences at least at an initial stage. The strength of anti-predator defence is an important parameter that may determine the ability of local communities to control the expansion of invasive populations. The African clawed frog, *Xenopus laevis*, is a globally invasive amphibian that has successfully established invasive populations on four continents. In its invasive distribution in western France, *X. laevis* encounters novel aquatic predators. Some may be related to the predators in the native range but others may belong to different taxonomic groups and not be functionally or ecologically equivalent. We tested whether naïve *X. laevis* tadpoles from the invasive French population exhibit anti-predator response to local predators, and whether the response depends on the degree of relatedness with predators encountered in the native range of the frog, or whether individuals may express generic neophobia to any cue they are not familiar with. We exposed naïve lab-reared tadpoles to a native non-predatory water snail, *Planorbis corneus*, a native predatory beetle, *Dytiscus dimidiatus*, and an invasive predatory crayfish, *Procambarus clarkii*. We found that *X. laevis* tadpoles innately reduce their activity when exposed to beetle and crayfish stimulus cues, but not to snails. Reducing activity can decrease the probability of being detected by predators. This demonstrates that invasive tadpoles respond to known and novel predators regardless of the evolutionary history. Whether the produced response is always effective against a totally novel predator remains to be tested.

5.2. Introduction.

Biological invasions often fail through lack of preadaptation to the new climate, enemies, and competitors encountered in the novel environment (Lodge, 1993; Moyle, 1986; Newsome & Noble, 1986). The presence of unknown predators can prompt the development or adaptation of traits in a similar manner as density, food availability, and temperature (Relyea & Hoverman, 2003; Gomez-Mestre et al., 2010; McCoy et al., 2011). For instance, the presence of predators induces deep tail fins and short bodies in larval gray treefrogs, *Hyla versicolor* (Van Buskirk & McCollum, 2000). These morphological responses to larval predators increase the probability of a tadpole surviving predation (Relyea & Hoverman 2003). Predators can also prompt behavioural adaptations such as vigilance or a decrease in activity in prey species (Nunes et al., 2013, 2014; Ferrari et al., 2014). For example, leopard frogs, *Rana pipiens*, reduce their activity in the presence of mudminnows, *Umbra limi*, and dragonfly larvae, *Anax* spp. (Relyea, 2001a). Such behavioural traits have recently been shown to be strongly affected, with performance consistently lower in the presence of alien species (Nunes et al., 2019). In aquatic environments, individuals acquire an abundance of information about the presence of predators via chemical cues (Wisenden & Chivers, 2006). Early detection of predator cues plays a key role in anti-predator behaviour. If a prey animal detects the predator first, it can evade an attack or simply avoid areas of higher predation risk. Numerous types of chemical cues give prey advance notice of the presence of the predator. In particular, the odours from injured conspecifics can accompany predator odours and act as an alarm cue for naïve prey (Ferrari et al., 2010).

Organisms with complex life cycles, such as amphibians, can show stage dependent responses to predator exposure. Earlier tadpole stages are smaller and more vulnerable to different types of predators than older, larger stages (Relyea, 2003). Phenotype, including body

size, can directly determine an individual's response to predators (Langerhans, 2009; Hettyey et al., 2010; Nunes et al., 2013). To evade possible fatal encounters with unknown predators, tadpoles may react in a neophobic manner. This is a simple, but costly, mechanism to control ecological plasticity in a novel environment due to avoidance of novel stimuli. Neophobic responses towards unfamiliar predators may offer naïve prey an adaptive mechanism to avoid the initial encounters by reacting to all new chemical stimuli associated with conspecific injury and alarm cues (Brown et al., 2013). However, prey species can also exhibit anti-predator responses toward a new predator type upon their first encounter if the predator species is related to predators present in the native range. For instance, both predators in the native and invasive ranges may release similar chemical cues that trigger the same behaviour in the prey. In amphibians, spontaneous predator avoidance may also result from learning of predator cues from the egg stage (Brown et al., 2013) allowing individuals to be responsive to potential predators at hatching.

The African clawed frog, *Xenopus laevis* (Daudin, 1802) originates from southern Africa. It is a globally invasive amphibian that has successfully established invasive populations on four continents (Measey et al., 2012). *Xenopus laevis* was introduced into the Deux-Sèvres department in western France from a breeding facility near Bouilllé-Saint-Paul at the beginning of the 1980s (Fouquet, 2001; Fouquet & Measey, 2006). Currently, *X. laevis* is present in five French departments (JS, unpublished data; Louppe et al., 2017). Some local predators in the novel environment may be functionally and phylogenetically similar to predators found in the native range. Alternatively, novel predators may be neither phylogenetically or functionally similar, nor otherwise ecologically equivalent, to predators they evolved with. We tested whether naïve *X. laevis* tadpoles from the invasive French population exhibit anti-predator responses to local predators, and whether the response depends on the degree of relatedness with predators encountered in the native range of the frog.

Alternatively, naïve tadpoles may express neophobia to any cue they are not familiar with. We exposed naïve lab-reared tadpoles to a non-predatory water snail, *Planorbarius corneus*, a native diving beetle, *Dytiscus dimidiatus*, and an invasive crayfish, *Procambarus clarkii*. In western France *X. laevis* and the native diving beetle *D. dimidiatus* have co-occurred since the introduction of *X. laevis* into France. Species of the genus *Dytiscus* are also present across southern Africa while no related species of crayfish occur in the native range of *X. laevis*. We predict innate anti-predator responses of *X. laevis* tadpoles to phylogenetically “familiar” predators as a result of coevolutionary history. A decrease in activity is expected as it is one of the most common and effective behavioural anti-predator responses that reduces detection and thus vulnerability (Nunes et al., 2019). We predict no response to the invasive crayfish due to the short interaction period between the two invasive species (~ 30 years). The red swamp crayfish, *P. clarkii*, is native to north-eastern Mexico and south-central USA (Hobbs et al., 1989). This species was introduced into Europe (southern Spain) in 1973 and is currently a widespread and abundant invasive species all over Europe (Habsburgo-Lorena, 1979). The first population in France was recorded in 1988 in the Charente-Maritime department. Since then the distribution has expanded into many different departments in metropolitan France, including four of the departments (between 1995 and 2001) where *X. laevis* is present (Changeux, 2003; Collas et al., 2007). The crayfish was not present in the Maine-et-Loire department in 2007, but was recorded in this department in 2014 due to their rapid distribution throughout the Loire basin (Collas et al., 2007, 2015). The species is not common in ponds across the study area. Yet, *X. laevis* and *P. clarkii* can be found in the same aquatic habitats. The frequency of sympatric ponds is difficult to assess, but it seems to be higher close to some river courses. The snail was used as a non-predator control. Water snails are present in southern Africa and France and co-occur with *X. laevis*.

5.3. Materials and methods.

5.3.1. Collection, care, and feeding of individuals.

Baited funnel traps were distributed in four different sites to collect *X. laevis*: site 1 (47°20'38"N, 0°45'46"W); site 2 (46°54'40"N, 0°21'11"W); site 3 (47°01'27"N, 0°19'10"W); and site 4 (47°00'38"N, 0°21'29"W) within the invaded area in western France (De Busschere et al., 2016). This experiment was conducted between 06/2017 and 07/2017. One breeding pair per site was collected. Breeding was induced using human chorionic gonadotropin hormone (hCG: Ovidrel®/Ovitrelle® 250 micrograms/0.5 mL). The males were injected with 250 IU of hCG for the first, second and third day of breeding and the females with 50 IU on the second day and 500 IU on the third day of breeding. The male and female of a breeding pair were kept separate and were only joined on the third day. After egg laying, adults were removed the following morning from the aquaria, and the eggs were counted and assessed for viability. Tadpoles were selected for the trials at pre-metamorphic stages 42 – 53 according to Nieuwkoop and Faber (1994). A naïve tadpole at an early and vulnerable stage was selected as when tadpole size increases, vulnerability to predation decreases, reducing the need for anti-predator responses (e.g. Nunes et al., 2013). Due to the selection of tadpole stage, we did not expect body size to display an effect. Individuals from each clutch were raised in tanks with a density of one tadpole per litre at 20 °C. Tadpoles were fed SERA Plankton tabs® (Sera GmbH, Heinsberg, Germany).

Experimental stimuli were collected using dip nets in outside ponds. Three species were collected: adults of a native water beetle predator, *D. dimidiatus* (n = 5; 1.66 ± 0.15 cm; 0.29 ± 0.12 g); juvenile invasive crayfish, *P. clarkii* (n = 6; 6.52 ± 1.20 cm; 5.61 ± 2.45 g); and a nonpredatory snail, *P. corneus*, (n = 5; 2.01 ± 0.22 cm; 2.59 ± 1.90 g). These species are common although the crayfish is rarely caught with *X. laevis* during trapping. Captures were

conducted outside the invasive range of *X. laevis* to prevent the potential release of conspecific alarm cues during tests. Dietary or conspecific cues were intentionally not added here to test whether tadpoles display an innate response. Snails, beetles, and crayfish were kept separately in 10 L tanks and fed every second day with pond invertebrates for beetles and crayfish, and lettuce leaves for the snails. Water in tanks for stimuli and tadpoles were changed every second day before the experiment, and for tadpoles every day during the experiment. Individuals of the same kind were placed together after measuring each individual. For the duration of the experiment, water containing stimulus cues was sampled from the stimulus tank water.

5.3.2. Behavioural test.

An adaptation of the protocol to measure activity of tadpoles in response to predators designed by Ferrari et al. (2010) was used. The experimental protocol consisted of two types of tests with two phases. For the type-1 test, tadpoles were exposed to water only during the first phase, as a control, and to the olfactory cues of one stimulus type during a second exposure phase. For the type-2 test, tadpoles were presented with olfactory cues of the same stimulus type on the first and second exposure phases. Type-1 tests were conducted as control to assess whether exposure phase rather than cue has an effect on response. Type-2 tests were conducted to assess the initial and second response of *X. laevis* tadpoles to stimulus cues.

Tadpoles from the same clutch were raised together at a density of ~ 10 tadpoles per litre. When tadpoles reached the desired stage for the experiment, 60 tadpoles from each clutch ($n = 4$) were removed and divided randomly into 3 groups of 20 for each stimulus type. Tadpoles were further divided into type-1 test ($n = 40$ per snail, beetle, and crayfish) and type-2 test ($n = 40$ per snail, beetle, and crayfish). All tadpoles from every group for each stimulus type were placed individually in 1 L plastic cups (5 cm radius) filled with aged tap water, where

they were left to acclimate for 24 h. The test was structured as a five minute pre-stimulus period, the one minute injection period when the cue was added, and a five minute post-stimulus period (Ferrari et al., 2010). In the first exposure phase, we injected 5 ml of water only as a control for all tadpoles for the type-1 test and 5 ml of water containing the olfactory stimulus cue for the type-2 test. Tadpoles exposed to the stimulus cue from type-2 tests are referred to as “experienced” and those exposed to water only from type-1 tests as “naïve”. After the first phase trials for each type of test, tadpoles were moved to new plastic cups with new water that contained no olfactory stimulus cues. Tadpoles were fed and left 24 h to acclimate between phases. For the second phase, we followed the same protocol as for type 1 test (naïve) and type 2 test (experienced) tadpoles (5 min pre-stimulus, 1 min injection, 5 min post-stimulus period). Cups containing experienced tadpoles were re-exposed to the same type of olfactory cues again, and the set of “naïve” tadpoles were exposed to olfactory cues for the first time.

Two lines were drawn under the cup to form four quadrants. The number of lines crossed by moving tadpoles was recorded for each period. We considered that a tadpole crossed a line when its entire body (tip of nose to the tip of the tail) was on the other side of the line (Ferrari et al., 2010). We recorded simultaneously sets of 10 tadpoles using a video camera (recorded at 30 fps) placed above the cups. Tadpoles were euthanized by immersion in MS-222 (ethyl 3-aminobenzoate methanesulfonate) after the completion of the tests. Linear measurements of snout-vent-length, tail length, full length (head to tail tip), head width, head depth, and tail depth were measured using ImageJ analysis software (v 1.52a, NIH, Washington, D.C., USA).

5.3.3. Statistical analysis.

To obtain a synthetic measurement of body size, we conducted a principle component analysis on the six measurements of tadpole morphology. The first principal component accounted for 79.4% of the variance between individuals, and all variables had negative loadings. Thus, the first axis is a global measurement of body size. We compared tadpole activity with snail, crayfish and beetle after the introduction of the olfactory cues and considered the pre-stimulus injection period as habituation. Some individuals did not cross any line during both testing sessions ($n = 19/120$ naïve tadpoles; $n = 18/120$ experienced tadpoles). We discarded them from further analyses and only kept tadpoles that moved at least once during one period. Generalized mixed models were used with post-stimulus line crosses as a response variable with stimulus type and body size (PC1) as fixed effects. Site, *i.e.* tadpole pond, was considered as a random effect. We compared different models including zero inflated, and hurdle models with different distribution using the package *glmmTMB*. Models with a generalized poisson distribution and a log link had the lowest AIC values and were selected thereafter. All analyses were carried using the statistical software R 3.4.1 (R Core Team, 2018).

5.4. Results.

No effect of stimulus type, body size, and their interaction was observed for naïve tadpole activity. The minimum model with only the intercept and the random effect presented the lowest AIC for the response after the first (water only: $\Delta\text{AICc} = 1.4$) and second tests (first exposure to an olfactory stimulus: $\Delta\text{AICc} = 2.0$) phase and was thus the best model. For experienced tadpoles, the lowest AIC value was observed for the minimum model ($\Delta\text{AICc} = 1.4$) for the first exposure phase. In contrast, for the second exposure phase, *i.e.* “experienced”

tadpoles, the best model included stimulus type only (Fig. 5.1). The second best model ($\Delta AICc = 1.7$) additionally included body size. Following the principle of parsimony, we retained the first model, that also differed from the null model that displayed no effect of body size ($\Delta AICc = 3.4$). The number of line crossings was the highest in tadpoles exposed to snail olfactory cues (Figure 1). Activity was significantly reduced for individuals exposed to olfactory cues of beetle ($n = 32$, $z = -2.514$, $p = 0.012$) and crayfish ($n = 37$, $z = -2.072$, $p = 0.038$). No significant difference of activity between tadpoles exposed to beetle and crayfish were observed ($n = 69$, $z = 0.588$, $p = 0.556$).

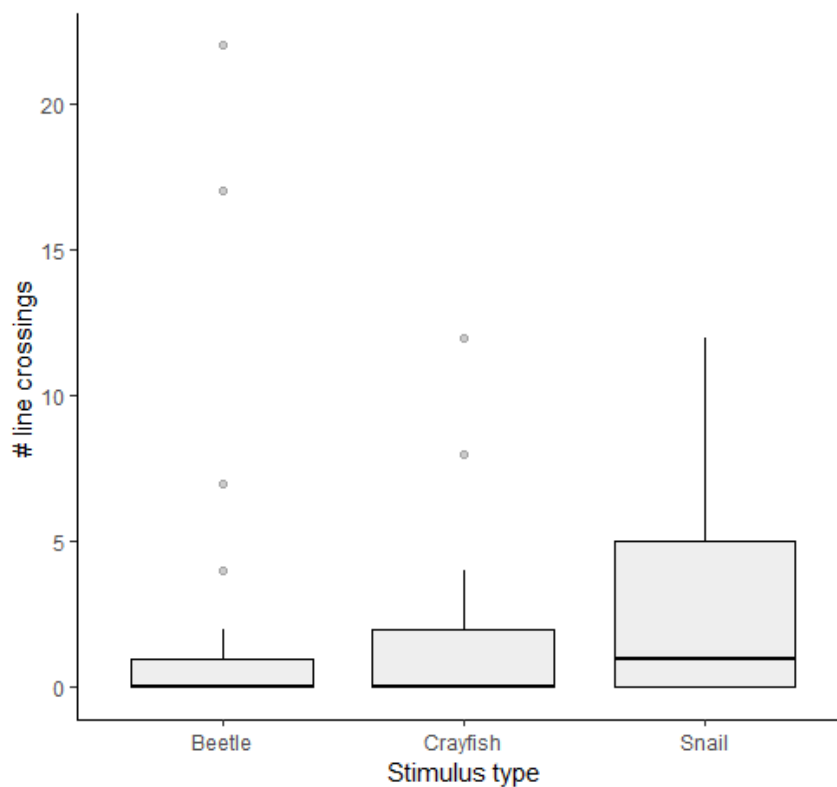


Figure 5.1. Boxplot of activity expressed as the number of line crossings by naïve *Xenopus laevis* tadpoles exposed to the olfactory cues of a dytiscid beetle, *Dytiscus dimidiatus*, a crayfish, *Procambarus clarkii*, or a water snail, *Planorbarius corneus*.

5.5. Discussion.

The variation in tadpole activity following exposure to different stimulus types was only observed on the second test in individuals already exposed to the same stimulus. A single exposure to predator cues without association with conspecific cues was insufficient to detect an anti-predator response. Previous studies have used predator cue accompanied by conspecific cues when testing the anti-predator response of organisms (e.g. Relyea, 2001a; Relyea & Hoverman, 2003; Ferrari et al., 2014; Lucon-Xiccato et al., 2018). Their association induced the learning of predation risk in tadpoles (Ferrari et al., 2010). In our protocol, the absence of conspecific cues may have prevented conditioning to occur. The lack of initial response to predator cue alone has been previously reported for tadpoles (e.g. Marquis et al., 2004). Most likely, habituation to the experimental conditions was not complete before the first exposure, and behavioural differences could only be detected on the second test. Nevertheless, we observed behavioural differences in the second exposure phase of experienced tadpoles. Olfactory cues of the two predators were enough to reduce the activity of “experienced” individuals. Such a decrease in locomotor activity is consistent with the literature on tadpoles of other species exposed to aquatic predators regardless of invasion status of either predator or prey (e.g. Nunes et al. 2019). Reduction in activity is a widespread and important form of anti-predator response in the presence of predators (Lima & Dill, 1990). Moving and being active increases probability of being detected by predators. Beetles and crayfish were captured outside the colonised area which discounts the possibility that tadpoles perceived dietary cues of conspecifics consumed earlier by the predator. Thus, our results support the presence of innate anti-predator behaviours to dysticid beetles and crayfish in *X. laevis* tadpoles.

Dytiscus dimidiatus belongs to a predator lineage with which *X. laevis* shares a long evolutionary history. This species is not present in southern Africa but other diving beetle species from the family Dytiscidae, which are functionally similar to *Dytiscus* species, can be

found (Alarie et al., 2017). The response to *D. dimidiatus* may indicate pre-adaptation to dytiscid predation in any part of the world where frogs and beetles may come into contact. Alternatively, *X. laevis* may have developed a response to this species, absent from southern Africa, since its introduction into France. This may not be the most plausible explanation.

The innate response of tadpoles suggests that they were not naïve to the crayfish. This result was unexpected because of the lack of coevolutionary history; the first contact between these two lineages in the African clawed frog's native range dates back less than 30 years, and sympatry between *P. clarkii* and *X. laevis* still appears uncommon today. Yet, the crayfish seems to be recognized as a threat by tadpoles which suggest three hypotheses. First, *X. laevis* specifically evolved an evolutionary anti-predator response to *P. clarkii*, due to the recent invasion of the crayfish into the invasive distribution of *X. laevis*. Regardless of coexistence time, the presence of *P. clarkii* can induce an immediate response in native Perez's frog, *Pelophylax perezi*, tadpoles in Portugal, where both naïve tadpoles and those from long-term invaded populations displayed reduced activity at initial exposure (Nunes et al., 2014). Second, the odours that *P. clarkii* releases are similar to the cues released by freshwater crabs of the genus *Potamonantes*, which are predators of *X. laevis* in southern Africa (Gutsche & Elepfandt, 2007). This cue type may be shared by a broader taxonomic range of species than initially expected. Third, *X. laevis* tadpoles exhibit an innate generic response to any predator cue. For instance, crayfish and beetles may have consumed other amphibian larvae in their pond of origin which provided alarm cues for *X. laevis* tadpoles. However, previous studies investigating the effect of the presence of unfed invasive *P. clarkii* on the behaviour of a native naïve amphibian species showed an initial decrease in activity, a possible neophobic response (Nunes et al., 2013). The release of cross-species alarm and dietary cues accompanied by predator cues is especially effective when alarm cues are from the same prey guilds, as they share similar predators (e.g. Adams & Claeson, 1998; Fraker et al., 2009). Careful effort was

made to ensure tadpoles were not exposed to cues from items predators had consumed before their capture. Therefore, tadpoles may have used more general cues indicating the presence of an organism with a carnivorous diet. The hypothesis of a generic response is only partly consistent with the neophobia hypothesis (Ferrari et al., 2010) as we did not expect to detect differences between stimulus types if *X. laevis* reduced its activity in response to any unknown cue. We cannot currently select one hypothesis but our results raise questions about the mechanisms by which invasive populations may express anti-predator behaviours to unknown predators, and reduce predation rate in novel environments. This property is probably desirable for a successful invader such as the African clawed frog.

In conclusion, invasive *X. laevis* tadpoles reduce their activity in the presence of diving beetles. We cannot conclude yet whether the response is due to a long-term evolutionary process in the native range, a rapid *in situ* response that evolved in the colonised range, or a generic anti-predator recognition system. Regardless of the mechanism, our results suggest that such an anti-predator behaviour may limit the ability of this predator type to control *X. laevis* populations at the larval stage. Predicting the consequences of expressing an analogous response to crayfish is not straightforward. The possible generic anti-predator responses displayed by tadpoles may not be adaptive against *P. clarkii* due to the different foraging behaviours observed between crayfish and diving beetles. For instance, a mud snail expressed the same anti-predator response against invasive crayfish as that displayed for fishes, which resulted in a higher predation rate on the snail (Sih et al., 2010). *Procambarus clarkii* is known to impact amphibian populations where it is introduced (e.g. Cruz et al., 2006; Souty-Grosset et al., 2016), and invertebrates (especially crayfish) were found to have the biggest effect on native tadpoles (Nunes et al., 2019). However, the presence of *P. clarkii* in Zhoushan Archipelago, China, was shown to mitigate the effect of invasive *Lithobates catesbeianus* on native amphibian populations, thus highlighting the importance of considering complex

interactions between co-invaders (Lui et al., 2018). Altogether, this study shows that *X. laevis* tadpoles tend to behave in a similar way to most other amphibian larvae towards sympatric predators. This may not confer strong control of invasive African clawed frog populations by local predators. The actual interactions between *X. laevis* and native predators or invasive crayfish deserve further attention to understand how the pond trophic network maybe reshaped by the accumulation of these different major invaders.

Chapter 6: General conclusion.

This thesis, consisting of four inter-dependent studies, intends to increase our understanding of the role tadpoles play in the invasion success of amphibians. The larval stages of the African clawed frog were used as a study system as the tadpole development, and morphology is well described (Nieuwkoop & Faber, 1994; Segerdell, 2008). Additionally, *X. laevis* is also a globally successful invasive amphibian (Measey et al., 2012). The findings of this thesis suggest that the tadpole stage can contribute to the invasion potential of *X. laevis* during the invasion process: (1) The tadpoles from the source populations of invasive *X. laevis* in France are phenotypically plastic. The high phenotypic plasticity of larval morphology and development can provide the necessary variation for the colonisation of new areas. (2) Invasive *X. laevis* tadpole phenotypic traits are decoupled from dispersal-relevant adult traits. Thus, enhancement of adult dispersal through differentially allocating resources to reproduction does not negatively affect the tadpole stage. This can be beneficial during range expansion. (3) The level of phenotypic plasticity of invasive *X. laevis* tadpoles from the core and the periphery of the range do not differ and can acclimate to warmer temperatures. This can facilitate their spread into France due to climate change (Ihlow et al. 2016). However, tadpoles displayed limited ability to acclimate to cooler temperatures. Thus, possibly limiting their spread into Continental climates. (4) The young naïve tadpoles of invasive *X. laevis* can identify and respond to known and unknown predators. This can increase the probability of young larvae to reach metamorphosis and can therefore facilitate the invasion success.

Tadpoles from the summer rainfall and winter rainfall populations displayed morphological and developmental plasticity in a reciprocal exchange experiment (Chapter 2). However, plasticity was not enough for translocated tadpoles to always reach the same performance levels as local tadpoles. In addition, the morphological and developmental plastic

response of tadpoles generated a survival cost translocated individuals survived less than local tadpoles. Over time, elevated maintenance cost of plastic phenotypes in a newly colonized range, may favour less plastic phenotypes adapted to local conditions (Price et al., 2002). This phenotypic plasticity of the *X. laevis* tadpole stage can contribute to the introduction and establishment success when translocated (Baker, 1974; Gray, 1986). Future studies can address whether the admixture of the two lineages will affect the plasticity of *X. laevis* tadpole phenotypes and the associated cost of survival.

In the invasive distribution of *X. laevis* in western France the morphology and timing to metamorphosis of tadpoles did not differ between populations from the core and the periphery of the range (Chapter 3) whereas morphological and physiological changes in adults allow higher dispersal ability in peripheral populations (Louppe et al., 2017; Courant et al., 2017a; Courant et al., 2019a; 2019b). Thus, this study suggests that tadpole and previously described dispersal-relevant traits of adults are decoupled to a large extent. The decoupling of tadpole morphological and developmental traits can assist independent adjustment of adult phenotypes in variable environments and conserve the tadpole phenotype if necessary. We found no evidence that trade-offs between reproduction and dispersal at the periphery (Courant et al. 2017a) affected the tadpole phenotype, however, currently it is not known whether the decoupling of tadpole traits will negatively affect the adult stage. This can play role in the spread phase of the invasion process by enhancing or limiting dispersal in the adult stage without affecting the morphology and development of the tadpole stage.

We plotted the measured snout-to-vent length (SVL) of tadpoles from the summer and the winter rainfall regions in the native distribution of *X. laevis* (Chapter 2) and of tadpoles from France (Chapter 3) (Fig. 6.1). The tadpoles from the summer rainfall region were found to be significantly larger at the later stages of the development before metamorphosis than the

tadpoles from the winter rainfall region (Chapter 2). The SVL of tadpoles from France does not differ from both native groups in the initial stage but displays an intermediate SVL between the larger tadpoles from the summer rainfall region and the smaller tadpoles from the winter rainfall region. The intermediate SVL may reveal the admixture between the two phylogeographic lineages detected by a previous genetic study (De Buscherre et al., 2016).

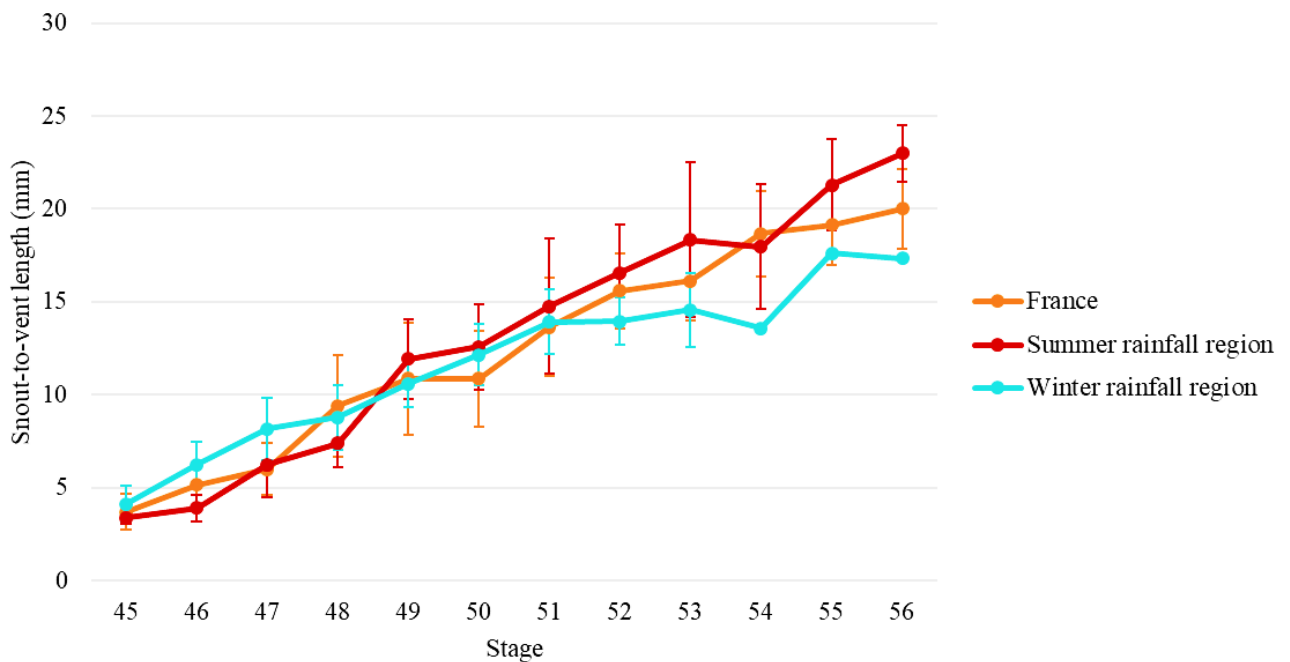


Figure 6.1. Snout-to-vent length variation of native *Xenopus laevis* tadpoles from the summer (solid red line) and the winter rainfall region (solid blue line) in southern Africa and invasive tadpoles from western France.

Higher phenotypic plasticity was expected at the periphery of the invasive population. However, no differentiation of performance in response to differing developmental temperatures could be identified between the *X. laevis* tadpoles at the core and at the periphery (Chapter 4). In other words, all tadpoles in the invaded range reacted to acute temperature change when reared in the same developmental temperature. The current lack of differentiation between the core and the periphery suggests that there was no independent evolution of

plasticity at the core and at the periphery. Colonisation of *X. laevis* into western France is quite recent (~ 40 years) and differentiation might still occur. The responses, however, differed between developmental temperatures. All tadpoles reared in warmer temperatures performed better in warmer temperatures and displayed limited ability to acclimate to cooler temperatures. This can limit their ability to expand into Continental climates as the temperature is more variable and characterised by colder weather in the winter. Current and projected distribution models do not show *X. laevis* expanding into this climatic region (Rödder et al., 2017). However, climate change and subsequent increasing temperature can possibly assist the spread of *X. laevis* into Europe.

We plotted a TPC of the pre-liminary results of maximum velocity of tadpoles from both rainfall regions in South Africa with the collected maximum velocity data from the core and the periphery of the invasive population in France (Chapter 4). Interestingly, the preliminary plot (Fig 6.2) indicates a shift of optimum thermal performance of tadpoles originating from France to colder temperatures. This shift in thermal performance is also seen in the adults of *X. laevis* of the invasive population in France (Araspin et al., 2020). The climate in France is colder than in South Africa and the climatic niche differs significantly (Rödder et al., 2017). This can indicate a rapid adaptation of thermal physiology for both adults and tadpoles in a short period (~ 40 years), subsequently enhancing their invasion success in France.

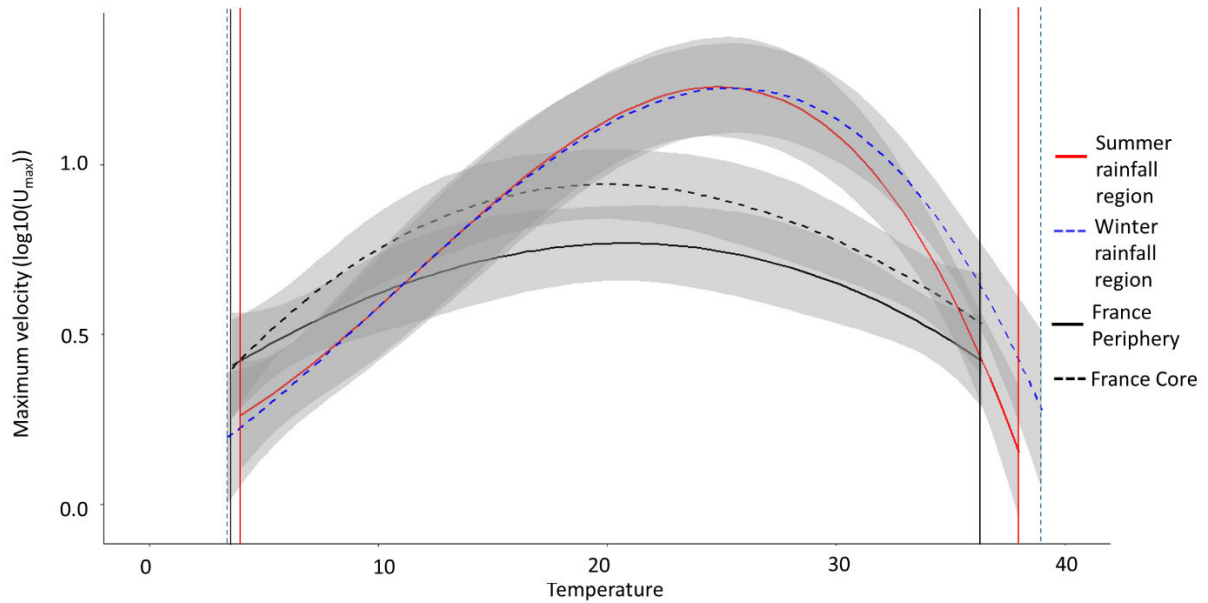


Figure 6.2: Thermal performance curves (TPC) of maximum velocity (U_{\max}) of core (*black dashed line*) and periphery (*black solid line*) tadpoles from France and the summer rainfall region (*red solid line*) and winter rainfall region (*blue dashed line*) tadpoles from South Africa. Tadpoles were reared at 22 °C. Indicated as black points on the curve is thermal optimum (T_{opt}) at maximum performance (P_{max}). Critical thermal minimum (CT_{min}); and critical thermal maximum (CT_{max}) are indicated as grey lines. *Grey envelope* represent 95% confidence interval. Black lines and asterisks indicate significant differences

When exposed to known and novel predator cues the tadpoles significantly decreased their activity compared to when tadpoles were exposed to the cue of a non-predator (Chapter 5). This illustrated the rapid development of an anti-predator strategy in the invasive distribution of *X. laevis* in western France. However, the efficacy of this strategy is unknown, thus future studies can aim to determine whether this anti-predator response of decreased activity is effective against the novel predator. This rapid development of the anti-predator response to novel predators can contribute to the invasion as predators are likely to be naïve to *X. laevis* and their tadpoles, especially in the initial stages.

Overall, the thesis used an array of methodological approaches to understand the invasion dynamics of the African clawed frog, *Xenopus laevis*. Additionally, the thesis

generated novel insights transferable to other taxa and the invasion dynamics of amphibians. An important, yet neglected, facet of amphibian invasion is the integration of the different stages of the complex life cycle. The findings of this thesis contribute to our understanding of the role larval stages play during the invasion process. Additionally, this project aids in the identification of the life-history traits that play a role in the invasion success of *X. laevis* and amphibians in general. The life-history traits of invasive species have been of interest due to their potential predictive power (Sakai et al., 2001). The life-history traits of larval stages can play a significant role in overcoming barriers that act as ‘filters’ during all phases of the invasion process. The findings of this study can provide information on the life-history traits that predispose a species to become invasive. Future studies may include adding more populations of invasive *X. laevis* potentially from another European country such as Italy for instance to determine the relevance of these life-history traits in other environments. Additionally, cross-species’ studies can shed more light on the important life-history traits across taxa. For instance, identifying whether the decoupling seen in *X. laevis* is a general feature in most invasive amphibians? This thesis illustrates that a predisposition is not necessarily responsible for invasion success and population spread. The adaptive ability of all stages of an invasive amphibian can enhance the invasion potential. This study highlights the rapid development of predator recognition, morphology and growth rate of larval stages to be important contributors to invasion success. Additionally, this study describes the impact of range expansion on the evolution of reaction norms within the invaded range that can enhance the spread into novel environments. Finally, life-history traits can be decoupled between adults and larval stages if genes coding for morphological traits of the larval stages and adults are stage-specific. The decoupling of traits can result in the independent enhancements of traits in each stage. Overall, this thesis describes mechanisms whereby larval can contribute to the invasion success of invasive amphibians.

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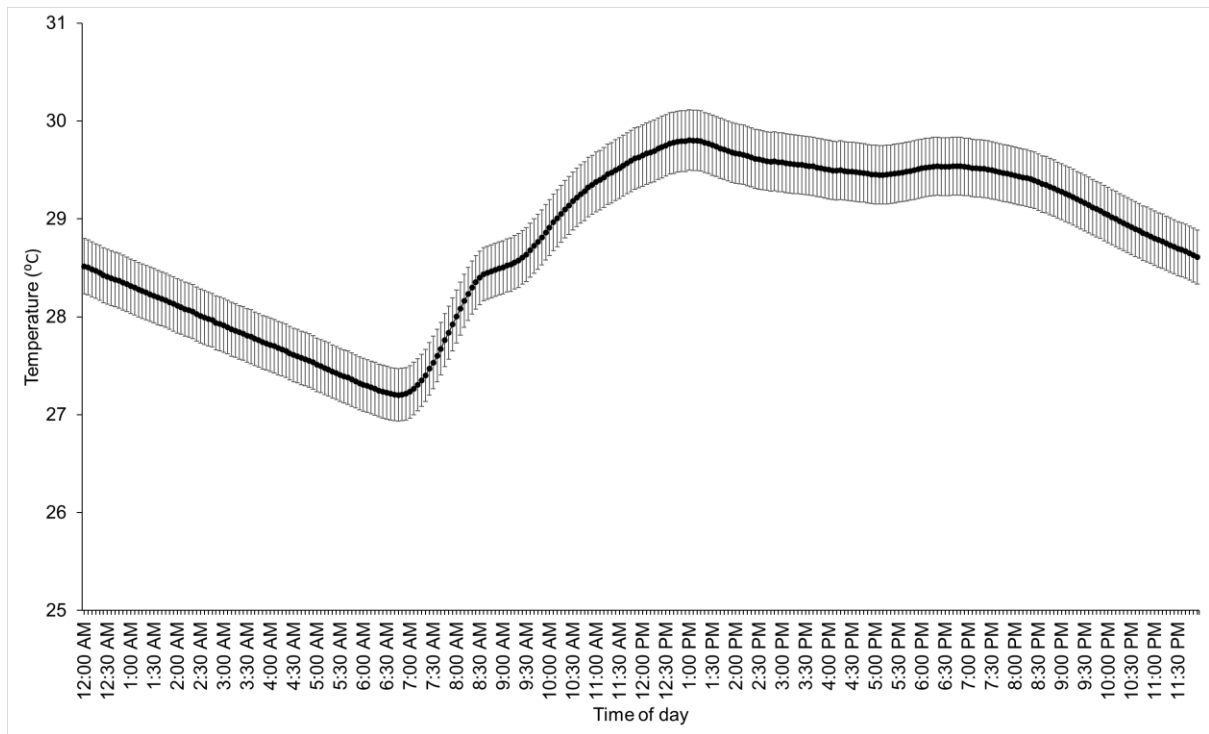
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Supplementary information.

Supplementary Table 2.1: Results of principal component analyses for morphology of *Xenopus laevis* larvae. The values represent body size (PC1). In all cases, PC1 described most of the variation.

	Proportion of variance	Cumulative variance	Standard deviation
Larvae			
PC1	0.939	0.939	2.166
PC2	0.024	0.963	0.349

PC1 loadings	Larvae
Head width	0.442
Snout-to-vent length	0.450
Maximum body depth	0.453
Tail depth	0.448
Tail length	0.443
Femur length	



Supplementary Figure 3.1: Temperature profile in the laboratory microcosms (*black dashed line*), and the outdoor mesocosms (*solid black line*) with standard deviations from the average (*dotted grey line*) for two days.

Supplementary Table 3.1: Full model-averaged parameter estimates and standard error for GLMM (Tadpole and climax individuals' snout-to-vent length).

Predictors	Tadpole Snout-to-vent length	
	Estimate	Standard error
Position	-0.190	0.260
Stage:		
Stage 46	0.454	0.174
Stage 47	0.682	0.164
Stage 48	1.341	0.134
Stage 49	1.523	0.167
Stage 50	1.585	0.151
Stage 51	1.919	0.185
Stage 52	2.208	0.168
Stage 53	2.335	0.150
Stage 54	2.836	0.114
Stage 55	2.925	0.119
Stage 56	3.109	0.129
Stage 57	3.468	0.107
Position x Stage:		
Periphery x Stage 46	0.279	0.378
Periphery x Stage 47	0.241	0.332
Periphery x Stage 48	0.201	0.276
Periphery x Stage 49	0.237	0.321
Periphery x Stage 50	0.206	0.291
Periphery x Stage 51	0.291	0.392
Periphery x Stage 52	0.249	0.336
Periphery x Stage 53	0.203	0.285
Periphery x Stage 54	0.097	0.170
Periphery x Stage 55	0.135	0.204
Periphery x Stage 56	0.161	0.233
Periphery x Stage 57	-0.012	0.109

Predictors	Climax snout-to-vent length	
	Estimate	Standard error
Position	-0.484	0.839
Stage:		
Stage 59	-0.044	0.719
Stage 60	-1.123	0.871
Stage 61	-1.330	0.897
Stage 62	-3.883	1.154
Stage 63	-4.256	1.019
Stage 64	-4.176	1.420
Stage 65	-5.981	1.185
Position x Stage		
Periphery x Stage 59	0.094	0.824
Periphery x Stage 60	0.470	0.992
Periphery x Stage 61	0.164	0.987
Periphery x Stage 62	1.148	1.431
Periphery x Stage 63	-0.238	1.263
Periphery x Stage 64	0.422	1.545
Periphery x Stage 65	0.855	1.477

Appendix 3.1.

Statistical analysis: For larvae, we used snout-to-vent length (SVL), head width, maximum body depth, tail length and tail depth. For climax individuals and metamorphs, we used SVL, head width and maximum body depth. The first principal component accounted for 94.2%, 92.9%, and 86.2% of variation in larvae, climax individuals and metamorphs, respectively. All loadings were positive for larvae and metamorphs and negative for climax animals. We retain only the first PCA axis in our analysis that represents a global measurement of body size (Table S3.2).

Supplementary Table 3.2: Results of principal component (PC) analyses of the morphology of *Xenopus laevis* larvae, climax individuals and metamorphs. The values represent body size (PC1). In all cases, PC1 describes most of the variation.

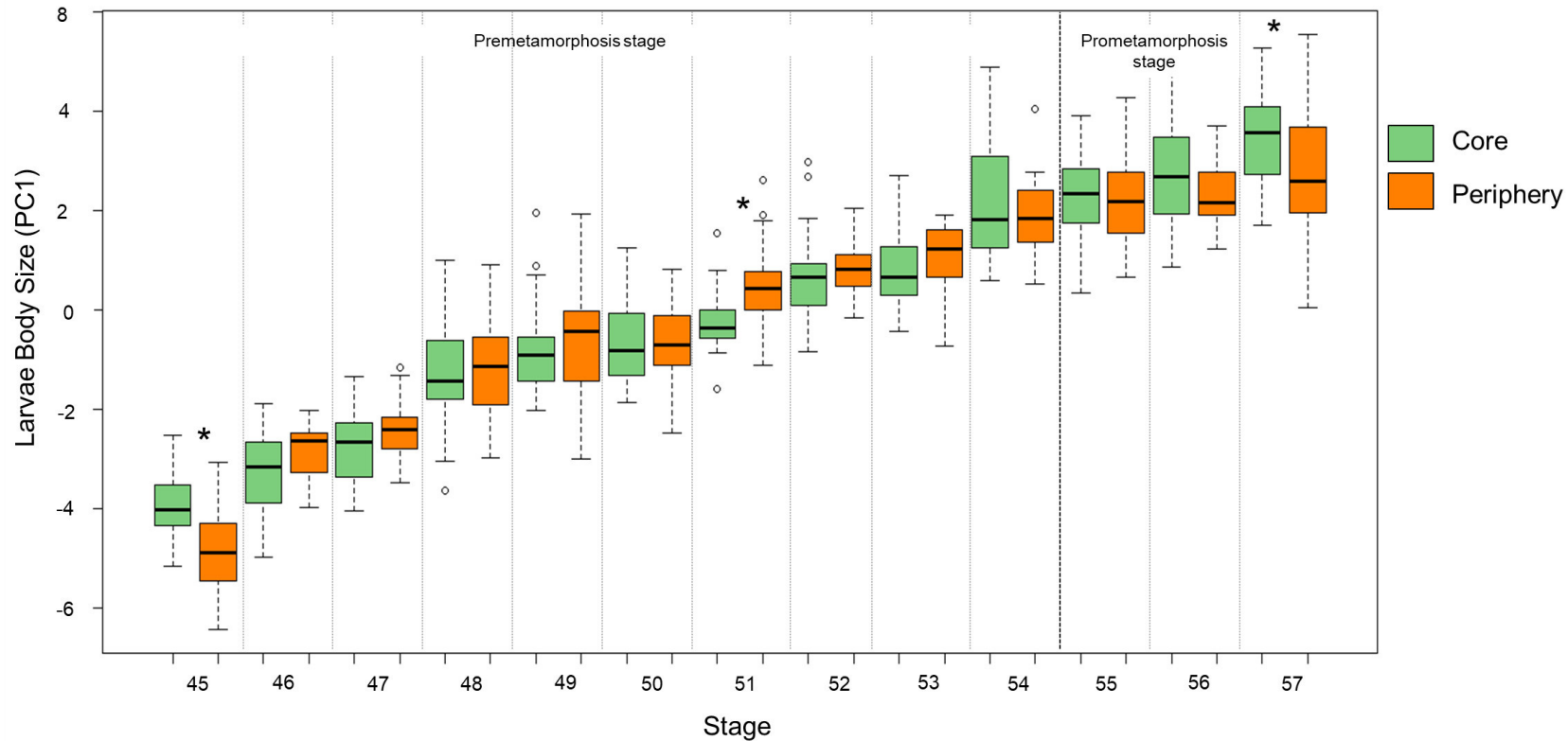
	Proportion of variance	Cumulative variance	Standard deviation
Larvae			
PC1	0.942	0.942	2.158
PC2	0.027	0.969	0.367
Climax			
PC1	0.929	0.929	2.950
PC2	0.050	0.979	0.683
Metamorphs			
PC1	0.862	0.862	2.683
PC2	0.084	0.946	0.839
PC1 loadings			
	Larvae	Climax	Metamorph
Head width	0.438	-0.261	0.253

SVL	0.454	-0.921	0.924
Maximum body depth	0.452	-0.289	0.287
Tail depth	0.444		
Tail length	0.447		

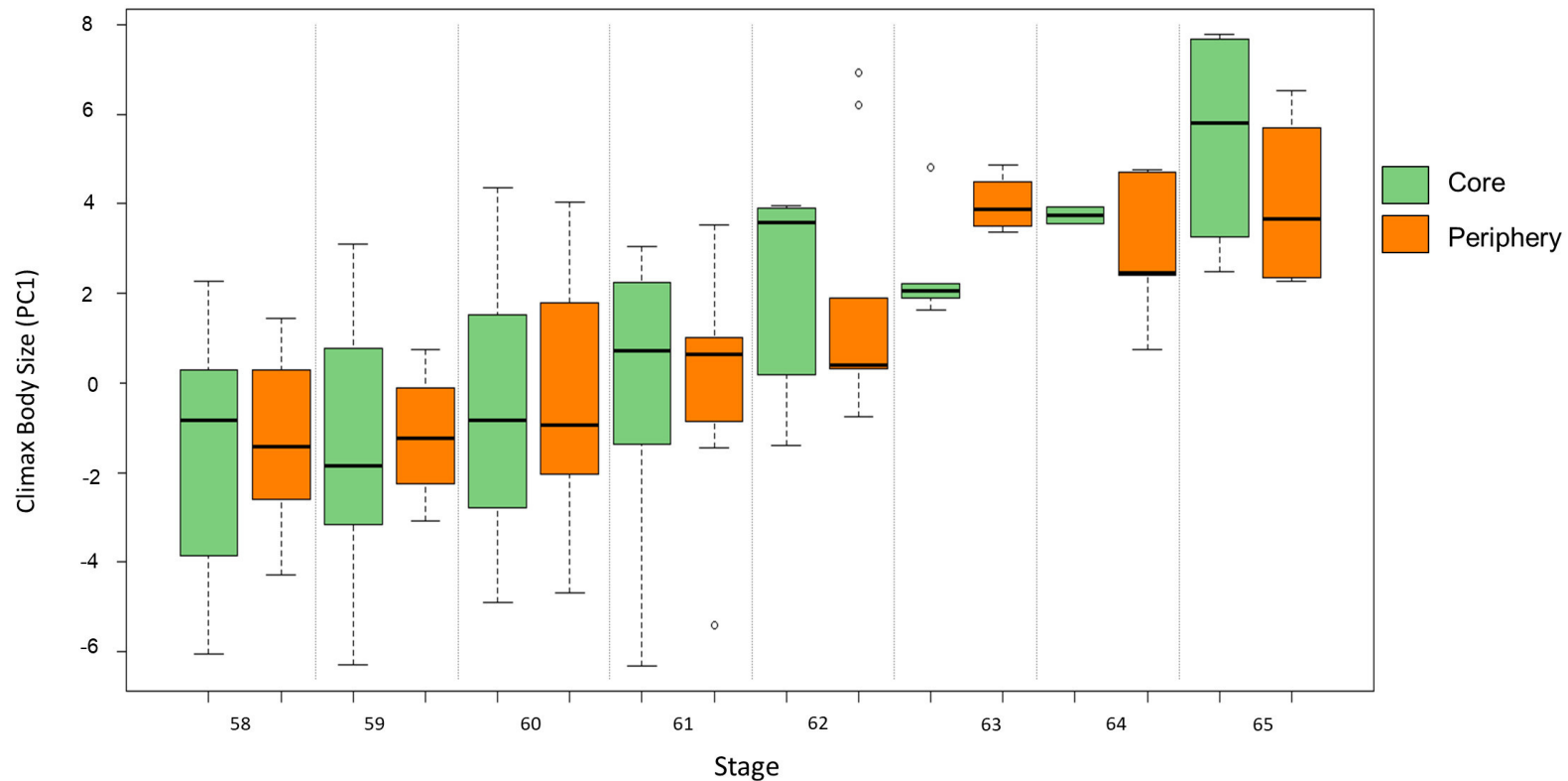
Results: Significant effect of position on body size was observed for larvae in the mesocosms. The model with stage interacting with position as fixed effect was chosen as the best fit (Table S3.2, $\Delta AICc = 5.8$). However, position only had a significant effect in stage 45 (estimate: 0.896, $p = 0.002$) and stage 57 (estimate: 0.799, $p = 0.003$) with core individuals larger than periphery individuals, and stage 51 (estimate: -0.769, $p = 0.001$) with periphery individuals larger than core individuals. Indicating no clear pattern (Fig. S3.2). For climax individuals no significant effect of position was observed in the mesocosms. The model with stage as fixed effect was chosen as most suitable (Fig. S3.3, Table S3.2, $\Delta AIC = 2.16$). No significant effect of position was observed for metamorph body size. The model with intercept was chosen as most suitable due to parsimony (Fig. S3.4, Table S3.2, $\Delta AIC = 0.42$).

Supplementary Table 3.3: 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. Only models with $\Delta\text{AICc} < 2$ are shown in the table. Predictors 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.

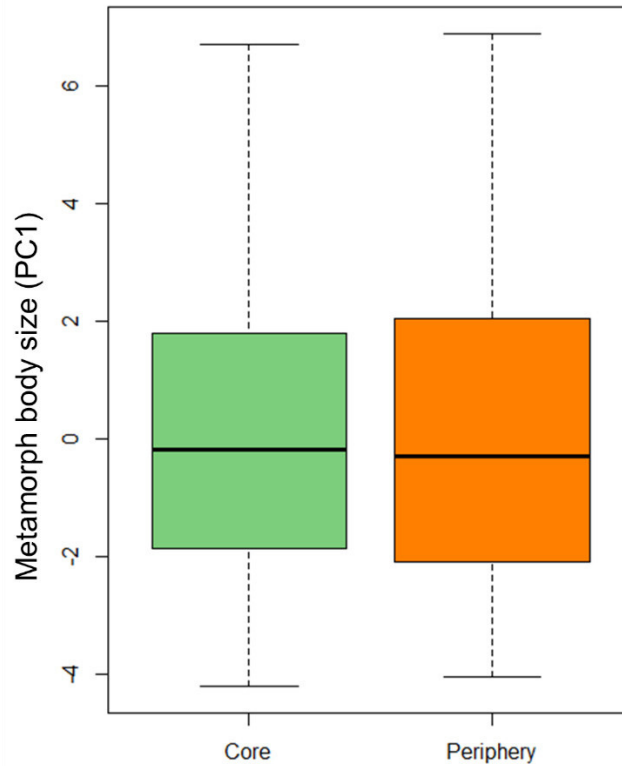
Response	Model parameters	logLik	k	AICc	ΔAICc	W_i
Larval body size (PC1)	Position x Stage	-1118.8	29.0	2297.7	0	0.9
Climax body size (PC1)	Stage	-330.8	19.0	705.2	0	0.7
Metamorph body size (PC1)	Null	-129.5	4.0	267.8	0.4	0.2
	Position	-128.9	5.0	269.0	1.58	0.1



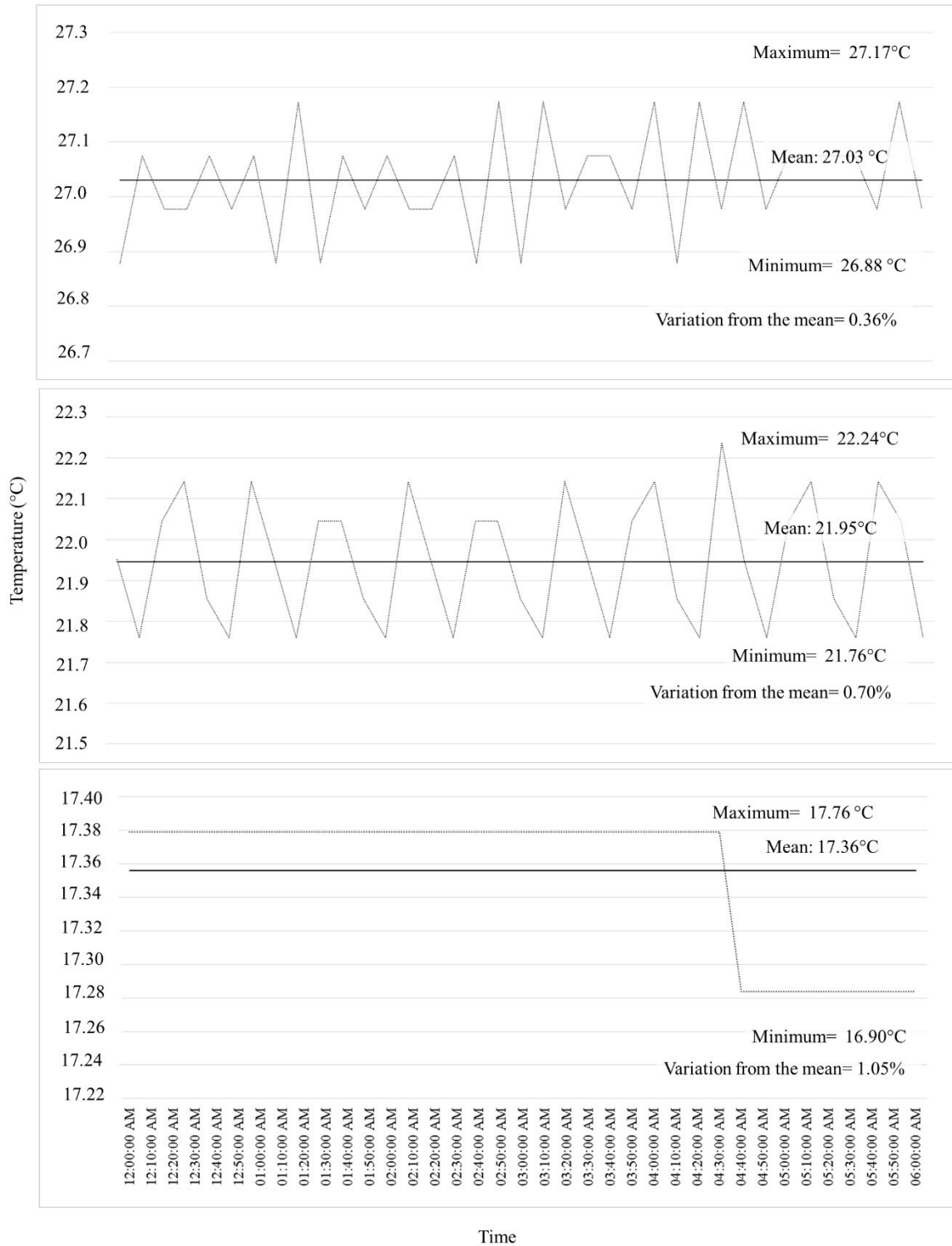
Supplementary Figure 3.2: Body size (PC1) variation during the larval development of larvae (NF stage 45-57) from the core and the periphery of the invasive range of *X. laevis* in Western France as measured from individuals raised in outdoor mesocosms. Asterisks indicates significant differences between core and periphery for specific stages. 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 indicates the 10th and 90th percentile. Points above and below the whiskers indicate outliers above and below the 10th and 90th percentile.



Supplementary Figure 3.3: Body size (PC1) variation during the larval development of climax individuals (NF stage 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. 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 indicates the 10th and 90th percentile. Points above and below and the whiskers indicate outliers above and below the 10th and 90th percentile.



Supplementary Figure 3.4: 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 outdoor mesocosm. 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 indicates the 10th and 90th percentile. Points above and below and the whiskers indicate outliers above and below the 10th and 90th percentile.



Supplementary Figure 4.1: Temperature profiles for high (27 °C), intermediate (22 °C), and low (17 °C) temperature acclimation tanks. On the graph maximum, average, minimum, and variation from the mean are indicated.